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Two New Corneal Diseases Characterized by Recurrent Erosions

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Cover illustration: Coat of arms and location of the two Swedish counties Småland (lat. Smolandia) and Hälsingland (lat. Helsingia or Helsinglandia) wherefrom the two corneal disorders described in this thesis originate.

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To my family and in memory of my father

Turmoil accompanies every great change.

The dragon Saphira to Eragon in the novel *Brisingr* by Christopher Paolini

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LIST OF PAPERS

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals:

- I. Hammar B, Björck E, Lagerstedt K, Dellby A and Fagerholm P (2008): A New Corneal Disease with Recurrent Erosive Episodes and Autosomal Dominant Inheritance. *Acta Ophthalmol* 86:758-763.
- II. Hammar B, Lagali N, Ek S, Seregard S, Dellby A and Fagerholm P (2008): Dystrophia Smolandiensis - recurrent corneal erosions with a novel morphological picture. *Acta Ophthalmol* accepted for publication 2009-02-10
- III. Hammar B, Björck E, Lind H, Lagerstedt K, Dellby A and Fagerholm P (2008): Dystrophia Helsinglandica - A New Type of Hereditary Corneal Recurrent Erosions with Late Subepithelial Fibrosis. *Acta Ophthalmol* 2008 Aug 11. [Epub ahead of print]
- IV. Neira W, Hammar B, Holopainen JM, Tuisku I, Dellby A, Tervo T and Fagerholm P: Dystrophia Helsinglandica - corneal morphology, topography and sensitivity in a hereditary corneal disease with recurrent erosive episodes. Manuscript.



ABBREVIATIONS

<i>CHST6</i>	Carbohydrate sulfotransferase 6 gene
<i>COL8A2</i>	Collagen, type VIII, alpha 2 gene
<i>DCN</i>	Decorin gene
<i>GSN</i>	Gelsolin gene
<i>KRT3</i>	Keratin 3 gene
<i>KRT12</i>	Keratin 12 gene
<i>M1S1</i>	Membrane component, chromosome 1, surface marker 1 gene
<i>PAX6</i>	Paired box gene 6
<i>PIP5K3</i>	Phosphatidylinositol-3-phosphate 5-kinase, type III gene
<i>SLC4A11</i>	Solute carrier family 4 (sodium borate cotransporter), member 11 gene
<i>STS</i>	Steroid sulfatase (microsomal), isozyme S gene
<i>TGFBI</i>	Transforming growth factor, beta-induced gene
<i>UBLAD1</i>	UbiA prenyltransferase domain-containing protein 1 gene
<i>ZEB1</i>	Zinc finger E box-binding homeobox 1



INTRODUCTION

The morphology and physiology of the cornea

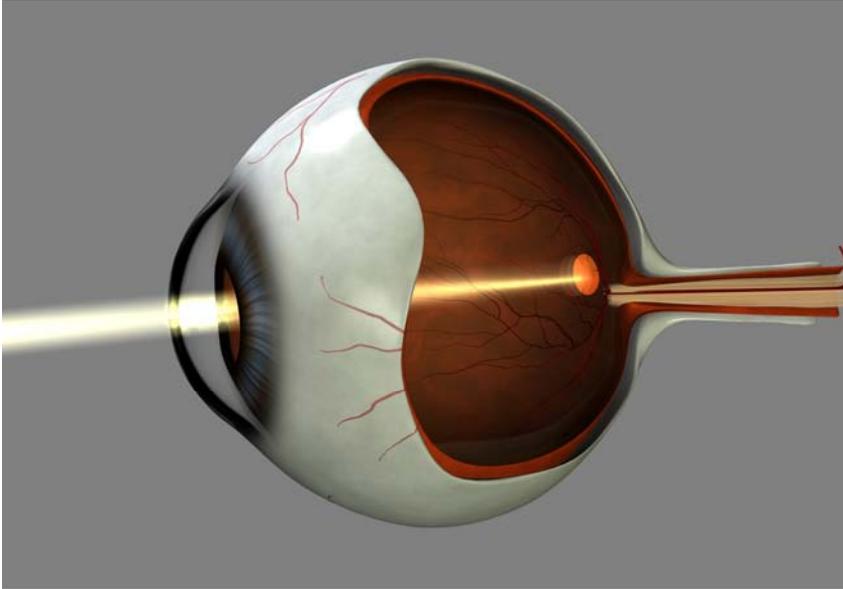


Figure 1. Exterior and interior aspects of the human eye (Courtesy of Dr. Fredrik Ghosh, Dep. of Ophthalmology, Lund, reproduced with permission.)

If the eye is the window to the soul, this is mainly dependent on the clarity of the cornea. With a clouded cornea the eye is dead and the soul invisible. Cornea fulfils its two primary functions: to focus the images of the world around us on the retina and to protect the inner eye, with admirable economy of means and unsurpassed precision of structural design (Fig. 1).

The avascular and convex cornea is covered by tear film on its anterior side and by the aqueous humor on its posterior side. Alterations in the components of tear fluid or the aqueous humor may result in pathological changes in the cornea. The tear film has nutritive and protective functions and also supplies factors essential for corneal healing (Malecaze et al. 1997; Kumagai et al. 2002; Maguen et al. 2002).

In the adult, the corneal surface is slightly oval and approximately 11 mm in diameter and 0.5 mm thick (Mishima 1968). The optical properties of the cornea are determined by its transparency, surface smoothness, convexity, and refractive index. A slight change of any of these parameters will affect clarity and function of the cornea. The transparency is due to the unique composition of the cornea allowing light to pass through it without scattering. The corneal smoothness is maintained by the corneal epithelium and the tear film, and in individuals with dry eyes, e.g. Sjögren syndrome, the cornea often has a rough and pitted corneal surface. Pathological conditions that change the corneal contour will lead to regular or irregular astigmatism.

The cornea consists of three different cellular layers: the epithelium, the stroma, and the endothelium, and two interfaces: Bowman's layer, and Descemet's membrane (Fig. 2). Each layer consists almost exclusively of a single type of highly differentiated cells that are essential for proper functioning of the cornea, and include epithelial cells, keratocytes (corneal fibroblasts), and endothelial cells. The cornea is densely innervated with sensory and autonomic nerve fibers.

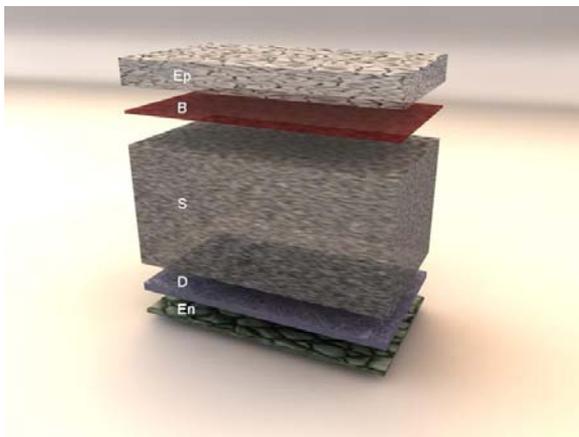


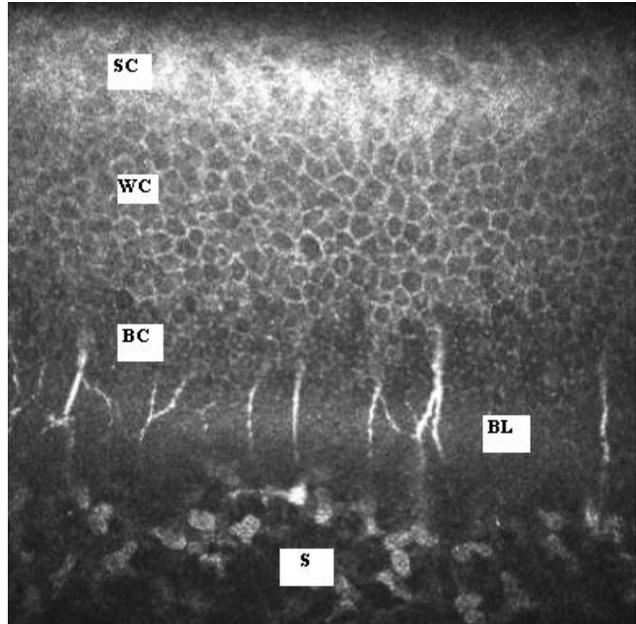
Figure 2. The layers and interfaces of the cornea. From top to bottom: epithelium (Ep), Bowman's layer (B), stroma (S), Descemet's membrane (D), and endothelium (En). (Courtesy of Dr. Fredrik Ghosh, Dep. of Ophthalmology, Lund, reproduced with permission.)

Epithelium

The corneal epithelium is composed of nonkeratinized, stratified squamous epithelial cells and is approximately 50 μm thick (10% of the total thickness of the cornea). It consists of five or six layers of three different types of epithelial cells: two or three layers of superficial cells, two or three layers of wing cells, and a monolayer of columnar basal cells, the latter of which adhere to the basement membrane adjacent to Bowman's layer (Fig. 3). Generally confined to the peripheral part of the epithelium Langerhans cells can be found. These dynamic and reactive cells have the capacity of antigen presentation (Mayer et al. 2007). Superficial cells are flat and covered with microvilli which greatly increase the surface area of each cell promoting the exchange of oxygen, nutrients and metabolic products (Collin & Collin 2006). In their outermost cell membrane, glycocalyx, oligosac-

charide-containing molecules interact with the mucinous layer of the tear film and help to maintain its trilayered structure. Damaged glycocalyx and injured superficial epithelial cells provide specific sites for attachment of bacteria that may lead to infection (Latkovic & Nilsson 1995). The presence of junctional complexes between adjacent corneal epithelial cells prevents passage of substances and microbes into the deeper layers of the cornea.

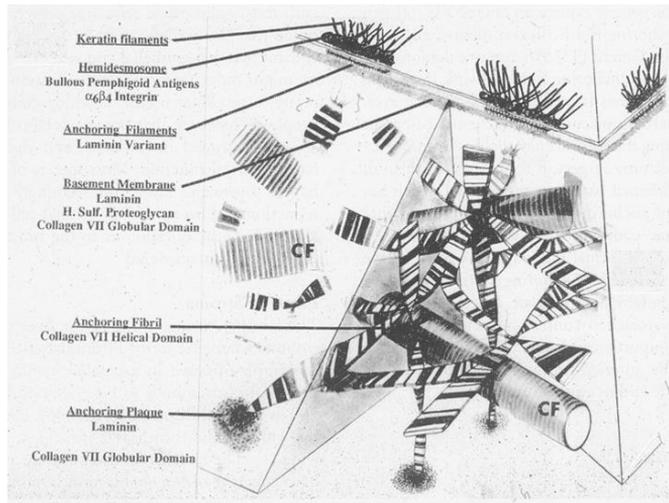
Figure 3. Cross sectional view of the corneal epithelium by HRT3 laser scanning confocal microscopy showing (SC) superficial cells, (WC) wing cells, (BC) basal cells, (BL) Bowman's layer, and (S) anterior stroma (Courtesy of Dr. Neil Lagali, Dep. of Ophthalmology, Linköping, reproduced with permission.)



The basal cells are the only ones that can proliferate, and their daughter cells differentiate into wing cells and subsequently into superficial cells, gradually desquamating into the tear film. This process takes about 7 to 14 days (Hanna et al. 1961). New epithelial cells come from a pool of stem cells at the limbus of the cornea (Davanger & Evensen 1971; Daniels et al. 2001), and then migrate towards the central corneal surface. A balance of mitosis, migration, and desquamation maintains the steady state.

The basal cells synthesize type IV collagen and laminin, which are the major components of the basement membrane. The presence of the basement membrane between the basal epithelium and the underlying Bowman's layer determines the polarity of the epithelial cells and provides a matrix on which cells can migrate and is thought to be important for maintenance of the stratified and well-organized corneal epithelium. Fibronectin is thought to provide a temporary matrix over which cells can attach and migrate during this transition period. During wound healing, the epithelial cells express integrins, i.e. adhesion receptors inducing binding to fibronectin. Thus, although type IV collagen and laminin are essential for maintenance of the steady state, fibronectin and integrins appear to play important roles under conditions of acute injury to the corneal epithelium (Nishida et al.1984).

Figure 4. Diagram of the anchoring complex. Anchoring fibril network with insertions into basement membrane below hemidesmosomes (HD). Branching and anastomosing cross-banded fibrils splay among type 1 collagen fibrils (CF), and some anchoring fibrils insert into dense patches of extracellular matrix which are composed of non-helical domains of type VII collagen molecules and other basal lamina components, so called anchoring plaques (AP) (adapted after Gipson et al. 1987).



The anchoring complex is a three-dimensional network of different linked structures holding the epithelium tightly to the stroma (Fig. 4). This complex consists of anchoring fibrils, anchoring plaques, and hemidesmosomes. The long fibrils of type VII collagen aggregate to form wirelike structures, anchoring fibrils, which extend from the hemidesmosomes in the basement membrane and into Bowman's layer. In the stroma the anchoring fibrils form anchoring plaques together with type I collagen (Gipson et al. 1987).

Bowman's layer

Bowman's layer is the anterior portion of the corneal stroma and is an acellular membrane-like zone of randomly arranged collagen fibers and proteoglycans which is 12 μm thick. Its function is unclear and it does not regenerate after injury.

Stroma

The stroma constitutes more than 90% of the corneal thickness. It consists of extracellular matrix, keratocytes, and nerve fibers. The total volume of the stroma is mostly made up of collagen and glycosaminoglycans, and only 2% to 3% of cells (Otori 1967). The collagen fibers form about 300 lamellae that are parallel to the surface of the cornea and between them spindle-shaped keratocytes are scattered (Hamada et al. 1972). The glycosaminoglycans have the ability to absorb and retain large amounts of water. Keratocytes synthesize collagen and glycosaminoglycans among others, and also collagen-degrading enzymes like matrix metalloproteinases. The regulation of corneal hydration is accomplished predominantly by an endothelial pump; it is also influenced by the epithelial barrier, surface evaporation, intraocular pressure, and stromal swelling pressure.

Descemet's membrane

Descemet's membrane is the basement membrane of the corneal endothelium, 8-10 μm thick, and composed of collagen type IV, laminin and fibronectin. It reflects any change in the shape of the stroma. Thus a swelling of the stroma will result in the folding of Descemet's membrane.

Endothelium

A single layer of corneal endothelial cells covers the posterior surface of Descemet's membrane. The cells are polygonal, 5 μm thick and 20 μm wide, and the cell density decreases with age. The most important physiological function is regulation of corneal hydration (Maurice 1972).

Corneal nerves

The cornea is one of the most densely innervated and most sensitive tissues in the body. The density of nerve endings in the cornea is about 300 to 400 times greater than that in the skin (Rózsa & Beuerman 1982).

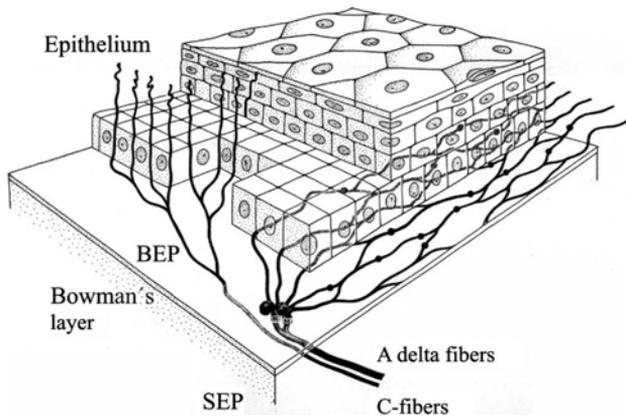


Figure 5. Drawing of corneal epithelial innervation; SEP, subepithelial plexus; BEP, basal epithelial plexus (adapted after Stachs et al. 2006).

The cornea is mainly innervated by sensory nerves derived from the ciliary nerves of the ophthalmic branch of the trigeminal nerve, but also by autonomic nerves. The long ciliary nerves provide the perilimbal nerve ring. Nerve fibers penetrate the cornea in the deep peripheral stroma and then run radially parallel to the epithelium. The nerve fibers lose their myelin sheath within a short distance into the cornea. They turn towards the surface, penetrating Bowman's layer and basement membrane forming sub-basal plexa, and finally terminate at the wing cell level as nerve endings (Müller et al. 2003)(Fig. 5). Loss of the superficial corneal epithelium results in exposure of the nerve endings and consequent pain.

The development of the cornea

The development of the anterior segment of the eye is complex. The corneal epithelium is derived from surface ectoderm, while the stroma and endothelium are derived from cranial neural crest cells (Beauchamp & Knepper 1984).

Congenital eye defects such as cataract, microphthalmia, aniridia, and coloboma are relatively common. 20 million children under the age of 16 years in the world have cataract. Knowledge about the molecular regulation of eye development is important to understand congenital diseases. During the past 10 years several master control genes that regulate pathways of development and differentiation have been identified, e.g. *PAX6*.

These genes are expressed early in the embryogenesis and initiate a cascade of gene expression that result in specific cell lines, transcription factors, proteins etc. Mutations in the master control genes, usually give rise to severe congenital eye defects (Lee 2008), whereas mutations in other genes, e.g. *KRT3*, *TGFBI*, result in hereditary diseases of less severe consequence, such as corneal dystrophies like Meesmann's dystrophy and Reis-Bücklers' dystrophy. In recent years the understanding of how specific genes are involved in the pathogenesis of corneal disorders has improved, leading to a different classification of inherited corneal disorders.

Corneal dystrophies

Corneal dystrophies are a group of disorders defined as corneal alterations that are commonly bilateral and progressive, occur after birth, and are not inflammatory (Waring 1978). Located in the central cornea, they are generally less evident at the limbus. They usually only involve one corneal layer. Most corneal dystrophies are autosomal dominantly inherited. The clinical manifestations of the corneal dystrophies depend largely on which layer of the cornea is affected. Therefore corneal dystrophies are commonly subdivided depending on its specific location within the cornea, but that is currently changing towards a classification based on a combination of clinical and genetic features (Weiss et al. 2008). The epithelial dystrophies are typically symptomatic with recurrent corneal erosions and/or the presence of irregular corneal astigmatism resulting from abnormalities in the epithelium, the corneal basement membrane, or Bowman's layer. The stromal dystrophies cause opacification from deposition of generated abnormal material. The endothelial dystrophies are characterized by the development of edema due to dysfunctional endothelial hydration regulation (Maurice 1972).

Corneal erosions

A superficial corneal wound occurs when there is partial or total loss of the epithelial layer. It is one of the most common ocular injuries and especially prevalent among contact lens users. Studies have estimated that corneal abrasions account for 10% of new patient visits to the eye emergency room (Chiapella & Rosenthal 1985), and that the third most common primary ocular diagnosis is corneal abrasions (Carter & Miller 2001). Symptoms of pain, epiphora, blepharospasm, photophobia, foreign body sensation, and redness are typical. Visual acuity varies from normal to foggy, or even severely reduced (when the erosion is centrally localized). The goal of therapy is to reduce discomfort, promote reepithelialization and wound healing, and prevent complications. Small erosions may require no specific treatment as they usually heal without complications within 1-3 days. Larger erosions are typically treated with a lubricant for a few days, usually a topical antibiotic to prevent secondary infection. Additionally, cycloplegic agents are used to minimize ciliary spasm, and eye patching is used to improve comfort. In severe cases topical anesthetics are used initially for continued pain control but with caution, since they can reduce healing and cause secondary keratitis. The most common complications are bacterial keratitis and recurrent corneal erosions.

Recurrent corneal erosions

Recurrent superficial corneal wounds or recurrent corneal erosions were first described in the scientific literature at the end of the 19th century by Hansen (Hansen 1872). He reported six cases of recurrent corneal erosions, all with an initial trauma. He thought that the corneal nerves were injured and resulted in a traumatic neuralgia, and therefore called it "intermittent Keratitis neuralgica vesicularis". The term recurrent erosions was coined in 1874 by von Arlt (von Arlt 1874), and von Szily gave the first detailed account of the signs and symptoms of the condition in 1900 (von Szily 1900). Chandler subdivided recurrent corneal erosions into a macroform with severe attacks, large epithelial loss but low frequency that may take 1 to 21 days to resolve, and a microform characterized by high frequency, less severe attacks and short duration (1-4 days) (Chandler 1945; Brown & Bron 1976). The microform was most common in spontaneous cases, and the macroform most commonly seen secondary to trauma (Brown & Bron 1976). However, the two forms can co-exist and there is no sharp distinction between the two forms. Bron & Burgess defined recurrent corneal erosions in two ways, pathologically as a defective adhesion of the corneal epithelium to the underlying Bowman's layer, and clinically as a symptom complex including early morning waking with pain, difficulty in opening the lids, followed

by watering of the eyes, photophobia, and irritation (Bron & Burgess 1981). There are few data on the incidence and prevalence of all types of recurrent corneal erosions. The incidence of recurrent corneal erosions following trauma has been estimated at 1 in 150 cases (Jackson 1960). In 2003 at the Lund University Hospital 2.4 % (1012 visits out of a total of 43 431) of all outpatient visits were associated with superficial corneal wounds or corneal erosions. Of these 1012 cases, 17 % (172) progressed to become recurrent superficial corneal wounds or recurrent corneal erosions. Aggravating or precipitating factors can be tiredness, menopause, menstruation, eye rubbing, barbiturates, and alcohol (Brown & Bron 1976; Hope-Ross et al. 1994).

Recurrent corneal erosions most commonly arise following a trauma, in association with various corneal dystrophies, or are idiopathic (Öhman & Fagerholm 1998). They may also occur secondary to corneal degenerations (Salzmann's nodular degeneration, bandkeratopathy), herpes virus infections, lagophthalmus, keratoconjunctivitis sicca, bacterial ulcers, and systemic diseases (diabetes mellitus, epidermolysis bullosa) (Das & Seitz 2008) (Table 1).

Table 1. Etiologies of Recurrent Corneal Erosions

I. Primary	
a)	Epithelial basement membrane dystrophy
b)	Dystrophy involving Bowman's layer Reis-Bücklers' dystrophy, Thiel-Benke dystrophy
c)	Stromal dystrophy Lattice, Macular, and Granular dystrophies
d)	Endothelial dystrophy
II. Secondary	
a)	Degeneration Band keratopathy, Salzmann's nodular degeneration
b)	Trauma Epithelial abrasion, Chemical and thermal injury
c)	Eyelid pathology Entropion, ectropion, lagophthalmus, Meibomian gland dysfunction, blepharitis
d)	Following ocular infection Bacterial and viral keratitis
e)	Following refractive surgery Laser in situ keratomileusis, photorefractive keratectomy
f)	Systemic causes Diabetes mellitus, epidermolysis bullosa, juvenile x-linked Alport's syndrome
g)	Miscellaneous Keratoconjunctivitis sicca, bullous keratopathy, idiopathic, Münchhausen syndrome

Modified after Das & Seitz 2008

Hereditary recurrent corneal erosions typically occur as a result of corneal dystrophies, but also secondary to inherited systemic diseases. In 1928 Franceschetti described a family in which six successive generations had a tendency towards recurring erosions of the cornea. The disease generally becomes manifest at the age of 4 to 6 years. The first attack is often secondary to a small corneal trauma. With increasing age these attacks, which gen-

erally occur in the morning, become rare and less intense, being exceptional after the age of 50 years. Similar reports like the one Franceschetti made in 1928 have been made by other authors (Berardi & Motolese 1938; Wales 1955; Holt 1956; Remler 1959; LeGrand 1963; Shindo 1968; Bron & Burgess 1981), the latter authors also proposed a classification for these disorders (Table 2).

Table 2. Classification of Inherited Recurrent Corneal Erosions

- I. Primary epithelial dystrophies
 - a) Franceschetti type
 - b) Epithelial rosette dystrophy
 - c) Basement membrane disorder
 - d) Meesmann's dystrophy

 - II. Secondary to other corneal dystrophies
 - a) Anterior limiting membrane dystrophies
 - b) Stromal dystrophies
 - c) Endothelial dystrophies

 - III. Secondary to inherited systemic disease
 - a) Epidermolysis bullosa
 - b) Other
-

Modified after Bron & Burgess 1981

The precise pathogenesis of recurrent corneal erosions is unclear, but is thought to be explained by pathologic changes involving different levels or molecules of the anchoring complex, e.g. a defect in collagen fibrils secondary to trauma or a defect at the level of the basement membrane in epithelial basement membrane dystrophy (Chen et al. 2006). Also degradation by matrix metalloproteinase enzymes of the anchoring complexes is believed to cause some of the pathologic changes (Garrana 1999).

Recurrent corneal erosions is typically diagnosed on the basis of clinical history of repeated episodes of ocular symptoms that develop with or without a prior corneal erosion or injury. These episodes are interspersed with asymptomatic periods. Clinically visible corneal changes are epithelial lesions (epithelial loss or separation, epithelial microcysts, or other specific epithelial lesion).

Several changes and morphological alterations in the cornea have been reported in association with recurrent corneal erosions. The ultrastructural changes include abnormal basal epithelial cell layer, abnormal epithelial basement membrane, absent or abnormal hemidesmosomes, and loss of anchoring fibrils (Brown & Bron 1976; Tripathi & Bron 1972). Binucleated cells and multinucleated giant cells have been found in the corneal epithelium (Aitken et al. 1995). The segmental absence of hemidesmosomes and in some cases the absence of entire thickness of basement membrane are main electron micro-

scopic characteristics of recurrent corneal erosions, but also thinning and splitting of basement membrane can be present (Tripathi & Bron 1972).

In individuals with recurrent corneal erosions and no associated corneal disorders, confocal biomicroscopy has revealed (Rosenberg et al. 2000):

- deposits in basal corneal epithelial cells
- microfolds of the basement membrane
- streaks in the basal epithelial cell layer
- damaged subbasal nerves
- altered morphology of the anterior stroma

Treatment of recurrent corneal erosions syndrome is aimed at the same goals as for corneal erosions, and the same therapeutic modalities are used initially. In recalcitrant cases, bandage soft contact lenses can be applied for 6 to 8 weeks; these protect the cells that are reepithelializing from the contact of the eyelids and reduce pain. Tetracycline inhibits matrix degrading enzymes (metalloproteinase-9) (Dursun et al. 2001) and can be used as adjuvant therapy. The invasive forms of treatment for recalcitrant recurrent corneal erosions include various surgical techniques, such as anterior stromal micropuncture and superficial keratectomy. In the former technique a needle or laser stimulates corneal bindings that adhere loose segments of epithelium to the stroma (Geggel & Maza 1990). In the latter one the epithelium is removed mechanically or by laser to activate a regeneration of basement membrane and anchoring complexes (Watson & Barker 2007).

Corneal wound healing

The epithelial wound healing can be divided into three phases – the latent phase, cell migration, and cell proliferation (Lu et al. 2001).

The preparatory, or latent, phase (four to six hours following injury) is a period of build up in the epithelial cells adjacent to the corneal wound, and it is necessary for the launch of the next phase (Crosson et al. 1986). A superficial wound triggers a cascade of preparatory events consisting of degradation and removal of necrotic cells and debris (Holopainen 2003), modulation of cell cytoskeleton (Anderson 1977), reduction of intercellular attachments (Kurpakus 1991), and formation of cellular extensions, so called pseudopodia. Fibronectin is deposited on the denuded surface immediately after epithelial or stromal injury and provides a temporary matrix for epithelial cell migration (Nishida 1983). Cell

surface proteins, integrins, mediate changes of cell cytoskeleton and cell adhesion to the matrix (Gipson et al. 1993).

The hallmark of the next phase is cell migration and cell adhesion that usually lasts 24-36 hours depending on the size of the corneal wound (Crosson et al. 1986). The pseudopodia move towards the center and drag the leading edge of epithelial cells forward. The cells detach and reattach in a cyclic manner until the epithelial defect is closed, then inhibition signals end cell migration and restoration of normal epithelial anchoring begins that can continue for 6-8 weeks (Khodadoust et al. 1968).

In the final phase epithelial cells proliferate to fill in the remaining wound defect, and the remodeling can continue for as long as several months depending on the severity of the wound. Affected sensory innervation of the cornea results in impaired epithelial wound healing (Baldwin & Marshall 2002), because of decreased mitotic rate, increased permeability, and impaired cell attachment. Regenerated corneal nerves have a different orientation and appearance, and often decreased corneal sensitivity (Rosenberg et al. 2000; Patel & McGhee 2008). Activation of stromal keratocytes can lead to collagen and proteoglycan deposition resulting in scar formation (Jester et al. 1999).

Genetics

A landmark in the history of genetics was when Watson and Crick discovered the structure of deoxyribonucleic acid (DNA) in 1953 (Watson & Crick 1953). When a cell divides, DNA is densely packed into chromosomes that can be visualized during cell division. A normal human cell has 46 chromosomes.

The genome is the complete DNA sequence that contains all the genetic information of a species. The number of genes in man has been debated but has recently been estimated to 20 000-25 000. The nucleus of every cell contains a copy of the genome, but only a fraction of the genes are actively expressed in a given cell. Genes can influence the disease panorama in different ways, by directly causing disorders through alterations (mutations) in genes or through interactions with environmental factors.

Diseases afflicting the cornea have been mapped to several chromosomes and genes, and Online Mendelian Inheritance in Man (OMIM 2009) lists over 100 hereditary corneal disorders, including systemic and developmental disorders. Advancements in molecular genetics have generated new knowledge that will hopefully lead to a better understanding of disease mechanisms behind vision-threatening corneal dystrophies and change the criteria of classification. For example, Meesmann's dystrophy can be caused by mutations in two different genes whereas mutations in *TGFBI* gene lead to at least six different phenotypes.

At present molecular genetic studies of corneal dystrophies suggest that at least 9 chromosome loci are involved: 1, 2, 5, 9, 10, 12, 16, 17, and 20. In these 9 chromosomes specific mutations in 13 genes have been identified in approximately 50 corneal dystrophies: *KRT3*, *KRT12*, *TGFBI* (*BIGH*), *GSN*, *MIS1*, *CHST6*, *UBLAD1*, *DCN*, *PIP5K3*, *COL8A2*, *ZEB1*, *SLC4A11*, and *STS* (Weiss et al. 2008). Currently, genetic studies on inherited diseases affecting the cornea have provided insight into some of these disorders at a basic molecular level and it has become recognized that distinct clinicopathologic phenotypes can result from specific mutations in a particular gene, as well as some different mutations in the same gene. A molecular genetic understanding of inherited corneal diseases is leading to a better appreciation of the pathogenesis of these conditions. This knowledge has made it imperative to revise the classification of inherited corneal diseases, and to use clinical and genetic features simultaneously.

AIMS OF THE STUDY

The general aim of this thesis was to investigate two hereditary corneal diseases with recurrent erosions in order to find out if they had been described before. The specific aims were to

- describe the clinical picture in the families with autosomal-dominant recurrent corneal erosions
- describe the morphological changes in the families with autosomal-dominant recurrent corneal erosions
- differentiate them from other known autosomal dominant corneal dystrophies with a clinical resemblance
- exclude genetic linkage to known corneal dystrophies with autosomal-dominant inheritance and a clinical resemblance



SUBJECTS AND MATERIALS

All subjects were treated in accordance with the tenets of the Declaration of Helsinki, and approval from the local Ethics Committees at the University of Linköping, the Karolinska Institutet, and Uppsala University was obtained. Informed consent was obtained from all patients.

Dystrophia Smolandiensis – Papers I and II

A six-generation family, consisting of altogether 171 individuals was studied. Forty-four individuals (26 female and 18 male) were affected, of which 37 were alive. Twenty-eight individuals were examined and interviewed extensively (21 affected and seven unaffected) at the University Hospital in Linköping. Medical records from the patients who had undergone corneal grafting and from the affected family members who were not examined were collected. A further 16 affected family members who were not examined were interviewed to obtain their medical history. A general and ophthalmological medical history was obtained, including 35 non-affected family members, and a pedigree was established.

Four corneal tissue samples following penetrating keratoplasty and diagnostic biopsy were obtained from three affected individuals and were subsequently processed for routine histology and immunohistochemistry. Additionally, seven affected and two unaffected individuals were examined by in-vivo confocal microscopy. Four affected and two healthy members were examined using a slit-scanning system. Another three affected individuals were examined using a laser-scanning system.

Dystrophia Helsinglandica – Papers III and IV

A seven-generation family including 342 individuals, of whom 84 were affected (41 females and 43 males) was studied. Of the affected individuals 64 were alive. Forty-three individuals of the family were examined and interviewed extensively (32 affected and 11 unaffected individuals) at Bollnäs Hospital and the University Hospital in Linköping, Sweden.

Nine affected and nine unaffected individuals underwent an additional comprehensive ophthalmic examination including slit-lamp biomicroscopy, videokeratography, corneal

sensitivity measurement, and in-vivo confocal microscopy of the cornea using a slit-scanning system. One eye from each individual (the most affected eye in the affected individual and the eye chosen by the patient in the non-affected) was included in the study.

METHODS

Medical history and clinical ophthalmological examination – Papers I,II,III, and IV

The ophthalmological history included details concerning age at onset, characterization of attacks, intensity of the disease over time, visual capacity, and attempts at therapy. Visual acuity and refraction were measured, and recording of intraocular pressure was made. The cornea was examined carefully in the slit-lamp biomicroscope, and photography of all abnormalities was performed. The fundus was examined by indirect ophthalmoscopy.

Pedigree analysis – Papers I and III

One method for determining the pattern of inheritance of any trait (a physical attribute or a disease) is to study its occurrence in several individuals within a family, spanning as many generations as possible. To determine who is affected, living family members have to be examined for a disease trait. The same information may be difficult to obtain, and is often incomplete, about more distant and dead relatives.

Once the family history is determined, the information is drawn up in the form of a diagram or family tree showing the relationships between individuals using a particular set of standardized symbols, a pedigree. In a pedigree (see e.g. page 759 of Paper I), males are represented by squares \square and females by circles \circ . An individual who exhibits the trait in question is represented by a filled symbol \blacksquare or \bullet . A dead individual is represented with a dash over the symbol, e.g. $\overline{\circ}$. A horizontal line between two symbols represents a mating $\square-\circ$. The offspring are connected to each other by a horizontal line above the symbols and to the parents by vertical lines. Roman numerals (I, II, III, etc.) symbolize generations. Arabic numerals (1, 2, 3, etc.) symbolize birth order within each generation. In this way, any individual within the pedigree can be identified by the combination of two numbers (i.e., individual II:3).

A pedigree analysis can determine whether the disease is inherited and establish the mode of inheritance. This information can then be used to predict recurrence risk in future generations, and to estimate gene penetrance and gene expressivity.

In-vivo confocal microscopy – Papers II and IV

In-vivo confocal microscopy has overcome the limitations of the conventional microscope to provide high-contrast imaging in thick, light-scattering specimens such as biological tissue. Minsky tried limiting sample illumination to a point and focusing the collected light with a lens identical to the objective lens onto a pinhole. He found that scattered light from outside the focal plane at the sample was blocked by the pinhole, allowing only light from the focal plane to pass through to a detector (Minsky 1988). Because both lenses are arranged to have symmetric (or 'conjugate') focal planes, the microscope was termed 'confocal'. The result was a dramatic sharpening of the image and an increased depth resolution that enabled optical 'slicing' of samples for three-dimensional imaging, but with the trade-off that the sample needed to be scanned point-by-point to build up an image. In early instruments this scanning was achieved by means of a rotating disk with a spiral hole pattern (Nipkow disk) used to raster-scan the sample, and later a more complex, symmetric version of the disk gave rise to the tandem-scanning confocal microscope (Fig. 6).

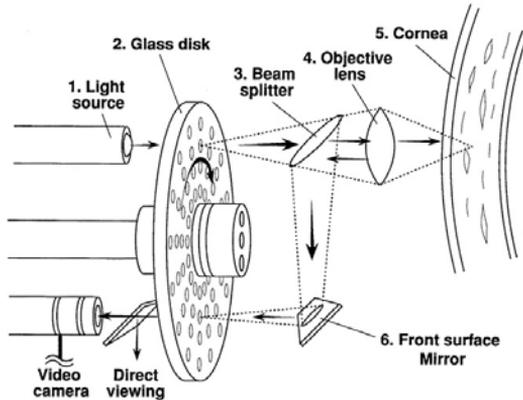


Figure 6. Tandem scanning confocal microscope (adapted after Cavanagh et al. 2000).

Further technological improvements in video-rate imaging, the use of slit-scanning and laser-scanning techniques have provided increased image quality, real-time scanning capability, and in the case of the laser scanning confocal microscope, the highest possible depth resolution and diffraction-limited lateral resolution. These developments have enabled confocal microscopy in-vivo at a cellular and subcellular level with sufficient magnification and resolution which before had only been possible with light or ultrastructural microscopy on fixed dead tissue preparations, so-called histopathological specimens.

Light and ultrastructural microscopy not only introduce artefacts in interpreting the function of living cells in tissues, but they are also static techniques.

The easy accessibility of the cornea for examination, combined with its optical transparency and thickness makes it an ideal tissue for in-vivo confocal examination. Several investigators have provided extensive reviews of the exploding field of confocal microscopy of the cornea (Jalbert et al. 2003; Patel & McGhee 2007; Zhivov et al. 2008). Confocal microscopy provides for non-invasive optical sectioning and observation of the corneal epithelium, epithelial basal lamina, stromal keratocytes, and nerves as well as the corneal endothelium (Fig. 7). At a practical level, proper orientation of the cornea during confocal examination is critical in obtaining flat-field images in which accurate three-dimensional epithelial, keratocytes or endothelial cell shapes, areas, and interactions can be verified. Because the eye is often moving, continuous manual adjustment by an operator is needed to keep the objective perpendicular to the cornea.

In this study, both the slit-scanning system (ConfoScan P4 , Tomey Corporation, Erlangen, Germany; ConfoScan 3, Software Version 3.4, Nidek Technologies, Vigonza, Italy) and the laser-scanning system (HRT3-RCM; Heidelberg Retina Tomograph III Rostock Corneal Module, Heidelberg Engineering GmbH, Heidelberg, Germany) were used.

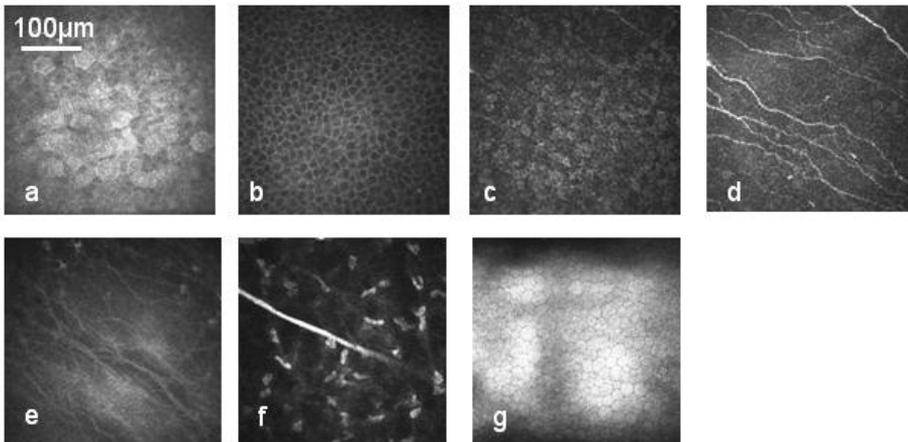


Figure 7. In-vivo confocal microscopy of a normal human cornea. Images of superficial cells (a), wing cells (b), basal cells (c), subbasal nerve fiber bundles (d), Bowman's layer (e), stroma,(f), and endothelium (g) are shown. (Courtesy of Dr. Neil Lagali, Dep. of Ophthalmology, Linköping, reproduced with permission.)

Noncontact gas esthesiometer – Paper IV

Pathological changes in the cornea may affect corneal nerves and thereby alter the corneal sensitivity. Corneal sensitivity can be measured in different ways. One technique is the Belmonte modified non-contact esthesiometer (Cooperative Research Center for Eye Research and Technology, Sydney, Australia) (Belmonte & Giraldez 1981). With a non-contact gas esthesiometer the mechanical sensitivity is measured by a series of short pulses of warmed air with varying flow rates to the surface of the central cornea. The lowest reported sensation is then recorded.

Statistics – Paper IV

The sensation thresholds between affected eyes versus unaffected were compared with the Wilcoxon signed rank t-test (SPSS, Version 6.0 Northampton, MA, USA). The Wilcoxon signed-rank test is a non-parametric statistical hypothesis test for the case of two related samples or repeated measurements on a single sample. The Wilcoxon test involves comparisons of differences between measurements, so it requires that the data are measured at an interval level of measurement.

Histopathology – Paper II

Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. The most commonly used stain in histopathology is a combination of hematoxylin and eosin.

The tissue specimen obtained from affected individuals were fixated in 10 % formalin, then embedded in paraffin, and finally sectioned to a thickness of 4 μm . The sections were stained with haematoxylin-eosin, where hematoxylin is used to stain nuclei blue, while eosin stains cytoplasm and the extracellular connective tissue matrix pink. The following stains were also used: van Gieson for collagen, the periodic acid-Schiff (PAS) to mark carbohydrates (e.g. glycogen, glycoprotein, proteoglycans such as those found in basement membranes, keratocytes, and Goblet cells), and Congo Red to detect amyloid. The stained specimens were inspected in the light microscope (Axophot, Zeiss, Germany).

Immunohistochemistry – Paper II

Immunohistochemistry enables the visualization (using light or confocal microscopy) and the exact localization of expressed proteins in cells or extracellular matrix of a tissue. It uses the principle of antibodies binding specifically to antigens in biological tissues (Ramos-Vara 2005). Immunohistochemical staining is widely used in the diagnosis of abnormal cells and in visualizing the distribution and localization of biomarkers and proteins in different parts of a tissue. Two examples important for this thesis are polyclonal affinity-purified rabbit anti-human antibodies against S100A4 (A 5114) and Fibronectin. S100A4 is a calcium-binding protein that modulates cell shape and motility by interacting with components of the cytoskeleton. Studies suggest that S100A4 may be involved in the interconversions that occur between keratocytes, fibroblasts, and myofibroblasts during corneal wound healing (Ryan et al. 2003). Fibronectin plays an important role in corneal wound healing and appears at wound sites immediately after epithelial or stromal injury.

Tissue specimens were stained with polyclonal affinity-purified rabbit anti-human antibodies against S100A4 (A 5114) and Fibronectin. Polyclonal affinity-purified rabbit anti-human antibodies against S100A4 (A 5114) and fibronectin (A 245) were obtained from DakoCytomation (Glostrup, Denmark). Briefly, slides to be stained for S100A4 and fibronectin were pretreated with pronase, and after blocking of endogenous peroxidase, slides were incubated for 1 hour at room temperature with the respective primary antibody diluted to 1:250 in Tris buffered saline (TBS) containing 10 % normal bovine serum. The slides were then incubated for 30 min at room temperature with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) diluted 1:200 in 10 % normal goat serum and TBS and further incubated with the avidin-biotin complex (Vector Laboratories) according to the manufacturer's instructions. Detection was performed with 3-amino-9-ethylcarbazole (Sigma-Aldrich, St Louis, MO, USA) after which the slides were counterstained with hematoxylin.

Videokeratography – Paper IV

Videokeratography, also known as corneal topography, is a non-invasive computerized imaging technology for mapping the surface curvature of the cornea, to produce a detailed description of the shape and power of the cornea. Since the cornea is normally responsible for about two thirds of the eye's refractive power, its topography is of critical importance in determining the quality of vision. Videokeratography can also detect cor-

neal changes and conditions invisible to most conventional testing. The patient is seated facing a concave screen containing an illuminated pattern of concentric rings. The pattern is focused on the anterior surface of the patient's cornea and reflected back to a digital camera at the centre of the screen. The topography of the cornea is revealed by the shape of the reflected pattern. A computer provides the necessary analysis, typically determining the position and height of several thousand points across the cornea, and calculating the surface regularity index, surface asymmetry index, potential visual acuity, simulated keratometry, and cylinder as corneal indices (Busin et al. 1989).

Polymerase chain reaction – Papers I and III

With polymerase chain reaction it is possible to make multiple copies of a specific DNA fragment, which enables detailed genetic studies (Saiki 1988; Mullis 1987). Microsatellite markers of affected patients were analysed with polymerase chain reaction. Microsatellite markers were analysed closely located to genes causing known autosomal-dominant corneal dystrophies with a clinical resemblance.

RESULTS AND DISCUSSION

The previously described Franceschetti-type dystrophies or Epithelial Recurrent Dystrophies (Weiss et al. 2008), all share recurrent corneal erosions as a common dominating symptom. In none of these epithelial recurrent dystrophies, progressive corneal opacifications have been described. None of these dystrophies have been genetically mapped. As our two families developed different types of progressive opacification and different patterns of opacification, we found it important to further characterize the phenotypes.

Dystrophia Smolandiensis – Papers I and II

The investigation that led to the characterization of Dystrophia Smolandiensis started with two patients from Småland, a mother and her daughter, who sought help for recurrent corneal erosions. They had previously been diagnosed as Reis-Bücklers' corneal dystrophy, but on clinical examination with a slit-lamp the corneal changes were not typical of that diagnosis or any other known corneal dystrophy. Only non-specific corneal changes associated with corneal wound healing were found. This initiated further studies and a pedigree analysis of the whole family was performed. A six-generation pedigree was established that included 171 individuals, of which 44 were affected (26 female and 18 male). Differentiating between affected and non-affected family members was never difficult because of the distinct symptoms of the disorder. The pedigree showed a clear autosomal dominant hereditary pattern with many affected family members.

The next step was to gather as much information about the phenotype as possible, and therefore twenty-eight individuals were examined and interviewed (21 affected and seven unaffected) at the University Hospital in Linköping. The ophthalmologic examination was thorough and included visual acuity, corneal photography, and slit-lamp examination. The interviews were based on a questionnaire that covered different topics of general and specific ophthalmological medical history, e.g. symptoms, treatment, precipitating factors, other diseases, or course of symptoms over time. Besides the two first individuals, another 16 affected family members who were not examined and 35 non-affected family members were interviewed. Additionally, medical records from the patients who had undergone corneal grafting and from affected family members who were not examined were collected. The data that were collected showed that the disease started early in life, typically during the first year of life. And that the symptoms were most pronounced up to the early twenties. The symptoms were typical of corneal erosions. The episodes usually lasted

up to a week and were preceded by eye irritation. The sometimes protracted episodes of erosive symptoms did not differ from other diseases with recurrent corneal erosions (Brown & Bron 1976). Visual acuity was usually reduced in affected eyes for 2–7 days during an episode, but it returned to normal between episodes, except in affected individuals whose visual acuity was reduced because of development of corneal opacities.

The majority of the affected individuals stated that an attack often followed a common cold or exposure to intense sunlight or draught. During pregnancy, the affected women generally reported more frequent and severe attacks if the subsequently born child turned out to be affected. Hormonal events, such as menstruation, pregnancy, and menopause, have been identified as precipitating factors that aggravate the recurrent corneal erosions and increase their number (Brown & Bron 1976; Hope-Ross et al. 1994); many female individuals affected with *Dystrophia Smolandiensis* confirmed this.

In approximately half of the affected individuals centrally located permanent subepithelial opacities developed, varying from subepithelial fibrosis to protruding keloid-like formations. These findings are in line with a non-specific corneal response to pathologic processes associated with corneal opacity and other disturbances in transparency (Cameron 2005). Opacities were observed over a wide age range, the youngest being 7 years old, but the tendency was towards increased frequency in the elderly. No changes consistent with epithelial basement membrane dystrophy or any other previously described corneal dystrophy were observed. Histopathological examination of excised corneal buttons showed epithelial hyperplasia, subepithelial fibrosis, and a total loss of Bowman's layer in affected areas of the cornea. Local variations in epithelial thickness due to epithelial hyperplasia is a common feature seen after superficial corneal trauma and has the function of maintaining ocular surface shape (Cameron 2005). Immunohistochemistry revealed an active wound healing process in the same affected areas indicated by abundant fibronectin present in the central subepithelial stroma, and distinct immunoreactivity for S100A4 by keratocytes described in the regenerating cornea. These findings correspond with results from other studies (Filenius et al. 2003; Ryan et al. 2003). Another tissue sample of a keloid-like formation in a non-grafted cornea stained positive for amyloid using the Congo Red stain and that is strongly suggestive of actual keloid material and also pointing towards a non-specific tissue response. Amyloid accumulates in the cornea in a wide variety of disorders, both localized and systemic, many of which are genetically determined (Kenyon et al. 2005). We postulate that the keloid-like structures as well as the subepithelial fibrosis were formed as a reaction to the recurrent erosive events.

A total of seven affected individuals underwent examination by in-vivo confocal microscopy. Confocal microscopy of the corneas in affected individuals revealed a pathologic epithelium and absence of Bowman's layer in affected areas, confirming

histopathological findings. The pathologic changes in the epithelium consisted of increased cytoplasmic reflectivity in wing and basal cells and possible additional deposition of reflective extracellular material. Similar findings of intracellular accumulation and increased reflectivity of epithelial cells have been done by in-vivo confocal microscopy in cases of epithelial basement membrane dystrophy and primary recurrent corneal erosions (Hernández-Quintela et al. 1998; Rosenberg et al. 2000). The nerve alterations included a total absence of subbasal plexus or a sparse distribution which were in accordance with findings in previous studies of recurrent corneal erosions (Hernández-Quintela et al. 1998, Rosenberg et al. 2000). The two examined non-affected family members showed normal corneal morphology.

Generally, affected individuals did not seek medical attention because they had found out that there was no permanent cure. The majority obtained some symptomatic relief from conventional therapy, i.e. mainly lubricants. Interestingly, the most effective treatment seemed to be B-kombin Forte N® (ACO, Täby, Sweden), containing thiamine, riboflavin, nicotinamide, pyridoxine, calcium pantotenate, cyanocobalamine, and folic acid. Many family members, but not all, who used this drug to prevent or alleviate attacks, stated that they experienced minimal symptoms or none at all. Vitamin B complex treatment has also been used successfully to treat recurrent aphthous stomatitis, which is characterized by recurring ulcers in the oral mucosa (Wray et al. 1978; Nolan et al. 1991). Because affected individuals with Dystrophia Smolandiensis and individuals with recurrent aphthous stomatitis had fewer recurrences after vitamin B complex treatment, there may exist a common pathological pathway for both disorders. Other therapeutic techniques including bandage soft contact lenses and phototherapeutic keratectomy have had little success. In nine affected individuals corneal opacities impaired vision to such a degree that penetrating corneal grafting was performed. The procedure was performed at a mean age of 44 years (range 19–63 years). All of the grafted patients had early, slowly progressing, recurrences of opacities in the periphery of the graft, probably reflecting the pattern of invasion of epithelial cells, keratocytes and reinnervation into the graft from the host. The visual outcome was satisfactory in the majority of the individuals.

We wanted to rule out other corneal dystrophies with an autosomal dominant inheritance and a clinical resemblance (Klintworth 2003), and therefore the ones mapped to *TGFBI* (Epithelial basement membrane dystrophy, Avellino corneal dystrophy, corneal dystrophy of Bowman layer type I and II, Groenouw type 1 corneal dystrophy, and lattice dystrophy type I and IIIA), *G3N* (Lattice corneal dystrophy type II), *KRT3* (Meesmann's corneal dystrophy), *KRT12* (Meesmann's corneal dystrophy), and *COL8A2* (Fuch's endothelial dystrophy 1) were analyzed through haplotype analysis. It was performed on four individuals of the family. As no haplotype flanking was shared in affected individuals, these genes could be excluded as candidate genes causing disease in this family. Therefore, the present family has a disease that most likely is a separate entity.

Dystrophia Helsinglandica – Papers III and IV

The characterization of Dystrophia Smolandiensis directed renewed attention on hereditary recurrent corneal erosions, and eventually old data about a family in Hälsingland, Sweden with recurrent corneal erosions and a large, but sketchy, pedigree was brought forward and re-examined. The initial information, sparse medical records, pointed towards a similar, but different phenotype than Dystrophia Smolandiensis. No previous diagnosis, besides recurrent erosions had been noted.

A pedigree analysis was initiated that showed a seven-generation family including 342 individuals with 84 (41 females and 43 males) affected family members. There was no inter-connection with the pedigree of Dystrophia Smolandiensis. The mode of inheritance was found to be autosomal dominant and there was never any problem differentiating between affected and non-affected individuals.

Haplotype analysis, i.e. analysis of genetic microsatellite markers located in the vicinity of *COL8A2* (Fuch's endothelial dystrophy 1), *TGFBI* (Epithelial basement membrane dystrophy, Avellino corneal dystrophy, corneal dystrophy of Bowman layer type I and II, Groenouw type 1 corneal dystrophy, and lattice dystrophy type I and IIIA), *GSN* (Lattice corneal dystrophy type II), *KRT3* (Meesmann's corneal dystrophy), *DCN* (Congenital stromal dystrophy), and *KRT12* (Meesmann's corneal dystrophy) was performed to rule out previously mapped corneal dystrophies with an autosomal dominant inheritance and a clinical resemblance.

The next step was to thoroughly describe the phenotype, and medical examinations and interviews of forty-three individuals of the family were performed at Bollnäs Hospital and the University Hospital in Linköping, Sweden. The onset of signs and symptoms was found to be between ages of 4 and 7 years with two to five episodes per year, and from the late 20s the recurrences tended to decrease in frequency so that among affected family members over the age of 37 years recurrences were rarely seen but did not cease completely. The episodes varied in length from 1 to 10 days and also in intensity, and were characterized by the classical signs and symptoms of corneal epithelial erosion. Affected individuals often stated that an episode prevented them from performing normal daily activities, and notably symptoms did not become less severe with increasing age. The dominating precipitating factor was minor trauma, but other factors were upon awakening, draught, foreign body, common cold, lack of sleep, spontaneously (at night), cold feet or fingers, intense sunlight, allergic conjunctivitis, and menstruation. The medical history of affected individuals gave no indication of any other related disorder, neither systemic nor ocular. Visual acuity decreased during the episodes, but was normal between episodes

of recurrent erosions. No statistical differences in visual acuity were found between affected and non-affected individuals.

The affected individuals represented different stages of corneal changes from a nearly normal cornea to progressive fairly discrete subepithelial fibrosis of the central cornea. In late stages of disease progression small jellylike corneal irregularities could be seen. The unaffected members of the family were normal upon biomicroscopical examination. All of the affected individuals aged 37 years or older had permanent subepithelial opacities; the youngest with corneal opacities was 16 years old. The corneal opacities could be divided into three stages (Table 3).

Table 3. Corneal stages of Dystrophia Helsinglandica	%	Mean age (SD)
No corneal changes	23	
Stage I - Discrete localized subepithelial fibrosis in the periphery or mid-periphery	12	36
Stage II - Widespread subepithelial fibrosis, mainly in the mid-periphery	31	36
Stage III - Subepithelial fibrosis engaging the central cornea	34	64
a) gelatinous superficial elevations		
b) iron lines		

No changes consistent with epithelial basement membrane dystrophy or any other corneal dystrophy were observed on examination by slit-lamp microscopy. No fibrosis or other corneal changes were found in the unaffected family members. The fibrosis never got serious enough to merit corneal grafts or superficial keratectomy, hence no histopathological specimen exists.

To further characterize and describe the corneal morphology, nine affected and nine non-affected family members were brought to Helsinki University Hospital for additional testing and examinations.

Videokeratography showed irregular astigmatism in all subjects, no significant statistical difference between the two groups.

In-vivo confocal microscopy in affected eyes demonstrated superficial epithelial cells with elongated cell bodies, irregular basal epithelium, and abnormal subbasal nerve plexa (typically absence of subbasal nerves). Fibrosis was observed in all affected eyes, and could contribute to obscuring nerve fibers. The morphology of the stromal nerve fibers appeared altered with more reflective and thicker nerve bundles in affected than in control eyes, and in some affected corneas multiply-branched, abnormally tortuous stromal nerve fibers. Non-contact esthesiometry showed that the sensitivity was significantly lower in affected eyes, which confirmed that the corneal nerves were affected. All affected family members

showed morphological alterations in the corneal stroma, i.e. varying degrees of abnormally dense extracellular matrix, along with altered (highly reflective sometimes named activated) keratocytes. Endothelial findings were compatible with age changes in control and affected eyes. In two affected eyes endothelial pigment or guttata changes were observed and one eye showed marked polymegatism. None of the control eyes exhibited any abnormalities in the epithelium.

Nonsurgical therapy included topical lubricants, antibiotics, anesthetics, sodium chromoglicate, hypertonic agents, or local steroids and usually had little effect. Surgical modalities like etching or phototherapeutic keratectomy had little or no effect. None of the affected individuals had undergone corneal grafting. The traditional treatment had been to stay in bed in a darkened room with closed eyes and dabbing one's eyelids frequently with a cloth soaked in cold water and to move one's eyes slowly on waking before gradually opening them. Between attacks some individuals wore eye glasses to avoid minor trauma and involuntary rubbing of the eyes.

COMPARISON OF DYSTROPHIA SMOLANDIENSIS AND DYSTROPHIA HELSINGLANDICA

Table 4. Comparison of DS and DH	<i>Smolandiensis</i>	<i>Helsinglandica</i>
Onset (years)	≤1	4-7
Age of decrease of recurrences (y)	30-40	20-30
Duration of recurrence (d)	1-21	1-10
Frequency of recurrence/year	3	5
Precipitation of recurrence	Draught, sunlight, common cold	Minor trauma
Response to treatment	Vitamine B	
EBMD	No	No
Corneal grafting	Yes	No

The pedigrees were large and included 171 and 342 individuals in at least six generations. The two pedigrees never interconnected. Based on family history the type of heredity was found to be autosomal dominant in both diseases. Both diseases were dominated by recurrent corneal erosions, but the phenotypes differed. For example, the onset in Dystrophia Helsinglandica was usually at the age of 4-7 years, but in Dystrophia Smolandiensis the symptoms often started within the first year of life. The number of recurrences per year was highest from the onset and for about 20-30 years in D. Helsinglandica, and a little longer in D. Smolandiensis. Duration of recurrence was usually up to about a week, but in D. Smolandiensis the duration of recurrence could stretch up to 21 days (Table 4).

The frequency of recurrences was variable in the diseases from continuous symptoms to once a year and tended to decrease later in life. The risk of having recurrences did not disappear completely with age. A precipitating factor of recurrence was typically a minor trauma in D. Helsinglandica, as opposed to draught and common cold in D. Smolandiensis. In D. Smolandiensis about two thirds of the affected individuals responded well to oral vitamin B treatment, but no other therapy has so far been successful in either family. In D. Helsinglandica vitamin B supplementation still has to be tried. Corneal grafting has only been performed in D. Smolandiensis, and recurrences were seen in all grafts. Corneal opacifications or secondary scarring of varying type and degree were found in the families. In Dystrophia Smolandiensis opacifications were first noted at the age of about 7 years, but usually first seen at the age of 20-40 years. We believe that the opacifications are reactive corneal changes to repeated erosive events. The corneal buttons from the D. Smolandiensis family showed epithelial hyperplasia, partial or total loss of Bowman's layer, and subepithelial fibrosis in the light microscope. The deeper stroma, Descemet's

membrane, and endothelium were normal. Confocal microscopy confirmed loss of Bowman's layer and revealed that the corneal nerves either were normal in their sub-basal plexa or showed signs of regeneration. None of the morphological findings were specific.

The similarities of the two disorders were recurrent corneal erosions. They were most active in children and young adults. Morphologically the lack of or altered nerves were a common factor.

The dissimilarities were varying time of onset for symptoms, different types of corneal opacifications, different response to treatment. The phenotypes are different. The genotypes of the two diseases are presently being investigated.

CONCLUSION

We have described the phenotypes including morphological changes in two hereditary corneal conditions characterized by recurrent erosions. The two phenotypes differ in several aspects. We have excluded genetic linkage to known corneal dystrophies with autosomal-dominant inheritance and clinical resemblance. In our opinion the two diseases represent two new and separate disease entities.

According to the recent ICD3-classification, Dystrophia Smolandiensis and Dystrophia Helsinglandica belong to Epithelial Recurrent Dystrophies (ERED) (Weiss et al. 2008), and this group of dystrophies are probably much more common than is acknowledged today. Molecular genetic characterization of each of the corneal dystrophies with wide genome search will in the future hopefully lead us to more information about what mutations cause these phenotypes, and help us to accurately diagnose, counsel, and manage the diseases in affected patients.



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