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REVIEW

An erroneous glycosaminoglycan metabolism leads to corneal opacification in macular corneal dystrophy

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Macular corneal dystrophy (MCD) is a rare, potentially blinding disease whose fundamental genetic defect and exact pathogenesis are yet to be elucidated. It is, however, an especially interesting pathology, which highlights how an erroneous glycosaminoglycan or proteoglycan metabolism can induce physical symptoms in a specific connective tissue. Based on immunochemical data, MCD is a heterogeneous condition, and at least two types of the disease have been identified. The cornea, cartilage, and serum from MCD type I patients all contain an unsulphated form of keratan sulphate. In contrast, these tissues contain normally sulphated keratan sulphate in MCD type II patients. A normal population of keratan sulphate proteoglycans (and chondroitin/dermatan sulphate proteoglycans) in the cornea seems to be a requirement of corneal transparency. However, a clinical diagnosis of MCD is unable to distinguish between the keratan sulphate positive and negative types of MCD. The histopathology of MCD is fairly well established, and various corneal aberrations—such as fibrillogranular and glycosaminoglycan deposits, abnormal diameter collagen, and collagen-free lacunae—result in a breakdown of the regular corneal architecture that presumably contributes to the subsequent corneal opacification.

Keywords: cornea, glycosaminoglycans, proteoglycans

Introduction

Macular corneal dystrophy (MCD) is a rare systemic disorder, first recognized by Groenouw [1] and later described by Fuchs [2] around the turn of the century, in which an erroneous glycosaminoglycan metabolism is manifested clinically as progressive corneal opacification [3–5]. Its mode of inheritance is autosomal recessive, with more prevalent MCD populations existing in certain societies such as Iceland [6], Saudi Arabia [7] and parts of North America [4]. Despite its low occurrence in the general population, MCD has been studied extensively because it provides much information regarding the involvement of glycosaminoglycan abnormalities in the mechanisms which regulate corneal ultrastructure and, hence, the loss of corneal transparency in the diseased state. This review of MCD aims to collate our current knowledge regarding the pathogenesis of this intriguing condition.

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Corneal anatomy

Prior to focusing on MCD it may be beneficial to provide a brief overview of corneal ultrastructure; a fuller description can be found in one of several

medical texts [8, 9]. Basically, the cornea is composed of five layers: the epithelium, Bowman's membrane, the stroma, Descemet's membrane and the endothelium.

The epithelium consists of two layers of surface cells, two or three layers of wing-shaped cells and a single layer of columnar basal cells which are separated from the underlying connective tissue by a basement membrane. Posterior to the epithelial basement membrane in many species lies Bowman's layer (or membrane); this is not a true membrane but is an acellular compaction of disorganized collagen fibrils constituting approximately 2% of corneal thickness. The bulk of the cornea is stroma, a connective tissue composed of various collagens, glycoproteins and proteoglycans populated by stromal fibroblasts (keratocytes). Descemet's membrane – the basement membrane of the corneal endothelium – lines the posterior stroma. The anterior banded portion of this membrane is present at birth and the whole membrane thickens throughout life as a posterior non-banded zone is continuously deposited by the endothelium. The innermost layer of the cornea is the endothelium, a single layer of hexagonal cells with minimal regenerative ability in humans, which separate the stroma from the aqueous humour, and play an important role in regulating stromal hydration.

Stromal transparency

The corneal stroma is a connective tissue, unique by virtue of its transparency. Within the stroma, collagen fibrils, regularly spaced and of uniform diameter, lie parallel to the surface of the cornea in wide thin sheets or lamellae – approximately 200 of these lamellae are stacked and constitute the stroma. In his classic 1957 paper, Maurice [10] discounted the idea that corneal transparency arises because the collagen has the same refractive index as the ground substance in which it is embedded, and proposed instead that 'the [collagen] fibrils are arranged in a regular lattice so that the scattered light is destroyed by mutual interference'. Later workers supported Maurice's hypothesis, and further demonstrated that a perfect lattice of collagen fibrils is not a strict requirement for transparency – a 'lattice-like' structure will suffice [11, 12]. Indeed, radial distribution functions of corneal electron micrographs have shown that the fibril lattice exhibits only short-range order, with the fibril positions being correlated over only a few nearest neighbour distances [13]. More importantly from the clinical standpoint, the early calculations of Benedek [12] and Goldman *et al.* [14] revealed that any fluctuations in the lattice-like arrangement of collagen fibrils—the so-called stromal 'lakes' whose size exceeds half the wavelength of light ($\approx$ 200–250 nm) will contribute to light scattering. The upshot of this transparency theory is that any pathological process or hydration change results in disruptions of the stromal architecture above a certain threshold will contribute to corneal opacification.

Regulation of the stromal architecture

Basically, two conditions need to be satisfied if the regular architecture of the corneal stroma is to be maintained. (i) The collagen fibrils should be of a uniform diameter, and (ii) the collagen fibrils should be regularly spaced. Given the complex interrelationships of the various stromal components, it seems reasonable to assume that the molecular mechanisms which satisfy each one of these conditions are not mutually exclusive. A physiological interaction which limits collagen fibril diameter, for example, could well influence the spacing of the fibrils.

Collagen fibrils in the mature human corneal stroma have diameters of 26 nm ($\pm$2.4 nm) when imaged in the transmission electron microscope [15] (synchrotron X-ray diffraction analysis of human corneas, a technique that does not require specimen dehydration, reveals that the true value is closer to 31 nm [16]). This remarkable uniformity of size has been attributed to several factors, including the intrafibrillar proportion of type V collagen in the hybrid collagen type I/V stromal fibrils [17, 18], intermolecular hydroxylysine-linked glycosides [19] and interactions with corneal proteoglycans [20–24]. The mean centre-to-centre fibril spacing in normally hydrated human corneas, quantified using synchrotron X-ray diffraction techniques, is 61.9 nm ($\pm$4.5 nm) [16]. Proposed modulators of this uniform spacing include corneal proteoglycans [25–27], the non-fibril-forming type VI collagen [28–30] and type XII collagen [31].

Corneal proteoglycans

Probably the most important group of non-collagenous corneal macromolecules is the stromal proteoglycans. These often exist in intimate association with the stromal collagen fibrils [32, 33], and

much evidence exists which implicates them in the maintenance of the stromal architecture [20–27].

The small, interstitial proteoglycans of the corneal stroma are hybrid molecules composed of a leucine-rich protein core to which glycosaminoglycans and oligosaccharides are covalently attached via a linkage region. A vast body of evidence exists concerning the biochemistry of the proteoglycans [34–38], and an appreciation of their structure and function can greatly aid our understanding of numerous corneal dystrophies and mucopolysaccharidoses.

Glycosaminoglycans, which in the past were termed acid mucopolysaccharides, are composed of repeating disaccharide units containing alternating hexosamine and uronic acid (or galactose) moieties. Importantly, in the normal cornea, sulphate groups are *O*-linked to various sites on the hexosamine and/or the uronic acid (although not the glucuronic acid) portion of a particular glycosaminoglycan, and the pattern of sulphation varies between different types of glycosaminoglycan [34]. The most abundant glycosaminoglycan in the mature cornea is keratan sulphate, a greater proportion of which is thought by some to exist in the posterior (bovine) stroma [39, 40], possibly as a result of the relatively low oxygen tension that exists in this region of the cornea in large mammals [41, 42]. The distributional heterogeneity of keratan sulphate proteoglycans which has been correlated directly with the degree of collagen fibril organization in human corneas [20], could well have some physiological significance related to the water sorptive and retentive properties of keratan sulphate versus other extracellular matrix constituents [43].

The repeating disaccharide of the corneal keratan sulphate glycosaminoglycan is composed of *N*-acetylglucosamine and galactose, linked 1–3 and 1–4 [34]. Multiple core protein isoforms exist in vertebrate corneas to which keratan sulphate can attach itself [44, 45], thereby forming the keratan sulphate proteoglycan termed lumican. The deduced amino acid sequence of chick corneal lumican documents five potential keratan sulphate attachment sites [46], and generally between one and three of these contain keratan sulphate chains [41, 48].

Decorin is the other main proteoglycan in the corneal stroma. This molecule consists of a protein core [49] to which a sole glycosaminoglycan is bound [34, 48]. The hexosamine portion of the repeating disaccharide in this case is *N*-acetylgalactosamine, and the uronic acid portion varies between iduronic acid and glucuronic acid. Strictly speaking, because the glycosaminoglycan contains some iduronic acid (approximately 10% for bovine cornea [50]) it should be classed as dermatan sulphate. However, since the majority of the uronic acid is in the form of glucuronic acid, this corneal glycosaminoglycan is also referred to as glucuronic acid-rich dermatan sulphate [34] and chondroitin/dermatan sulphate [41, 49].

Studies on day 18 chicken embryos have revealed that, in addition to their glycosaminoglycan chains, lumican contains 2–3 *N*-linked oligosaccharides, and decorin contains 1–3 *N*-linked oligosaccharides and one *O*-linked oligosaccharide [48].

Work employing cDNA clones has indicated that lumican and decorin are different but related gene products [46, 49] and are independently regulated at both the translational and post-translational levels during corneal development [51]. The interaction of decorin [21] and lumican [24] with the hybrid type I/V stromal collagen fibrils occurs via their protein cores, and it appears that disulphide bridges within the protein cores play a major role in this interaction [24, 52].

Clinical presentation of MCD

Classically, MCD presents within the first decade of life. Typically, the initial stromal clouding is accompanied by the appearance of small, scattered, white opacities in the central superficial cornea which subsequently extend posteriorly and peripherally as the condition becomes symptomatically advanced by the third decade [3] (Figure 1). The central cornea tends to be thinner than normal in MCD [53, 54], a finding attributed to the compaction of normal-diameter collagen fibrils [55]. Visual impairment due to MCD is such that a corneal graft is normally warranted by the third to fifth decade [3], although occasionally MCD patients in their eighth decade have had corneal transplantations [56, 57]. This procedure has a high success rate, although there are occasional reports of MCD recurring in the donor tissue, sometimes many years following the original graft [58–64]. The selective rarity of MCD recurrence and the extreme variability in the time course of its recurrence are somewhat puzzling. Histopathologically confirmed recurrences of MCD may be due to diseased keratocytes migrating from the host tissue into the grafted tissue and producing abnormal glycosaminoglycans [61, 63]. Alternatively, since

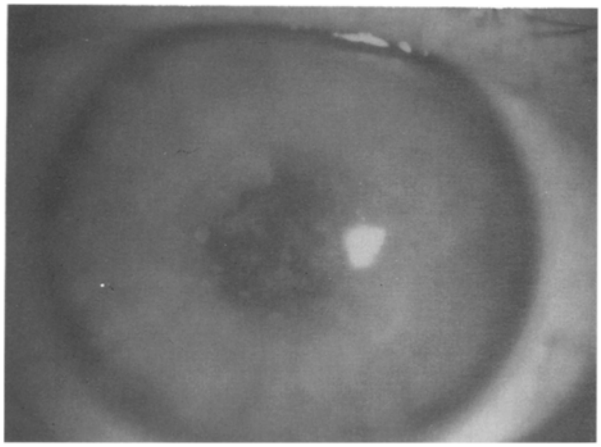

Figure 1. The typical clinical appearance of a macular corneal dystrophy cornea prior to corneal transplantation. Scattered, white opacities are observed with intervening stromal clouding. This particular patient suffered from macular corneal dystrophy type I, although the clinical presentation of type II patients is identical (see text).

keratocytes in donor tissue in which MCD has apparently recurred have been reported to synthesize normal glycosaminoglycans [62], the recurrence may be due to abnormal glycosaminoglycans migrating into the graft from the adjacent host tissue [60, 62].

Glycosaminoglycans synthesized in MCD

The early histological work on MCD reported a deposition of acid mucopolysaccharides (glycosaminoglycans) in the corneal stroma [58, 59, 65–67]. The suggestion that the glycosaminoglycan accumulations in MCD were the result of a mucoid degeneration of collagen [65] were soon refuted as new evidence favoured a cellular disorder [58, 59, 67].

In 1964 Klintworth & Vogel [58] proposed that MCD is a metabolic storage disease restricted to the cornea, and characterized by an intracellular and extracellular accumulation of glycosaminoglycans. They further postulated that the glycosaminoglycan deposits form intracellularly and are stored prior to their expulsion into the extracellular matrix. Later work supported their hypothesis, and the present-day histopathological diagnosis of MCD is based on the fact that the glycosaminoglycan deposits stain positively with Hale's colloidal iron, alcian blue, periodic acid–Schiff and metachromic dyes [58, 65, 67–69].

A significant breakthrough in our appreciation of the pathogenesis of MCD occurred when studies of MCD corneas in organ culture taught us that, in addition to synthesizing markedly decreased levels of keratan sulphate, they synthesize an unusual glycoprotein not found in normal corneas [70–74]. This novel glycoprotein has an immunologically identical protein core to the keratan sulphate proteoglycan found in normal corneas, but is slightly smaller [71–73, 75]. In their respective corneas both molecules are synthesized in similar amounts [73]. Further studies revealed that the novel glycoprotein in MCD resembles a keratan sulphate proteoglycan whose glycosaminoglycan moieties are not sulphated [75, 76]. Since the onset of transparency in the embryonic chick cornea is concomitant with the initial production of sulphated keratan sulphate as opposed to the unsulphated form, which is produced earlier, it has been suggested that MCD may represent an error in development in which MCD patients synthesize an embryonic form of lumican [51].

Sulphated keratan sulphate can be identified in an enzyme-linked immunosorbent-inhibition assay (ELISA) which makes use of a monoclonal antibody specific for a sulphated epitope common to both corneal and skeletal keratan sulphate [77]. In the mid-1980s studies employing the aforementioned ELISA revealed that MCD patients whose corneas lacked sulphated keratan sulphate also possessed serum which was deficient in this molecule [77–79]. Since most of the keratan sulphate-containing molecules present in serum are thought to be products of the degradation of proteoglycans in cartilage, the absence of sulphated keratan sulphate in serum from MCD patients whose cornea lacked this molecule suggested that their cartilage was also deficient in sulphated keratan sulphate; this contention which has now been supported by the direct analysis of a cartilage biopsy [56]. This work indicates that the phenotypic expression of the basic defect in MCD is not restricted to cells in the cornea; however, unlike cornea, the lack of sulphated keratan sulphate in cartilage has no obvious symptomatic manifestations.

Efforts to identify the abnormal MCD keratan sulphate have largely overshadowed the biochemical characterization of decorin in MCD corneas. However, MCD corneal cells which do not synthesis sulphated keratan sulphate do synthesize more chondroitin 6-sulphate than normal [70], and the chondroitin/dermatan sulphate proteoglycans are larger than normal and oversulphated [75]. In

addition, there is some evidence which suggests that the chondroitin/dermatan sulphate glycosaminoglycans may be incorporated into the extracellular deposits of abnormal keratan sulphate [75].

Until about 6 years ago all the evidence supported the type of MCD glycosaminoglycan synthesis pathway as outlined above, however in 1988 a surprising heterogeneity in the immunochemical expression of keratan sulphate within a population of MCD patients was reported [80].

Heterogeneity of MCD

As stated, up to 1988 the primary defect in MCD was assumed to occur in the synthetic pathway of the keratan sulphate-containing proteoglycans and was thought always to involve an incomplete glycosaminoglycan sulphation process. However, our view of MCD changed when Yang *et al.* [80], in evaluating the levels of sulphated keratan sulphate in a population of MCD patients, discovered that the cornea and serum of some MCD patients contained normal keratan sulphate glycosaminoglycans as identified immunochemically. Their finding was soon confirmed by other groups [81, 82]. Thus, immunochemical criteria dictate that MCD patients fall into one of two main subgroups that are clinically indistinguishable. The cornea and serum of the majority of MCD patients contain no normally sulphated keratan sulphate and are designated as suffering from MCD type I (keratan sulphate negative) [80, 81]. Some MCD patients demonstrate immunochemically detectable, if reduced, levels of sulphated keratan sulphate in their serum and cornea and are thus classified as MCD type II (keratan sulphate-positive) sufferers [80, 81]. One study [81] has reported two MCD patients who lacked serum keratan sulphate but who had positive corneal immunostaining for keratan sulphate – a finding which suggests that further groupings may be necessary. However, the situation is still somewhat unclear since an evaluation of almost 100 MCD patients by Klintworth [5] did not encounter such a case.

Histopathology of MCD

The underlying defect in the synthetic pathway of the MCD glycosaminoglycans, whatever its exact nature, seems ultimately to be responsible for the disruptions to the regular architecture of the corneal stroma, and hence the attenuated corneal clarity. The structural abnormalities exhibited by MCD stromas have been fairly well documented. Individual collagen fibrils appear well preserved, and, when positively stained using heavy metal salts in the transmission electron microscope, display a typical collagen banding pattern [55, 57–59, 66–68, 83]. X-ray diffraction evidence supports this finding by revealing that the axial electron density of stromal collagen in MCD is not significantly different from normal [83].

The stromal keratocytes and endothelial cells often contain fibrillogranular material contained in membrane-bound vacuoles [56, 57, 84, 85]. Morphologically similar structures are also observed within the extracellular stromal matrix [56, 57, 84, 85], where, presumably, they exist either as products extruded from cells or as remnants of cell death. Another striking feature of transmission electron microscope images of MCD stromas is the ubiquitous presence of small, electron-lucent, collagen-free lacunae [55, 59, 83, 84]. Such structures are also noted in the posterior non-banded region of Descemet's membrane, but not the anterior non-banded region which is present at birth [68, 84, 85]. When MCD corneas are chemically fixed in the presence of cuprolinic blue, a cationic, copper-based stain specific for sulphated glycosaminoglycans under certain conditions, such stromal [55, 83] and Descemet's (A. J. Quantock, unpublished observation) lacunae are often observed to contain congregations of abnormally large electron-dense filaments of various sizes. The posterior-most Descemet's lacunae tends to contain more electron-dense filaments than the more superficial Descemet's lacunae (A. J. Quantock, unpublished observation). In the stroma of a cuprolinic blue-stained MCD type II cornea, electron-dense filaments associate predominantly with the margins of extracellular fibrillogranular vacuoles [57] (Figure 2). Interestingly, regions of MCD stromas adjacent to abnormal proteoglycan deposits often exhibit 'pockets' of abnormally large collagen fibrils [57] (Figure 3). Such regions of abnormally sized collagen fibrils, which have also been documented in corneal mucopolysaccharidosis type I-S (Scheie's syndrome), may well contribute to corneal opacification, and their proximity to the abnormal proteoglycan complexes is suggestive of a collagen diameter regulating role for stromal proteoglycans in both Scheie's syndrome and MCD.

Synchrotron X-ray diffraction work has revealed that the collagen intermolecular spacing in both

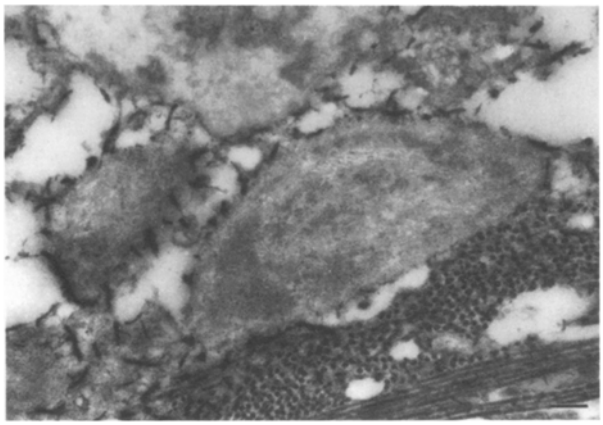

Figure 2. The margins of extracellular fibrillogranular vacuoles in the anterior stroma of a macular corneal dystrophy type II patient are lined by abnormally large electron-dense proteoglycan filaments. Stained for sulphated proteoglycans with cuprolinic blue and for collagen with phosphotungstic acid and uranyl acetate. Bar = 200 nm. (Reproduced with permission from ref. 57.)

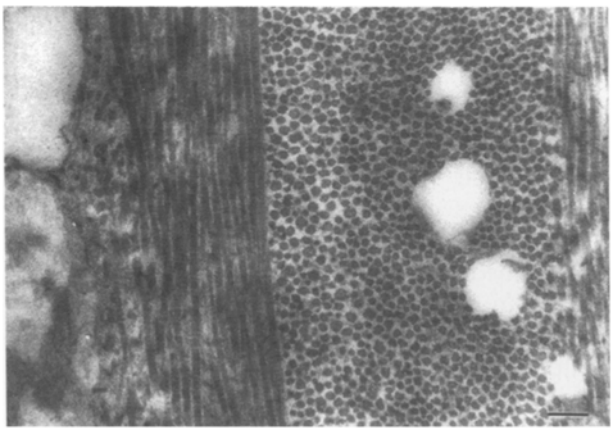

Figure 3. A region of the anterior stroma of a macular corneal dystrophy type II patient just posterior to a layer of extracellular fibrillogranular vacuoles which lie to the left of the image. The lamella viewed in cross-section clearly displays some electron-lucent lacunae and an abnormally large range of collagen fibril diameters which is more prevalent towards the left half of the lamella. Stained for sulphated proteoglycans with cuprolinic blue and for collagen with phosphotungstic acid and uranyl acetate. Bar = 200 nm. (Reproduced with permission from ref. 57.)

MCD type I and MCD type II corneas increases with hydration from the dry state in a similar fashion to the intermolecular spacing of normal human corneal collagen [86]. In addition, a regular structure with a periodic repeat in the region of 0.46 nm was identified in the stroma of MCD type I and MCD type II corneas. Such a structure is not present in any other human corneas, either normal or pathologic [86]. The original supposition, that abnormal glycosaminoglycans or proteoglycans contained the unique 0.46 nm ultrastructure, was confirmed recently [87].

The identity of the abnormal proteoglycan filaments present in MCD type I and MCD type II has not been fully elucidated but, in the stroma at least, their susceptibility to chondroitinase ABC has shown them to possess a chondroitin/dermatan sulphate component [83]; this, however, may not be the sole component of the abnormal MCD deposits. Some organ culture studies [75] have suggested that in MCD type I the chondroitin/dermatan sulphate proteoglycan is probably incorporated into the extracellular deposits of unsulphated keratan sulphate. In addition, results from recent X-ray diffraction work are consistent with the interpretation that in MCD type I and MCD type II the chondroitin/dermatan sulphate-containing deposits possess, or interact with, a lumican component [87]. That some form of hybrid macromolecule (composed of abnormally sulphated chondroitin/dermatan sulphate and abnormally sulphated keratan sulphate, and displaying a unique 0.46 nm periodically [86, 87]) exists in MCD corneas [75, 87] is an intriguing concept which requires further study to confirm (or disprove) the presence of such a molecule and ascertain what form the interaction may take.

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