A novel mutation in KRT12 associated with Meesmann’s epithelial corneal dystrophy

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Background: The molecular basis of Meesmann’s epithelial corneal dystrophy (MECD) has recently been attributed to mutations in the cornea specific keratin genes KRT3 and KRT12. The mechanisms by which these mutations cause the Meesmann’s phenotype are not clear. This study presents new data, examines clinical, histological, ultrastructural, and molecular aspects of MECD, and compares the features seen in this condition with those observed in other well studied keratin diseases such as epidermolysis bullosa simplex.

Methods: A two generation family with typical features of Meesmann’s epithelial corneal dystrophy (MECD) was studied. All family members were examined under a slit lamp. Biopsy material from elective keratoplasty was studied by histopathological and ultrastructural analysis using standard techniques. Direct automated sequencing of genomic DNA was used for mutation detection, mutations were confirmed by restriction digest analysis.

Results: The abnormal corneal epithelium was acanthotic and contained numerous dyskeratotic cells and intraepithelial vesicles. By electron microscopy abnormally aggregated and clumped keratin filament bundles were detected in basal and suprabasal keratinocytes from the centre of the cornea. Direct sequencing of the patients’ genomic DNA revealed a novel missense mutation (423T>G) in exon 1 of the cornea specific keratin 12 (KRT12) gene. This mutation predicts the amino acid change N133K in the mature corneal epithelium.

Conclusions: A clinical, histopathological, and ultrastructural study of a previously unreported family with MECD is presented. In this family the disease is ascribed to a novel mutation in KRT12. A molecular mechanism is proposed for MECD based on the comparison with other well characterised keratin diseases.

Meesmann’s epithelial corneal dystrophy (OMIM 122100; MECD) is inherited as an autosomal dominant trait and was initially described in a German family. MECD is a bilaterally symmetrical disorder of the corneal epithelium with a characteristic slit lamp appearance of myriad fine round epithelial cysts of uniform size and shape, which become visible by 12 months of age and increase in number throughout life. Patients are usually asymptomatic until adulthood when rupture of the corneal microcysts may cause erosions, producing clinical symptoms such as photophobia, contact lens intolerance, intermittent diminution of visual acuity and, in some cases, permanent visual impairment.

Keratins are expressed specifically in the cytoplasm of epithelial cells where they form a dense meshwork of 10 nm intermediate filaments. Keratins are expressed as obligate heterodimers of type I/type II pairs in a tissue and differentiation specific fashion. In 1991, the skin blistering disorder, epidermolysis bullosa simplex (EBS) was recognised as a human keratin disease. In EBS, mutations in either K5 or K14 genes render basal epidermal keratinocytes less resilient to mechanical stress, leading to increased skin fragility, which is manifest as blisters. Following this initial breakthrough, the total number of keratin genes associated with diseases (of the skin, hair, oral mucosa, and cornea) has risen to 18. The highly specialised corneal epithelium has evolved to combine the key function of providing structural protection to the eye while maintaining transparency. In keeping with this requirement, the keratin filament network of corneal keratinocytes appears less tightly bundled than that of cornified stratified epithelium such as the epidermis. K5 and K14 are expressed in limbal cells, but K3 and K14 mRNAs are undetectable beyond the limbus. As corneal keratinocytes approach terminal differentiation, K3 and K12, first expressed in the three to four layered developing cornea at 12–13 weeks’ gestation, become the dominant keratin pair in all layers of the mature corneal epithelium.

The molecular basis of MECD was elucidated when the first mutations in the cornea specific keratins K3 and K12 were reported in three families, including Meesmann’s original family. Subsequently, another group has confirmed these findings with mutations in K12 in four families with MECD. Here we report a clinical, histological, ultrastructural, and molecular study of a previously unreported family with MECD.

Materials and Methods

A two generation family with three affected individuals was studied. All individuals were examined under a slit lamp by an experienced ophthalmologist (SIM/GCMB). Corneal tissue was obtained from the proband following lamellar keratoplasty. Samples were processed for routine histology and transmission electron microscopy. All subjects were treated in accordance with the tenets of the Declaration of Helsinki, and local institutional ethics committee approval was obtained. Specimens obtained at keratoplasty were fixed in formalin, placed in 10% formal saline and processed on a Shandon Hypercentre. Paraffin wax embedded sections were cut at 3–4 µm and cut sections stained with haematoxylin and eosin, periodic acid Schiff, and Masson’s trichrome. For electron microscopy, specimens were fixed in 2% glutaraldehyde in Sörensen’s buffer and then 2% osmium tetroxide using a standard protocol. Semithin (1 µm) sections were cut on an Ultracut E (Reichert-Jung) and stained with toluidine blue.
Tissue was embedded in Araldite resin (Araldite resin embedding kit-E009 T aab). Electron microscopy was performed on a Philips EM410.

Most of exon 1 of the KRT12 gene was amplified from affected and unaffected family members using primers K12e1.L (5’ ATG GAT CTC TCC AAC AAC ACC ATG 3’) and K12e1.R (5’ GTA ATC GCT CTG TGA AGC ATC TGC 3’), as described previously.

Polymerase chain reaction (PCR) products were purified using QIAquick columns (Qiagen, Chatsworth CA, USA), sequenced with both primers using the ABI PRISM Ready Reaction system (Perkin-Elmer, Foster City, CA, USA) and analysed on an ABI 377 automated sequencer. The 423T>G mutation generates an Afl II restriction site. To confirm the mutation within the family and exclude it from 100 normal unrelated chromosomes, PCR products generated with primers K12sp1 (5’ CTC TAG GTA TTC TCT CGC GCA ATG 3’) and K12sp2 (5’ TGC TGC AAG TAC AGC TAA ATT GGA 3’) were digested overnight with 1.5 U Afl II and analysed on 3% Seakem/TBE minigels.

RESULTS AND DISCUSSION

All affected individuals had the characteristic slit lamp appearance of multiple fine round epithelial cysts (Fig 1A). Individuals in the second generation had a relatively symptom free presentation. The proband had a more problematic clinical course with a history of soreness in both eyes from his late teens, especially marked in the morning. The symptoms had progressed with age, necessitating medical intervention. At age 40 he noted gradual bilateral deterioration in visual acuity and bilateral epithelial debridement was performed at age 41 years. Unfortunately he experienced recurrence of symptoms and clinical signs within 1 month. Excimer laser PTK was performed to the left (amblyopic) eye, but again there was recurrence of symptoms within 1 month. Because of persisting symptoms a non-matched lamellar keratoplasty to left eye was performed with relief of symptoms for 3 months, but full recurrence within 3 months. At age 45 years he underwent donor limbal stem cell allograft to the right eye from a tissue matched donor. This eye pursued a similar course to the left. Eventually, his symptoms recurred necessitating long term bandage therapeutic contact lens wear, although his corneas still only showed a moderate degree of clinical recurrence.

By light microscopy abnormal epithelium from the centre of the cornea was thickened and comprised five to six cell layers (Fig 1B). The overall architecture of the epithelium seemed disordered with many keratinocytes containing periodic acid Schiff (PAS) positive fibrillar material (PAS stain, ×200). Ultrastructural changes were noted in all layers of the corneal epithelium and most prominent changes were seen in the outer epithelial layers, with thin walled vesicles clearly apparent (Fig 1C). The cytoplasm of many keratinocytes varied in size, shape, and electron density (Fig 1D).
MECD has many features in common with other well-characterised keratin diseases affecting other epithelia. The multiple cysts seen clinically on slit lamp examination are analogous to the intraepithelial cytolysis in other keratin disorders but are seen more easily in the transparent corneal epithelium. The histological appearance of thickened and disorganised epithelium seen in MECD is characteristic of a keratin disorder. The cytolysis and cell degeneration seen histologically in all keratin disorders is manifest in the cutaneous context as epidermolysis (in EBS, bullous congenital ichthyosiform erythroderma and ichthyosis bullosa of Siemens). Here, in the corneal context, cytolysis is manifest as cyst formation. We believe the PAS positive clumps noted by previous authors and demonstrated in this study represent aggregates of keratin filament clumps and associated cellular debris. Although keratins are not generally regarded as glycoproteins, it has been shown that keratins K8 and K18 have short O linked sugar side chains. Glycosylation of K3 and K12 has not been reported. In addition, intermediate filament aggregates have been shown to contain chaperone proteins of the small heat shock family, offering another explanation for the PAS reactivity observed. Unfortunately, frozen biopsy material was not available to confirm this association by immunohistochemistry.

The ultrastructural observation of tonofilament clumping in MECD is again reminiscent of findings in other keratin diseases. In the most comprehensively studied human keratin disease, the Dowling-Meara form of EBS (EBS-DM), ultrastructural tonofilament clumping was first suggested as being indicative of keratin disease as early as 1982, and these clumps were formally identified as being composed of K5/K14 aggregates by immunohistochemistry in 1991. The clumped tonofilaments seen here are presumably K3/K12 aggregates although we were unable to confirm this by immunoelectron microscopy owing to the method of preparation of the archival tissue. The multiple dyskeratotic cells are not seen in other keratin disorders and presumably reflect the unique nature of the corneal epithelium. The appearance of breaches in the basement membrane is also atypical.

In conclusion, MECD shares many molecular, histological, and ultrastructural features with other keratin disorders but also has specific characteristics, probably because of the unique morphology of the anterior corneal epithelium. Further work to more clearly define the mechanisms underlying the development of the “peculiar substance” is planned. This will shed light on the role of cornea specific keratins in the maintenance of epithelial integrity and in the aetiology of conditions associated with its breakdown.
References