NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Br J Ophthalmol. Author manuscript; available in PMC 2009 July 15.

Published in final edited form as:
Br J Ophthalmol. 2008 October ; 92(10): 1434–1436. doi:10.1136/bjo.2008.139527.

Sulfation patterns of keratan sulfate in different macular corneal dystrophy immunophenotypes using three different probes

T Saito[1], **K Nishida**[2], **J Nakayama**[3], **T O Akama**[4], **M N Fukuda**[4], **K Watanabe**[1], **A J Quantock**[5], **N Maeda**[1], **H Watanabe**[1], and **Y Tano**[1]

[1] Department of Ophthalmology, Osaka University Medical School, Osaka, Japan

[2] Department of Ophthalmology, Tohoku University School of Medicine, Sendai, Japan

[3] Department of Pathology, Shinshu University School of Medicine, Matsumoto, Japan

[4] Glycobiology Program, Burnham Institute for Medical Research, La Jolla, CA, USA

[5] School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

Macular corneal dystrophy (MCD) is subdivided into three immunophenotypes, MCD types I, IA and II, based on the reactivity of serum and corneal tissue to an antibody that recognises sulfated keratan sulfate (KS).[1] In MCD type I (MCD-I), antigenic KS is undetectable in both serum and cornea, while in MCD-II it is present at normal or subnormal levels in serum, and is evident immunohistochemically in the corneal stroma. In MCD-IA, antigenic KS is absent from serum and extracellular stromal matrix, but is detected in keratocytes. Mutations of the carbohydrate sulfotransferase gene, CHST6, have been identified as causative for MCD.[2] This study investigated the distribution of differentially sulfated KS in cornea in the three main MCD immunophenotypes.

Materials And Methods

Serum and postoperative corneal tissue from three unrelated individuals with MCD from three Japanese families were studied. As control tissue, two paraffin blocks of eyes resected for malignant melanoma and containing intact, undiseased cornea were used. Relevant ethical committees approved the protocol.

Serum KS was quantified using the anti-KS monoclonal antibody 5D4 (Seikagaku Kogyo, Japan) in an enzyme-linked immunosorbent assay (ELISA).[1] This antibody was also used to immunolocalise antigenic KS in cornea using published methods.[3]

PCR-direct sequence was performed according to a previous report.[2] Deparaffinised serial sections were stained with Alcian Blue for acidic substrates including sulfated KS. Tomato lectin (LEL, Vector Laboratories, Burlingame, CA) was used to detect more than three repeat Galβ1→4GlcNAc residues (ie, an unsulfated form of KS), and high-iron diamine (HID) to detect low sulfated KS.[4]

Correspondence to: Professor K Nishida, Department of Ophthalmology, Tohoku University School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai, 980-8574, Japan; E-mail: knishida@oph.med.tohoku.ac.jp.

Competing interests: None.

Ethics approval: Relevant ethical committees approved the protocol.

Patient consent: To be provided.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Results

Patients 1 and 2 had serum KS levels below the detection limit of the ELISA and were categorised as MCD-I or IA. Patient 3 had normal KS levels and was designated as MCD-II (table 1). Patient 1 showed no immunoreactivity for 5D4 in cornea and was further subclassified as MCD-I; positive 5D4 label in epithelial cells and keratocytes in patient 2 indicated MCD-IA (fig 1A). In MCD-I, the superficial epithelium, stroma and endothelium all stained with LEL, pointing to a widespread distribution of poly-N-acetyllactosamine, or unsulfated KS. In MCD-IA and -II, the superficial epithelium, endothelium and keratocytes were positive for LEL, but, with the exception of focal, subepithelial deposits, stromal lamellae did not stain. Thus, as in normal cornea, the stromal matrix in MCD-IA and MCD-II contains little or no unsulfated KS, whereas the stroma of MCD-II does. In all immunophenotypes, HID staining for low-sulfated KS was seen in the stromal matrix and endothelium, but not the epithelium. In MCD-II, HID staining was most prevalent in keratocytes (fig 1A).

A previously reported missense mutation was found in MCD-I.[2] In MCD-IA, a new replacement mutation was discovered in the coding region of CHST6 in one allele. MCD-II showed no mutation in the CHST6 coding region (table 1).

Comment

Sulfated KS in MCD cornea has been studied using the anti-KS antibody 5D4 at the light- and electron-microscopic levels.[1 5] Unsulfated KS has also been examined by electron microscopy using the lectin *Erythrina cristagalli* agglutinin (ECA),[6] and the anti-i antibody.[5] Here, we used probes to sulfated, low-sulfated and unsulfated KS to establish their tissue distribution in the three main MCD immunophenotypes (fig 1A). This is summarised, along with genetic information, in fig 1B. The mutation in MCD-IA does not cause a frame shift, but the number of amino acid increases, which might lead to changes in the conformation of C-GlcNAc6ST, the product and a decrease in enzymatic activity. We predict that in MCD-II, unfound mutations in the promoter/enhancer region of CHST6 occur, and that the activity of C-GlcNAc6ST itself remains, but with abnormalities in the distribution pattern of KS.

Acknowledgments

Funding: Thanks to the Sasakawa Foundation for grant support.

References

1. Klintworth GK, Oshima E, Al-Rajhi A, et al. Macular corneal dystrophy in Saudi Arabia: a study of 56 cases and recognition of a new immunophenotype. Am J Ophthalmol 1997;124:9–18. [PubMed: 9222226]
2. Akama TO, Nishida K, Nakayama J, et al. Macular corneal dystrophy type I and type II are caused by distinct mutations in a new sulfotransferase gene. Nat Genet 2000;26:237–41. [PubMed: 11017086]
3. Shiozawa T, Tsukahara Y, Nakayama J, et al. Immunohistochemical reactivity of antikeratan sulfate monoclonal antibody 5D4 to various conditions of human endometrial tissues and its application as a useful marker for identifying endometrial epithelia. Gynecol Obstet Invest 1991;32:239–42. [PubMed: 1778517]
4. Spicer, SS.; Horn, PG.; Leppi, TJ. Histochemistry of connective tissue mucopolysaccharides. In: Wagner, BM.; Smith, DE., editors. The connective tissue. Baltimore: Williams & Wilkins; 1969. p. 251-303.
5. Young RD, Akama TO, Liskova P, et al. Differential immunogold localisation of sulphated and unsulphated keratan sulphate proteoglycans in normal and macular dystrophy cornea using sulphation motif-specific antibodies. Histochem Cell Biol 2007;127:115–20. [PubMed: 16944190]

6. Lewis D, Davies Y, Nieduszynski I, et al. Ultrastructural localization of sulfated and unsulfated keratan sulfate in normal and macular corneal dystrophy type I. Glycobiology 2000;10:305–12. [PubMed: 10704529]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

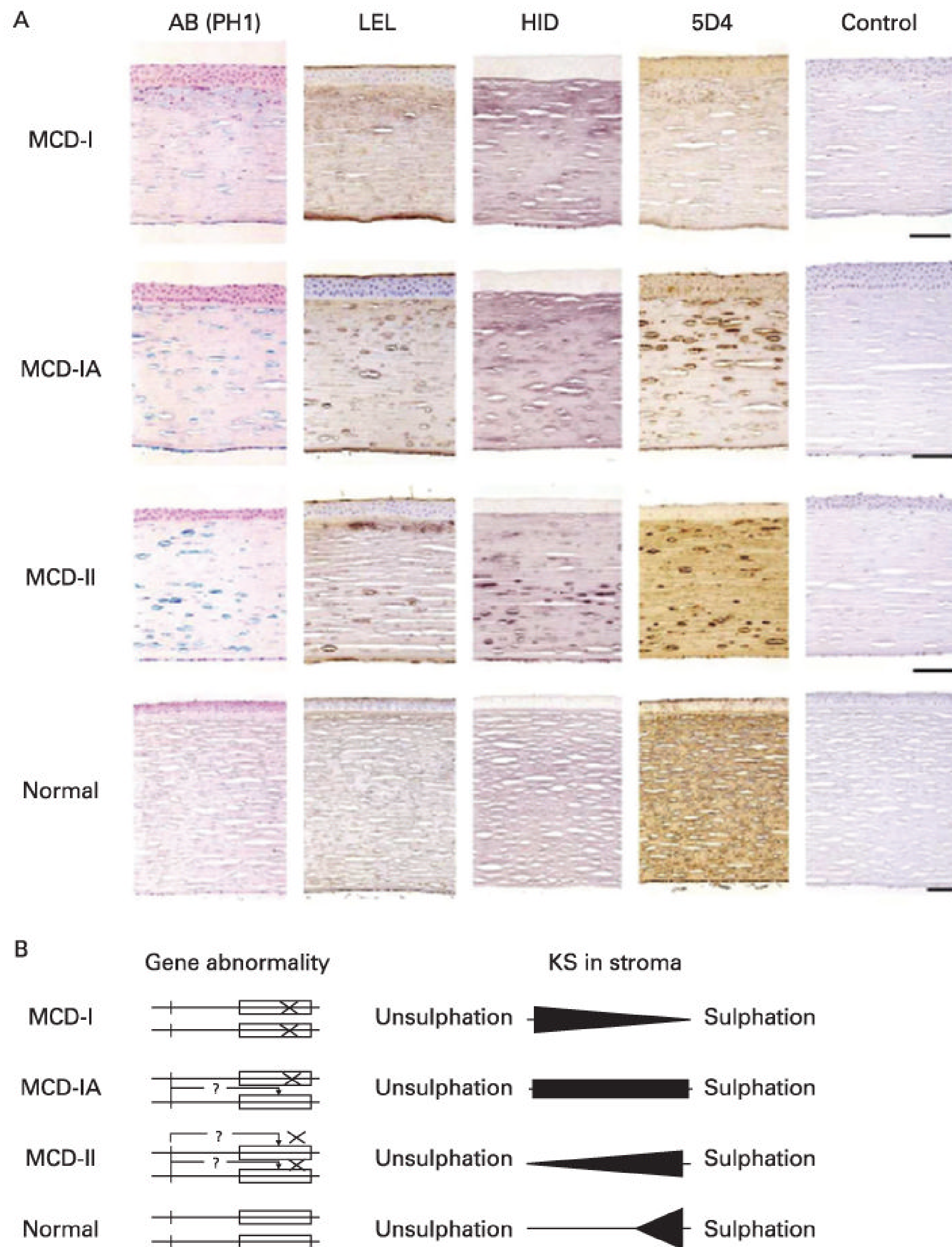

Figure 1.

(A) Distribution of poly-N-acetyllactosamine (ie, unsulfated keratan sulfate (KS)), low-sulfated KS and sulfated KS in normal, acular corneal dystrophy (MCD)-I, IA and II corneas. Tissue sections were stained with Alcian Blue (AB) and with tomato lectin (LEL) for poly-N-acetyllactosamine, HID for low-sulfated KS and 5D4 for sulfated KS. Bar, 100 μm. (B) Scheme indicating the genetic and stromal KS differences in the three MCD immunophenotypes. Sulfated KS refers to that identified by 5D4, and unsulfated KS to poly-N-acetyllactosamine identified by LEL. The open box in gene abnormality indicates the carbohydrate sulfotransferase gene (CHST6) coding region. “X” indicates an abnormality suspected based on the findings of a previous paper.[2]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Patient profile, immunophenotype and mutational status

Patient no.	Age/gender	Sulfated keratan sulfate in serum (μg/ml)	Immunophenotype	Mutation 1	Mutation 2
1	45/male	<0.15	Type I	G1512A (E274K)	G1512A (E274K)
2	40/male	<0.15	Type IA	Replacement mutation #1 (#2)	N
3	63/male	276	Type II	N	N

#1, 981 CAACGTCTCCC 991→981 TCAAGACTTCGTCCAGGAATGCA 1003;

#2, 328-NVSQ-332→328-QDFVQECK-335;

N, no mutations in the coding region.