# Familial limbal stem cell deficiency: clinical, cytological and genetic characterization

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**Sir**

Limbal stem cell deficiency (LSCD) is characterized by a decreased ability to replenish the population of corneal epithelial cells, resulting in corneal neovascularization, surface defects, scarring and replacement of the corneal epithelium with conjunctival epithelial cells. Familial LSCD with minimal or no other ocular pathology is an extremely rare condition. In some but not in all families coding or splicing pathogenic mutations in the paired box gene 6 (*PAX6*, MIM *607108) have been detected [1, 2].

The proband first developed ocular symptoms at the age of 3 years, when she had been examined for redness and tearing of the right eye (RE). Peripheral superficial corneal vascularization, rough uneven corneal surface and scarring in the RE were documented in historical notes. At the age of 12 years her best corrected visual acuity (BCVA) in the RE was recorded to be hand motion. Apart from decreased BCVA, her main subjective symptoms have been recurrent periods of light sensitivity and foreign body sensation associated with profound redness occurring often in the RE and occasionally in the left eye (LE). Since 25 years of age, these symptoms have been managed by daily contact lens wear in the RE. In addition to the recommendation to administer regularly artificial tears, she has been prescribed intermittently mild steroid drops, topical cyclosporine A and anti-VEGF therapy (bevacizumab); however, these compounds provided little or no relief. Examination at 30 years of age revealed a marked asymmetry in her clinical features. In the RE, extensive vascularization of the entire cornea and stromal scarring with yellowish deposits was observed (Figure 1A). BCVA was hand motion. In the LE, a circular peripheral vascularization was observed that was most pronounced in the superior cornea where it extended about 3 mm from the limbus; the corneal stroma was otherwise clear. Uncorrected visual acuity in the LE was 1.0. Re-examination of the proband at the age of 36 years did not reveal any

progression (Figure 1D, E). Mild corectopia loss of the limbal palisades of Vogt was observed bilaterally (Figure 1A, B, D, E). Spectral domain optical coherence tomography (SD-OCT; Spectralis; Heidelberg Engineering GmbH, Heidelberg, Germany) scans of the right cornea showed irregular corneal thickness and thinning up to 251 µm (Figure 1C). SD-OCT imaging of the macula did not reveal any abnormalities in both eyes (Supplementary Figure 1).

The son of the proband was born from an uneventful pregnancy. He presented since the age of 1 month with bilateral tearing, light sensitivity, conjunctival injection and episodes of mucous discharge, for which he underwent lacrimal probing. Bilateral corneal epithelial defects were noted when he was 4 months old and topical treatment with acyclovir and artificial tears was started as he was suspected to suffer from herpetic keratitis. At the age of 5 months, ophthalmic examination was performed under general anaesthesia using an operating microscope. Bilateral corneal vascularization extending 1-2 mm from the limbus nasally and inferiorly, corneal epitheliopathy with punctuate fluorescent staining, and a haze of the entire corneal surface were found. No gross abnormalities of the iris or lens were present, intraocular pressure and fundus examination were also bilaterally normal. Subsequent re-examination under general anaesthesia at the age of 1 year and 1 month showed bilateral progression of the vascularization extending diffusely from limbus in all four quadrants over the corneal centre. At 2 years and 8 months of age, both of his corneas were entirely vascularized with irregular surface and central opacity (Figure 1F, G). The child has been photophobic since an early age, and for this reason could not undergo ophthalmic examination at the last follow-up at the age of 7 years. It was noted that his left eye was esotropic. He has been treated with artificial tears and topical cyclosporine A, however no major improvement of his symptoms was observed.

No systemic abnormalities were detected in the affected child who also underwent, before the age of 5 months, blood tests for general inflammatory response and serologic tests for infectious disorders known to cause congenital corneal clouding.

Impression cytology performed in both individuals confirmed the diagnosis of LSCD by histological detection of goblet and inflammatory cells in corneal imprints and by immunocytochemical visualization of conjunctivalization using KRT7 as a marker [3] (Supplementary Material and Supplementary Figure 2).

*PAX6* exons 1-13 and 5a (reference sequence ENST00000638914.1) were Sanger sequenced [4]. Next, whole exome sequencing (WES) of the proband was performed. Reads were aligned to the human reference sequence (Ensembl Genome browser hg19) and annotated (Supplementary Material). Minor allele frequency cut-off value ≤ 0.005 (population frequency databases used are listed in Supplementary Material) was chosen taking into consideration the rarity of the condition studied, and the presumed autosomal dominant inheritance with unknown penetrance. Copy number variation (CNV) analysis was also performed in the proband (Supplementary Material).

Investigation of the proband's WES data identified 81 rare heterozygous variants, of which 39 were unique. Cross referencing of this dataset with genes possibly implicated in anterior segment dysgenesis, corneal dystrophy and corneal vascularization (Supplementary Tables 1- 3) identified a unique heterozygous variant in *KRT12* (keratin 12; MIM *601687), c.1111G>C; p.(Asp371His) (reference sequence ENST00000251643.4). Mutations in *KRT12* have been reported in Meesman corneal dystrophy patients presenting with corneal neovascularization as an additional feature to the classical phenotype [5]. Sanger sequencing however showed that the affected son of the proband was wild type (Supplementary Figure

3), indicating that the *KRT12* variant in the proband is a benign polymorphism. No CNVs were detected.

Clinical findings in the two affected individuals were consistent with descriptions of familial LSCD without systemic abnormalities [2, 6]. To the best of our knowledge, this is the first report on the earliest onset of this condition at 1 month of age in the son of the proband. We also extended our understanding of the effects of LSCD on the corneal architecture through use of a combination of imaging and molecular approaches. Although we applied state-of-the-art techniques, including WES and CNV analysis we were not able to find the molecular genetic cause of LSCD in the family studied. This finding, in conjunction with the observation that the molecular defect is unknown for some familial cases of LSCD published to date [2], raises the possibility that, in addition to specific genes such as *PAX6*, the underlying pathogenic mechanisms of inherited and/or congenital LSCD with minimal or no iris abnormalities may be associated with non-coding regions of the genome and/or be multifactorial.

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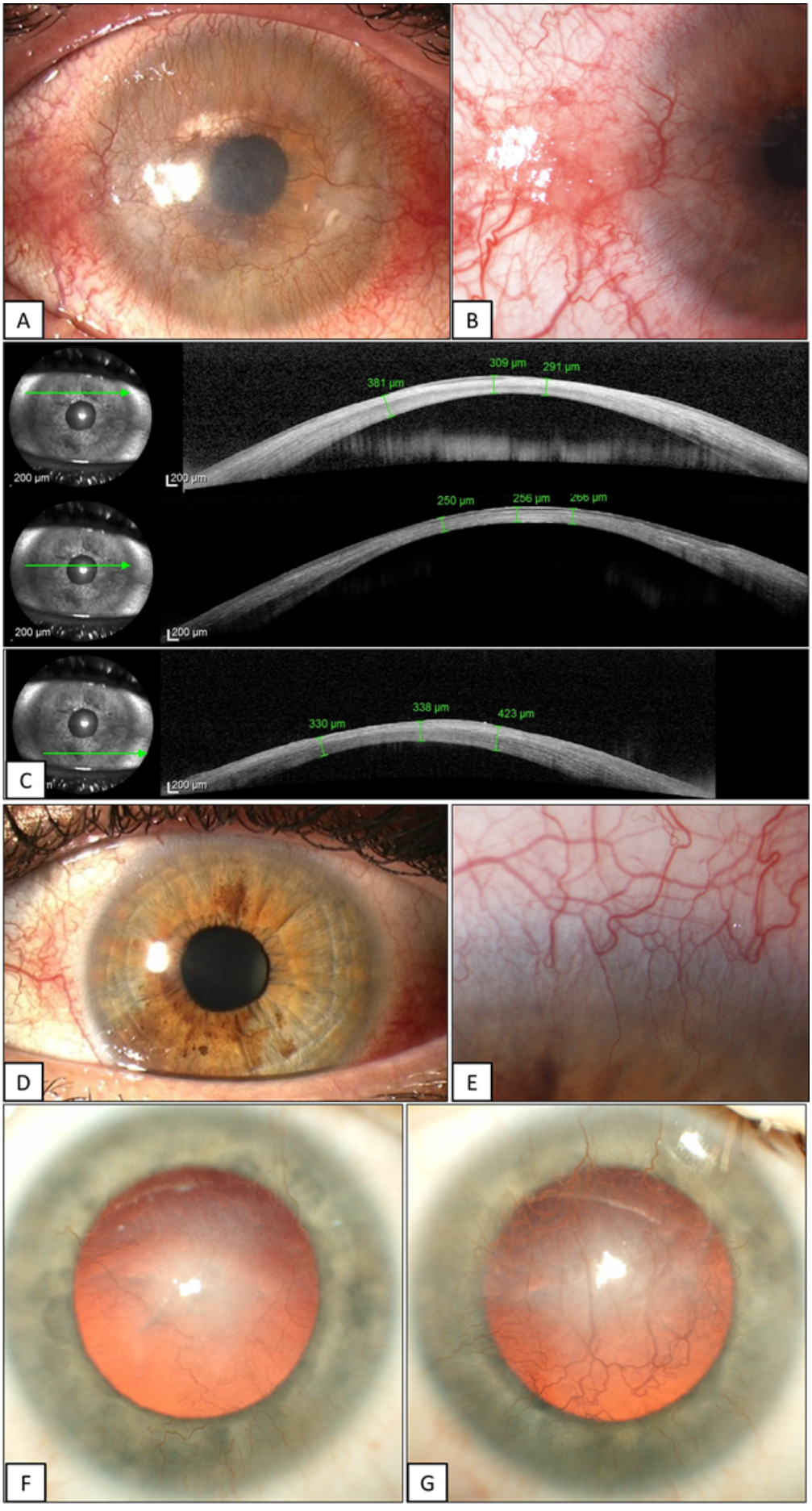
**FIGURE LEGEND**

**Figure 1. Clinical findings in familial limbal stem cell deficiency.** Anterior segment photograph of the proband's right eye (age 30 years) showing completely vascularized cornea, with the blood vessels located primarily in the anterior stroma, and stromal scarring with yellowish deposits [A]; temporal limbal area in detail (age 36 years) demonstrating loss of the limbal palisades of Vogt [B]. SD-OCT imaging documenting irregular thinning in the right cornea (age 36 years) [C]. Anterior segment photograph of the proband's left eye, note only mild perilimbal vascularization [D] and superior limbal area in detail [E]; (both at the age of 36 years). Anterior segment photographs of the son (age 2 years and 8 months) show advanced corneal vascularization and central opacity in his right [F] and left [G] cornea.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