ABSTRACT

Fuchs' Endothelial Corneal Dystrophy (FECD) is an age-related disorder characterized by corneal endothelial cell loss and guttae formation, leading to vision impairment. It occurs sporadically or via autosomal-dominant inheritance, with population-specific variability. Disease mechanisms include diverse single-nucleotide polymorphisms (SNPs) and gene dysregulation, leading to corneal edema and damaged endothelium. Though several genes and SNPs are associated with FECD, their relevance in the Indian population is limited. Discovering novel genes and establishing an animal model for FECD will enhance understanding of disease pathogenesis.

Here, we examined the genetic association of SNPs rs918980 and rs938350, previously identified in a Caucasian genome-wide association study, in an Indian FECD cohort. This study identified an association between the intergenic SNP rs918980 and FECD in the Indian population and showed upregulation of two nearby genes, Engulfment and cell motility protein 1 (*ELMO1*) and G protein-coupled receptor 141 (*GPR141*), in FECD corneal endothelium (CE). *ELMO1* and *GPR141*, involved in cytoskeletal reorganization and epithelial-to-mesenchymal transition (EMT), were also upregulated in FECD mouse CE, indicating their engagement in disease pathogenesis. The Phosphatidyl glycerophosphate Synthase 1 (*PGS1*) SNP rs938350 also exhibited significant association with FECD and concomitant *PGS1* overexpression. As a cardiolipin biosynthesis enzyme in the inner mitochondrial membrane, PGS1 maintains mitochondrial integrity. Linkage disequilibrium (LD) analysis with rs938350 identified 12 new SNPs linked to disease; notably, rs3817294 binds transcription factor YY1 via the protective allele 'A', while an elevated PGS1 expression is seen with the rs2292642 'TT' genotype. *In silico* analysis confirmed enhancer activity and possible promoter looping driving PGS1 overexpression.

Furthermore, an induced mouse model of FECD was successfully developed by controlled UVA exposure of the mouse cornea, reproducing FECD-like phenotypes within 90 days. A reproducible method for isolating the CE from mouse eyes was standardized, offering a powerful tool for molecular and functional analyses. Gene expression profiling of selected genes, including *ALDH3A1, BDNF, CACNA1D, CFTR, CLCA2, CLCN3, KRT12, SLC12A2, SEMA3E, SLC16A7, TACSTD2*, *TRPM3, TMC2, and TNFRSF11B,* in the isolated mouse CE identified differential regulation of fourteen genes, primarily membrane transporters, with four upregulated and ten downregulated. Validation in human FECD samples confirmed dysregulation of nine genes involved in chloride and calcium transport, highlighting altered ion homeostasis as a pathogenic feature. Moreover, cellular studies established necroptosis as an unreported mode of cell death in FECD, supported by elevated expression of the necroptosis marker, phosphorylated Mixed Lineage Kinase Domain Like protein (p-MLKL), specifically near the guttae region, along with reduced mitochondrial density.

Overall, this study elucidates FECD's genetic, molecular, and cellular basis by identifying novel genetic variants, implicating membrane transporters and necroptosis in disease pathogenesis, and establishing a FECD animal model to facilitate advanced therapeutic and mechanistic research.