

DISCOVERY OF NOVEL MUTATIONS

Expertise in Candidate Gene Analysis

Helps Scientists Identify Illusive Mutations in SCCD Patients

PROJECT OVERVIEW

The Pharmacogenomic Services division of TRANSGENOMIC aids researchers in the gene identification and discovery of novel disease causing mutations in **Schnyder Crystalline Corneal Dystrophy**. In-depth knowledge of mutation detection, positional cloning and linkage analysis guided this discovery and unveiled possible functions of the UBIAD1 gene.

Schnyder Crystalline Corneal Dystrophy (SCCD: OMIM 121800) is a rare autosomal dominant eye disease characterized by abnormal deposition of cholesterol and phospholipids in the cornea [Rodrigues et al., 1987] resulting in progressive bilateral corneal opacification and decreased visual acuity. The specific gene loci for SCCD in prior investigations have only mapped to a 2.32-Mbp candidate region in a genetic interval between markers D1S1160 and D1S2667 on chromosome 1. Of the 31 candidate genes in this interval, Transgenomic's team focused on three genes: ANGPTL7, FRAP1 and UBIAD1. Strong scientific evidence of their expression of these genes in the cornea was the basis. Furthermore, FRAP1 and UBIAD1 are functionally important because of their involvement in lipid metabolism.

OBJECTIVE

- Analyze candidate genes with input of Positional Cloning and Linkage Analysis data from a collaborator to identify specific gene involvement with SCCD
- Identify the specific mutations associated with SCCD on the gene employing mutation detection platforms
- Identify Phenotype-Genotype correlations
- Propose gene product structure and function
- Refine an assay to detect disease-causing mutations

METHODS AND MATERIALS

- Informed consent was obtained from patients from previous studies¹ totaling 20 unrelated pedigrees with clinically confirmed SCCD. The project also included 200 individual commercially available normal Caucasian DNA control samples of European ancestry (Coriell Cell Repositories, Camden, NJ courtesy).
- Ophthalmologic examination included assessment of visual acuity and performance of slit-lamp examination to assess corneal findings. Characteristics and location of the corneal opacity was documented for later phenotype-genotype correlation analysis.
- Analysis of FRAP1, ANGPTL7 and UBIAD1 was performed on subject blood samples by PCR-based DNA sequencing to examine protein-coding regions, RNA splice junctions and 5' untranslated region (UTR) exons. All control samples were examined for each mutation to ensure that mutations were novel, associated with SCCD disease and were not rare single nucleotide polymorphisms (SNPs).
- Retrospective review of slit lamp photos and gene mutation were compared between individuals from different families who share a mutation and between multiple individuals from the same family. Families with at least three affected individuals were used to make age-matched phenotypic comparisons.



CANDIDATE GENE ANALYSIS IN SCCD

DATA DISCUSSION–MUTATION ANALYSIS

- No mutations were found in FRAP1 or ANGPTL7 genes.
- Eight mutations were identified in the UBIAD1 gene.
- Only SCCD-affected members contained UBIAD1 mutations.
- There is no phenotype-genotype correlation. Affected family members with the same genotype presented different phenotypes contrasting with unrelated subjects with varying genotypes presented with matching phenotypes.
- Only the T175I mutation demonstrated a unique phenotype.

CONCLUSIONS

- The study narrows the candidate gene search for SCCD to the UBIAD1 gene and provides supporting evidence that the presence of UBIAD1 mutations are the cause of SCCD.
- Of the eight mutations found in the study, the 637 A>G mutation found in the protein product N102S, may be a mutation hot spot. This mutation is also located in a highly conserved region among species and in a structurally important domain.
- There was no definitive genotype phenotype correlation in the majority of patients examined in the study. However, only the T175I mutation demonstrated a unique phenotype of prominent diffuse corneal haze with minimal to absent crystals.
- All SCCD-affected subjects demonstrated predictable and gradual corneal changes with increasing age.
- A simple genetic assay can be designed to assess SCCD.
- While genetic testing may not be relevant for the patient with a typical presentation of SCCD, the study suggests that genetic testing may be especially useful in difficult to diagnose cases, genetic counseling and confirmation of young family members not yet presenting disease symptoms.

Pedigree	Mutation	Protein
1	861 G>A	G177R
2	637 A>G	N102S
3	637 A>G	N102S
4	637 A>G	N102S
5	637 A>G	N102S
6	637 A>G	N102S
7	637 A>G	N102S
8	637 A>G	N102S
9	637 A>G	N102S
10	637 A>G	N102S
11	637 A>G	N102S
12	693 C>T	L121F
13	693 C>T	L121F
14	685 A>G	D118G
15	888 G>A	G186R
16	856 C>T	T175I
17	843 T>C	S171P
18	861 G>A	G177R
19	861 G>A	G177R
20	1040 C>G	D236E

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