CHARACTERIZATION OF POSTERIOR CORNEAL DYSTROPHIES FROM PUNJAB POPULATION

[1]SHAGUFTA NAZ, [2]SAIMA SHARIF, [3]AFIA IQBAL, [4]HAIBAKAUL, [5]FARKHANDA MANZOOR

Department of Zoology, Lahore College for Women University, Lahore
E-mail: [1]shagufta6@gmail.com, [2]ssharif1978@yahoo.com, [3]falak_kainat@yahoo.com, [4]haibakaul@gmail.com, [5]doc_farkhanda@yahoo.com

Abstract - Corneal dystrophies are group of inherited corneal diseases causing changes in the different layers of cornea. Posterior corneal dystrophies are characterized by abnormalities of the corneal endothelial and descemet membrane. Three consanguineous families (PKCD001, PKCD004 and PKCD005) affected with CHED2 were enrolled in this study for identification of known genes of affected families to obtain the molecular basis of Posterior corneal dystrophies. Evidence of linkage to chromosome 20p was demonstrated with a maximum LOD score of 2.79 with θ=0 using microsatellite markers D20S889 and D20S181 in PKCD005.

Keywords - Corneal Dystrophy, Descemet membrane, LOD Score, SLC4A11

I. INTRODUCTION

Vision is a mode of skillful encounter with the environment and it mainly relies on one sensory organ-the eye. All vision is based on the perception of electromagnetic rays. These rays pass through the cornea in the form of light. Visual impairment is signified as the functional loss of the vision. Refractive errors crop up as a result of changes in the lens or cornea. The cornea is the outermost part of the eye and exceedingly important for clear vision and overall eye health. Rare diseases related to degenerations and alteration of cornea are entitled as corneal dystrophies. According to a National blindness survey 11.8% blindness in Pakistan is caused by corneal opacity [1]. .According to a latest finding 80% of world's blind people live in developing countries which comprises of 8%-25% blindness leading corneal diseases.

Corneal dystrophies have Mendelian mode of inheritance which may include a simple autosomal dominant, autosomal recessive or X-linked recessive mode. The corneal dystrophies can be classified into three groups based on the sole or prevalent anatomical position of the aberrations. Some affect primarily the corneal epithelium and its basement membrane or Bowman layer and the superficial corneal stroma (anterior corneal dystrophies), the corneal stroma (stromal corneal dystrophies), or Descemet membrane and the corneal endothelium (posterior corneal dystrophies). Most corneal dystrophies have no systemic manifestations and present with variable shaped corneal opacities in a clear or cloudy cornea and they affect visual acuity to different degrees. Different corneal dystrophies are caused by mutations in the CHST6, KRT3, KRT12, PIP5K3, SLC4A11, TACSTD2, TGFBI, and UBIAD1 genes [2].

The group of the posterior corneal dystrophies includes Fuchs corneal dystrophy (FECD), posterior polymorphous corneal dystrophy (PPCD), congenital hereditary endothelial corneal dystrophy (CHED) and X-linked endothelial corneal dystrophy (XECD). These diseases are characterized by abnormalities of the corneal endothelium and Descemet membrane. In most of them, a defective active fluid transport by the corneal endothelium causes excessive edema of the corneal stroma and this impairs the clarity of the cornea and reduces visual acuity. The prevalence of FECD differs markedly in different parts of the world. It is common and the most prevalent corneal dystrophy in the USA, where it affects. Approximately 4% of the population over the age of 40 years. PPCD and CHED are both rare, but CHED2 is more common than CHED1 [3]. XECD has been identified in a single large Austrian pedigree. Several distinct posterior corneal dystrophies exist a relationship between some of them has been suggested on the basis of molecular genetic studies. Phenotypic heterogeneity and allelic heterogeneity exists.

In this study, identification of known genes of affected families was performed to obtain the molecular basis of Posterior corneal dystrophies. These affected families were recognized from different Eye hospitals of Punjab. The phenotype of the disease was confirmed by genetic analysis to find gene mutation. The objective of this research was to make aware the Pakistani population about Posterior corneal dystrophies along with their risk factors.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Three families with affected members having CHED were enrolled in this study after approval by an ethics committee of the Institutional Review Board of the Lahore College for Women University Lahore. After informed consent was obtained, a slit lamp examination was performed for each study participant

to determine the affected status. CHED is characterized by bilateral corneal clouding at birth or prenatally. Symptoms consist of reduced vision, often leading to amblyopia. Nystagmus is another finding, presumably arising from corneal opacification (occlusive amblyopia) at an early age. On slit-lamp evaluation, CHED is characterized by diffuse corneal edema and thickening of Descemet's membrane affecting both eyes, usually symmetrically, presenting at birth. The corneal edema can vary from a blue-gray ground-glass appearance to total corneal opacification.

Detailed family and medical histories were recorded to assess the familial transmission pattern and to exclude any other health conditions segregating with disease. Pedigrees were drawn and blood samples were obtained for DNA Extraction. Genomic DNA was isolated from blood samples and buccal swabs according to a standardized protocol in LCWU, which includes: lysis of cell, protein digestion by proteinase K, proteins precipitation by saturated NaCl and DNA precipitation via Isopropanol.
Determination of DNA concentration was done by Agarose Gel DNA Quantitation and calculated the concentration of stock DNA.The linkage analysis method was used for genetic mapping. It involved the exclusion of reported loci\genes for autosomal recessive CHED and then a genome scan was conducted to map new loci\ genes implicated in PCD.
After PCR amplification all the samples were pooled along with fluorescently labeled three markers with the help of Hamilton. Genotyping was done commercially and the results of peaks were analyzed with the help of Genemapper program. The Genemapper results were transferred to data spreadsheets for haplotype and statistical analyses.

RESULTS AND DISCUSSION

Three consanguineous families (PKCD001, PKCD004 and PKCD005) affected with CHED2 were enrolled for this study. All the three families belonged to Punjab province of Pakistan. PKCD001 belonged to Lahore with three affected and three unaffected family members participated in this study. PKCD004 belonged to Pattokiand had three affected and 4 unaffected individuals. PKCD005belonged to Sahiwal with four affected and two normal individuals. Nineteen individuals including ten affected members participated in this study. The patients underwent detailed ophthalmic and clinical evaluation but no other disease was found segregating with CHED phenotype. Clinical assessment showed that all the affected members of thefamilies have bilateral opaque and swollen corneas since birth or shortly thereafter (Fig. 1). Parents of all the affected individuals were phenotypically unaffected, thus excluding the dominantmode of inheritance, that is, CHED1. The families were screened with microsatellite markers to confirm the linkage to the only gene (SLC4A11) reported to be implicated in CHED2. Evidence of linkage to chromosome 20p was demonstrated with a maximum LOD score of 2.21 at a recombination fraction of 0.0 using microsatellite marker D20S193 in PKCD001, LOD score of 2.18 at a recombination fraction of 0.0 using microsatellite marker D20S193 in PKCD004 and maximum LOD score of 2.79 with $\theta=0$ using microsatellite markers D20S889 and D20S181 in PKCD005 .The results showed homozygosity in all the affected members (Fig. 2,3 & 4); however, their normal family members were either heterozygous for the affected allele or were carrying both the wild type alleles. This prompted us to the conclusion that SLC4A11 could be the culprit gene responsible for CHED2 in these families.

Corneal dystrophies are uncommon conditions in which the cornea is modified without the presence of any inflammation, infection or another eye sickness.

Congenital hereditary endothelial dystrophy (CHED) is defined as a rare inheritable disorder of the corneal endothelium characterized by bilateral, symmetric, non-inflammatory corneal clouding (edema) seen at the time of birth or shortly thereafter [4]. It is assessed that roughly 1.5 million children throughout the world are blind and corneal dystrophies are the noteworthy reason for visual impairment in these children [5].The age of onset and progression of the disease and the degree of visual impairment can be variable, although some children with CHED can develop nystagmus [6].The current study was designed for molecular characterization of Posterior corneal dystrophies in Punjab population. It contained linkage analysis with three markers D20S181, D20S193, D20S889 of gene SLC4A11 in identified affected families. Evidence of linkage to chromosome 20p was demonstrated with a maximum LOD score of 2.79 with $\theta=0$ using microsatellite markers D20S889 and D20S181 in PKCD005.



Fig. 1: Affected individual of family PKCD004 with autosomal recessive congenital hereditary endothelial dystrophy (CHED2) depicting bilateral hazy corneas

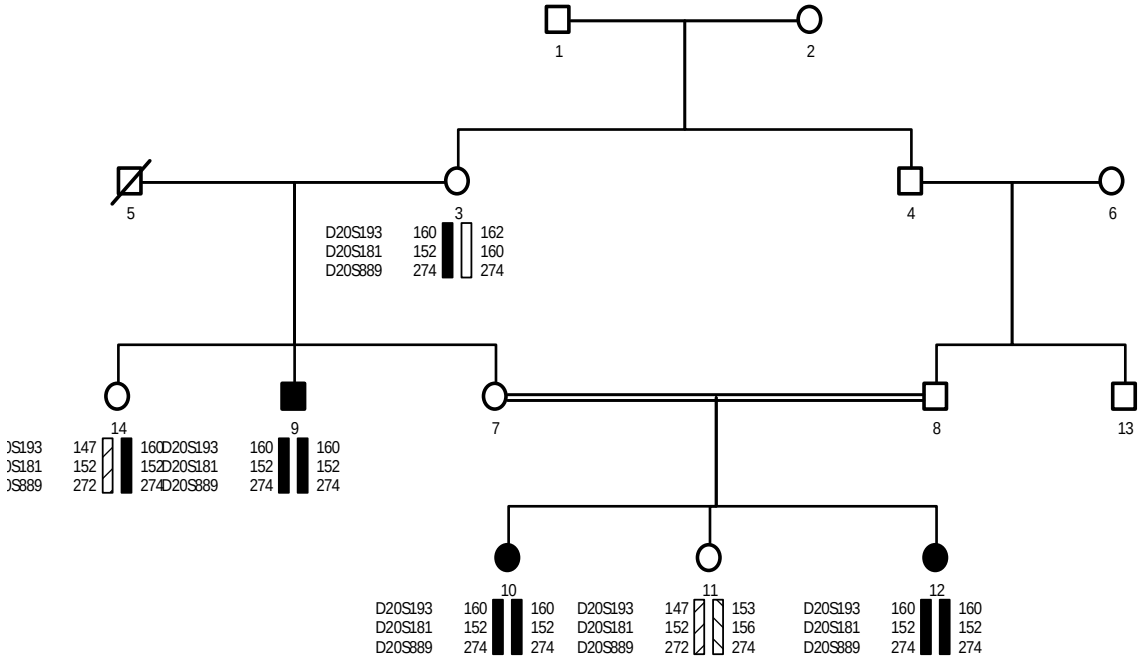


Fig. 2: Pedigree drawing of family PKCD001 affected with autosomal recessive congenital hereditary endothelial dystrophy. Filled symbols indicate clinically affected individuals, open symbols indicate unaffected individuals or those whose disease status was not established

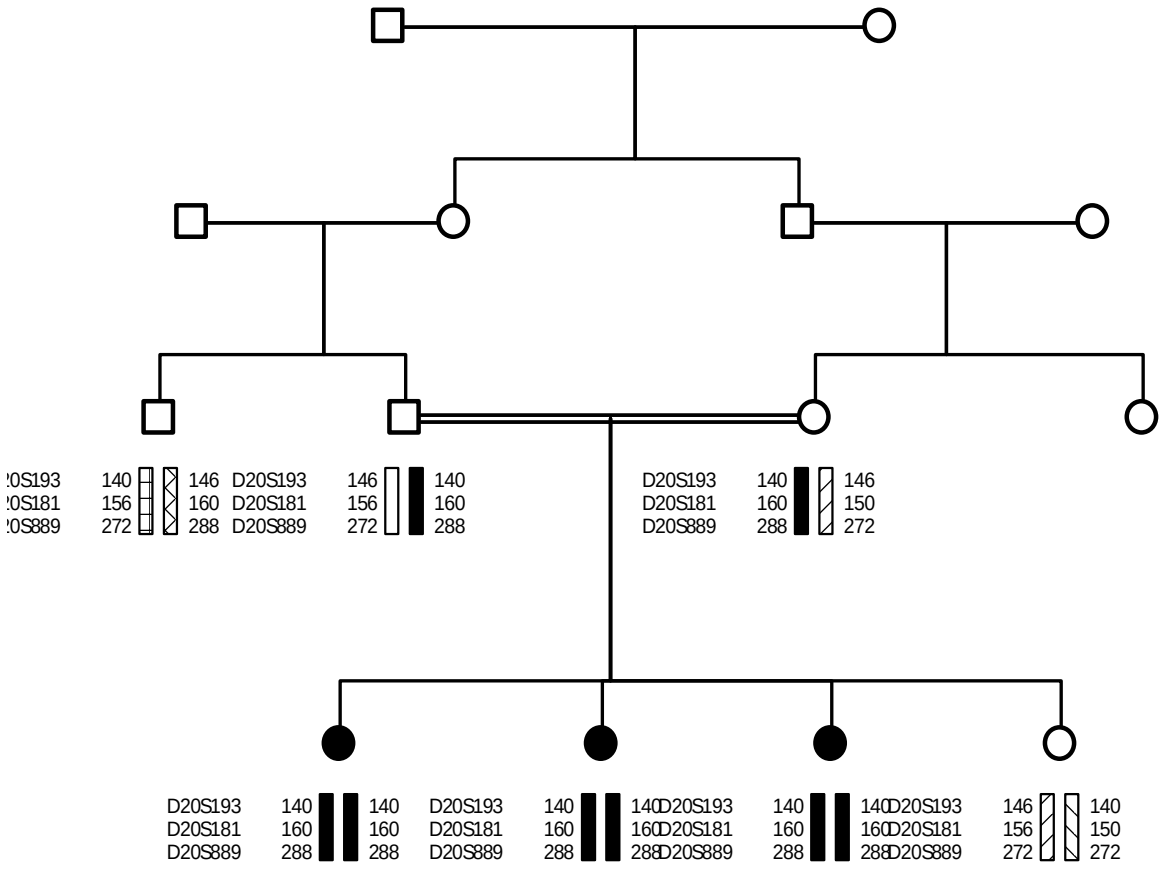


Fig. 3: Pedigree drawing of family PKCD004 affected with autosomal recessive congenital hereditary endothelial dystrophy. Filled symbols indicate clinically affected individuals, open symbols indicate unaffected individuals or those whose disease status was not established

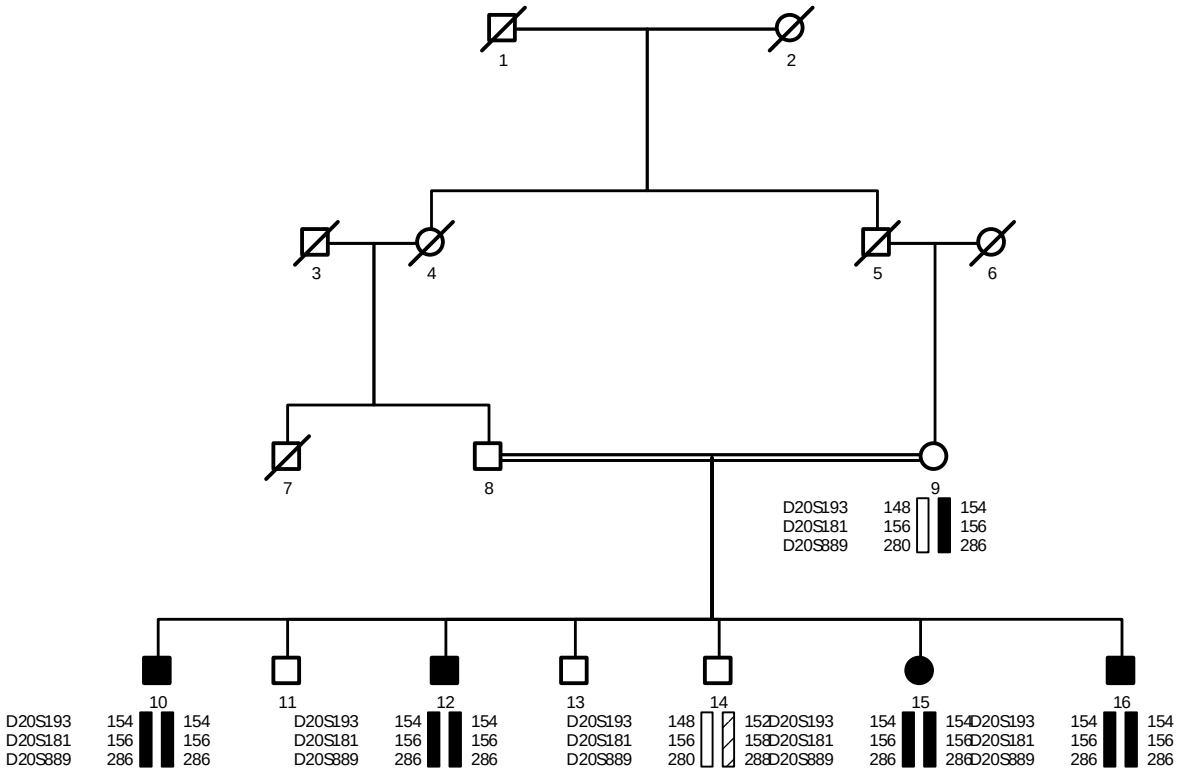


Fig. 4: Pedigree drawing of family PKCD005 affected with autosomal recessive congenital hereditary endothelial dystrophy. Filled symbols indicate clinically affected individuals, open symbols indicate unaffected individuals or those whose disease status was not established

ACKNOWLEDGEMENTS

We sincerely thank patients for their help in the clinical evaluation and data collection.I acknowledge the financial support of HEC (Ref No. 20-2211/NRPU/R&D/HEC/12) to carry out my research work with full satisfaction.

REFERENCES

[1] A. A. Khan, N. U. Khan, K. M. Bile, andH. Awan, "Creating synergies for health systems strengthening through partnerships in Pakistan – a case study of the national eye health programme", EMHJ Supplement, vol. 16, pp.61-68, 2010.

[2] G. K. Klintworth, "Corneal Dystrophies",Orphanet Journal of Rare diseases, vol. 4,pp.7, 2009.

[3] E. N. Vithana, P. Morgan, and P. Sundaresan, "Mutations in sodium-borate cotransporter SLC4A11 cause recessive congenital hereditary endothelial dystrophy (CHED2)", Nature Genetics, vol. 38, pp.755–757, 2006.

[4] P. Pilwal, and A. Sharma, "Relevance of Molecular Diagnosis of Corneal Dystrophies:,International Journal of Human Genetics, vol. 11,pp.1-14, 2011.

[5] D. C. Musch, L. M. Niziol, J. D. Stein, R. M. Kamyar, and A. Sugar, "Prevalence of Corneal Dystrophies in the United States: Estimates from Claims Data", Investigative Ophthalmology and Visual Sciences, vol. 52, pp.6959–6963, 2011.

[6] R. W. Weisenthal, and B. W. Streeten, "Posterior membrane dystrophies", In: Krachmer JH, Mannis MJ, Holland EJ, eds. Cornea. Philadelphia: Elsevier Mosby, pp.929–954.954, 2005.

★★★