Methodology:-

This study will be approved Desai Eye Hospital Ethics committee in accordance with the rules and regulations of the regulatory respective authorities in vogue, written informed consents will be obtained from recruited patients.

**Genes selected for present study**

SOX2, PAX6, OTX2, RAX, CHX10, FOXE3, CRYBA4, BAMP4 are the candidate genes selected for present study.

**Subjects**

The subjects recruited will be 20 children with congenital blindness due to A/M/C and normal. The study visit began with a brief history including current age, conformation of Anophthalmia/ microphthalmia/ coloboma. Informed consent will be obtained from all subjects whose blood and DNA samples will be used. Peripheral blood samples (5-10 ml) will be collected, and genomic DNA will be extracted by the extraction procedure.

**Primer designing:** primer for all seven genes will be designed using free online primer designing software simgene.com/Primer3.

**Conformation-sensitive-gel-electrophoresis:** Conformation sensitive- gel-electrophoresis (CSGE) will be used for initial gene screenings Conformation-sensitive gel electrophoresis is a heteroduplex-based method that is particularly well suited to high-throughput analyses. Its simplicity makes it amenable to various adaptations and modifications to enhance its applicability to genome wide mutation scans.

**Polymerase chain reaction (PCR) and DNA sequencing:** Standard polymerase chain reaction (PCR) will be performed on genomic DNA of affected patients, and healthy controls to amplify gene exonic sequences. PCR products will be separated by electrophoresis in an 8% polyacrylamide gel for detection of heteroduplexes and homoduplexes. Patient samples containing heteroduplexes will be selected for sequence analysis. Target sequencing will be done by Sanger method.

The sequence will be analysed for the mutation patterns using bioinformatics approach. sequence will analysed by mutation surveyor (www.softgenetics.com/mutationSurveyor.html) and other freely available software such as www.sanger.ac.uk/resources/software http://www.ccmb.res.in/bic/software_snsAlign.php

**Scope and limitation**

Scope of this study is to find out candidate gene for A/M/C mutation. Genetic screening of candidate genes and the identification of genetic factors influencing gene expression may help
to determine the susceptibility of various disease alleles in the occurrence of this severe form of eye malformation and also help to identify factors that may influence gene expression. The limitation of this study is the small cohort size screened. The small cohort size and diverse nature of the disease phenotypes studied in this cohort make it difficult to extrapolate the incidence rate of mutations in the general A/M/C population. However, it is difficult to collect large, homogenous sample sets of isolated A/M/C patients. In addition, different A/M/C populations need to be screened to better ascertain the percentage of A/M/C cases due to mutations. Continued recruitment of A/M/C patients is necessary for future genetic screenings.