Work plan and Methodology:

**Family ascertainment**
Patients with a positive family history of childhood cataract will be recruited from the Desai Eye Hospital, Pune, India. The family members will be interviewed to obtain a detailed medical, ophthalmic, and family history and will be included in the study based on their informed consent as approved by the ethics committee of the Hospital. Patients with a history suggestive of intrauterine infection such as rubella, complicated cataract, and traumatic cataract will be excluded from the study.

**Molecular analysis**
One affected representative individual from each of the identified families will be chosen for mutation analysis. Primers will be designed to amplify the entire coding exons and 10–30 bp of the flanking intronic sequences of the 10 crystallin genes - CRYAA, CRYAB, CRYBA1, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGC, CRYGD, and CRYGS (Devi et al., 2008). DNA will be extracted from blood using any of the isolation methods like phenol chloroform or salting out (Miller SA et al. 1998). Commercially available DNA isolation kits such as the DNA extraction kit from Agilent technologies may also be used for extraction of DNA.
Sequencing of the DNA will be performed using chain terminator chemistry with a Big Dye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI 3130 DNA analysis system.
The DNA sequences obtained will be analysed and compared with the reference sequence using the bioinformatics approach using the Mutation Surveyor software from Softgenetics Incorporation, USA. If a nonsynonymous sequence change that results in a change in the amino acid sequence of the protein is found, available family members will then be analysed for cosegregation of the genotype with the phenotype. If available, the presence or the absence of a restriction site will be employed to confirm the cosegregation of sequence variation among the family members and in the control population. In case no suitable restriction site is identified, direct sequencing will be performed to analyse the cosegregation of the genotype with the disease phenotype. (Devi et al., 2008).

**Homology modeling**
Modeling of the human βγ-crystallins, γC- and γS-crystallin, will be performed by homology modeling based on correspondent crystal coordinates for murine protein structures (Brookhaven Protein Database [PDB] files: 2v2u and 1zw0) as the structural
templates. The impact of the mutations on the conformations will be observed in order to study the gain/loss of functions in the crystallin proteins due to mutations (Devi et al., 2008).

**Scope and Limitations**

Currently, diagnosis of the childhood cataract is based on the physical and clinical examinations only. There is an ample scope for the development of the prognostic and diagnostic molecular tools based on the biomarker genes. Such biomarker genes and proteins can be identified through genetic studies and linking of the genotype to disease phenotype by undertaking mutation analysis of the causative genes.

**Utility:**

This research work will investigate the presence of further novel genes or sequence elements involved in the pathogenesis of congenital cataract in Maharashtrian families. It will also provide the platform to develop the prognostic and diagnostic tools to monitor, treat and prevent the childhood cataract.