2. AIM AND OBJECTIVES

2.1. Rationale and scope of the study

As far as cure for Retinoschisis is concerned, no treatment is currently available to arrest the natural progression of schisis. Surgical intervention or application of carbonic anhydrase inhibitors may help in managing the severe effect of the disorder, however, long-term rescue or complete cure is yet to be established. Although gene therapy has reached clinical trials, it involves potential risk factors which need to be addressed. One of the primary reasons behind the lacunae in efficacious disease management is the incomplete knowledge about the pathobiology of the disease. Although in vitro research has provided insights on basic molecular mechanism of mutant retinoschisin, the actual pathological events occurring in the affected eye has not been examined in detail. Besides, the holistic role and function of retinoschisin in eye as well as in other tissues still remain enigmatic.

Furthermore, there are several reports showing huge variability in the disease severity which has not been understood so far. Therefore, functional characterization of RS1 mutants is crucial to comprehend the variation in phenotype expression. Likewise, exploring the protein interaction network of retinoschisin and understanding the biochemical changes in the diseased eye might improve our knowledge on the pathophysiology of the disease. This would aid in designing effective drugs to combat the symptoms and in the development of a safe as well as successful gene/protein/cell therapy by overcoming its current limitations.

In addition, clinical diagnosis of these genetic disorders remains a challenge since the severity varies and at times the ocular presentations overlap with other clinical conditions. In such cases genetic testing may help in differential diagnosis
substantiating the clinical observation also benefiting the patient and family members in terms of prenatal diagnosis and carrier status detection during genetic counselling.

2.2. Aim of the study

The present study aims at investigating the clinical, genetic and molecular aspects of X-linked retinoschisis (XLRS) to understand the disease pathogenesis in Indian cohort, which may advance the development of an effective treatment modality.

To address this aim, the proposed specific objectives of the study were:

2.2.1. Specific objectives

- **Objective I:** To clinically recruit retinoschisis patients and evaluate their ocular manifestations by various diagnostic parameters.
- **Objective II:** To screen the patients for genetic defects by candidate gene screening approach or high-throughput genotyping platforms.
- **Objective III:** To investigate the molecular mechanism of the identified RS1 mutants by studying their expression and secretory profile in mammalian cell line.
- **Objective IV:** To understand clinical heterogeneity in XLRS by correlating the observed phenotype with the genotype of XLRS patients.
- **Objective V:** To employ *in silico* tools like molecular modelling along with dynamic simulation studies and *in vitro* experiments to explore the mechanism behind variable phenotype expression in XLRS.
- **Objective VI:** To probe the functional interactions of retinoschisin by examining its protein-protein interaction network and identifying its putative binding partners in Human retinal tissue.

- **Objective VII:** To analyze the proteome composition of the intraschisis fluid from XLRS patients (obtained during surgical intervention) using mass spectrometry in order to determine the dysregulated signalling pathways occurring in the course of the disease.

2.3. Work flow of the study

![Figure 2.1. Pictorial representation of the work flow and study methodology. OCT- Optical Coherence Tomography, ERG - Electoretinogram, SNP - Single Nucleotide Polymorphism, NGS - Next Generation Sequencing, IF - Immunofluorescence, WB - Western Blotting, IP - immunoprecipitation, IPA - Ingenuity Pathway Analysis]