5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Based on the present study, about 89% of the clinically diagnosed retinoschisis patients were found to harbour mutation in the RS1 gene. Functional characterization of the RS1 mutants showed intracellular protein retention as the major molecular mechanism that eventually results in loss of function. Phenotype-genotype correlation analysis revealed huge variability in disease severity even among patients exhibiting the same mutant secretory profile.

Detailed investigation on the structural and subcellular localization of various RS1 mutants led us to hypothesize that the phenotype heterogeneity in XLRS is not merely dependent on the secretory profile of the mutant, but, the precise localization and overall structural conformation of the mutant protein. On the whole, our findings suggests that the molecular mechanism of disease causation and severity may be depend on whether i) the mutant RS1 is expressed ii) mutant RS1 is secreted but nonfunctional iii) mutant RS1 is completely retained within the cell or iv) mutant RS1 is retained within the cell while a fraction of it is transported to the plasma membrane region. This knowledge might help in understanding the distinctive molecular mechanism and effect of each RS1 mutant that may affect the therapeutic efficacy of the wild type counterpart during gene therapy.

Protein-protein interaction analysis of retinoschisin identified several putative binding proteins that were indicative of the fact that RS1 might be involved in the regulation of MAP kinase signalling pathway that controls a broad range of cellular activities and physiological processes including immune response, cell survival and apoptosis.
RDH14 was found to be abundant in the intraschisis fluid of the patients, which is speculated to elicit the three canonical pathways viz., LXR/RXR activation, complement system and acute phase response signalling. The involvement of these pathways suggests that immune and inflammation might play a key role in the pathogenesis of XLRS and modulation of this might help in the management of the disease.

5.2. Highlights of the study

- This is the first study to molecularly characterize the novel mutations in an Indian cohort demonstrating the likely localization of mutant RS1 to the plasma membrane region although not secreted.
- Co-existence of XLRS with systemic defects such as developmental delay, sensorineural hearing loss and hypotonia in the same individual was a striking feature observed in our study sample.
- The findings on intraschisis fluid have provided insights on the actual proteomic changes occurring in the affected eye of the patient that was not completely examined in the past. This data might serve as a valuable source of knowledge for future studies that focus on identifying biomarkers of retinoschisis.
- The pilot data exploring the functional association between Retinoschisis and Norrie disease has demonstrated the lack of occurrence of a physical interaction between RS1 and NDP, thus eliminating the possibility of a direct protein level relationship between the two disorders.
- This study has provided evidence emphasizing the need for gene testing, which could help in differential diagnosis and have a direct impact on genetic counselling as it can detect the carrier status of the family members, thereby predict the risk of transmission of the disease to their offspring.
5.3. Limitations of the study

- Though the outcome of molecular characterization study has helped us in understanding the intricate pathophysiology of retinoschisis, the interpretations were arrived from *in vitro* experiments and hence, cannot be accounted in full for the *in vivo* cell machinery and effects.

- Owing to the small sample size (time period of sample collection was 3 years); it was not possible to elucidate a more elaborate phenotype-genotype correlation.

- All *in silico* predictions of this study have been correlated only with the monomeric form of retinoschisin although the functionally active form of the protein is known to attain a paired double octameric structure. Analyzing the mutant protein’s oligomeric conformation might involve extensive biocomputing software.

5.4. Future prospects

- The precise secretory phenomenon of wild type retinoschisin still remains to be explored. Understanding the sequence of events in the secretory pathway might throw light on the variation in phenotype exerted by different RS1 mutants.

- A recent report on the structural analysis of retinoschisin has shown that the wild type protein forms a paired double octamer ring, which forms the structural basis for its functional role. Hence, it would be a promising prospective study to examine whether the membrane-bound RS1 mutants form a double octamer structure and therefore are likely to be functional.

- The putative binding partners of wild type RS1, especially those involved in the MAP kinase pathway need to be studied in detail. Likewise, functional studies on the candidate proteins dysregulated in the disease condition (schisis fluid) might help in identifying probable biomarkers of the disease and substantiate the
involvement of inflammatory and immune responses in the pathogenesis of XLRS.

- One other challenging research subject would be investigating the actual source of the intraschisis fluid. The likely source of schisis fluid accumulation in the retina other than the infiltration of liquefied vitreous could be the seepage of plasma from the retinal blood vessels. And, this would involve collecting vitreous as well as plasma sample from the affected patient from whom the schisis fluid is drained.