



**University
of Victoria**

Graduate Studies

Notice of the Final Oral Examination
for the Degree of Master of Science

of

ANASTASIA LITKE

BSc (University of Victoria, 2015)

**“Investigating the Pathogenicity of Missense Mutations in *VSX1* and
their Association with Corneal Dystrophies”**

Department of Biology, Neuroscience

Wednesday, April 25, 2018

10:00 A.M.

Medical Science Building

Room 150

Supervisory Committee:

Dr. Robert Chow, Department of Biology, University of Victoria (Supervisor)

Dr. Leigh Anne Swayne, Division of Medical Science, UVic (Member)

Dr. Patrick Walter, Department of Biology, UVic (Member)

External Examiner:

Dr. Lisa Reynolds, Department of Biochemistry and Microbiology, UVic

Chair of Oral Examination:

Dr. Perry Howard, Department of Biochemistry and Microbiology, UVic

Abstract

Two corneal dystrophies, posterior polymorphous corneal dystrophy (PPCD) and keratoconus, have been associated with missense mutations found in a transcription factor-encoding gene *Visual System Homeobox 1* (*VSX1*). Despite this association, the pathogenic link between *VSX1* and these diseases remains controversial.

To address this issue, I utilized a variety of *in vitro* approaches to study the effects on transcriptional activity, protein expression levels and subcellular localization, seven missense mutations found in disease populations that span two highly conserved domains, the homeodomain (HD) and CVC domain, may have. I also carried out an *in vivo* investigation of one mutation by generating a mouse line carrying the *Vsx1* P247R mutation. In addition to investigating morphology in adult corneas through histology, *in vivo* whole eye confocal imaging was used to examine corneal curvature and thickness. Quantification of immunocytochemistry was used to characterize terminal marker expression in the inner retina compared to previously described phenotypes in *Vsx1*-null mice.

My results showed that mutations found in both the HD and CVC domain can alter transcriptional repression *in vitro* in *Vsx1*. However, my investigation showed that these subsequent changes are not the result of changes to protein expression or subcellular localization. Characterization of corneal and retinal phenotypes *in vivo* revealed no significant differences in my *Vsx1* P254R mice when compared to wild-type and *Vsx1*-null controls. Therefore, I concluded that P254R is not pathogenic for corneal dystrophies in a mouse model. However, my work does show that *Vsx1* mutations do have the ability to alter protein activity and therefore may still have the potential to be pathogenic in humans. Further investigation, however, is needed to determine whether *VSX1* mutations found in disease populations are in fact causative for corneal dystrophies.