

Prof. Natascia Tiso, PhD

*Associate Professor in Applied Biology
DeBio Zebrafish Facility Deputy Director*

Department of Biology

Via U. Bassi 58/b, I-35131 Padova, Italy

Tel: +39 049 8276302

E-mail: natascia.tiso@unipd.it

To the kind attention of:

Warsaw PhD School in Natural and BioMedical Sciences

Padova, 31 August 2026

Review of the Doctoral Dissertation of Razieh Amini

Institution: International Institute of Molecular and Cell Biology in Warsaw

PhD School: Warsaw PhD School in Natural and BioMedical Sciences

Title: Genetic & Physical Regulation of Ventricular Development & Reissner Fiber Formation in Zebrafish

Supervisor: Dr hab. Vladimir Korzh

Reviewer: Prof. Natascia Tiso, Department of Biology, University of Padova (Italy)

The doctoral dissertation submitted by Razieh Amini addresses the genetic, membrane-associated, and physical mechanisms regulating the development of the brain ventricular system (BVS) and the assembly of the Reissner fiber (RF) in zebrafish. The work was carried out under the supervision of Dr hab. Vladimir Korzh in the Laboratory of Neurodegeneration at IIMCB, Warsaw. The dissertation is presented in the form of a collection of four manuscripts/articles, accompanied by an extended introduction, a discussion section including unpublished data, and a chapter on future perspectives. The topic is original, timely, and clearly situated within the broader field of developmental neurobiology, at the intersection of ion channel physiology, membrane biology, developmental signalling, and biomechanics. The title accurately reflects the scope and content of the dissertation.

Form and general structure

The dissertation is written in clear, precise English. The overall structure (Introduction, Research articles in the collection, Discussion, Future perspectives, Materials and Methods, References) is logical and easy to follow. I particularly appreciated the way the Discussion chapter (Section 3) is explicitly organised around the three research questions stated in Section 1.9, using a clear question-and-answer format for each manuscript. This editorial choice makes the connection between research objectives and findings unusually transparent and should be commended; it is a structural strength that many multi-manuscript dissertations lack.

One formal issue deserves attention: Manuscript 2 (R. Rosa Amini, Ruchi P. Jain, Vladimir Korzh, "Body orientation affects brain ventricular system development and mechanosensory gene expression in zebrafish embryos") is included as a manuscript still "currently submitted to npj Microgravity," without final editorial data. This is notable because it is a manuscript in which the Candidate holds a prominent position as first author. While inclusion of unpublished/submitted work in a dissertation is common and acceptable practice, the Candidate should be prepared to comment on the current status of peer review for this manuscript, as any revisions requested by reviewers could affect the presentation of these results as final.

Introduction and Research Objectives

The Introduction is comprehensive and well-referenced, moving logically from the general architecture of the brain ventricular system (1.1) to the structure and function of the Reissner fiber (RF) (1.2), the biology of the subcommissural organ (SCO) (1.3), known regulators of RF assembly (1.4), physical/gravitational constraints (1.5), the molecular biology of Kv2.1/Kcng4 channels (1.6), and finally to the justification for using zebrafish as a model (1.7) and the evolutionary/translational relevance of the work (1.8). The three research questions stated in Section 1.9 are clearly formulated and map directly onto the three major experimental threads of the thesis (Kv2.1 channel biology, membrane/signalling crosstalk, and gravity-dependent mechanics). This is a well-designed, hypothesis-driven PhD project.

Research Articles in the Collection

The four manuscripts/articles collectively demonstrate an unusually broad and productive research output for a single PhD project. The Candidate is:

- First author on Manuscript 1 (published, *Frontiers in Cell and Developmental Biology*, 2026) and Manuscript 2 (submitted, *NPJ Microgravity*), both representing the core, hypothesis-driven contributions of the thesis;
- Second author on Manuscript 3 (Jain, Amini, Majewski, Korzh; bioRxiv preprint, under review), addressing the neurophysiological and behavioural consequences of Kv2.1/Kv6.4 deficiency;
- A contributing co-author on Manuscript 4 (Jędrychowska et al., published in *Developmental Biology*, 2024), addressing the role of Kcng4b in inner ear development.

Authorship of four manuscripts/articles, with two as first author, is a strong and mature publication record for a doctoral candidate, and clearly indicates the Candidate's central and independent role in generating the major findings of the thesis, as well as her collaborative engagement with parallel projects in the laboratory. The explicit designation of "major contribution" versus "minor contribution" manuscripts in the Discussion chapter is a commendable and transparent choice that aids evaluation of the Candidate's individual scientific contribution; a clarity that is not always present in cumulative dissertations.

Manuscript 1 convincingly demonstrates that Kv2.1, through the antagonistic activities of its Kcnb1 and Kcng4b subunits, negatively regulates RF formation at multiple levels, including the transcription of RF-associated genes, Sspo (SCO-spondin) secretion, SCO (subcommissural organ) apical morphology, and RF disassembly at the ampulla terminalis.

The staged model of RF assembly (floor plate-derived posterior RF; SCO-derived anterior RF; fusion at the flexural organ) is a valuable conceptual contribution in its own right, independent of the mutant analysis. Concerning the choice of mutant genotypes for the analysis, although their characterization has already been described in previous publications, I would appreciate it if the Candidate could comment further on the rationale for using homozygous versus heterozygous individuals, as well as on their viability and lifespan.

Manuscript 2 provides an elegant and technically sophisticated approach to studying gravity-dependent effects under normogravity, using fixed body orientation as a proxy for altered cerebrospinal fluid (CSF) redistribution. The finding that *chl1a/camel* transcript levels respond far more dramatically to altered orientation than the Hippo-pathway component *yap1* is a striking and potentially very useful result.

The unpublished data presented in Section 3.3 (Wnt/Shh signalling, cholesterol depletion, oxidative stress, AFRU antibody injection, and ciliary architecture) substantially strengthen the mechanistic narrative of the thesis, showing that RF assembly depends on a cholesterol-dependent membrane environment that is itself required for proper Wnt/Shh signalling output.

This is one of the most conceptually interesting parts of the thesis, although the Candidate might be prepared to comment on the imaging experiments, for which it is not clear whether pattern analysis was performed solely through signal quantification or also included software-based analysis of signal distribution and/or morphology.

Discussion and Future Perspectives

The Discussion is well organised and demonstrates mature scientific reasoning, correctly identifying both the strengths of the findings and the open questions they generate (e.g., the mechanism behind the duplicated anterior RF phenotype in *kcnb1* mutants). The Future Perspectives chapter proposes concrete, methodologically sound next steps (defining the RF initiation window, direct CSF flow imaging, separating local from systemic effects, and dissecting membrane/secretion mechanisms). This chapter demonstrates the Candidate's capacity for independent scientific planning beyond the immediate scope of the completed work.

Questions and suggestions for the defence

1. Regarding the cholesterol depletion experiments (Section 3.3.3): would the Candidate consider it informative to also test the opposite manipulation (increasing membrane cholesterol availability) to determine whether RF formation and/or Kv2.1-dependent phenotypes are similarly sensitive to cholesterol excess? If so, which genetic or pharmacological approaches could be used to increase membrane cholesterol availability in zebrafish embryos?
2. The role of Kv2.1 subunits was established primarily through loss-of-function mutant analysis. Would overexpression approaches (e.g., mRNA injection at the embryonic stage, assessed during the first 2–3 days of development) or pharmacological/microenvironmental manipulation of channel activity (if such tools exist) have offered a complementary or confirmatory line of evidence?
3. The data indicate that Kv2.1 influences RF formation, but do not clearly distinguish between its electrical/conductance-dependent functions and its non-conductive roles in membrane organisation, trafficking, and ER–plasma membrane junctions. What experiment would the Candidate consider the most decisive for distinguishing these mechanisms?
4. The phenotypes of *kcnb1* and *kcng4b* mutants are largely opposite and symmetrical (expanded/duplicated RF vs. reduced/absent RF). Does the Candidate interpret this primarily as a stoichiometric effect on channel composition (*Kcnb1:Kcng4b* ratio), or is there evidence supporting additional, channel-independent functions of either subunit?
5. Would cell-type-specific transcriptomic approaches, such as single-cell, spatial, or targeted transcriptomic profiling of SCO and floor plate/flexural organ cells, help resolve the molecular mechanisms underlying the observed RF phenotypes?
6. In Manuscript 2, embryos were immobilised in low-melting-point agarose within capillaries to fix body orientation. Has the Candidate considered or experimentally controlled for potential confounding mechanical effects of the agarose matrix itself (e.g. local confinement, pressure, or substrate stiffness) on mechanotransduction pathways, independently of gravitational orientation?

7. Given that chl1a/camel showed the strongest transcriptional response to altered body orientation among the genes tested (approximately 13-fold in the reversed-vertical condition), does the Candidate consider it a promising candidate marker of mechanically induced stress or altered mechanotransduction in the developing ventricular system, and how could this be tested under true microgravity conditions?

Final Conclusion

The dissertation presents original, methodologically diverse, and well-integrated research addressing a coherent set of questions concerning the molecular and physical regulation of Reissner fiber and brain ventricular system development in zebrafish. The Candidate has demonstrated strong independent scientific capability, evidenced by a first-author publication record, a second and a contributing authorship, and a mature, well-organised dissertation.

I evaluate this PhD dissertation very positively, and I consider it to be of exceptional quality, worthy of distinction, given the originality of the experimental design, the breadth and quality of the publication record (four manuscripts/articles, including two as first author), and the clarity and maturity of scientific reasoning demonstrated throughout the manuscript.

I, the undersigned, hereby state that the doctoral dissertation of Razieh Amini meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended).

I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Razieh Amini to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

I further consider that the doctoral dissertation of Razieh Amini should be awarded a **distinction**, in recognition of the originality and coherence of the research programme, the significant and independent contribution of the Candidate as demonstrated by her authorship record (first author on two manuscripts, including one published in a peer-reviewed journal, second author on a third manuscript, and contributing author on a fourth), and the overall scientific maturity, clarity, and rigor of the dissertation as a whole.

Date: 31 August 2026

Yours sincerely,
Prof. Natascia Tiso

Prof. Natascia Tiso, PhD

Firmato digitalmente da: Natascia Tiso
Organizzazione: UNIVERSITA' DEGLI STUDI DI PADOVA/00742430283
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