Predoctoral Fellowship

Applicant: Minton, Austin
Title: Contribution of Variants in the Titin Gene to the Pathology of Human Dilated Cardiomyopathy
Program: Predoctoral Fellowship
Institution: University of Kentucky Research Foundation
App #: 25PRE1375285

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Committee: 2025 Fellowship Clinical 1

Summary Statement

Percentile Rank: (Triage)

Reviewer Role: Reviewer 1

Brief Summary of the Proposal

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This is a predoctoral fellowship application by a graduate student currently in his 3rd year in the College of Medicine at the University of Kentucky. The applicant proposes to study the role of genetic mutations in Titin in the development of Dilated Cardiomyopathy (DCM). The applicant's mentor has established a patient sample repository with over 20,000 cardiac samples collected from 570 patients and organ donors. During the time in his mentor's lab, the applicant led the effort to obtain whole genome sequencing on samples from 350 of these patients. He has identified samples from 15 patients with DCM that show variations in Titin (TTNtv), 15 patients with DCM without TTNtv, and 15 healthy organ donors. Using these samples, the applicant proposes to test the hypothesis that TTNtv results in a DCM phenotype by overloading cellular decay pathways and integrating truncated titin filaments into the sarcomere. The applicant is a promising candidate who aspires to be a distinguished principal investigator involved in translational research. He has demonstrated an ability to make the most of scant research opportunities that were available to him as an undergraduate. The letter of support from his undergraduate mentor and the applicant's listed poster and oral presentations at local and National meetings are a testament to his efforts. The applicant's current mentor also rates him highly and credits him with developing the idea of the proposal entirely on his own. The mentor, Dr. Campbell is a highly successful independent investigator with several years of experience in studying sarcomere function in myofilaments. He is well published and well-funded and has included a detailed training plan. Enthusiasm for this application is dampened because the scientific portion of the application is very poorly written. The applicant will test the hypothesis that TTNtv results in a DCM phenotype by overloading cellular decay pathways and integrating truncated titin filaments into the sarcomere. Three specific aims are proposed to test individual hypotheses that samples with TTNtv will have higher UPF1 and EXOSC10 abundance (aim 1), will have higher titin ubiquitination and lipofusinogenesis (aim 2), and will incorporate truncated titin filaments into sarcomeres (aim 3). The experiments are clear,

but the rationale for each aim and the overall hypothesis are very poorly developed. It is frequently hard to understand the ideas that the applicant is trying to convey. There is excessive use of scientific jargon without any explanation. Several statements do not make logical sense. Methods of analysis are poorly described. Alternate approaches/interpretation if results are different from expected are not discussed. The patient populations to be studied though already identified is not described in terms of biological covariates, such as age, sex and diabetes status (mentioned in the application). These are several weaknesses that outweigh the strengths of the scientific proposal. It also suggests that the mentor did not work with the trainee on helping him polish his writing to clearly and logically present ideas. Similar lack of clear scientific understanding, or the ability to present scientific ideas clearly, is also obvious in the non-scientist summary. Overall, the application is strong in all other aspects but quite weak in the written scientific proposal.

Evaluation of the Applicant, Sponsor/Training Plan and Environment
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Strengths:

- The applicant is interested in a career in translational Science. He completed undergraduate degree in Kentucky Wesleyan College in 2022 with honors in Chemistry and Biology. During his undergraduate studies, he participated in the discovery of new antibiotics from soil bacteria. This is a popular hands-on research training program run by Tiny Earth. It has a world-wide network of instructors including many universities in rural area with scant research avenues for students. Students are provided opportunities to present their work in conferences. The applicant continued to work on this project for 5 semesters after his initial exposure. He went on to do chemical extraction and analysis on some of the isolates as a Wesleyan Fellow and was selected for oral presentations in the 2020 and 2021 meetings of the organization.

- The applicant has been enrolled in the University of Kentucky's graduate program since August 2022 where he has joined the laboratory of Dr. Kenneth Campbell in the College of Medicine. Dr. Campbell works on muscle sarcomere mechanics and mathematical modeling, now expanding to ventricular remodeling and organ-level function. The mentor has established a patient sample repository since 2008. It has over 20,000 cardiac samples collected from 570 patients and organ donors. During his first year, the applicant extracted DNA and RNA from 394 patients' (though unclear how these were selected) cryopreserved cardiac samples using a high throughput protocol. He coordinated submission of 350 samples for library prep and whole genome sequencing through genomics companies. To accomplish this, he needed to take sequencing depth, coverage, and enrichment methods into consideration. The applicant collated the whole genome sequencing data with the clinical data to identify patients with DCM and sequencing variations in the TTN gene. These results were presented as posters at local meetings at the University of Kentucky and an oral presentation at 2024 STEP-UP research symposium held at the NIH attended by participants of the Short-Term Research Experience for Undergraduate Persons (STEP-UP).

- Applicant's academic records are acceptable with a trend of improvement over time. Mentor notes that the applicant is proficient in generating figures in MATLAB and working on large dataset.

- All 3 reference letters and the mentor's assessment are highly positive.]

- The applicant was very effective in seeking and utilizing research opportunities available to him. While there are no publications or manuscripts in preparation that are mentioned, the applicant has presented posters and given oral talks at local and national meetings.

- Mentor notes that the applicant developed the project entirely by himself and improved it with feedback from the advisory committee.

- The proposed work is aligned with the ongoing work in Dr. Campbell's laboratory and utilizes the resource of banked samples available to the lab.

- The mentor, Dr. Campbell is a highly successful independent investigator who studies sarcomere function in myofilaments through biophysical, biochemical, and computational approaches. He is well published scientist and an MPI on at least 2 ongoing R01s and a third expected to be funded. In addition, he serves as a Co-I on two additional R01s. There is no funding concern to support the applicant's research proposal.

    - The mentor had completed a 12-hour training titled "Entering Mentoring". It included reading and discussions of the "Crucial Conversations" text and discussion of skills to maneuver mentor-mentee relationships.

    - Mentor leads a session titled "Assessing Knowledge" for the College of Medicine's mentor training program. He has 4 current PhD or MD/PhD trainees including the applicant and 2 postdoctoral trainees. Of these, one of the graduate students is supported by an AHA fellowship and another by an F31.

- Mentor's training plan is appropriate.

    - PhD advisory committee has 1 cardiologist and 3 muscle physiologists. The inclusion of a cardiologist is deliberate to promote training in translational research.

    - 4 training goals are identified:

        - Applicant should be able to independently perform publication-quality experiments measuring the contractile properties of chemically permeabilized human myocardium, trouble-shoot apparatus, analyze data, and conduct experiments independently. Mentor will spend 2 hours per week to train the mentee on operating and trouble shooting the muscle mechanics apparatus.

        - Applicant should be able to cast and run SDS-PAGE to quantify UPF-1 and EXOSC10, and ubiquitinated titin, and perform Sudan Black B staining to measure lipofuscin levels and use antibodies to quantify truncated titin.

        - Training in scientific writing through attending an online course offered by Duke University and by working with the mentor. Proficiency will be demonstrated through submission of F31/AHA applications and 2 first author and 2 middle author publications.

        - Networking and career development opportunities by targeting 2 talks and 8 posters from the proposed work.

- Training plan includes instruction in responsible conduct of research and rigor and reproducibility.
- Next step identified as Postdoctoral fellowship in a lab focused on translational research.
- The training plan is appropriate for the applicant's career goals and experience.

- The environment is suitable for training. No concerns about institutional commitment.

Weaknesses:

- There is no plan for training in statistical methods. This is particularly important when working with clinical samples that need to take covariates and missing data into consideration.

There is no specific mention of training on data management or human subject and animal use.

Evaluation of the Proposal
:
Strengths:

- The applicant will test the hypothesis that TTNtv results in a DCM phenotype by overloading cellular decay pathways and integrating truncated titin filaments into the sarcomere. Three clear aims are proposed. These aims test the hypotheses that samples with TTNtv will have higher UPF1 and EXOSC10 abundance (aim 1), will have higher titin ubiquitination and lipofusinogenesis (aim 2), and will incorporate truncated titin filaments into sarcomeres (aim 3).
- The applicant has access to a repository of cardiac samples from 570 patients and organ donors, Whole genome sequencing has been conducted on 350. Samples corresponding to 15 patients each with DCM and TTNtv or DCM without TTNtv, and healthy organ donor controls have been selected for the proposed work.
- The experiments are feasible given the mentor's expertise and resources available to the applicant.
- The proposal is aligned with AHA's mission to accelerate discovery to enhance/treat cardiovascular health.

Weaknesses:

- The proposal is very difficult to understand and full of statements that could have been written much more clearly. Scientific terms/concepts are used without being explained. E.g., “the pathological mechanisms of TTNtv in DCM remain controversial, including models of haploinsufficiency and poison peptide.”
- The applicant is advised to evaluate the rigor of prior research and summarize it for the reader. For example, how good is the prior evidence of titin variants playing a role in DCM and what exactly are the gaps in knowledge.
- The rationale for the hypothesis and aims is not presented clearly/developed. There seems to have been insufficient mentoring in grant writing.
- The patient population has been identified but there is no table showing how the biological covariates are distributed between groups. It is unclear how differences in biological covariates will be statistically handled.
- It is unclear if it will be possible to address sex as a biological variable since there are no details of sex distribution of samples provided.
- Methods of analysis and statistical tools are insufficiently discussed.
- Alternate approaches/interpretation, if results are different from expected, are not discussed.

Evaluation of the Non-Scientist Summary
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Strengths:

- DCM is well described in paragraph 1 and that it may be caused by mutations in Titin is well described.
- Some elements of the research questions in paragraphs 3 and 4 are good.

Weaknesses:

- It is unclear from the lay summary, what is already known and what will be investigated. The lay summary starts by stating that mutations in Titin makes the heart not function properly. But from the proposal it also seems to be the question being researched.
- In paragraph 2, the connection between DCM and how it decreases the ability of certain pathways that fix mutations to do their job is not clear.
- The overall impact and potential advances to the field could be better articulated.
- The summary does not relay how the proposal supports the mission of the AHA.

Reviewer Role: Reviewer 2

Brief Summary of the Proposal

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No response entered

Evaluation of the Applicant, Sponsor/Training Plan and Environment

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Strengths

- Strong applicant who has performed well academically and made significant progress in research to date.
- Primary mentor highly productive and well-funded laboratory with strong history of training.
- Detailed training and mentoring plan in place to support applicant's development.
- Supportive reference letters speak to potential of applicant.
- Environment at University of Kentucky is well suited to support the proposed studies.

Weaknesses

- Minor concern regarding applicant's early stage and still acquiring necessary proficiencies with proposed approaches.

Evaluation of the Proposal
:
Strengths

- Novel hypothesis that titin-truncating variant (TTNtv) results in dilated cardiomyopathy via overloading cellular decay pathways and integrating truncated titin filaments into sarcomere.
- Use of human myocardium from organ donors and dilated cardiomyopathy tissues with and without TTNtv.
- Leveraging existing human myocardial repository of >20,000 samples from 570 subjects.
- Preliminary studies support feasibility of proposed approaches.

Weaknesses

- Lack of pilot data to directly support proposed hypothesis.

Evaluation of the Non-Scientist Summary
:
Well written and nicely highlights need to understand how specific mutations contribute to dilated cardiomyopathy.

Reviewer Role: Reviewer 3

Brief Summary of the Proposal
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No response entered

Evaluation of the Applicant, Sponsor/Training Plan and Environment
:
Strengths

- The applicant is Austin Minton, a 3rd year graduate student in Physiology at U. Kentucky. His interest in muscle biology was spurred by his personal experience with his sister.
- The applicant has won several awards at his undergrad institution (Kentucky Wesleyan).
- The applicant has presented several conference abstracts.
- The letters of reference are supportive
- The mentor is Dr. Kenneth Campbell, who is professor Director of Translational Research in Cardiovascular Medicine at U. Kentucky. His lab has deep expertise in biophysical, biochemical, and computational methods to develop therapies for heart failure. He has published over 120 papers to date. He has trained over 50 trainees to date and even won mentor of the year in 2014. He is MPI on four R01 awards that can be used to support the proposed research.
- The training plan includes skills acquisition in experimentation (biophysical and biochemical experiments and analyses using human myocardium), scientific writing, and networking/career development. There is a mentoring committee in place that includes Drs. Gupta, Wen, McCarthy, and Dupont-Versteegden, in addition to Dr. Campbell. The training plan includes goals, plans, and metrics and is well designed. RCR is also included.
- The environment at Kentucky is excellent.

Weaknesses

- The applicant does not yet have any publications, which is not unsurprising at this stage of training, but would be a nice indicator of productivity.

Evaluation of the Proposal
:
Strengths

- The goal of the proposed project is to test the hypothesis that titin-truncating variants (TTNtv) result in a DCM phenotype by overloading cellular decay pathways and integrating truncated titin filaments into the sarcomere. This is based on these variants having the strongest association to DCM. The hypothesis will be tested in three Aims that will assess the involvement of UPF1 and EXOSC10, ubiquitination, lipofuscinogenesis, and force/tension experiments in TTNtv-mediated DCM in human tissues.

- Titin variants occur in 25% of DCM patients, making this project significant.

- Because of titin's large size, RNA/protein clearance pathways become faulty or overwhelmed, and truncated titin can be incorporated into sarcomeres, causing mutated titin (TTNtv). Pursuing the mechanisms of this idea is innovative and likely to move the field forward.

- Access to the human myocardial repository is a strength for the application. Having access to matching of patient samples for age, sex, and diabetes status is also a strength.

- The incorporation of functional mechanics measures in Aim 3 in human tissues is a strength.

- Variability in data obtained from humans and human tissues is recognized and addressed as well as can be expected by using appropriate controls, technical and biological replicates and potential confounds like sex, mutations, and zygosity. There is no discussion of how or whether that may be accounted for in this present study and how/whether the patient pharmacological profiles may impact the data interpretation.

Weaknesses

- Aim 1 is hinged upon UPF1 and EXOSC10 being key players in decay pathways in DCM; however, there is key preliminary data missing to support this in the context of the proposed work.

- Protein abundance in tissues are informative, but perhaps not representative of functional readouts. This reviewer isn't expert in clearance/lipofuscin/ubiquitination pathways, but are there other proteins in these pathways that can be measured in Aims 1 and 2 to further implicate these as THE primary drivers of the phenotype? Currently, the scope of experiments for those aims seem very light.

- There is no discussion of statistics (beyond one broad statement) or power analyses. It is recognized that n=15 per group may be all that the applicant has access to, but is that sufficient to observe expected differences?

- While potential confounds are considered broadly, there is no indication on how sex and other confounds will specifically be handled in the data collection and analysis. Will experiments be conducted in both sexes? Are they as matched as can be expected? If so, does that still give the applicant the statistical power to test the hypothesis?

Evaluation of the Non-Scientist Summary
:
Strengths

- The non-scientist summary is well written, but some parts of it are probably too complicated for a lay summary.

Weaknesses

- None noted.