# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Children's Hospital of Pittsburgh of the UPMC Health System

2. **Reporting Period (start and end date of grant award period):** 1/1/11 to 12/31/13

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees**): David H. Perlmutter, MD

4. **Grant Contact Person's Telephone Number:** (412) 692-6081

5. **Grant SAP Number:** 4100054844

6. **Project Number and Title of Research Project:** Project #1 Regulation of Aging by the Proteasomal Pathway

7. **Start and End Date of Research Project:** 1/1/11 to 12/31/13

8. **Name of Principal Investigator for the Research Project:** Arjumand Ghazi, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 327,174

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project | Cost |
|---|---|---|---|
| Ghazi, Arjumand | Principal Investigator | 10.77% Yr 1,2,3 | 23,288.25 |
| Gandhi Das, Francis | Post-Doctoral Fellow | 100%Yr 1,2;50% Yr3 | 93,525.91 |
| Holden, Kyle | Research Technician | 100% Yr 1,2;50% Yr3 | 57,438.66 |

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project |
|---|---|---|
| Zoffel, Michael | Research Grants Admin. | 5% Yrs 1-3 |
| Ratnappan, Ramesh | Post-doctoral fellow | 5% Year 2 |
| Keith, Scott | Research Technician | 5% Year 3 |
| Sarah Winston | Summer student | 100%; 1 summer Year 2 |
| Martin Echavarria | Undergraduate student | 50% for 1 semester Year 2 |
| Sarah Bass | Summer student | 50% for 1 summer Year 1 |
| Hetal Patel | Undergraduate student | 50% for 1 semester Year 2 |
| Laura Smith | Master's rotation student | 100% for 1 semester Year 2 |

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

| Type of Scientific Equipment | Value Derived | Cost |
|---|---|---|
| 21 cubic foot Freezer | Preserve sensitive samples and chemicals | 699.95 |
| 4.5 cubic foot Refrigerator | Preserve sensitive samples and chemicals | 199.95 |
| | | |
| | | |
| | | |
| | | |
| | | |

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

| A. Title of research project on grant application | B. Funding agency (check those that apply) | C. Month and Year Submitted | D. Amount of funds requested: | E. Amount of funds to be awarded: |
|---|---|---|---|---|
| | ☐NIH<br>☐ Other federal (specify:_____ _____)<br>☐ Nonfederal source (specify: _____) | | $ | $ |
| | ☐NIH<br>☐ Other federal (specify:_____ _____)<br>☐ Nonfederal source (specify: _____) | | $ | $ |
| | ☐NIH<br>☐ Other federal (specify:_____ _____)<br>☐ Nonfederal source (specify: _____) | | $ | $ |

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

The aging-regulatory E3 ligases identified through this project will be the subject of mechanistic studies to understand the relationship between protein homeostasis, reproductive status and aging. We plan to submit a proposal of these experiments as an NIH grant.

**12. Future of Research Project.** What are the future plans for this research project?

We are particularly interested in studying E3 ligases that allow an animal to balance the needs to protein homeostasis, quality of aging and reproductive demands. The genes identified through the screen developed in this project will form the basis of these studies.

**13. New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | 4 | 1 | | 2 |
| Female | | | | |
| Unknown | | | | |
| **Total** | **4** | **1** | **0** | **2** |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Hispanic | | | | |
| Non-Hispanic | 3 | 1 | | 2 |
| Unknown | 1 | | | |
| **Total** | **4** | **1** | **0** | **2** |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| White | 3 | 1 | | |
| Black | | | | |
| Asian | | | | 2 |
| Other | | | | |
| Unknown | 1 | | | |
| **Total** | **4** | **1** | **0** | **2** |

**14. Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

**15. Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This research project allowed us to develop a very important and exciting project while setting up a new laboratory, an undertaking that would have been difficult without the resources involved in the project. In addition, the preliminary data generated from these studies has led to increased interactions with other faculty in areas such as proteomics and genomics that we would not have otherwise encountered.

**16. Collaboration, business and community involvement.**

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

**17. Progress in Achieving Research Goals, Objectives and Aims.**

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

*Regulation of Aging by the Proteasomal Pathway*

This project attempts to understand how the reproductive status of an animal alters its rate of aging by modulating protein homeostasis mechanisms, especially the proteasomal pathway of protein ubiquitination. The main focus has been the identification of E3 ligase enzymes that change the rate of aging of an animal in response to signals from reproductive tissues. The nematode, *C. elegans*, is an ideal model organism for this project since it is particularly accessible to unraveling the relationship between reproduction and aging as well as protein homeostasis mechanisms. In worms, removal of germ cells increases lifespan by ~60% (1). The lifespan extension is not just a consequence of sterility; animals can be rendered sterile by multiple interventions but only when a pool of totipotent germline-stem cells (GSCs) is removed is its lifespan enhanced (2). This longevity is precisely regulated by, and dependent upon, the presence of a group of transcription factors. Two key transcription factors of this network include DAF-16, a conserved longevity determinant, and TCER-1, a transcription-elongation factor that our previous studies found to specifically promote life in response to germline loss (1, 3). Previously, we had discovered that the proteasomal pathway of protein degradation regulates the aging of *C.elegans* (4). Specifically, we had found that inactivation of the CUL-1/SKR-1,-2/F-Box E3 ligase complex abolishes the extended lifespan of mutants of the insulin/IGF-1 receptor *daf-2*, that also rely on DAF-16 for their longevity. Our data suggested that the targeted proteasomal modification of specific substrate proteins is crucial for the extension of lifespan in *C. elegans,* by mutations in the *daf-2* pathway (4). In this study, we attempted to identify proteasomal E3 ligases that mediate the longevity brought about by germline loss by employing large-scale RNAi screening. In addition to developing the RNAi screen, we planned to perform detailed secondary assays on the candidates obtained from the screen to identify bonafide lifespan-regulatory E3 ligases. Once identified, the E3 ligases could be used to discover the substrates whose proteasomal modification controls the long lifespan of germline-ablated worms. Following is a brief description of the progress achieved towards this end.

Specific Aim 1: To identify E3 ligases essential for lifespan extension brought about by germline removal

Aim 1 was partially achieved as reagents required for the E3-ligase screen were generated and the experimental set-up was standardized. Following are brief descriptions of the steps involved in this process.

*i) Developing a Fluorescence-based Screening Strategy to Replace Longevity Read-out*

To identify E3 ligase genes essential for lifespan extension mediated by germline loss, we proposed to undertake an RNAi screen, using mutants for a gene called *glp-1*. The *glp-1* gene product is required for germ-cell proliferation; temperature sensitive *glp-1* mutants lack germ cells (at non-permissive temperature), are sterile, long-lived and have proven to be a valuable surrogate for longevity produced by germline removal (2, 3). We first confirmed that *glp-1* mutants exhibited the lifespan extension attributed to them at the restrictive temperature in a new laboratory setting (the Ghazi lab was set up at the beginning of this project) and under dependence of *daf-16* and *tcer-1*. Based on the experience with the lifespan assays, we revised our strategy to reduce the time of the screen (*glp-1* mutants have a mean lifespan of ~28 days). We explored multiple options for designing a screen strategy in which the

molecular landmarks could be used as surrogates for longevity, including using transgenic worm strains carrying GFP-tagged DAF-16, TCER-1 as well as GFP expressed under control of their targets. Upon germline loss, DAF-16 becomes nuclear localized (5), TCER-1 levels rise transcriptionally (3) and the expression of both their targets is induced in intestinal cells (5, 6). Previously we had identified several genes that are up-regulated under control of DAF-16 and TCER-1 following germline ablation (3). These genes were studied using transgenic worm strains expressing GFP-tagged transcriptional fusion constructs that reported on the expression pattern of specific genes. Many of these genes exhibited striking increases in GFP levels in long-lived, *glp-1* mutants, especially on Day 2 of adulthood (this reduced the screen time from ~30 to ~4 days). We considered using a reporter construct for one such gene, *stdh-1*, a common target of *daf-16* and *tcer-1* that is induced in intestinal cells of *glp-1* mutants and is easily visible under the magnification provided by a stereo-microscope (3). However, we found that both DAF-16::GFP and TCER-1::GFP strains were not conducive for large-scale RNAi screens for independent reasons. TCER-1::GFP transgene is expressed at low levels that can only be observed under a compound microscope. Similarly, DAF-16::GFP nuclear localization was found to be variable and extremely sensitive to mild environmental perturbations. In addition, *Pstdh-1::GFP* was found to be regulated by multiple up-stream regulators, which made it as a non-specific reporter. However, in an independent project, in which we used *Pstdh-1::GFP* to screen a library of RNAi clones targeting worm nuclear hormone receptors (NHRs), we discovered a gene, *nhr-49*, that provided us with an ideal transgenic strain to screen for E3 ligases that mediate the reproductive control of aging.

*Generation of Transgenic Strain for RNAi Screen*

We found that NHR-49, the worm functional homolog of human PPARα proteins (7) is essential for the longevity of germline-less worms (Ratnappan et al., manuscript in preparation). NHR-49, similar to PPARα proteins, is an extremely important regulator of energy and fat metabolism (7) and its involvement in the germline-mediated control of aging raises interesting questions about the relationship between reproduction, fat and aging. As a part of the study that addressed the role of NHR-49 in regulating lifespan, we generated transgenic worms that express a GFP-tagged NHR-49 protein driven by its endogenous promoter (NHR-49::GFP). This construct was expressed in all somatic tissues of the worm in larval and adult stages and the levels of GFP rose when the animal lost its germline (Fig. 1). This strain expressed GFP at significantly higher levels than DAF-16::GFP or TCER-1::GFP strains and the fluorescence was clearly and widely visible under a stereo-microscope with a fluorescence attachment. In preliminary assays, we found that the transcriptional increase in expression of NHR-49 in germline-ablated worms is dependent on DAF-16 and TCER-1 (Fig. 1). This not only revealed to us the genetic pathway in which NHR-49 operates to mediate longevity, it also allowed us to standardize the conditions and timing under which NHR-49::GFP can be observed clearly.

*Genomic Integration of NHR-49::GFP Construct for RNAi Screen*

In the original transgenic worms, the NHR-49::GFP construct was not integrated in the worm genome permanently (but carried as an extra-chromosomal array made up of concatemers of the DNA-construct that can be lost during cell divisions) and this resulted in two issues:

*(a) Heterogeneity of expression levels:* since the number of DNA arrays could not be controlled within different cells, tissues and animals, there was a wide variation in the levels of GFP. As a result, the effects of any RNAi gene-inactivation on GFP levels was difficult to distinguish from the normal variation exhibited by a population unless very large numbers of worms were used.
*(b) Transgene transmission frequency*: the extragenic nature of the array results in only a fraction of progeny of any transgenic worm carrying the GFP construct. Consequently, the strain needs to be maintained by manually picking fluorescent worms in each generation. Although our NHR-49::GFP transgenic strain showed a high-frequency of transmission (>80%), we found that for the purpose of an undertaking as a screen, it was arduous to work with an un-integrated strain.

To circumvent the above two issues, we integrated the NHR-49::GFP construct into the genome. This was performed using a standard technique that relies on the use of UV or γ rays to irradiate the parents. The radiation introduces DNA breaks that can undergo recombination with the extragenic array to cause integration of the transgene at relatively random genomic locations, usually in single copies. We used both UV and γ radiation (independently) for our experiment. Irradiated parents were allowed to lay eggs and their GFP+ve progeny were picked onto individual plates. In the next generation, about 600 lines were set up from the progeny of these fluorescent worms and scored for plates where all the worms were green. We isolated a single integrant from screening 600+ lines. The integrant was outcrossed six times to the lab wild-type stock (to eliminate background mutations that may have arisen from the radiation) and tested for authenticity of the NHR-49::GFP construct by RNAi tests.

*ii) RNAi Screen Paradigm on 24-well Petri dishes*
While the integration of the NHR-49::GFP was in progress, we focused on developing a screen pipeline in which 24-well tissue-culture petri-dishes can be used to culture worms instead of regular 6cm nematode plates. This reduces the space and material requirements of the experiment and makes it possible to test much larger number of RNAi clones in a shorter duration. We determined that about 20-25 worms could be grown per well of a 24-well plate for 3-4 days without causing starvation or hypoxia or cross-contamination between wells. The increase in NHR-49::GFP upon loss of germline was found to be most striking on Day 2 of adulthood (~4 days of nematode culture) and this will be used as the major time-point for the screen. We are now conducting additional pilot tests (with RNAi sub-libraries targeting chromatin factors) to further fine-tune the screen conditions.

*iii) An Updated Proteasomal E3 Ligase RNAi Sublibrary*
Our lab has a repository of two genome-scale feeding RNAi libraries that cover >87% of the worm genome (The Ahringer Library and the Vidal Library) (8, 9), and the E3 ligase RNAi clones to be used in the screen will be derived from these. We had previously generated an RNAi 'sub-library' targeting proteasomal genes. However, in the last two years the gene annotations for many genes have changed (www.wormbase.org) and this has resulted in the necessity for a new, updated 'sub-library'. We are now up-dating the virtual RNAi library to reflect the latest gene annotations. Once the sub-library is updated and re-constructed, we will perform the RNAi screen to identify E3 ligases that influence NHR-49 expression, and through this and other functional assays discover E3 ligases critical for longevity of

germline-ablated worms.

*iv) Developing secondary tests to identify bonafide lifespan-regulatory E3 ligases*
Since protein homeostasis is such a fundamental aspect of survival, reduced function of most proteasomal genes is highly deleterious to animal health and causes death rapidly. Consequently, it is difficult to distinguish the direct longevity functions of proteasome genes as compared to their role in cellular maintenance. One means of addressing this issue is to perform RNAi-inactivation of genes only in adult animals after the genes have fulfilled vital developmental functions (4). We have developed an RNAi strategy (Adult Only RNAi) to facilitate this, but by itself it is not sufficient. We have focused on this issue extensively, as tests that will help us identify E3 ligases whose RNAi-knockdown accelerates aging (as compared to treatments that just cause sickness and general dysfunction) will be the most crucial aspect of this project. In addition to testing the positive clones obtained from the screen for their effect on the extended lifespan of *glp-1* mutants (by Adult Only RNAi), a series of morphometric assays, healthspan and stress-response tests can be used that reflect the overall health of the animal. Worms display characteristic age-related physical and functional deteriorations that are highly similar to features of human age-related decline, and can be quantified precisely (10, 11). Similarly, healthspan tests can be used to assess the rate of physiological aging. In addition, reduced resistance to environmental threats is a common feature of aging that is manifested in easily assayable manners in worms (12). Through exploratory pilot tests, we have finalized a series of healthspan tests that will evaluate the morphological and functional parameters of aging as well as stress-resilience of worms to give a comprehensive picture of the rate of aging of a population. RNAi clones identified from the screen that also reduce *glp-1* longevity will be tested for their effects on the healthspan parameters. Clones that negatively impact these measures of overall health will be the most attractive candidates that will be studied molecularly. The healthspan assays are listed here in brief.

*1. Morphometric Age-Associated Changes:* Worms exhibit a series of well-characterized morphological changes that reflect the aging of tissues, similar to humans (10, 11). RNAi clones that influence NHR-49::GFP will be evaluated for their effect on the rate of aging by examining the anatomic age-related declines.
*(a) Sarcopenia:* The deterioration undergone by muscle cells and nuclear architecture is easily evaluated using Nomarski optics (10, 11) and a population will be tested on Days 2, 3, 5 and 7 of adulthood.
*(b) Aging pigment accumulation:* Lipofuscin and Advanced Glycation End products (AGEs) together constitute the age-related increase in intestinal autofluorescence (10, 11). We observe the accumulation of aging pigment fluorescence under a fluorescence microscope by using the DAPI filter (Ex/Em: 340nm/430nm) on Days 2, 3, 5 and 7. As a control, we also acquire the spectra at Ex/Em: 290nm/330nm to detect the signal generated by aromatic amino acids that does not change with age

*2. Functional Age-related impairment:* Similar to humans and other animals, aging nematodes also experience declining functionality (10). We have found that the following measures of functionality are easy and reliable markers that can be used on our screen candidates.

*(a) Loss of mobility:* Closely associated with sarcopenia, reduced mobility can be directly correlated with the rate of aging, independent of the effect of a gene on length of life (10). We perform a chemo-attractant mobility assays on a population in which a group of age-matched worms are placed at the center of a culture plate, and the average time taken by the animals to reach a drop of chemoattractant placed at one end of the plate is calculated.
*(b) Pharyngeal Pumping:* Worms use a muscular pharynx to grind the bacteria they consume before it is transferred to the digestive tract. The rate of pumping undergoes a sharp decline with age (11). The pharynx is often compared to the human heart due to its muscle physiology, so this is a particularly useful assay to screen E3 ligases that influence aging. This test involves counting the number of pharyngeal pumps in a given interval of time (1min) for different members of a strain and comparing the averages between strains.

*3. Decline in stress-tolerance:* Longevity and stress-resistance are intimately related (12). Indeed, a large majority of long-lived mutants of various species are found to exhibit increased resistance to environmental stressors, including elevated temperatures, pathogens and atmospheric toxins (13). Alternatively, progeric mutants are extraordinarily sensitive to these threats (10, 11). Long-lived *glp-1* mutants are highly resistant to many stressors as compared to their normal, fertile counterparts (14). We have developed a compendium of stress assays that examine the resistance of a population to both biotic (pathogens) and abiotic (heat, oxidative stress etc.,), (Table 1) and RNAi clones identified from the screen will be evaluated for their effects on these paradigms.

*Abiotic Stress Tests:*
*(a) Osmotic Stress Test:* We test for tolerance towards osmotic shock by exposing worms to high salt (500mM NaCl) and scoring for animals that loose motility over a period of 15 minutes. The same animals are then observed for an extended period of 12 hours to score for animals that are able to recover from the osmotic shock and regain motility. In addition, we also run a lifespan on these high salt plates for 72hrs till all the control wild-type animals die. We find that *glp-1* mutants demonstrate greater resistance in each of these paradigms as compared to wild-type worms.
*(b) Oxidative Stress Test:* Worms are exposed to concentrations of tert-Butyl Hydroperoxide (t-BOOH) and Paraquat, known inducer of oxidative stress, that are highly toxic to normal worms. *glp-1* mutants are able to survive these treatments for significantly longer periods than wild-type worms.
*(c) Heat Stress Test:* We test for tolerance towards heat stress by exposing worms to high temperatures (35$^{o}$C) and documenting the length of survival time. *glp-1* mutants are highly resistant to heat stress as well.

*Biotic Stress Test:*
*Pathogen Stress Test:* To test for immune resistance in *glp-1* worms, we conduct a lifespan assay by transferring worms onto plates with pathogenic bacteria (*Pseudomonas aeruginosa*). *glp-1* survive longer periods on two pathogenic strains, PA14 and PA01, as compared to fertile worms.

*Uncoupling (Oxidative) Stress Resistance from Lifespan:*
In standardizing the stress assays described above, we made some startling discoveries.

Resistance to a variety of stressors such as oxidative agents, high temperature, pathogens and heavy metals, is found to be highly correlated with long life. Most long-lived mutants demonstrate extraordinary resilience towards one or more of these stressors (13). Our previous studies and those of others (14) had shown that this is true of GSC-ablated worms as well. We tested *daf-16* and *tcer-1* RNAi clones as positive controls in these stress experiments, since *daf-16* has previously been shown to underlie a majority of the worms' stress-resistance. However, we were surprised to observe that *tcer-1* RNAi did not abrogate the resistance of germline-ablated worms to an oxidative stress-inducing agent, t-Butyl Hydroperoxide (t-BOOH), while *daf-16* RNAi did (Amrit FG and Ghazi A, unpublished data). We found this to be particularly true for oxidative stress. This data is interesting as it suggests that there may be proteins (such as DAF-16) that confer both longevity and stress-resistance, whereas there may be other proteins that specifically alter the length of life, without any effect on stress-resistance (such as TCER-1). These implications could extend to the E3-ligases we are studying. Specifically, our healthspan assays will help us distinguish E3 ligases that influence longevity specifically, without altering stress-resistance, from those that simultaneously promote longevity and stress resistance. Of the latter class, it will be informative to identify E3 ligases that confer broad resistance to many stressors as well as those that are specific to individual stress paradigms.

Specific Aim 2: To identify substrates of E3 ligases whose controlled degradation is required for the extended lifespan of *glp-1* mutants
Aim 2 was partially achieved. The transcription factor, SKN-1/NRF2, was identified as a potential target of LIN-23/bTRCP, The details are described below.

Specific Aim 3: To perform detailed cellular and molecular analyses of identified substrates
Aim 3 was partially achieved. We examined the role of SKN-1/NRF2 in mediating lifespan extension as well as stress-resistance in worms and analyzed the genetic circuit in which it functions to mediate these functions.

*The LIN-23/βTRCP and SKN-1/NRF2 Pathway in Regulating Reproductive Control of Aging:*
In previous experiments, we had identified an F-Box adaptor protein LIN-23 as one of the E3-ligase components essential for the longevity of insulin/IGF-1 receptor, *daf-2*, mutants (4). LIN-23 is the worm homolog of a highly conserved E3-ligase protein in mammals, $\beta$TRCP. Both LIN-23 and $\beta$TRCP have been shown to function in the CUL-1/SKR-1/2 E3-ligase complex in worms and humans, respectively (15). In addition, $\beta$TRCP is also a key regulator of the human NRF2 transcription factor whose worm homolog is SKN-1 (16). We have explored this relationship between the worm LIN-23/$\beta$TRCP and SKN-1/NRF2 proteins. SKN-1, the worm homolog of NRF2 is a key transcription factor that promotes longevity and stress-resistance. We found that it is part of the transcriptional circuit that gets activated upon germline loss in intestinal cells, and is required for the longevity of *glp-1* mutants. We also discovered that LIN-23/$\beta$TRCP is essential for the up-regulation of SKN-1/NRF2 target genes that mitigate oxidative stress, and for the enhanced stress-resistance of long-lived worms (Fig. 2). We confirmed that SKN-1/NRF2 becomes nuclear localized following germ-cell loss and are exploring the relationship between the E3 ligase (LIN-23) and its substrate (SKN-1) that influence the reproductive control of aging.

*References*

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2. Arantes-Oliveira N, Apfeld J, Dillin A, Kenyon C. Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans. Science.* 2002. 295(5554):502-5.
3. Ghazi A, Henis-Korenblit S and Kenyon C. A transcription elongation factor that links signals from the reproductive system to lifespan extension in *Caenorhabditis elegans* (2009).*PLoS Genetics.* 2009. 5(9):e1000639.
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5. Lin K, Hsin H, Libina N, Kenyon C. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet.* 2001. 28(2):139-45.
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*Figures and Table*

Figure 1

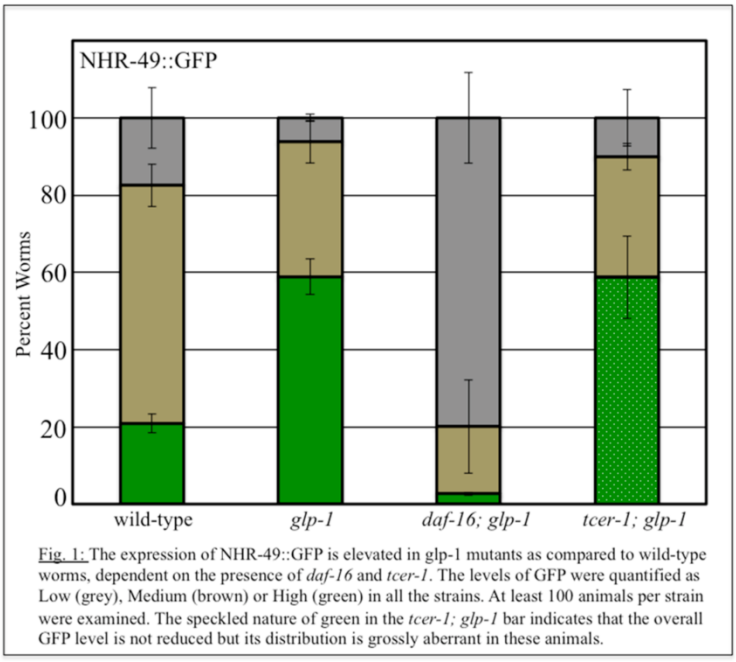


Fig. 1: The expression of NHR-49::GFP is elevated in glp-1 mutants as compared to wild-type worms, dependent on the presence of *daf-16* and *tcer-1*. The levels of GFP were quantified as Low (grey), Medium (brown) or High (green) in all the strains. At least 100 animals per strain were examined. The speckled nature of green in the *tcer-1; glp-1* bar indicates that the overall GFP level is not reduced but its distribution is grossly aberrant in these animals.

Table 1: Stress assays to examine the effect of E3-ligases gene inactivation on the healthspan and rate of aging of a population

| Stress Type | Stress Agent | Experimental Conditions | Average Lifespan of wild-type worms |
|---|---|---|---|
| Oxidative Stress | Paraquat | 7mM continuous exposure | 4-6hrs |
| | t-BOOH | 6.2mM for 1hr | 4–8hrs |
| Thermal Stress | Heat | 35°C for 2hrs | 7-9hrs |
| Osmotic Stress | Sodium Chloride | 500–700mM | 18-24hrs |
| Pathogenic Bacteria | *P. aeruginosa* PA14<br>PA01 | Young adults transferred to pathogen plates and examined at regular intervals (15min for PA01 and 3hrs for PA14) | PA14: 3-4 days<br>PA01: 4 hours |

Figure 2

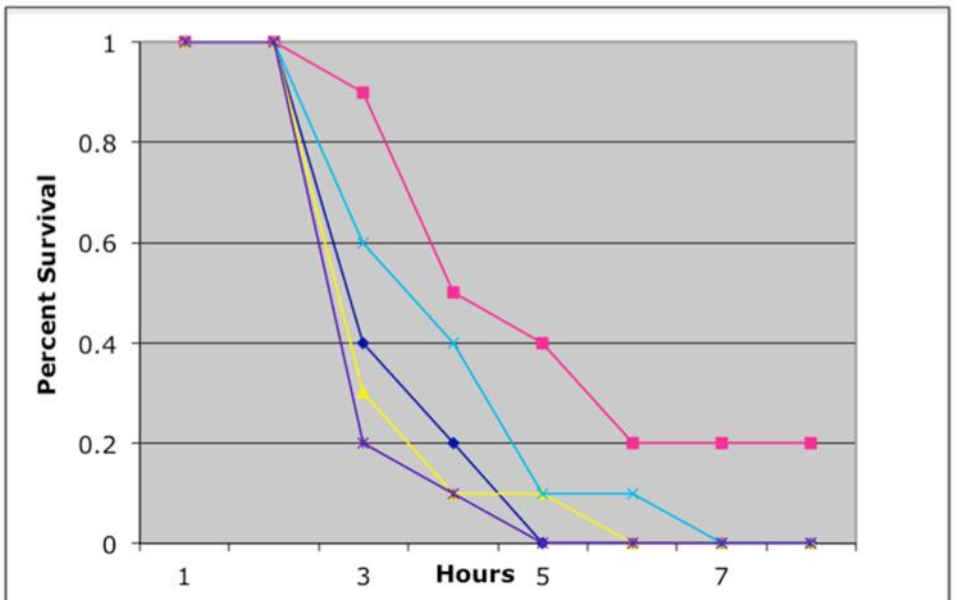


Fig. 2: The E3-ligase LIN-23/bTRCP and its substrate SKN-1/NRF2 are both essential for the oxidative stress resistance of long-lived mutants. Survival of Day 2 young adults exposed to 7mM Paraquat was compared between wild-type worms (blue) and *daf-2* mutants grown on empty vector (RNAi control pink), *lin-23* RNAi (yellow), *skn-1* RNAi (light blue) and *daf-16* RNAi (purple). Control *vs. lin-23* RNAi, $p<0.01$; Control *vs skn-1* RNAi $p<0.05$; Control *vs daf-16* RNAi $p<0.001$; wild-type *vs daf-2* control $p<0.01$

*Meeting Abstracts:*

1. Kyle Holden[$], Emmanuel Schrieber, Mani Balasubramani and Arjumand Ghazi*. The E3 Ligase LIN-23/βTRCP Influences SKN-1/NRF2 Activity and Reduces Toxic Proteins in *daf-2* Mutants.
Department of Pediatrics, Rangos Research Centre, University of Pittsburgh School of Medicine. [$]Presenting Author; *Corresponding Author: Arjumand.Ghazi@chp.edu
Presented at 19th *International C. elegans Meeting*. University of California, Los Angeles, June 2013.

2. Kyle Holden[$], Emmanuel Schrieber, Mani Balasubramani and Arjumand Ghazi*. The E3 Ligase LIN-23/βTRCP Influences SKN-1/NRF2 Activity and Reduces Toxic Proteins in *daf-2* Mutants.
Department of Pediatrics, Rangos Research Centre, University of Pittsburgh School of Medicine. [$]Presenting Author; *Corresponding Author: Arjumand.Ghazi@chp.edu
Presented at Annual Aging Day of the Aging Institute of University of Pittsburgh. 2013.

3. Kyle Holden[$] and Arjumand Ghazi*. Identification of substrates of E3 ligases that promote longevity

Department of Pediatrics, Rangos Research Centre, University of Pittsburgh School of Medicine. [$]Presenting Author; *Corresponding Author: Arjumand.Ghazi@chp.edu
Presented at Rangos Research Day of the Rangos Research Center, Children's Hospital of Pittsburgh. 2013

4. Sarah Winston[$], Francis A. Gandhi and Arjumand Ghazi*. The Regulation of Stress-Response Pathways by the Germline in *C. elegans*
Department of Pediatrics, Rangos Research Centre, University of Pittsburgh School of Medicine. [$]Presenting Author; *Corresponding Author: Arjumand.Ghazi@chp.edu
Presented at Summer Student Research Presentation. Children's Hospital of Pittsburgh, July 2012.

**18. Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

**If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

**Note**: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for

refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. The *C. elegans* Healthspan and Stress-Response Assay Toolkit | Scott Alexander Keith, Francis Raj Gandhi Amrit, Ramesh Ratnappan and Arjumand Ghazi | Methods | February 2014 | Published |
| 2. The *C. elegans* Lifespan Assay Toolkit | Francis Raj Gandhi Amrit, Ramesh Ratnappan, Scott Alexander Keith, and Arjumand Ghazi | Methods | February 2014 | Published |

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X___ No_____
If yes, please describe your plans:

We have one manuscript in preparation that addresses the relationship between the E3-ligase LIN-23/βTRCP and its substrate SKN-1/NRF2 in the context of aging. A second manuscript that addresses the uncoupling of stress-resistance and aging will be written after additional experiments are concluded. In addition, we plan to submit the screen proposed in this project as an article once it is completed.

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.**
Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___XX___

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

## Biographical Sketch

| NAME<br>**GHAZI, ARJUMAND** | POSITION TITLE<br>Assistant Professor of Pediatrics,<br>of Developmental Biology and Cell Biology |
|---|---|
| eRA COMMONS USER NAME **ARJUMAND** | |

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| St. Ann's College, Osmania University, Hyderabad, India | B.S. | 06/93 | Microbiology, Chemistry, Zoology |
| Hyderabad Central University, Hyderabad, India | M.S. | 06/95 | Biotechnology |
| National Centre for Biological Sciences (NCBS), Tata Institute for Fundamental Research (TIFR) Centre, Bangalore, India | Ph.D. | 11/01 | Developmental Biology, Genetics |
| University of California, San Francisco (UCSF) | Postdoc | 06/02-05/07 | Genetics of Aging |

**Positions and Honors**

**Positions and Employment**

Aug.- Dec. 1999 Visiting Fellow, Institut de Genetique et de Biologie Moleculaire et Cellulaire (IGBMC), Strasbourg, France

Apr. 2001-Apr. 2002 Visiting Fellow, National Centre of Biological Sciences (NCBS), TIFR Centre, Bangalore, India

June 2002-June 2007 Postdoctoral Scholar, University of California, San Francisco (UCSF)

July 2007- Jan. 2010 Associate Research Specialist, University of California, San Francisco (UCSF)

Dec. 2010 Visiting Professor, University of Pittsburgh School of Medicine

Jan 2011-present Tenure Track Assistant Professor, University of Pittsburgh School of Medicine

**Honors**

2013 Session Chair, 'Aging and Stress' Session, 19th International *C. elegans* Meeting

2012 Ellison Medical Foundation's New Scholars in Aging award

2012 American Heart Association Beginning Grant-in-Aid award (declined)

2012 Competitive Medical Research Fund (CMRF) award of the University of Pittsburgh (declined)

2012 Invited to submit application for Basil O'Connor award of March of Dimes foundation (declined)

2012 Session Chair, *C. elegans* meeting on 'Aging, Stress, Pathogenesis and small RNAs'

2011 Research Advisory Committee Children's Hospital of Pittsburgh New Investigator grant

2011 American Federation for Aging Research (AFAR) grant

2011 Kimble Chase New Lab Start-up grant
2011 University of Pittsburgh Clinical and Translational Science Institute Translational Technologies Cores Pilot Program
2010 Pennsylvania Department of Health, Health Research Formula Funds
2009 Publication recommended by Faculty of 1000 (Ghazi et al., 2009; *http://www.f1000biology.com/article/id/1163908/evaluation)*
2008 Best Poster Prize, 15th International *C. elegans* Meeting, UCLA
2006 Larry L. Hillblom Foundation for Aging Postdoctoral Research Grant
2004 Indian National Science Academy (INSA) Young Scientist Award
2003 American Federation for Aging Research (AFAR) Postdoctoral fellowship
2002 Scientific Advisory Board Assistant, Science of Aging Knowledge Environment of the journal *'Science'*
2002 Cold Spring Harbor Laboratory fellowship (to attend the course '*C. elegans'*)
2001 Best Poster Prize, International Cell and Developmental Biology Symposium, Bangalore, India
1999 Institute of Genetics and Molecular and Cell Biology (IGBMC) research support
1999 WoodWhelan Research Fellowship, International Union of Biochemists and Molecular Biologists
1999 Journal of Cell Science Fellowship, Company of Biologists
1998 Best Poster Prize, International Cell Cycle Meeting, Ullal, India
1998 Cold Spring Harbor Laboratory Fellowship (to attend 'Neurobiology of *Drosophila'*) (Declined)
1998 Surdna Foundation Scholarship, Marine Biological Laboratory (MBL), Woodshole
1995 Ranked 31st (99.8 percentile) All India Graduate Aptitude Test in Engineering conducted by the Indian Institutes of Technology (IITs)
1995 Council for Scientific and Industrial Research (CSIR), India, Graduate Fellowship (Declined)
1994 Centre for Cellular and Molecular Biology (CCMB), India, Summer Research Fellowship
1993 Department of Biotechnology, India, Master's in Biotechnology Scholarship
1993 Valedictorian, St. Ann's College, Osmania University, Hyderabad, India

**PUBLICATIONS**

1. **Ghazi A.** Transciptional networks that mediate signals from reproductive tissues to influence lifespan. ***genesis***, **The Journal of Genetics and Development** doi: 10.1002/dvg.22345. 2013.
2. **Ghazi A,** Henis-Korenblit S, Kenyon C. A transcription elongation factor that links signals from the reproductive system to lifespan extension in *Caenorhabditis elegans*. ***PLoS Genet*** 5(9):e1000639, 2009. PMID: 19749979.
   *(Recommended by Faculty of 1000) www.f1000biology.com/article/id/1163908/evaluation*
3. **Ghazi A***, Henis-Korenblit S*, Kenyon C. Regulation of *Caenorhabditis elegans* lifespan by a proteasomal E3 ligase complex. ***Proc Natl Acad Sci USA*** 104(14):5947-5952, 2007. PMID: 17392428 *equal contribution
   *Focus of 'Dispatch' Article: Bruce Bowerman (2007). C. elegans Aging. Proteolysis cuts both ways*. ***Current Biology*** (2007) *17(13): R514-16.*
4. **Ghazi A**, Paul L, VijayRaghavan K. Prepattern genes and signaling molecules regulate stripe expression to specify *Drosophila* flight muscle attachment sites. ***Mech Dev*** 120(5):519-528, 2003. PMID: 12782269

5. **Ghazi A**, VijayRaghavan K. Muscle Development in *Drosophila*. ***Proc Ind Natl Sci Acad*** 5:691-702, 2003.
6. **Ghazi A**, Anant S, VijayRaghavan K. Apterous mediates development of direct flight muscles autonomously and indirect flight muscles through epidermal cues. ***Development*** 127(24):5309-5318, 2000. PMID: 11076753
7. **Ghazi A**, VijayRaghavan K. Developmental biology. Control by combinatorial codes. ***Nature*** 408(6811):419-420, 2000. PMID: 11100709

*Manuscripts under review*

8. Amrit FG, Ratnappan R, Keith SA and **Ghazi A.** The *C. elegans* Lifespan and Healthspan Toolkit. Invited review article for the journal *Methods*
9. Amrit FG, Ratnappan R, Keith SA and **Ghazi A.** The *C. elegans* Lifespan and Healthspan Toolkit. Invited review article for the journal *Methods*=

*Additional publications relevant to the field*

10. **Ghazi A.** (2002). Puzzling over research on Aging. Crossword Puzzle on Aging Research. *SAGE KE, Perspectives.*
11. **Ghazi A** & Kenyon C. Cells of the somatic gonad that promote *C. elegans* longevity. Manuscript in preparation.

*Manuscripts in preparation from work at the University of Pittsburgh*

12. Ratnappan R, Amrit FG, Ward J, Gill H, Holden K, Olsen CP, Yamamoto K and **Ghazi A**. Reproductive Signals Deploy NHR-49/PPARα to Enhance Fatty Acid β-Oxidation and Desaturation and Increase Lifespan
13. Amrit FG, McClendon B, Ratnappan R, Arora A, Benos T, Yanowitz J and **Ghazi A**. TCER-1/TCERG1 and DAF-16/FOXO Balance Fertility and Longevity in Response to Germline Signals by Controlling Genes Involved in Reproduction and Lipid Homeostasis
14. Holden K, Keith SA, Schreiber M, Balasubramani M and **Ghazi A**. Proteomic Analysis of LIN-23/βTRCP Reveals Novel Targets and that Influence Longevity and Stress-Resistance through Regulation of SKN-1/NRF2
15. Gill H, Amrit FG, Stolz DB and **Ghazi A**. Use of Scanning Electron Microscopy (SEM) to document age-related cuticular deterioration in *C. elegans*.
16. Ratnappan R, Amrit FG, Keith SA and **Ghazi A. Reproductive Regulation of Aging.** Invited review for the journal *Current Genetic Medical Reports*

**C. Research Support** (ongoing, pending and completed projects)

**Current:**

Ellison Medical Foundation, New Scholars in Aging award
miRNAs and lipophilic-hormonal pathways that relay reproductive signals to control aging
7/1/2012-6/30/2016 $400,000
This project explores the role of miRNAs and lipophilic-signaling genes in controlling longevity.

**Completed:**

American Federation of Aging Research (AFAR), AFAR Research Grant
Study of reproductive signals that regulate aging
7/1/2011-6/30/2013 $100,000
This project focused on identification of new genes involved in the reproductive control of aging.

Children's Hospital of Pittsburgh, Research and Advisory Committee New Investigator grant
Control of Longevity Genes by 'Paused' RNA Polymerase II and Regulated Transcript Elongation.

1/1/2012-12/31/2013 $64,000
This project focused on the study of paused RNA polymerases in aging.

Pennsylvania Department of Health, Health Research Formula Funds
Regulation of aging by the proteasomal pathway
1/1/2011 – 12/31/2013 $327,174 (part of start-up funds)
This project focused on proteasomal E3 ligases involved in reproductive control of aging.

University of Pittsburgh Clinical and Translational Science Institute, Translational Technologies Cores Pilot Program
Proteomic analysis of F-Box substrates that promote longevity
7/1/2011 – 6/30/2012 $15,000 (core services)
This project used proteomics to identify E3-ligase substrates that regulate aging of *daf-2* mutants.

Larry L. Hillblom Foundation for Aging Research Grant, Regulation of aging by the proteasomal pathway of protein degradation
6/1/06-5/30/09 $180,000
This project focused on the proteasomal regulation of lifespan.

American Federation of Aging Research Grant, Role of the somatic gonad in the regulation of *C. elegans* aging
7/1/2003-6/30/2005 $50,000
This project aimed to identify cells of the somatic gonad that promote longevity in worms.