BIOGRAPHICAL SKETCH

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NAME: Ashraf, Sadia

eRA COMMONS USER NAME (credential, e.g., agency login): SASHRAF2

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Leeds, UK	M.Res.	06/2010	Membrane and Systems Biology
University of Padua, Italy	Ph.D.	08/2014	Cell Biology

A. Personal Statement

My long-term goal is to create my own research program to study the pathophysiology of cardiovascular diseases associated with obesity and diabetes. My immediate objective is to acquire theoretical and practical training in the areas of cardiovascular physiology and metabolism, and to develop the organizational and mentoring skills that will best prepare me best to become a successfully funded independent investigator in the academic setting. To achieve these goals I am pursuing my postdoctoral training in the laboratory of Dr. Romain Harmancey in the Department of Physiology and Biophysics at the University of Mississippi Medical Center. Dr. Harmancey has a solid track record of NIH-funded research investigating the molecular mechanisms underlying cardiac dysfunction in diabetes. My secondary mentor Dr. John E. Hall is a world leading expert on the neurohormonal changes affecting the cardiovascular-renal system in obesity, and he has successfully mentored trainees over more than 40 years. The department of Physiology and Biophysics at UMMC offers an ideal environment to train in the field of cardiovascular physiology and metabolic diseases, and provides multiple opportunities to meet and interact with skilled scientists affiliated to the Cardiovascular-Renal Research Center, the Mississippi Center for Heart Research, and the Mississippi Center for Obesity Research. Using the excellent mentoring and technical support of my institution, I have initiated a novel project that will investigate the role played by the transcription factor NR4A2 in cardiac remodeling in response to chronic pressure overload, and how this response is impaired with obesity and diabetes. My project will be successfully carried out by combining my past research expertise in molecular biology with my current training in generation of small animal models of cardiovascular diseases and echocardiographic evaluation of heart structure and function. With the support of this fellowship, my mentors and my institution, I intend to become a well-rounded investigator with a unique expertise in an area of research that will expand our knowledge on the molecular alterations contributing to pathological remodeling of the heart during obesity and chronic hypertension.

B. Positions and Honors

Positions and Employment

2010	Research Technician, University of Leeds, UK
2011-2014	Ph.D. Graduate Student, University of Padua, Italy
2014-2016	Lecturer, Lahore University of Management Sciences, Lahore, Pakistan

2016-present Postdoctoral Fellow, University of Mississippi Medical Center, Jackson, MS

Other Experience and Professional Memberships

2016- Member, American Heart Association
2016- Member, American Physiological Society

Honors and Awards

2003 Roll of Honour, Lahore College
2006 Distinction in M.Sc (Microbiology and Molecular Genetics), Punjab University
2008 Faculty of Biological Sciences Award towards MRes, Leeds University
2010 Travel award, CRG Symposium Barcelona
2011 Doctoral Program Scholorship-Cariparo, University of Padua
2015 Faculty Grant, Higher Education Commission Pakistan
2017 Best poster award-UMMC Research Day
2019 Travel award, Experimental Biology 2019

C. Contributions to Science

The contributions below are listed starting with the most recent ones

1. Molecular control of cardiomyocytes growth and cardiac structural remodeling

Over the past 4 years, my research work as a postdoctoral fellow has essentially focused on investigating the molecular mechanisms that control the growth of adult ventricular myocytes, and how those mechanisms may contribute to pathological cardiac hypertrophy associated with obesity and diabetes. I have demonstrated that the Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2) is specifically induced by β-adrenergic stimulation in adult rat ventricular myocytes, and that NR4A2 then acts as a negative feedback regulator of the pro-hypertrophic adrenergic signalling through DUSP-mediated inhibition of the ERK/S6K1 protein synthesis pathway (**a**). In another study, I demonstrated how the metabolic and hormonal dysregulations caused by consumption of a high-fat and high-carbohydrate Western diet cause overactivation of AKT and ERK signalling pathways in response to β-adrenergic stimulation (**b**). These data have laid the groundwork for the current grant application.

a. **Ashraf S**, Hegazy YK, Harmancey R. (2019) NR4A2 inhibits activation of ERK signaling and cell growth in response to beta-adrenergic stimulation in adult rat cardiomyocytes. *American Journal of Physiology: Cell Physiology* 317(3):C513-C524. PMID 31188636

b. **Ashraf S**, Yilmaz G, Chen X, Harmancey R. (2020) Dietary fat and sugar differentially affect beta-adrenergic stimulation of cardiac ERK and AKT pathways in C57BL/6 male mice subjected to high-calorie feeding. *The Journal of Nutrition* 150(5):1041-1050. PMID 31950177

2. Role of insulin resistance in cardiac adaptation and maladaptation in obesity and diabetes

Over the course of my postdoctoral training, I have also contributed to the research work led by my primary mentor on the identification of the molecular mechanisms by which insulin resistance affects cardiac and whole-body adaptation to the metabolic and hormonal dysregulation of obesity and diabetes. One specific protein investigated by my mentor is the mitochondrial protein uncoupling protein 3 (UCP3), which he previously found to be down-regulated with obesity and type 2 diabetes due to hyperinsulinemia. Using rats that were genetically modified to decrease UCP3 levels to the levels seen with type 2 diabetes, we demonstrated that UCP3 insufficiency contributes to worsening of cardiac functional recovery at reperfusion following ischemia (**a**). More recently, we also found in that animal model that complete loss of UCP3 attenuated weight gain and insulin resistance in response to Western diet feeding. We linked this unexpected finding to a compensatory increase in

glutathione synthesis and improved metabolism in brown adipose tissue (**b**). Altogether, those findings highlight the importance of UCP3 in maintaining the oxidative stress balance and energy homeostasis in the tissues that express this protein.

a. Edwards KS, **Ashraf S**, Lomax TM, Wiseman JM, Hall ME, Gava FN, Hall JE, Hosler JP, Harmancey R (2018) Uncoupling protein 3 deficiency impairs myocardial fatty acid oxidation and contractile recovery following ischemia/reperfusion. *Basic Research in Cardiology* 113(6):47. PMID 30374710.

b. Lomax TM, **Ashraf S**, Yilmaz G, Harmancey R. (2020) Loss of uncoupling protein 3 attenuates Western-diet induced obesity, systemic inflammation and insulin resistance in rats. *Obesity (Silver Spring)* Epub ahead of print. PMID 32716607.

3. Mechanisms of action of novel scorpion neurotoxins

In 2011, I was awarded one of 15 merit scholarships among a pool of more than 500 international candidates to pursue a Ph.D. degree in the laboratory of Dr. Ornella Rossetto at the University of Padua in Italy. My Ph.D. project was focused on understanding the mechanism of action of scorpion neurotoxins in the cleavage of SNARE proteins (**a**). Until recently SNARE proteins had only been shown to be targets of clostridial neurotoxins. My studies provided evidence for the presence of a unique class of metalloproteinases in the venom of various species of scorpions of the genus *Buthidae* (**b**). I also showed that these toxins are antarease-like proteins capable of entering intact mammalian neuronal cells and to cleave the SNARE proteins VAMP2 and SNAP 25 in a mechanism similar to that of *botulinum* toxins (**c**). This study was important in three different aspects. Firstly, it provided the groundwork for the generation of novel antivenoms. Secondly, the specificity of these metalloproteinases towards two SNAP25 and VAMP2 could be used for treatment of certain medical conditions such as pathological muscle hyperactivities. Thirdly, further analysis of these unique proteases could provide deeper insights into the biology of scorpions, their preying habits, geographical habitats and phylogeny.

a. **Ashraf S**. (2013) Study of mechanism of action of scorpion neurotoxins. Thesis Library. University of Padua, Italy. (http://paduaresearch.cab.unipd.it/6435/)

b. **Ashraf S**, De Lima ME, Montecucco C and Ornella R. (2012) Study of mechanism of action of bacterial and scorpion neurotoxins. Venoms 2012 (Oxford, UK).

c. Pirazzini M., Maria V., **Ashraf S**, and Montecucco C (2011) Tetanus and botulinum toxins need double anchorage to the membrane for entry into neurons. ABCD congress, cell biology and differentiation. 8-10 September, 2011. Ravenna, Italy.

4. Proteomics analyses in the design of novel anti-Chlamydial drugs

Between 2008 and 2010, I pursued a M.Res. degree in the laboratory of Dr. Stephen Baldwin at the University of Leeds in UK. There, I worked on devising strategies to overexpress the nucleotide transporter CtNTT1, a protein located in the membrane of *Chlamydia trachomatis*, for the development of novel anti-Chlamydial drugs. My work led to the generation of a novel araBAD promoter-based vector, pBAR-0550-C0H, which was used to generate high yields of recombinant CtNTT1 to perform structural analysis of this protein. Structural models of CtNTT1 were successfully generated based on my work in molecular cloning and the concomitant analysis of the crystal structure of the bacterial lactose transporter LacY. I was also involved in site-directed mutagenesis experiments used to validate this structural model (**a**). The generation of the CtNTT1 structural model was a key step in the subsequent determination of the mechanism of action of nucleotide transporters in *C. trachomatis* (**b**). Ultimately, my work contributed to the identification of the substrate binding site

of CtNTT1, information which will be critical for identification of small molecules inhibitors that could be developed as novel anti-Chlamydial drugs.

a. **Ashraf S**. (2010) Adenine nucleotide transporters as potential targets for novel anti-chlamydial drugs. Thesis Library. University of Leeds, UK.

b. **Ashraf S,** Postis V, Baldwin S (2010) Adenine nucleotide transporters as potential targets for novel anti-chlamydial drugs. CRG Symposium (Barcelona, Spain).

5. Impact of spontaneous mutations in bacterial resistance to vaccination

From 2004 to 2006, my M.Sc. in Microbiology and Molecular Genetics involved the study of capsular antigen proteins of *Pasteurella multocida*. The aim of this work was to amplify, clone and compare sequences of capsular proteins in different strains of bacteria to identify potential mutations in the antigenic regions which might lead to ineffective response of vaccines (**a, b**).

a. **Ashraf S**. (2006) Molecular Characterization of capsular protein genes of *Pasteurella multocida* isolated from different localities in Pakistan. Thesis Library. University of Punjab, Pakistan.

b. **Ashraf S**. (2005) Molecular Characterization of capsular protein genes of *Pasteurella multocida* isolated from different localities in Pakistan. Proceedings of Pakistan congress of zoology, volume 25.

Complete List of Published Work in My Bibliography
https://www.ncbi.nlm.nih.gov/myncbi/sadia.ashraf.1/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

NIH R01 HL136438-01A1 Harmancey (PI) 04/01/2018-03/31/2023
"Molecular Basis of Postischemic Maladaptation in the Insulin Resistant Heart"
The goals of this project are to identify the mechanisms by which UCP3 deficiency may impair contractile recovery of the insulin resistant heart after myocardial infarction and to apply this knowledge to develop therapeutic strategies for improving the recovery of diabetic patients at reperfusion.

P20 GM 104357 JE Hall (PI) 09/05/13 – 04/30/23
NIH/NIGM Cardiorenal and Metabolic Diseases Research Center
This grant supports a Center of Biomedical Research Excellence focused on obesity, cardiovascular, renal and metabolic diseases.

Completed Research Support

Ph.D Fellowship Ashraf (PI) 01/02/2011-02/02/2014
Fondazione Cassa di Risparmio di Padova e Rovigo
The goal of this fellowship was to provide three years of salary support for the principal investigator and to cover supplies required for completion of the dissertation project.

Scholastic Performance

Year	Title	Percentage Marks
2005	Biochemistry	78
2005	Cell Biology	70

2005	Microbiology	75
2005	Genetics	78
2005	Virology	82
2005	Microbial Genetics	76
2005	Biostatistics	81
2005	Molecular Biology	75
2006	Immunology	85
2006	Environmental Microbiology	80
2006	Food Microbiology	84
2006	Recombinant DNA Technology	70
2006	Human Genetics	76
2006	Gene Therapy	90
2006	Industrial Microbiology	82

PhD

2012	Electron Microscopy	Pass
2012	Protein Crystallization	Pass
2012	Scientific Writing	Pass
2013	Optogenetics	Pass
2013	Bioimaging	Pass

Except for PhD courses all course marks are in percentages. PhD courses were marked as either fail or pass. Students were required to attend or clear at least three courses.