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# On the Cutting Edge of Medicine

Katherine Wallerius  
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# **On the Cutting Edge of Medicine**

A thesis submitted to  
Regis College  
Honors Program  
in partial fulfillment of the requirements  
for Graduation with Honors

Katherine Wallerius  
Honors Thesis  
May 2016

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Director, University Honors Program



## **Table of Contents**

<b>List of Figures</b>	<b>vii</b>
<b>Acknowledgements</b>	<b>ix</b>
<b>Introduction – Mountains and Medicine</b>	<b>1</b>
<b>Chapter 1 – Getting Into Academic Medicine</b>	<b>5</b>
<b>Chapter 2 – Defining “The Process” and Finding Your Way</b>	<b>19</b>
<b>Chapter 3 – My Introduction to Academic Medicine: Distribution of EC-SOD affects collagen deposition in the development of pulmonary diseases</b>	<b>37</b>
<b>Chapter 4 – Reflections on Academic Medicine</b>	<b>62</b>
<b>List of Abbreviations</b>	<b>70</b>
<b>References</b>	<b>71</b>

## List of Figures

<b>Figure 1: Fetal Circulation</b>	<b>31</b>
<b>Figure 2: Dismutation of Superoxide</b>	<b>48</b>
<b>Figure 3: Heparin Binding Domain</b>	<b>49</b>
<b>Figure 4: Right Ventricular Hypertrophy</b>	<b>50</b>
<b>Figure 5: Trichrome Stain of Collagen</b>	<b>51</b>
<b>Figure 6: Pircosirius Red Stain of Collagen</b>	<b>52</b>
<b>Figure 7: Overexpression and Loss of EC-SOD</b>	<b>53</b>
<b>Figure 8: Cononical TgfB Pathway</b>	<b>54</b>
<b>Figure 9: R213G SNP Alters Binding Affinity</b>	<b>55</b>
<b>Figure 10: R213G SNP worsens collagen deposition following chronic hypoxia</b>	<b>56</b>
<b>Figure 11: Active Tgf<math>\beta</math> decreases in response to hypoxia</b>	<b>57</b>
<b>Figure 12: R213G SNP mitigates the development of fibrosis after IT bleomycin treatment</b>	<b>58</b>
<b>Figure 13: R213G SNP attenuates collagen following treatment with bleomycin</b>	<b>59</b>
<b>Figure 14: Trend towards increased active Tgf<math>\beta</math> in WT but not R213G mice</b>	<b>60</b>
<b>Figure 15: Variability in Gapdh house-keeping gene</b>	<b>61</b>

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*What you are in love with,  
what seizes your imagination, will affect everything.  
It will decide  
what will get you out of bed in the morning,  
what you do with your evenings,  
how you spend your weekends,  
what you read, whom you know,  
what breaks your heart,  
and what amazes you with joy and gratitude.  
Fall in love, stay in love,  
and it will decide everything.*

- Fr. Pedro Arrupe, SJ



## **INTRODUCTION**

### **Mountains and Medicine**

On Longs Peak's Boulder Field, a cobbled path for giants, we leapt across the huge boulders while carrying the stretcher with the ashen-faced man. The forceful wind from the chopper blades buffeted us as we hurried toward the Flight For Life helicopter. The man groaned when we hoisted him in. I prayed he would survive as I traced the little orange dot through the sky until it disappeared over the ridge of the adjacent peak. It was powerful to witness how quickly life can change on a mountain.

I often think of that man and about how fragile life can be. However, I am comforted by the knowledge that I did what I could to help him, as did the Flight For Life pilots, the doctors and nurses he came across, and the other professionals who have followed a path to dedicate their lives to healing. And I know that it is my path too.

When I think of medicine, I think of caring for the patient in front of you. This past summer, however, I realized that medicine can extend beyond caring for this one patient, from this one mountain, at this one time. I had the incredible opportunity to conduct lung disease research in Dr. Eva Grayck's Cardiovascular and Pulmonary lab at the University of Colorado School of Medicine, and I discovered the field of academic medicine.

Academic medicine is unique in that its three-part mission includes not only 1) providing excellent clinical care to patients, but also 2) a commitment to driving life-changing research, and 3) educating the next generation of physicians. This mission called to me—I realized that I could become a doctor who makes valuable contributions to people’s health and well being, while making a more far-reaching impact through research.

Upon becoming a physician, you may enter into private practice or academic medicine. Due to the research component, physicians who pursue academic medicine often face stressors on top of those associated with being a private practice doctor. Many academic medical centers are currently facing challenges as they struggle to balance their triple mission of providing medical education, driving life-changing research, and providing clinical care to the communities they serve. More than ever, there is also a magnifying glass on healthcare in both traditional and social media regarding cost and reputation. The public requires more transparency and accountability than ever before for pricing, health outcomes, and community impact.

As an aspiring doctor, I wanted to learn more about academic medicine to help determine if I want to pursue this path. I interviewed two leading doctors in both research and medicine to more fully understand the pros and cons of a career in this field. I interviewed my research mentor, Dr. Eva Grayck, a Pediatric Critical Care attending at Children’s Hospital Colorado; and Dr. Cassidy Delaney, a Neonatologist at Children’s. As I listened to their stories, I began to think about

how the three-part mission of academic medicine truly pushes you to be on the cutting edge.

In the pages that follow, you will find biographies of Dr. Grayck and Dr. Delaney with specific focus on the experiences that led them to academic medicine. I then describe my experience working in Dr. Grayck's lab and present the findings from my research. Throughout, I explore how pursuing a career in academic medicine can help the physician to seek the *magis* and live out their calling in medicine.



## **CHAPTER 1**

### **Getting into Academic Medicine**

Dr. Cassidy Delaney motioned towards her windowsill, which was lined with pictures. She pointed to a picture of a young boy with a tracheotomy tube, his name was Aiden. “I was at his delivery,” she said. “I was there with his first time mom and dad when they learned he had severe congenital diaphragmatic hernia.”

Can you image the terror Aiden’s first-time parents felt upon hearing their son’s diagnosis? It was Dr. Delaney’s job to educate Aiden’s parents and treat their son’s illness. More than that it was her personal mission to support and care for Aiden and his parents. Dr. Delaney became very close with Aiden and his family during his year and a half of life; Aiden had such an impact on her that she kept his framed picture on the windowsill where she would see it everyday.

...

Dr. Delaney is an Assistant Professor at the University of Colorado School of Medicine and a Neonatology specialist at Children’s Hospital Colorado. I benefitted tremendously from interviewing Dr. Delaney to understand what draws her to academic medicine. Her personal mission and compassion resonated deeply with me and I think she is an excellent example of a physician scientist.

She believes that academic medicine pushes her to be on the cutting edge, in research, education, and patient care. She says, “My colleagues in academic medicine are always thinking about the next level, the next scope, where we can bring things.” They push in order to help bring medicine forward. It is the focus on building connections and giving back that draw Delaney to the field.

She says that in academic medicine, “The fact that I’m a good doctor doesn’t get me promoted. The fact that families love me doesn’t get me promoted. That fact that I have publications and grants gets me promoted. Your expectation is that you’re a good doctor; everybody’s should be, right?” In academic medicine, it is what you do beyond that that earns you a promotion. “So it’s this ladder and constant stress,” Delaney said. “But I think without that then you just become complacent.” Academics align you with the cutting edge of medicine because you are always learning and striving for better patient care, both in the clinic and in the laboratory.

### **A Physician’s Path**

Dr. Cassidy Delaney grew up in Southern Florida. Though no one in her family was in the medical field, she found herself drawn toward the sciences and medicine. In college, she pursued the pre-medical requirements, but felt that she didn’t have the self-confidence to go to medical school, so she also majored in physical therapy and planned to go to physical therapy school. “If it wasn’t for my



mom saying, you can do this, then I probably wouldn't have become a doctor," she said.

Delaney found a mentor and her calling during her third year of medical school. After graduating with her bachelor's degree, she taught for a year before beginning her medical education at the University of Miami. During a rotation in her third year of medical school, she fell in love with the Neonatal Intensive Care Unit. She was fascinated by the physiology and the critical care nature as well as the long-term relationships with infants and their families. Delaney remembers when one of her role models, Eileen Secenco, a Neonatologist at the University of Miami, spoke to Delaney and her classmates about changes in infant viability. Secenco presented a slide showing the drastic improvement in neonatal outcomes in the past 10 years. Delaney remembers thinking, "This is what I want to do. I want to be a part of this impact."

Delaney first gained experience in the research setting during her fellowship at the University of Colorado School of Medicine. One day, Delaney was meeting with her mentor, who just by accident had her file cabinet opened with a little manila folder reading "Selective Serotonin Reuptake Inhibitors in Pulmonary Hypertension" peaking out. Delaney had just read a New England Journal article about how SSRIs, a common treatment for depression, taken during pregnancy might cause lung disease and pulmonary hypertension in newborns. Delaney wondered how this would impact mothers who are struggling with depression during pregnancy.

“If I hadn’t seen that manila folder peaking out, I don’t think I would be where I am now,” Delaney said. For the last 7 years, she has studied the effects of serotonin on the fetal circulation. “It is funny how something as small as that can change your life,” she said.

...

How do you decide which area of medicine to go into? Delaney says that, “Throughout your Fellowship you ask yourself, do I want to do private practice? Do I want to be a clinician in an academic setting? Do I want to do research in an academic setting as well as clinical?” Personally, she really enjoyed and wanted to continue her research and she also wanted to continue teaching and taking care of babies. She remembers, “I wanted to do all of that and I wanted to be with like-minded people who wanted to do all of it as well, rather than people who were solely focused on clinical care, on coming in, doing their work, then going home. I feel like in academics it’s part of your life and your lifestyle, it becomes you rather than it just being a facet of who you are.”

Delaney has found like-minded people in academic medicine, and especially in Pediatrics. “In this field, you’ve already taken away a big subset of people that went into medicine for the financial aspects of what medicine can bring. In Pediatrics, I’ve found that you are surrounded by people who love to teach.” She says, “If you choose General Pediatrics, for example, you are educating families all day. You are educating pre-teens about their bodies and their changes; you are educating teenagers about pregnancy and health. It is a

lot of anticipatory guidance, education, as well as a lot of policy work in Pediatrics. This includes big endeavors like seatbelt use and bike helmet use, where from a policy standpoint you can significantly impact children everywhere.”

“This is another aspect of medicine that I find fascinating! You can go into the field and want to take care of children, but then realize, oh I love the policy, I want to impact every 5 year old in the country. Through policy you can say that every 5 year old should all be seated in this car seat until they’re 7 because it will save 2 million children a year.” In the clinic you have the opportunity to impact one child at a time. “For some people, impacting that one child and knowing that that has changed their life and their family’s life is good, most days that’s all I need,” Delaney says.

“But sometimes I want bigger, I want more,” she said of her ability to make a more far-reaching impact through research. “So I think that’s the nice part about medicine, you can evolve and change all the time based upon opportunities for what you want to do. I do find that people in academics think big picture.”

There are tradeoffs, however. Delaney described how people in academics are often expected to perform equivalent clinical work with that of a private practice physician, in addition to their research commitment. Delaney says “Sometimes you work harder in academics, because you have your job in the clinic and your job in the lab.” Though this balance is difficult, Delaney wouldn’t be satisfied if she didn’t pursue more than clinical care. She explained to

me how when you're going through training, you find role models who inspire you such that you aspire to be like them. "Trying to emulate them was important to me," she said, "and staying in this setting, whether you work a little bit harder sometimes, to be around like minded people is really important. I didn't necessarily feel that working in a setting where being a physician felt like more of a job would be as gratifying."

She added that in academics you often have a lot more control over how you practice. "Sometimes in private practices your responsibilities are dealt out and you're just following a regimen. You see kids, and then turn them around. It's about producing a revenue." "Here," she said, indicating the University of Colorado, "it's not about that."

"In your research, while there are big stressors like grant funding, and publishing papers, it is about more than being published. It is also about going to a conference to hear what someone else is doing, even if it doesn't necessarily relate to what you're doing but is just incredibly interesting. Seeing someone succeed and helping them to succeed, that is where you gain your rewards from."

### **The Role of Clinical Care in Academic Medicine**

"In an ideal world, you get to choose exactly what you want. In reality, there are also decisions based upon where your spouse wants to be, based upon where your kids would be happier, based upon where the jobs are. Maybe there

are no jobs here when you finish so you can't stay in academic medicine. There are so many things that impact your decision, and sometimes it is difficult to figure out what you want," said Dr. Delaney. "I just knew that I was happy here at Colorado, so I stayed to figure out if this is the right life for me."

Delaney's focus is on neonatal pulmonary hypertension. "I initially chose to study pulmonary hypertension because of my interest in serotonin. Then I became fascinated by the physiology of this disease that impacts a huge number of the babies that I take care of." While some therapies exist, including nitric oxide, and benefit a huge number of patients, these therapies don't help all babies. Dr. Delaney is passionate about helping these babies.

During our conversation, I asked whether any patients in particular have influenced her research or clinical practice. This is when she pointed to her windowsill, which was lined with pictures of her children and her patients. Aiden's picture sat there, and Dr. Delaney described to me how he ended up with a tracheotomy and very bad pulmonary hypertension and heart disease. He was in the hospital for a year and a half; he died about a month after going home from the hospital. "During that time I became very close to their family," Dr. Delaney said, "As my team and I tried therapies to help him survive. You know when they get to be a year and a half and they have a personality and they're bonding with their family. We want to keep these babies alive so their families get to know him. After a year and a half, they know him, he has an identity."

“But I think there’s also a difficult balance in that. A baby who dies shortly after birth is an incredibly painful loss. But you don’t know his personality and what he got from you. Losing a one and a half year old after you’ve gotten to know him is absolutely devastating.”

“I could sit and talk to moms for hours and just talk about their life and their families and their babies and delivery,” Delaney said. “I think my favorite part of being a clinician is bonding with families. For me, it’s something that you don’t know until you’re there, but you are allowed into parts of human connection with these families that they don’t let anybody else into. If a baby is born with or diagnosed with a terrible illness, you are the one to do that. It is in these moments that you connect with them on a deep deep level. You are there for a family when their baby is dying and sometimes you are the only one they want there. You witness the strength mothers and fathers have during that time. I think that is intangible. It’s just powerful, incredibly powerful. And it’s a huge honor to be that person. For me that connection is unlike anything else I’ve ever experienced. It impacts your life a lot when you leave there because you don’t stop thinking about them. Those moments are worth all of the training and everything you do.”

There are also challenges of being allowed into families’ lives. Delaney says, “I think you are also allowed into parts of a family’s life where you see how some babies are mistreated or are set up for necessary failure in the families that they will go home to. This is really hard for me. Sometimes, no matter how hard

you try to work through something with a family, if they see it a certain way you just won't change their minds."

"I don't deal with any of the billing, or the money, or the insurance. If I did I would hate it and that would be my least favorite part of my job. But working in a group practice in a hospital in an ICU, you don't do any of that stuff. I've never wanted to have my own practice because I don't want to deal with business or finance, and by working in a group practice in a hospital ICU, I don't have to."

Dr. Delaney works in the ICU for 12 weeks per year and about 20 weekends a year. She is on call at night probably around 7-8 times a month. If you're on call in the hospital, you do 24-36 hour shifts fall.

### **The Role of Research in Academic Medicine**

Another piece of Dr. Delaney's work is in the laboratory. She says, "In the lab it is a completely different side of me. In the lab it's quieter, and there's less immediate stress. It is the more cerebral part where you're learning, you're pushing, and you're adding to the literature but you're also in control. In the clinic you're not in control. I like the variation."

"My research is driven by my clinical interests and relating it back to the babies. If I didn't have that connection it would be very hard for me to do research. In basic science I study very broad strokes. For example, this signaling molecule affects pulmonary hypertension, and I can treat PH with this. PhD scientist, on the other hand, might study a very specific pathway and its

regulation. There's no way I could do that. And I'm not trained well enough to do that either. It's never going to be me and I'm okay with that."

Delaney is also interested in treating babies who are born with hypoxic brain injury. Through clinical research, she and her group determined that, for babies born with hypoxic brain injury, cooling their body temperatures for three days after they are born will improve their survival and decrease their risk of neurodevelopmental impairment at 2 years of age. Children's Hospital Colorado is a big catchment for children born with hypoxic brain injury throughout the region, including neighboring states and they receive a lot of patients. Dr. Delaney was involved not only with the clinical research to determine to what temperature and for how long the babies body temperatures should be cooled, she is also involved quality improvement research to write protocols so that babies who arrive from around the region are all treated in the same way. When she first began, only about 40% of babies arrived with their body temperature in the suggested range. Their work helped to bring this number to closer to 100%.

Of her research, she says, "I think my most favorite part about it is being a part of a bigger group, there's a collegiality in it. I enjoy the thought process and brainstorming with other people. And finding out something that nobody else in the entire world knows is really cool. When I did the hypoxic brain injury studies, it was all in-vivo physiology in sheep. I remember leaving here on a Saturday being totally exhausted. I hadn't seen my kids and it felt like I had been working for so long. But as I was leaving, I thought, I know something nobody else does. I



remember thinking, this is incredibly cool, and I want to share this with everybody. One day it could be in a book that I discovered this.”

She said that, on the other hand, her least favorite part of being in the lab is probably the writing. Delaney said, “In order to be good at research you have to be good at and enjoy writing. So for me that’s been an evolution over the last few years. I’ve been trying to take away a lot of my anxiety around writing and have some confidence in it.” In academics, you need to have funding. The problem is that you have to have papers in order to get funding. But you need funding in order to do the science and to get the papers out. Funding has become scarcer, especially as hospitals debate their role in funding research.

Limited funding makes competition for research dollars stressful. I have realized that as the leader of your lab, you are not just paying for your materials and your salary; you are also responsible for the people you work with. If you don’t have funding, you are responsible for firing your team. This can place an enormous burden on one’s heart.

### **Supporting Education in Academic Medicine**

Dr. Delaney believes that educating young physicians is among most important aspects of academic medicine. The emergence of evidence-based medicine has radically changed the field. She says, “When I was a first year medical student in 1999, we had a course on evidence based medicine. Before this type of medicine emerged, physicians relied on antidotal medicine, where

everything was ‘Okay, well I saw patient A 10 years ago and we did this so now I’m going to treat patient B based on that patient I saw 10 years ago.’” In comparison, evidence based medicine, in a randomized controlled trial, involves researching how one variable impacts another to determine how to improve outcomes.

For example, the National Neonatal Resuscitation Program (NRP) provides a strict algorithm for resuscitating newborns. Just recently, the NRP published new guidelines. A number of the things that they changed were based upon either new evidence or no evidence that the previous guidelines were effective. Dr. Delaney says, “It is people in academics who are constantly thinking about those changes, about what’s better and what’s worse for patients.”

“We are constantly thinking about why things are the way they are,” Dr. Delaney says. “By challenging current practices we’re not just doing the same thing every day that we did 20 years ago.” Dr. Delaney this aspect of medicine—that she is both educating young physicians and learning herself.

Capitalizing on her love of teaching, I asked Dr. Delaney for advice as I begin my journey in medicine. She said, “I think the most important part is being honest with yourself about who you are. Do what you want to do, don’t do it for somebody else to fulfill his or her expectations of you. Think about why you do or don’t want to go into a field. Be honest with yourself. If you don’t want to do something, is it because you are fearful or don’t think that you can succeed or is it because you don’t like that field.”

“Everybody in academics wants people to stay in academics. As an academic physician, your goal is to train academic doctors who will learn from you and continue the cycle. You want to train those people who are like-minded to you. But only 20% of people are going to stay in academics, a lot of people are going to go into private practice. Some people that stay in academics even though it’s not for them because they don’t want to let somebody down.”

“So really just be true to who you are. Ask for a lot of advice. Enjoy it. Enjoy the whole process; don’t just look for the end. The process is really really rewarding and just fun.”



## **CHAPTER 2**

### **Defining “The Process” and Finding Your Way**

The typical path into academic medicine involves either a dual MD/PhD degree or a fellowship that includes research. The research is wide-ranging and can impact on many areas of health including treatments for cancer, stroke, and heart disease to name but a few. Academic medicine is a very competitive field and clinical academics comprise around 5% of the medical consultant workforce. If interested in this field, one needs to be an academic high achiever with a good record of success at medical school. You also need to be passionate about your chosen area and driven to push boundaries and make new discoveries in your field.

Some students apply to medical schools that offer a PhD alongside the medical degree, leading to an MD/PhD. However, many people undertake a PhD later on in their career and it is not essential to gain experience of research during your time at medical school. Some people develop this interest later on and pursue research during their residency and fellowship.

### **Getting Into Academic Medicine**

From a young age becoming a doctor was among the list of things Dr. Eva Grayck wanted to be when she grew up. Upon entering college at the University

of Colorado in 1979, however, she was unsure of which path to take. Guided by the idea that she liked science, she dabbled in Anthropology and Environmental Biology before choosing to major in Molecular, Cellular, and Developmental Biology. The field of Molecular Biology was beginning to take off at that time. Molecular biology overlaps with biology and chemistry and in particular, genetics and biochemistry. A key area of molecular biology is understanding how various cellular systems interact, with specific focus on DNA, RNA, and protein synthesis function.

“The field really resonated with me,” she said of biology and as an undergraduate Dr. Grayck chose to pursue an independent project in cancer biology. She worked in this lab for a year and fueled by the experience, sought a summer internship at Cold Spring Harbor Laboratories, a private, non-profit institution with research programs focusing on cancer, neuroscience, plant genetics, genomics and quantitative biology, located in New York. James Watson directed Cold Spring Harbor—one of the co-discoverers of the structure of DNA. Grayck remembers how it was “incredibly cool to feel like you met someone who discovered DNA and won a Nobel Prize.” Cold Spring Harbor attracted scientists from around the world. Renowned investigators would visit Cold Spring Harbor to speak on topics from signal transduction to gene regulation. Grayck was able to attend many of these seminars, where she caught the scientist’s infectious excitement for research.

Dr. Grayck's research project was on the hormonal regulation of a protein found in rat urine. Though her team didn't know the protein's function, they knew that it was hormonally regulated. Their goal was to sequence the protein to determine the specific sequence responsible for its regulation. At the time sequencing was performed using long, tedious gels. Grayck remembers how, "On the one hand, I was incredibly inspired by the science and by the opportunity to learn from people who were doing incredible research. But on the other hand, I was taken aback by not knowing the purpose of our experiment and felt that fundamental biology was not necessarily my calling." She wanted whatever she did to make a difference in someone's life. So, upon returning to the University of Colorado for her fourth year, she began thinking about going to medical school. Medical school, she thought, would allow her to combine her research interests with her desire to help people.

She applied to medical school and, in what she describes as a best-case scenario, she was waitlisted. Dr. Grayck was still ambivalent about whether she wanted to pursue a PhD or an MD. The committee liked her application but they too were unable to tell whether she was really committed to medicine or if she knew what she was getting herself into. They recommended she gain some kind of clinical exposure and re-apply if she discovered that medicine was right for her.

After graduating with her Bachelors degree, Dr. Grayck was able to get a job in the same lab at Cold Spring Harbor and she returned to her research

project. “I began volunteering at a hospital,” she said, “but it was really a worthless experience. I ran errands and wheeled patients to radiology, both of which were less than fulfilling.” During that time she thought about her strengths, and about what she liked in medicine and in research. She chose to reapply to medical school and was accepted at the University of Colorado School of Medicine.

The medical field held many options for Grayck—from becoming a general family practitioner in the mountains to doing research at a university. For Grayck, the first two years of medical school were similar to her undergraduate education with the addition of an interviewing and a physical exam class. It wasn’t until her third year that she gained her first clinical exposure working in the hospital. She entered every clinical rotation trying to envision herself in that field.

She loved almost all of the rotations, but found her calling in her last rotation—pediatrics. “If you change the life of a child,” Grayck said, “they have their whole life ahead of them.” Children encountered things that were developmental or that they were born with—they didn’t do anything wrong to become sick. It suited her. Grayck knew doctors who didn’t want to go into pediatrics because they thought that parents were too protective, but she found that parents were protective for all of the right reasons and really just wanted to know that was going on with their child.

Choosing pediatrics, Grayck said, was one of the easiest decisions she ever made. In her fourth and final year of medical school, it became clear to her



that she had made the right decision to pursue medicine. “It took me a long time to say this is the perfect combination of everything that resonates with who I am and what I like to do and what excites me,” she said. As a fourth year student, she gained exposure in the field of critical care, and though it was interesting she didn’t think of it as a career, because, at the time it wasn’t an official subspecialty. Boards for Critical Care were offered for the first time a couple years after she graduated from medical school.

At the time Grayck was interested in infectious disease. She had visited Thailand on a self-guided 6-week public health rotation, where she visited several hospitals and tracked the different patients seen as well as how the systems were organized, who took care of patients, and how doctors were trained. In Thailand she encountered diseases she had never seen before; this experience was a catalyst for her interest in infectious disease and public health. She chose to study infectious disease and began her residency at the Duke School of Medicine, which had a very strong infectious disease program.

During her residency, Grayck began working in the Intensive Care Unit (ICU) and realized that for her, the ICU was an ideal environment. She didn’t have to choose which organ system she studied. “I felt that it was okay to give up the long term relationships with families because I built short relationships with families at the most important time in their life,” she said. In the ICU, Grayck quickly became interested in what caused some patients to become critically ill

with an infection while others simply had an ear infection or a bad cold. That interest ultimately led her to subspecialize in critical care.

She chose, however, to do research in infectious disease and began looking for fellowships. She chose to pursue a fellowship because she thrived in the academic environment. I loved “the teaching, the smart and amazing people I came across, loved learning something new everyday and loved being on the forefront of knowledge,” she said. Dr. Grayck chose to pursue her Fellowship in Pediatric Critical Care at the Duke University School of Medicine.

### **The Oxygen Paradox**

Rarely do we think of oxygen being toxic. The paradox of aerobic life, or the “Oxygen Paradox,” is that eukaryotic aerobic organisms depend upon oxygen, but oxygen is inherently dangerous to their existence.<sup>1</sup> This ‘dark side’ of oxygen comes from the unpaired electron that each oxygen atom has in its outer valence shell, making it a free radical.<sup>2</sup> Free radical reactive oxygen species and reactive nitrogen species are generated in some of the body’s natural reactions. Oxidants are a by-product of normal metabolism, for example. In metabolism, the mitochondrial electron-transport chain reduces oxygen to produce water. This is a fairly safe process, however the reduction of oxygen generates reactive intermediates, which can cause extensive damage DNA, proteins, and lipids.<sup>3</sup> When excess oxidants overwhelm a cell’s normal state, the cell falls into

oxidative stress. Oxidative stress is the result of too many oxidants or too few antioxidants, which as their name suggests, combat oxidant's deleterious effects.

...

During her Fellowship, one of the populations that required Dr. Grayck's attention was the neonatal population, specifically neonates who had the life-threatening condition, persistent pulmonary hypertension of the newborn (PPHN). These babies, who were otherwise born on time and healthy, would develop perinatal stress. Babies can be born with stress, due to, for example, infection, aspirated meconium (fetal bowel movements are inhaled into the lungs before birth), and/or intrauterine stress not detected during the pregnancy.

Before birth, a baby's blood circulates differently while in the uterus (Figure 1). The fetal circulatory system uses two shunts, which are small passages that direct blood that needs to be oxygenated to bypass certain body parts—in particular, the lungs and liver—that are not fully developed while the fetus is still in the womb.<sup>4</sup> The shunt that bypasses the lungs is called the foramen ovale.<sup>5</sup> It moves blood from the right atrium of the heart to the left atrium and as a result, only a small amount of the blood continues on to the lungs. Most of this blood is bypassed or shunted away from the lungs through the ductus arteriosus to the aorta.<sup>6</sup> At birth, the umbilical cord is clamped and the baby no longer receives oxygen and nutrients from the mother. With the first breaths of life, the lungs begin to expand; the ductus arteriosus and the foramen ovale both

close.<sup>7</sup> The pulmonary vascular resistance decreases, which allows blood to flow through the lungs to become oxygenated.

With PPHN, however, the baby does not change over from fetal to normal newborn circulation.<sup>8</sup> Instead of the pulmonary vascular resistance dropping like it is supposed to with the first breath, babies with PPHN have elevated pulmonary artery pressures. Blood is forced away from the lungs due to high blood pressure in the arteries that go to the lungs and decreasing the body's supply of oxygen. As a result, these babies are hypoxic (extremely low oxygen levels) and experience constriction in the small pulmonary arteries.<sup>9</sup>

The process seems illogical at first. Low oxygen levels should theoretically lead to increased blood flow to the lungs to receive increased gaseous exchange. However, it is explained by the fact that constriction leads to redistribution of blood flow to better-ventilated areas of the lung, which increases the total area involved in gaseous exchange.<sup>10</sup> This improves arterial oxygenation, but is not helpful in the case of long-term whole-body hypoxia. For these babies, PPHN is a very lethal, terrible disease.

The goal of PPHN treatment is to improve oxygen levels in the blood, relax the blood vessels in the lungs and maintain a normal blood pressure.<sup>11</sup> For these treatments, babies were given bicarbonate infusions and were ventilated to give oxygen. Ventilation, however, often caused severe lung damage and as a result, many of these babies died. Then a brand new treatment program

emerged, called the Extracorporeal Membrane Oxygenation program, ECMO for short.

ECMO is a procedure that uses a machine to take over the work of the lungs and sometimes the heart.<sup>12</sup> Extracorporeal means that the blood circulates outside of the body with the help of a machine. Membrane oxygenation, referred to as the “artificial lungs,” means that oxygen is put into the blood and carbon dioxide is taken out. ECMO treatment ensures that the child’s body has enough oxygen while giving the lungs and heart time to rest and recover.<sup>13</sup> A child can be on ECMO for several days up to a few weeks. When the lungs or the heart have healed and can work on their own, the support from ECMO is gradually removed. There are, however, risks associated with taking babies off of ECMO. To do so, the carotid vessel must be tied off, potentially causing perfusion to the brain or increasing risk of stroke in adulthood. Grayck said of ECMO, “it was a life-saving machine, but it was also really rough, high tech equipment.”

In her first year of fellowship Grayck treated 25 babies with ECMO. The next year she treated 50. She said there were always two, sometimes three babies receiving treatment with ECMO per day. Then, a little appreciated molecule, nitric oxide, began to emerge as a potential treatment to PPHN.

Long thought of as an air pollutant, nitric oxide was found to play a crucial role in such fundamental biological processes as regulation of blood pressure, functioning and malfunctioning of the immune system, and activation of mechanisms in the central nervous system.<sup>14</sup> Scientists were fascinated that this

colorless, odorless gas could act as a signaling molecule. Nitric oxide was named “Molecule of the Year” in 1992 by the journal *Science*, and 6 years later, three US scientists—Robert F. Furchgott, PhD, Louis J. Ignarro, PhD, and Ferid Murad, MD, PhD—received the 1998 Nobel Prize for Physiology and Medicine for their discoveries surrounding nitric oxide.<sup>15</sup>

“I just happened to be in Piantadosi’s lab when nitric oxide—long thought of as an air pollutant or waste product—was discovered to be made by the body in the blood vessels, specifically in the endothelial cells lining these vessels,” Grayck said. Nitric oxide was found to be a potent vasodilator and it was critical in transitioning the fetal circulation to allow blood to go through the lungs.<sup>16</sup> Researchers and physicians brought this new discovery to the bedside and began to treat babies with nitric oxide. There was hope that it would prevent lung disease. What it did do was decrease the amount of children that required ECMO. Nitric oxide, combined with a new mode of ventilation, totally changed how many patients ended up on ECMO.

Dr. Grayck says, “I was fascinated by nitric oxide, by the idea that a toxin and an air pollutant is also made by the body and is essential for life.” She joined Claude Piantadosi’s project related to nitric oxide. The project was to understand how nitric oxide might have opposite effects depending on its concentration and the amount of reactive oxidative species (ROS) present.

Nitric oxide plays many roles: it is simultaneously a vasodilator, a neurotransmitter, and part of the immune system. The body is always searching

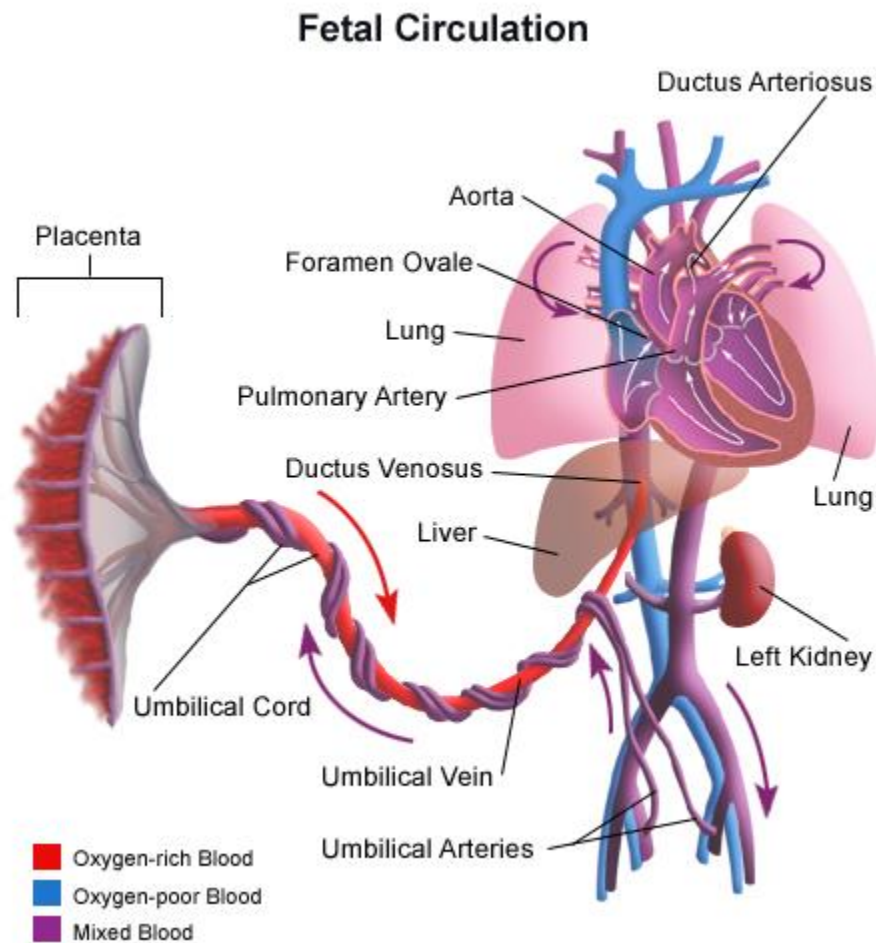
for the balance. In low concentrations, nitric oxide can positively affect the pulmonary circulatory system to cause blood vessel vasodilation, but only if there are low levels of the ROS superoxide. In these conditions, nitric oxide binds to heme, a component of red blood cells, allowing heme to vasodilate blood vessels. When there are high levels of ROS superoxide, however, nitric oxide will react with the superoxide to form peroxynitrite. Most of the cytotoxicity attributed to nitric oxide is actually due to peroxynitrite. Peroxynitrite interacts with lipids, DNA, and proteins to trigger harmful cellular responses including modifications of cell signaling, oxidative injury, and cell death.<sup>17</sup> Nitric oxide can react with iron, resulting in vasodilation, or with superoxide, resulting in toxic peroxynitrite. The oxidative stress in the body regulates which direction the reaction goes.

In humans, the generation of peroxynitrite has many medical implications, including stroke, chronic heart failure, cancer, and neurodegenerative diseases.<sup>18</sup> Therefore, being able to administer nitric oxide while removing peroxynitrite could be a powerful therapeutic tool.

In clinical trials, physicians were administering nitric oxide to babies with PPHN, to cause vasodilation. They were beginning to notice, however, that not every baby responded to nitric oxide. Perhaps only the babies who had deficiencies in nitric oxide benefited from this treatment. Nitric oxide is also part of the immune response. If the babies had an infection and therefore many inflammatory cells, they would be making tons of nitric oxide.

Grayck was interested in the yin and yang of reactive oxygen and nitrogen species. Some nitric oxide and ROS are necessary, but as with all things, everything in moderation. Another challenge is that nitric oxide or superoxide in one area or compartment does not have the same effect as in another compartment of the body. To address a problem, you have to understand where the superoxide is.





**Figure 1:** Fetal circulatory system – Before birth, a baby’s blood circulates differently while in the uterus. The fetal circulatory system uses two shunts to bypass the lungs and liver, which are not fully developed while the fetus is still in the womb.<sup>19</sup> The shunt that bypasses the lungs is called the foramen ovale.<sup>20</sup> It moves blood from the right atrium of the heart to the left atrium and as a result, only a small amount of the blood continues on to the lungs. Most of this blood is bypassed or shunted away from the lungs through the ductus arteriosus to the aorta.<sup>21</sup> At birth, the umbilical cord is clamped and the baby no longer receives oxygen and nutrients from the mother. With the first breaths of life, the lungs begin to expand; the ductus arteriosus and the foramen ovale both close.<sup>22</sup> The pulmonary vascular resistance decreases, which allows blood to flow through the lungs to become oxygenated. Retrieved from: <https://www.urmc.rochester.edu/encyclopedia/content.aspx?ContentTypeID=90&ContentID=P02362>

## **Finding the Balance**

A major antioxidant enzyme that combats oxidative stress in the lung and vasculature is extracellular superoxide dismutase, or EC-SOD. When Eva Grayck finished her fellowship, she chose to stay at Duke to continue her research. During her first year on faculty, she was invited to write a project for a big multi-group Program Project Grant (PPG). Though the PPG ultimately wasn't successful, Grayck says what came from the project was that she made an antibody<sup>1</sup> for the rabbit against EC-SOD. EC-SOD is involved in the pulmonary circulation because it decreases superoxide and thus prevents nitric oxide from becoming dangerous peroxynitrite. EC-SOD is the focus of Grayck's current research.

At the time, Grayck performed a couple of studies about how EC-SOD expression changes with birth. She found that as the lung is being prepared for breathing room air, a number of antioxidants, including EC-SOD, are upregulated. Before birth, the fetus is in a hypoxic environment in the mother's womb. In utero, the baby does not need antioxidants because oxygen is provided from the mother. When the baby is born, however, it needs antioxidant defenses because room air is toxic for a lung that is used to hypoxia. So antioxidant enzymes, including EC-SOD, get unregulated around birth.

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<sup>1</sup> Antibodies are host proteins that the immune system produces in response to foreign molecules that enter the body. The ability of animal immune systems to produce antibodies capable of binding specifically to antigens can be used to manufacture probes for detection of molecules of interest in a variety of research and diagnostic applications.

At this point, Grayck chose to apply for her first NIH grant. She worked in collaboration with her fellowship mentor, Piantadosi, and Howard Hughes Investigator, Jonathan Stamler, to create physiologic preps to show that if you treated lungs with a nitric oxide donor, you could prevent their vasoconstriction by agents like serotonin or phenylephrine. She wrote a mentored grant with Stamler and Robert Lefkowitz, who later won the 2012 Nobel Prize for Chemistry for discovering G-protein coupled receptors, to look at whether nitric oxide was able to block adrenergic receptor signaling by nitrogen protein modification, called nitrosylation. This would have implications for treating infection. In sepsis, for example, patients have very low blood pressure. These patients also make tons of nitric oxide because they have a huge inflammatory response. Grayck, Stamler, and Lefkowitz hypothesized that this inflammatory response prevented some patients from responding to inotropes like epinephrine or adrenaline. They thought that if sepsis patients are making a lot of nitric oxide, which is binding to and shutting down the receptors, and then the inotropes would be ineffective at binding to the

receptors. Grayck took this idea back to her own research and hypothesized that it might be the opposite in hypoxia, that if you didn't have enough nitric oxide, and then you didn't have the normal nitrosylation. She thought this might be a potential reason why patients in hypoxia experienced vasoconstriction.

Grayck found herself in "this incredible environment, studying a very specific question with inspiring people," but she realized she was more interested in pulmonary circulation than the mechanism of nitrosylation. She was the only person in her section at Duke doing basic science research and she felt as though she was working in a vacuum. She happened to email Kurt Stenmark, the division head of Pediatric Critical Care Medicine and director of Cardiovascular Pulmonary Research at the University of Colorado School of Medicine.

"This was one of those times where life takes an unexpected twist," Grayck said when she recalled that on the day before she emailed Stenmark, it just so happened that one of Stenmark's senior clinical faculty had told him that she was taking another job. Stenmark was very excited about Grayck's research and offered her the position. Grayck had been on faculty at Duke for 10 years when she accepted the position to move back home to Colorado. Her children were going into 2<sup>nd</sup> and 4<sup>th</sup> grade at the time and her husband was doing contract work in IT. She found it to be the perfect opportunity to move.

She returned to the University of Colorado, where she had attended medical school. She spent her first two years there trying to get the project from Duke to work, but she didn't have the infrastructure. Stenmark was really interested in the work she had done with EC-SOD and how its reactions in the adventitia, which is the outermost tissue that covers an organ or vessel, were important in a life-threatening lung disease called pulmonary hypertension. The adventitial fibroblast, where EC-SOD is located, is a key sensor of hypoxia.<sup>23</sup>

Grayck chose to further her investigation of EC-SOD by looking at how it regulates inflammation and fibrosis in pulmonary hypertension. Her first NIH Research Project Grant (R01) showed that levels of EC-SOD are decreased in pulmonary hypertension. She also found that the overexpression of EC-SOD protects against pulmonary hypertension, while decreased EC-SOD leads to worsened pulmonary hypertension. Her current project, and the one that I got to be a part of, is to look in more detail at why EC-SOD is so important in the vasculature, why its specific location within the vasculature is so important, and why antioxidant enzymes haven't worked as treatment for pulmonary hypertension. Administering a general antioxidant is ineffective. You need to get the antioxidant to the exact location of the oxidative stress and not just anywhere in the body. The antioxidant also has to be against the right species and at the right time. There are many factors to consider, but this complexity is what first captured Grayck's interest in pulmonary circulation. It is all about finding the balance.





## **CHAPTER 3**

### **My Introduction to Academic Medicine: Distribution of EC-SOD affects collagen deposition in the development of pulmonary diseases**

#### **Goals of my research**

My primary research goal was to understand how the distribution of EC-SOD affects collagen deposition in the development of pulmonary diseases. The deposition of collagen is implicated in many pulmonary diseases, including interstitial lung disease (ILD) and pulmonary hypertension (PH), which were the focus of my project. Collagen is the most abundant protein in the human body.<sup>24</sup> It is found primarily in connective tissue, where it provides support and structure. Though collagen is good and necessary, too much collagen, like anything, is bad, especially in the lungs.

How do we get too much collagen? If, for example, you fall and scrape your arm on the sidewalk, your body immediately reacts to minimize the damage. Your body sends clotting factors to the site of injury to clot and stop blood flow. The scrape will scab over and eventually, it will scar. A similar process takes place in the lungs. When injury occurs in the lungs, the body works to protect itself. Inflammation is a typical response, followed by scabbing and scarring, known as fibrosis. In the lungs, this scarring is due to the buildup of collagen. While scarring helps your lungs to treat the injury, it makes your lungs stiff.



Scarring due to collagen deposition can also occur in the pulmonary arteries, making them stiff as well.

Interstitial lung disease is characterized by fibrosis due to collagen deposition within the lung. Pulmonary hypertension is characterized by vascular remodeling, which is due, in part, to collagen around the pulmonary artery. In both of these diseases, oxidative stress is a major contributor to increased collagen deposition. As previously discussed, oxidative stress is the imbalance in a cell's normal redox state due to either the increased production of reactive oxygen species (ROS) or to decreased scavenging by antioxidants. The major antioxidant in the lung and vasculature is extracellular superoxide dismutase, or EC-SOD.

EC-SOD protects against oxidative stress in the lungs by facilitating the breakdown of oxidant species, superoxide (Fig. 2). EC-SOD is highly localized in the vasculature due to its positively charged heparin-binding domain, which allows it to bind to the negatively charged extracellular matrix (Fig. 3). This allows EC-SOD to protect against the fibrosis caused by oxidative stress in the lungs and vasculature.

One way to study protein function is by using a mouse model. By introducing a new piece of DNA into the mouse genome, you can alter a protein's expression levels, which in turn, can tell you about the function of that protein. Depending on the goal of the experiment, the transgenic mouse will exhibit over-expression of the protein, or a complete loss of that protein.

## Background

We are interested in EC-SOD's role in the development of fibrosis and hypertension in response to 1) chronic hypoxia and 2) bleomycin. Chronic hypoxia is associated with inflammation, vascular remodeling, and ultimately the elevation of pulmonary vascular pressure (i.e. pulmonary hypertension), which also results in right ventricle hypertrophy<sup>2</sup> (Fig. 4). Mouse models show that loss of EC-SOD exacerbates pulmonary hypertension while overexpression of EC-SOD mitigates the development of PH (Fig. 5).<sup>25 26</sup>

Bleomycin is a chemotherapeutic drug that was identified to cause pulmonary fibrosis. Thus, bleomycin or bleo, has been adopted as a model of interstitial lung disease. Similarly, loss of EC-SOD worsens bleo-induced fibrosis whereas overexpression of EC-SOD attenuates bleo-induced fibrosis (Fig. 6).<sup>27 28</sup>

In summary, overexpression of EC-SOD protects against collagen deposition while loss of EC-SOD exacerbates collagen deposition. Therefore, we are interested in whether EC-SOD plays a role in the mechanism of collagen deposition. To answer this question, we looked at a major molecular regulator of collagen synthesis, transforming growth factor beta, or Tgf $\beta$  (Fig. 7).

In the canonical Tgf $\beta$  pathway, Tgf $\beta$  is synthesized in a latent, or inactive, form. This latent Tgf $\beta$  can be activated, allowing it to bind to the Tgf $\beta$  receptor, which can then send signaling complexes to the nucleus to participate in the regulation of target gene expression, ultimately resulting in collagen production

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<sup>2</sup> Right ventricle hypertrophy occurs when blood does not flow well from the heart to the lungs, placing extra stress on the right ventricle. This can ultimately lead to heart failure and death.

(Fig. 8). Under conditions of oxidative stress, ROS upregulate Tgf $\beta$  activation, leading to an increase in collagen gene expression and synthesis.

## **Hypothesis**

So given the role of EC-SOD in combating ROS, and the role of ROS in the Tgf $\beta$  signaling pathway, we hypothesized that EC-SOD regulates collagen deposition through regulation of the Tgf $\beta$  pathway.

## **Experimental Design**

### ***Mouse strains***

To test how the location of EC-SOD affects collagen deposition we used lung tissue from two mouse strains, wild typed (WT) mice and R213G mice. Wild type mice have normal tissue binding of EC-SOD. R213G mice, on the other hand, exhibit decreased tissue binding of EC-SOD due to a unique single nucleotide polymorphism, or SNP, in the enzyme's heparin binding domain. This SNP is based on a naturally occurring human polymorphism that is associated with increased risk of cardiovascular disease. This SNP in EC-SOD leads to an arginine to glycine amino acid substitution at position 213 (R213G) in the heparin-binding domain.

In recent human genetic association studies, this SNP reduces the risk of lung disease, but increases the risk of cardiovascular disease. By capitalizing on the complete sequence homology between human and mouse in the heparin-

binding domain, we created an analogous R213G single-nucleotide polymorphism knockin mouse. The R213G single-nucleotide polymorphism did not change enzyme activity, but shifted the distribution of EC-SOD from lung and vascular tissue to extracellular fluid (e.g. bronchoalveolar lavage fluid and plasma) (Fig. 9). Mice harboring the R213G SNP develop worsened PH.<sup>29</sup>

### ***Models of injury***

Pulmonary fibrosis was induced by a one-time intratracheal installation of bleomycin, which causes airway inflammation followed by fibrosis. Exposure to hypoxia was used to cause vascular remodeling and pulmonary hypertension. To produce chronic hypoxia, 8-week-old-mice were placed in a hypobaric hypoxia chamber at a simulated altitude of 18,000 ft (395 mmHg) for up to 35 days. Lung tissue was harvested at several time points from normoxic and chronically hypoxic mice.

### ***Outcome measures***

We evaluated how location of EC-SOD affects the regulation of three genes known to be critical to the development of fibrosis, transforming growth factor (Tgf $\beta$ ), its receptor (Tgf $\beta$ r2), and collagen type 1 alpha 1 (Col1A1). In addition, we tested for Tgf $\beta$  activation. The results of our study provide insight into how the distribution of extracellular superoxide dismutase affects collagen deposition in the development of pulmonary diseases.

### ***Immunohistochemical fibrosis analysis***

Lung sections at 5um thickness were stained for collagen and subsequent fibrosis scoring using a trichrome stain according to the manufacture's protocol (Sigma-Aldrich, cat# HT15-KT). Lung sections were dried, coverslipped, then fibrosis was scored using the Ashcroft scoring method by a scientist blinded to animal identifiers.

### ***mRNA expression***

mRNA expression for 3 key regulators of the Tgf $\beta$  pathway, Tgf $\beta$ , Tgf $\beta$  r2, and collagen 1A1 (Col1A1), was determined by quantitative RT-PCR. Briefly, mRNA was isolated from the lung using the Qiagen RNeasy Plus Mini Kit. Gene copy numbers were normalized to hypoxanthine-guanine phosphoribosyl transferase (Hprt) for the chronic hypoxia model, and to Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) for the bleomycin model. Gene copy numbers were expressed relative to each groups control mice.

### ***Protein isolation***

Pulverized lung tissue was homogenized in T-PER (Thermo Scientific) containing protease inhibitor cocktail (Sigma-Aldrich) and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich) and centrifuged to remove tissue debris.

Protein concentration was determined using the Pierce 660-nm protein assay reagent (Thermo Scientific).

### ***Tgfβ ELISA***

Lung tissue homogenized in T-PER buffer was diluted to 0.5ug/1ul. Active Tgfβ was measured in each sample using the DuoSet ELISA Mouse Tgfβ1 protocol according to the manufacture's notes (R&D Systems). A total of 50ug of protein was loaded into each well and each sample was measured in duplicate. Concentrations of active Tgfβ were determined using a 4-PL standard curve.

### ***Statistical analysis***

Data (expressed as mean ± SE) were analyzed according to two-way ANOVA, followed by Bonferroni *post hoc* analysis using Prism (GraphPad Software, La Jolle, CA). Significance was defined as  $P < 0.05$ .

## **Results**

### ***R213G SNP exacerbates collagen deposition in chronic hypoxic PH***

To evaluate Tgfβ regulation, we measured gene expression for three key regulators of the Tgfβ pathway, Tgfβ, it's receptor Tgfβr2, and collagen type 1A (Col1A). Tgfβ mRNA did not change significantly in response to hypoxia, although R213G mice tended to express less Tgfβ than WT mice (Fig. 10, A). There were no significant changes for the Tgfβ receptor (Fig. 10, B). R213G mice

had sustained Col1A expression whereas WT mice had decreased collagen expression (Fig. 10, C).

### ***Active Tgf $\beta$ decreases in response to hypoxia***

Active Tgf $\beta$  decreased over time in WT and R213G mice (Fig. 11). Though we predicted greater activation of Tgf $\beta$  in R213G mice in accordance with the increased collagen deposition described by Hartney et al 2014, our data reveal no difference between strains.

### ***R213G SNP protects against collagen deposition in bleomycin-induced fibrosis***

Unexpectedly, we saw the opposite effect on collagen deposition in the R213G strain when exposed to bleomycin instead of hypoxia. In the bleomycin model, the R213G SNP actually protects against collagen deposition. 21 days after treatment with the fibrosis-inducing drug, bleomycin, WT mice experience an increase in collagen whereas the R213G mice do not (Fig. 12). There were no significant changes for Tgf $\beta$  or Tgf $\beta$ r2 mRNA expression in the lung (Fig. 13, A-B). However, there was a trend towards increased Col1A expression in WT mice that didn't occur in the R213G mice (Fig. 13, C).

### ***Active Tgf $\beta$ increases in WT but not R213G mice following treatment with bleomycin***

In accordance with the decreased collagen deposition in the R213G mice seen in Fig. 14, our data reveal a trend towards increased active Tgf $\beta$  in WT but not R213G mice.

### **Discussion**

In this study, we demonstrate that the R213G SNP has opposite effects on collagen deposition in chronic hypoxia and bleomycin models. In the hypoxia model, Hartney et al. demonstrated that the R213G SNP exacerbates collagen deposition around the pulmonary artery after 35 days of hypoxia (Hartney et al. 2014).<sup>30</sup> While we did not see significant changes in Tgf $\beta$  or Tgf $\beta$ r2 expression over the course of hypoxia, mRNA expression for Col1A1 mirrors the trend described by Hartney et al such that collagen expression was sustained in R213G mice but decreased in WT mice. While we predicted that active Tgf $\beta$  would follow this same trend, we saw decreased active Tgf $\beta$  in both WT and R213G mice over the course of hypoxia. These results suggest that the R213G SNP alters Tgf $\beta$  signaling at the level of gene expression, but not at the level Tgf $\beta$  activation.

In bleomycin, on the other hand, the R213G SNP seems to protect against collagen deposition in the lung such that R213G mice experience less collagen



than WT mice 28 days after IT installation of bleomycin (Fig. 3). In accordance with this, we saw less Col1A1 in R213G mice than in WT mice at each time point following IT installation of bleomycin. Also consistent with this, we saw less Tgf $\beta$  activation in R213G mice than in WT mice. We were surprised that post-hoc analysis of the mRNA expression data revealed significance. That led us to look back at the raw data, where we noticed significant variability in our house-keeping gene, Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) (Fig. 6). It appears that Gapdh was affected by bleomycin treatment. Additional PCR using a different housekeeping gene is needed.

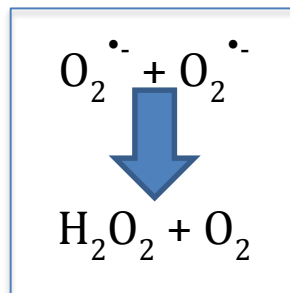
## **Conclusions**

We conclude that the distribution of EC-SOD, based on its binding affinity, has opposite effects on collagen deposition in different disease states. It appears that EC-SOD's ability to modulate collagen deposition depends on the compartment in which the injury occurs, and therefore where the ROS superoxide is being produced.

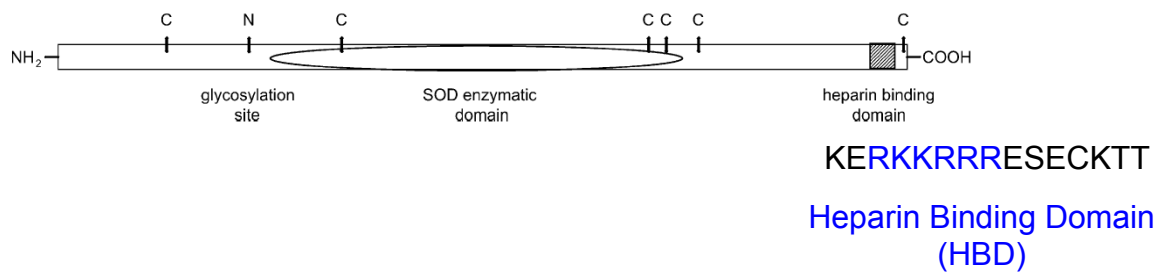
For this reason, we speculate that the R213G SNP was protective in the bleomycin model because EC-SOD is redistributed to the lavage fluid where it may combat the ROS produced by inflammation. In contrast, in hypoxia, the inflammation and ROS production is in the vasculature, and lack of EC-SOD binding worsens the vascular injury.

There are important therapeutic implications associated with understanding how the distribution of EC-SOD affects collagen deposition in pulmonary diseases. The lab is currently working on a method of targeted delivery of EC-SOD. If we can determine where EC-SOD needs to be localized in different disease states, we hope we can then selectively restore EC-SOD activity in the appropriate compartment and improve outcomes.

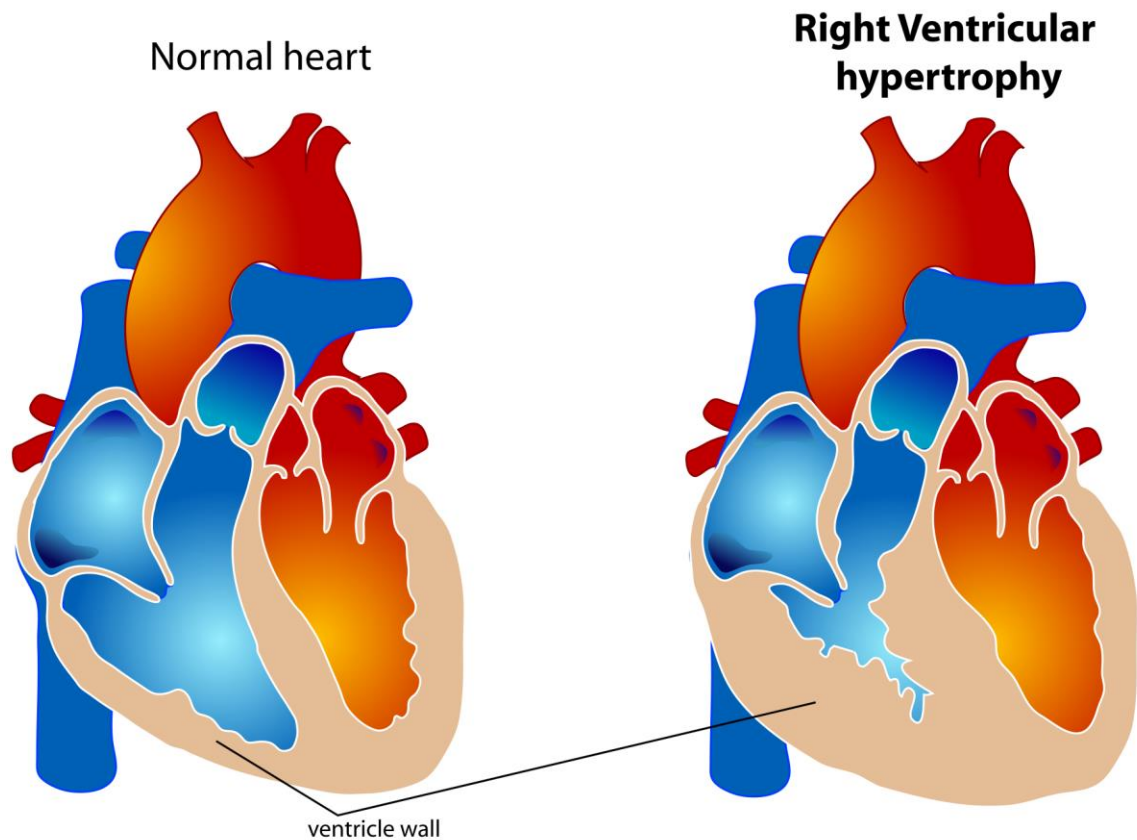
## Dismutation (SOD)



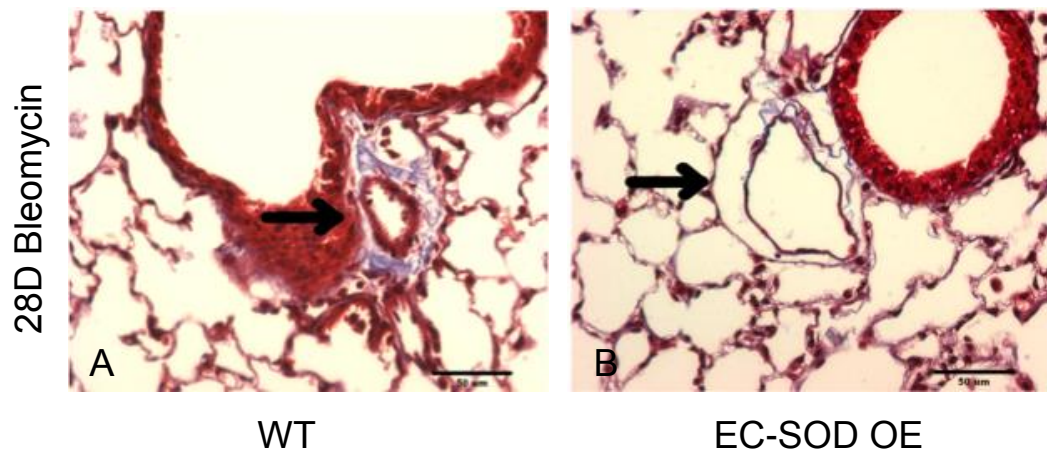
**Figure 2.** Dismutation of Superoxide – EC-SOD is the sole extracellular defense against the ROS superoxide through the dismutation, or breakdown, of superoxide to hydrogen peroxide and oxygen.



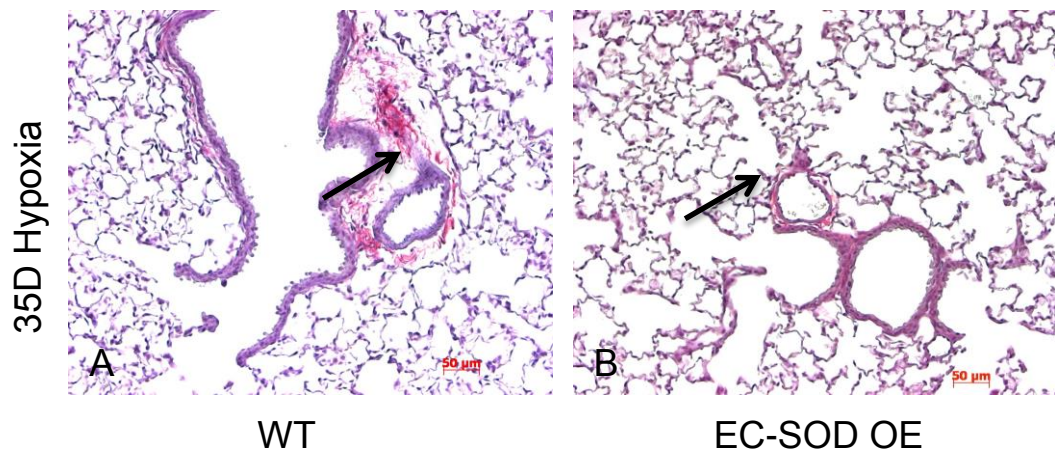
**Figure 3.** Heparin Binding Domain – EC-SOD is highly localized in the vasculature due to its positively charged heparin binding domain (shown in blue), which allows it to bind to the extracellular matrix. This allows EC-SOD to protect against fibrosis in the lung and vasculature.



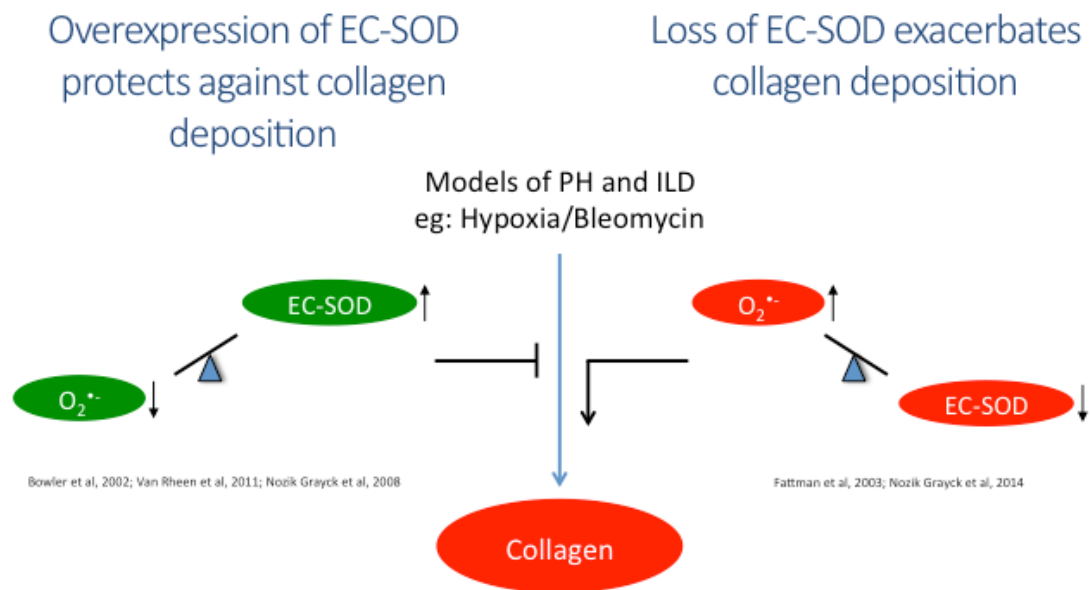
**Figure 4.** Right Ventricular Hypertrophy – The walls of the right ventricle wall thicken in pulmonary hypertension. Blood travels through the right ventricle to the lungs via the pulmonary arteries. In pulmonary hypertension, where the pulmonary circuit decreases, meaning blood does not flow well from the heart to the lungs; extra stress can be placed on the right ventricle. This can lead to right ventricular hypertrophy. Retrieved from: [https://en.wikipedia.org/wiki/Right\\_ventricular\\_hypertrophy](https://en.wikipedia.org/wiki/Right_ventricular_hypertrophy)



**Figure 5.** Trichrome Stain of Collagen – Overexpression of EC-SOD protects against bleomycin-induced fibrosis. 28 days after bleomycin installation, A) the wild type mouse experiences collagen around the pulmonary artery, shown in blue. B) The mouse overexpressing EC-SOD does not develop collagen deposition. (Bowler et al, 2002; Van Rheen et al, 2011)

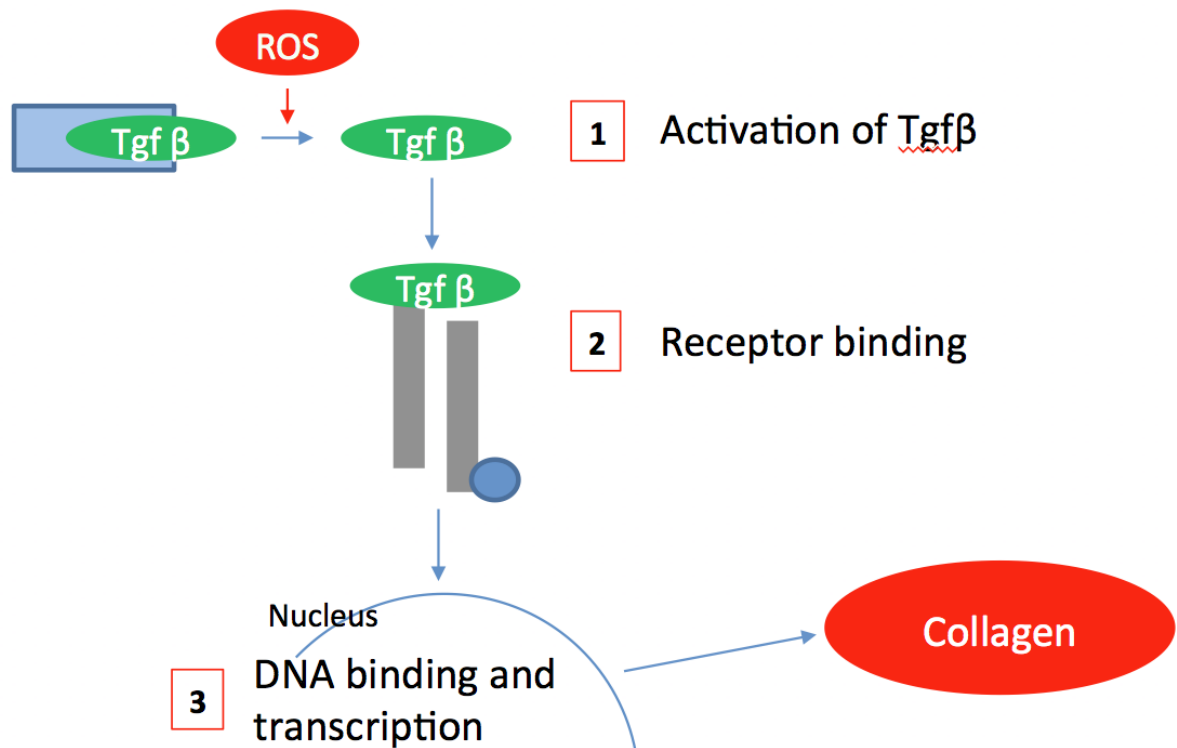


**Figure 6.** Picrosirius Red Stain of Collagen – Overexpression of EC-SOD protects against chronic hypoxia induced pulmonary hypertension. After 35 days of chronic hypoxia, A) the wild type mouse develops collagen around the pulmonary artery, shown in pink. B) The mouse overexpressing EC-SOD does not develop collagen deposition. (Nozik-Grayck et al, 2008)

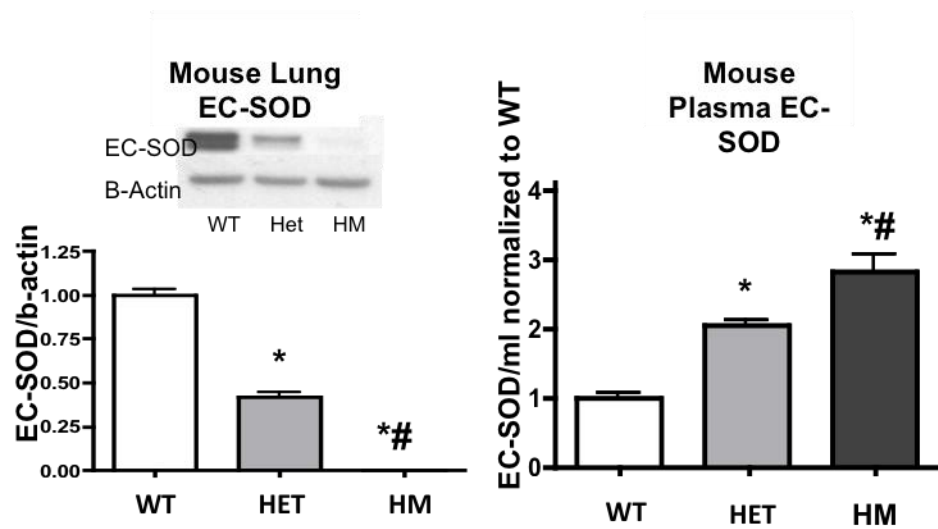


**Figure 7.** Overexpression and Loss of EC-SOD – In summary, overexpression of EC-SOD protects against collagen deposition while loss of EC-SOD exacerbates collagen deposition.

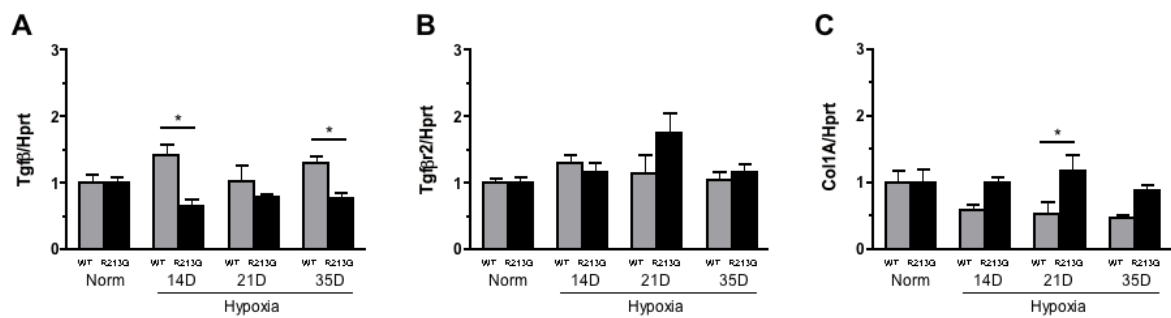




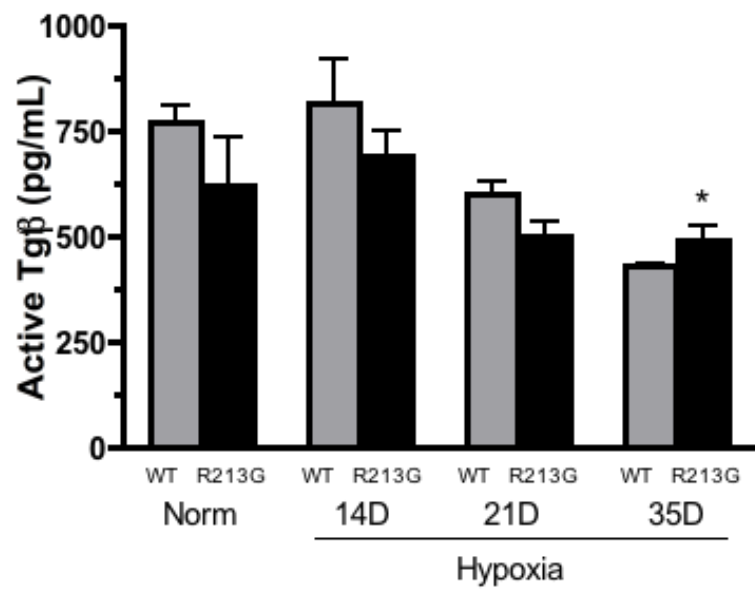
**Figure 8.** Canonical TGFβ Pathway – In the canonical TGFβ is synthesized in a latent form. This latent TGFβ can be activated, allowing it to bind to the TGFβ receptor, which can then send signaling complexes to the nucleus to participate in the regulation of target gene expression, ultimately resulting in collagen production. Under conditions of oxidative stress, ROS upregulate TGFβ activation, leading to an increase in collagen gene expression and synthesis.



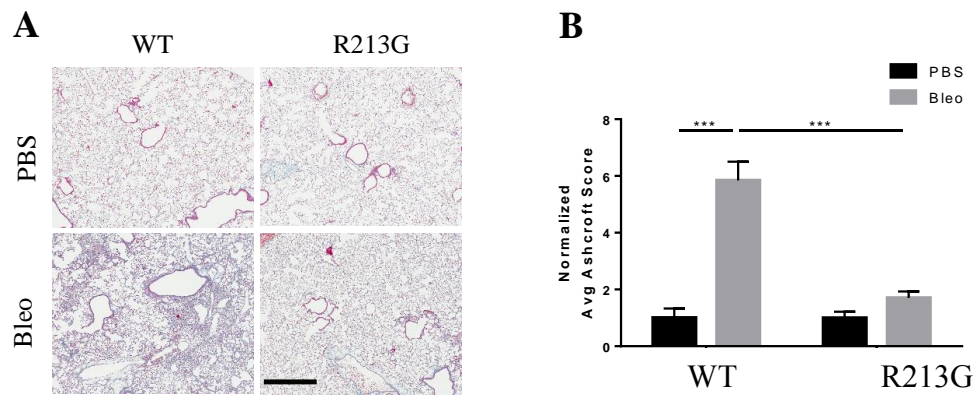
**Figure 9.** R213G SNP Alters Binding Affinity – The R213G SNP alters the binding affinity of the EC-SOD to the extracellular matrix, causing the redistribution of EC-SOD from the lung and vascular tissue to the plasma and lavage fluid.



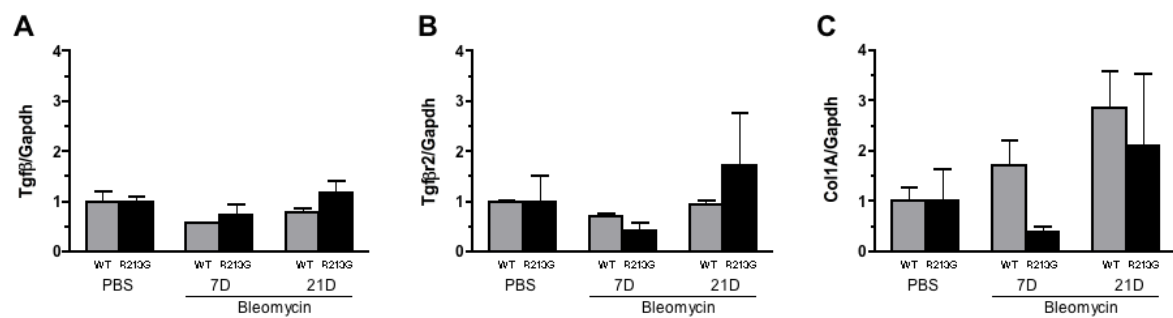
**Figure 10.** R213G SNP worsens collagen deposition following chronic hypoxia. **A**,  $Tgfb$  mRNA does not change significantly in response to hypoxia, although R213G mice tended to express less  $Tgfb$  than WT mice. **B**,  $Tgfb2$  mRNA does not change significantly in response to hypoxia. **C**, R213G mice exhibit sustained Col1A expression whereas WT mice have decreased Col1A expression. \* $P < 0.05$  WT vs R213G by 2-way ANOVA,  $n = 6$



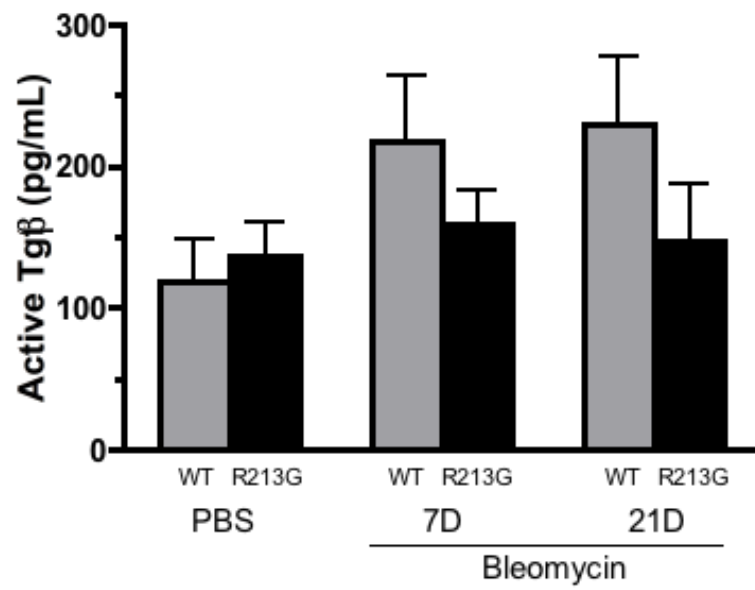
**Figure 11.** Active Tgβ decreases in response to hypoxia in R213G and WT mice. \*P<0.05 WT vs R213G by 2-way ANOVA, n = 6



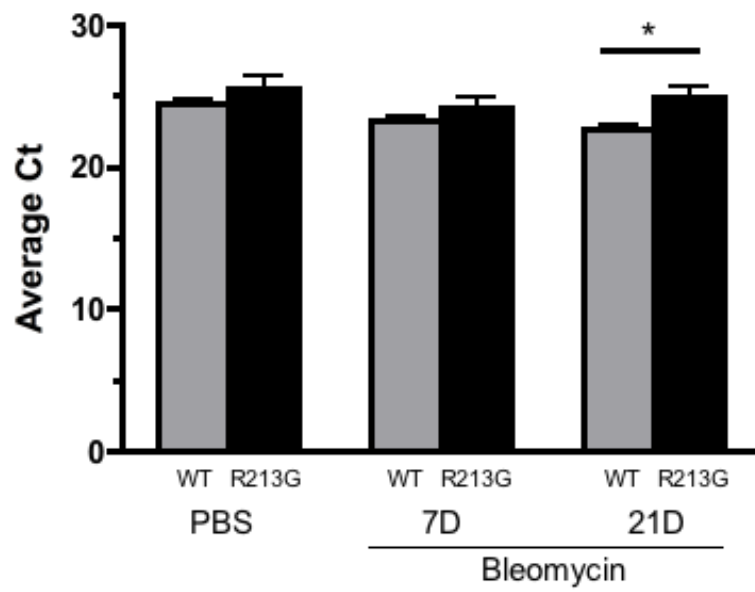
**Figure 12.** The R213G SNP mitigates the development of fibrosis 21 days after IT bleomycin treatment. **A**, Representative trichrome immunostaining 21 days after PBS and Bleomycin treatment. **B**, Average Ashcroft score for fibrosis based on blinded analysis of trichrome immunostained lung sections. \*\*\*P < 0.0001



**Figure 13.** R213G SNP attenuates collagen following treatment with bleomycin. **A-B**, There were no significant changes for Tgfb $\beta$  or Tgfb $\beta$ 2 mRNA expression in the lung. **C**, There was a trend towards increased Col1A expression in WT mice that didn't occur in the R213G mice. n = 6



**Figure 14.** Trend towards increased active Tgβ in WT but not R213G mice. n = 6



**Figure 15.** Variability in Gapdh house-keeping gene. \*P<0.05, n = 6



## **CHAPTER 4**

### **Reflections on Academic Medicine**

“Some people pick a disease and then they’ll study anything that has to do with that disease to understand the disease better,” Dr. Grayck described. “I’ve taken sort of a different approach. I’ve been interested in oxidative stress and redox signaling and this particular enzyme I think is important in fibrosis and inflammation. I’ve studied pulmonary hypertension because EC-SOD seems to be really important in that disease process.” As a result, she has developed an expertise in this enzyme that not many people study.

Grayck believes academic medicine is “absolutely essential.” She says, “It is rare for one person to make an amazing discovery all alone. More often it is a collective knowledge and sharing of ideas and resources to come to an understanding over time.” Our uniqueness drives our passions and together clinicians with diverse passions add their own pieces to the medical puzzle.

Grayck loves the clinical aspect of her role, but believes that research is what allows her to contribute more to the field. “You also get very rapid responses in the ICU,” she says, “It is a lot of positive reinforcement right away. It is an amazing place to work. The team is incredible, the nurses and respiratory therapists; everyone comes together in the unit to work so well.” However, Grayck says, “we really know so little about the body, there are so many

questions, and a lot of what we do is based on the best that we can do but it's not perfect." She appreciates being able to move from the clinic to the lab where she can ask questions that no one has asked and set up experiments that are controlled. She can learn something new and talk to people who approach problems or have a different knowledge set that may help you to better understand your problem and learn new things. "It is incredibly satisfying," she says.

Beyond research contributions, academic medicine furthers medicine by educating future leaders and clinicians. It addresses problems we have in treating patients to come up with a new understanding of diseases. This, in turn, allows us to come up with better therapies, monitor outcomes, report them, and change the health of the population. "Academic medicine crosses every discipline," Grayck says, "It has clinical, educational, and research missions within it. It is essential because if you don't have those centers, then you don't ever change what you're doing."

It is, however, very challenging to balance the triple mission. Sometimes your mind says yes to the challenge but your heart says no. Being a doctor can place an enormous burden on the heart. Going rounds with Dr. Grayck in the Pediatric Intensive Care Unit Children's Hospital was a powerful experience for me. When I saw one mom and grandma start to cry, I had to excuse myself and I also started to cry. Dr. Grayck said that especially during her training, she would

cry every day, but helping families during this very critical time in their lives is where she finds meaning in her life.

On the research side, limited funding makes competition for research dollars stressful. As the leader of your lab, you are responsible for paying the researchers who work in the lab. This places tremendous pressure on securing funding, which is never guaranteed.

So what does it take to stay in academics? Above all else, Dr. Grayck and Dr. Delaney said that academic medicine takes a passion, you have to find meaning in your work and through the combination of clinical care, research, and education. This reminds me of Father Bart, who says, “The only way to happiness is finding meaning in one’s life at the end of the day.” What makes us happy can change, but if we search for meaning, we will find happiness.

### **The Radical Mastectomy**

In concluding, I would like to give an example of the importance of academic medicine by sharing the horrific story of what happened in its absence. For most newly diagnosed breast cancer patients, the option of breast conserving surgery, known usually as a simple mastectomy, is taken for granted. But that was not always the case. Forty years ago the radical mastectomy was the standard of care. William S. Halsted, an accomplished young surgeon at Johns Hopkins, first performed the “radical mastectomy” in 1882.

Halsted believed that cancer grew in a slow, orderly way, starting in the breast and growing outward in a circular pattern. With surgery as a breast cancer patient's only option, a woman treated by Halsted (and the many surgeons who followed his method) not only had her entire breast removed, but also the surrounding tissue. Halstead believed that the more extensive the surgery, the less likely the cancer would be to return. He began to remove more and more tissue surrounding the breast including the lymph nodes, and then even including the pectoral muscle. This left women grossly disfigured with a sunken chest and the inability to move their arm outward or back.

In the *Emperor of All Maladies*, Siddhartha Mukherjee writes, "Halsted and his disciples would rather evacuate the entire contents of the body than be faced with cancer recurrences. 'Radicalism' became a psychological obsession, burrowing its way deeply into cancer surgery. Even the word *radical* was a seductive conceptual trap. Halsted had used it in the Latin sense of 'root' because his operation was meant to dig out the buried, subterranean roots of cancer. But *radical* also meant 'aggressive,' 'innovative,' and 'brazen,' and it was this meaning that left its mark on the imagination of patients." (69)

Halsted and other surgeons persisted with their mammoth operations because they genuinely believed they could relieve the dreaded symptoms of cancer. "But they lacked formal proof," Mukherjee writes, "and as they went further up the isolated chambers of their own beliefs, proof became irrelevant and trials impossible to run. The more fervently surgeons believed in the inherent

good of their operations, the more untenable it became to put these to a formal scientific trial. Radical surgery thus drew the blinds of circular logic around itself for nearly a century.” (70)

With the blinds drawn, the pendulum of cancer surgery swung desperately between terminal optimism and terminal desperation. It wasn't until 1971 that physician scientist Bernard Fisher organized the first academic and randomized controlled trial in America that compared radical mastectomy with simple mastectomy or simple mastectomy followed by radiation therapy. Published in 1974, its early results based on findings from 1,700 patients enrolled at 34 institutions, showed that the survival outcomes for patients were the same regardless of which type of treatment was performed.

This disproved Halsted's “one size fits all” model of treatment. For women whose cancer had already spread, the radical mastectomy could never be enough. And for women whose cancer hadn't spread, simple mastectomy could remove the cancer and spare the gruesome disfigurement.

It was evidence-based academic medicine that disproved the radical mastectomy, which persisted for more than a century. In medicine, and in life, there is danger in an unchecked pendulum that swings back and forth between extremes. The goal of academic medicine is to always question to continually move things forward. We must humble ourselves and know that just because something is good does not mean it is the best.

So in my title, when I describe academic medicine as the cutting edge, I do not mean the cutting edge of the surgeon's knife, but rather the cutting edge of the mind, of collaboration, passion, and of knowledge.

### **Finding the Jesuit Values in Academic Medicine**

My new classmates and I had arrived at Regis University only a few hours earlier and were surprised when Father Bart began to ask us profound questions about our hopes and dreams. He asked us for what and for whom we were living our lives. Then he asked, "What are the names of your great grandparents?" I think my classmates and I were all taken aback by the simplicity of this question. While it should have been an easy question to answer, no one raised his or her hand to do so. Not one of us sitting in the chapel knew the names of our great grandparents. Then Father Bart asked us again, "For what and for whom should we live our lives?" if not even our own family will remember who we are. Since my arrival at Regis University, I have been asked to contemplate the question, "How ought we to live?"

I believe my mission is to care for and advocate for others. I want to be a doctor who makes a valuable contribution to people's health and well-being, while making a more far-reaching impact through research. I know I will encounter people unlike myself and situations that are uncomfortable and difficult, but that is also the beauty of the human race—we are all unique. Being a

doctor is so much more than being “book smart,” there must be an intrinsic level of compassion that values our differences.

The Jesuit values can help me to more fully live out the mission of academic medicine. In my first thesis chapter Dr. Delaney describes her relationship with Aiden by saying, “You are allowed into parts of human connection with families that they don’t let anyone else into.” For me, this resonates deeply with the Jesuit value of *cura personalis* and is a meaningful example of caring for the whole person instead of just treating an illness.

Another Jesuit value, the *magis*, drives physicians to seek more in the combination of a career in clinical care with one in research. Academic clinicians become men and women in the service of others by mentoring young physicians and scientists. Even when it is incredibly challenging to balance the three-part mission of academics, physicians who seek unity of heart and mind may find purpose and happiness in their work.

At Regis we have all been blessed with a most powerful gift of receiving an education, taught in the Jesuit tradition. We must use this gift to go out and do good in the world. Father Fitzgibbons once said to me, “I want you to go out into the world and be successful and do well. But if you do not go out into the world and change it and make it a better place, then Regis has failed you.” The mark of our success at Regis is measured by how we serve others and change the world.

Father Fitzgibbons told me that as graduates of a Jesuit university, when we walk across the stage at commencement, the diploma we receive does not

actually belong to us. Certainly we have earned it, but the degree we're awarded and the skills and knowledge we've acquired truly belongs to those who we will serve with it. The education we have received belongs to the lives that we will touch, the people we will impact. No matter what our occupation, each of us is called to be active, engaged, and compassionate within our community.

As I graduate and leave Regis University, I will keep this design in my heart. I vow to take the amazing gift of my education and lead positive change in the world around me. I will strive to be mindful in my actions, gracious in my successes, present in my community, and impactful in my service. I will go forth to “set the world on fire.”



## **List of Abbreviations**

Col1A1	Collagen type 1 alpha 1
DNA	Deoxyribonucleic acid
EC-SOD	Extracellular superoxide dismutase
ECMO	Extracorporeal membrane oxygenation program
ICU	Intensive care unit
ILD	Interstitial lung disease
NIH	National Institute of Health
PCR	Polymerase chain reaction
PH	Pulmonary hypertension
PPHN	Persistent pulmonary hypertension of the newborn
R213G	Arginine to glycine amino acid substitution at position 213
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
Tgf $\beta$	Transforming growth factor beta
Tgf $\beta$ r2	Transforming growth factor beta receptor 2
WT	Wild type

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