inquiro

Volume 9 • 2015

UAB's UNDERGRADUATE RESEARCH JOURNAL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

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Founded and staffed by undergraduates at the University of Alabama at Birmingham, *Inquiro* is an annual journal produced with the mission of highlighting the contributions of undergraduates to UAB's many outstanding research programs and supporting the development of these student researchers by providing an outlet for them to publish their work. Any students who have conducted research at UAB, including those visiting from another institution, are invited to submit research manuscripts, short reports derived from scientific posters, or personal narratives regarding their research experiences. Authors retain full rights to their work, and are free to submit to other journals after publishing in *Inquiro*.

"The scientist does not study nature because it is useful; he studies it because he delights in it, and he delights in it because it is beautiful." In the present era of "big science," extreme competition for both jobs and funding in research, and ever more-obvious impacts of technology on our lives and our world, these words from the brilliant French mathematician Henri Poincaré may seem to be less true than when he wrote them over a century ago. Yet they are also more important than ever before. Whatever its practical implications, science always has been and always will be a vehicle for our inherent curiosity, desire for beauty, and yearning to know the story of ourselves and our world. These are the things that first draw us to science and that sustain us even when work is difficult and results elude us. As the challenges and opportunities associated with science become ever greater, so does our need to visit these inexhaustible wells of inspiration and perspective.

If science is partly about knowing the story of our universe and ourselves, then it also needs good storytellers. Thanks to scientific communication in its many forms, both the beauty and the practical rewards of science are now more accessible to more people than they have ever been. I am proud to say that *Inquiro* has its own small part in this global conversation, helping both to support the development of young scientists at UAB and to raise awareness and enthusiasm for science within our community. During my time as Editor of the journal, I have tried to keep these dual missions always in mind and I have had the privilege and challenge of exploring new ways to accomplish them. Many of the changes I have overseen have had to do with expanding the journal's accessibility to potential readers. For example, this issue marks a notable increase in *Inquiro***'s** online presence—on Facebook, Twitter, and our own website—as well as a wider distribution of printed copies across the UAB campus. We have also made strides to ensure that our general interest content—such as news, book and film reviews, interviews, and more—is more original, relevant, and understandable without compromising scientific accuracy. Other changes have involved making *Inquiro* more representative of the diversity that is one of UAB's great strengths. To this end, we have sought to ensure that both our editorial board membership and our technical research articles encompass a wider array of academic backgrounds than in the past.

It is with great excitement that I invite you to dive further into this issue of *Inquiro* and see for yourself what we have been up to and what we have to offer to the UAB community. Whatever your interests or level of scientific knowledge, there is something here for you. If you would like to know more about how your own learning and memory work at a molecular level, or about the tiniest of subatomic particles—hundreds of billions of which pass through your body every second without ever striking anything—or about prime numbers and how they help keep digital information secure, our editorial board has broken down these and other topics in excellent and accessible reviews. For a look at how science and technology intersect with culture, check out our article on scientific accuracy in films and television and our exploration of national and cultural differences in STEM education. You can also hear firsthand about some of the amazing work being done right here at UAB by your own classmates, professors, and colleagues: in this issue you will find interviews with Forrest Satterfield, a student who has founded his own startup to develop more affordable prosthetics, and Professor Steven Austad, whose work on aging has been both widely cited in the scientific literature and frequently discussed in popular media. Finally, we encourage you to explore this issue's collection of original research articles prepared by undergraduate scientists at UAB. There is no better way to understand a novel experiment than from the original literature, and you might find that it is easier than you think. There is much more to be discovered in this issue, and no matter what catches your interest we hope you enjoy it, learn something new, and perhaps even feel inspired to get more involved with science.

Of course, none of this content would be possible without a host of people who have helped *Inquiro* to succeed and grow throughout this year and its nearly ten years of operation. Our staff—entirely made up of undergraduates and including fifteen editorial board members, my co-Editor Maggie Collier, and Assistant Editor Josh Purvis—have worked diligently and enthusiastically on every aspect of the journal and have continually provided new ideas on how it might be improved. Undergraduate researchers have contributed the scientific articles that are *Inquiro's* reason for existing, and the journal has been made as beautiful as the science inside it thanks to the student artists who provided the cover designs and to Ryan McAnulty and Tammy Bracknell of UAB Printing Services. Numerous members of the faculty have volunteered their time to serve as expert reviewers on each research article, working with the authors to make each manuscript as strong as it could be. Faculty members like Mike Sloane, Peter O'Neil, Diane Tucker and Cristin Gavin have also offered their assistance and advice on the journal. Vital financial and administrative support has been provided by Gareth Jones, Libba Vaughan, Suzanne Austin, and Robert Palazzo. To all of these individuals, and to our readers, we offer our sincere thanks.

As my own time working on *Inquiro* draws to a close, it is easy to reflect on how satisfying the work has been. I have loved to talk about science to anyone who would listen since I was a young child, and I have also been an undergraduate researcher struggling to learn the ropes in lab, get results, and communicate them effectively. *Inquiro* has allowed me to share the beauty and joy I find in science with the community I have called home, as well as to lend a hand to other researchers just starting their careers by making opportunities available for them that are often out of reach for undergraduates. I and the rest of the staff have certainly enjoyed putting this issue together and learned a lot in the process, and I hope you have the same experience in reading it. Most of all, I hope you find in it something that makes you stop, look at the world around you, and wonder.

John Decker

Chief Editor, 2015–2016

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Predicting Schizophrenia with Computers

Hriday Bhambhvani

The lack of objective clinical tests in psychiatry, relative to other areas of medicine, has been a persistent challenge for the field. Despite considerable progress in characterizing the pathophysiology of many neuropsychiatric illnesses, markers that reliably differentiate psychiatric health from illness in individual patients remain elusive. Recently, however, the seemingly disparate field of computer science has begun to be recognized as a potential source of answers. Its increasingly sophisticated approaches to characterizing and predicting human behavior are already widely used in industry—as in computerized job screens and essay scoring, for example—but their applications to diagnosis and prognosis in psychiatry are only now beginning to be explored.

In a study published on August 26, 2015, in the journal *Schizophrenia* by researchers at Columbia University, the New York State Psychiatric Institute, and the IBM T.J. Watson Research Center, an automated speech-analysis program administered at the start of the 2.5-year study period predicted with 100% accuracy which of the 34 young participants would experience a psychotic episode during the remaining time. This program managed to outperform more traditional advanced screening technologies, such as EEG recordings and neuroimaging biomarkers (e.g. ^{1}H magnetic resonance spectroscopy to gauge glutathione).

The study began with the participants undergoing open-ended, narrative interviews in which they were encouraged to describe significant life events or lifestyle changes they had experienced and the impact of these events. Interviews were transcribed and analyzed for semantics (meaning) and syntax (structure); tone and inflection were also important variables. While a psychiatrist may intuitively pick up on signs of disorganized thought, a machine can have the advantage of quantifying these parameters in a rigorously consistent way. Following baseline interviews, participants were observed for two and a half years.

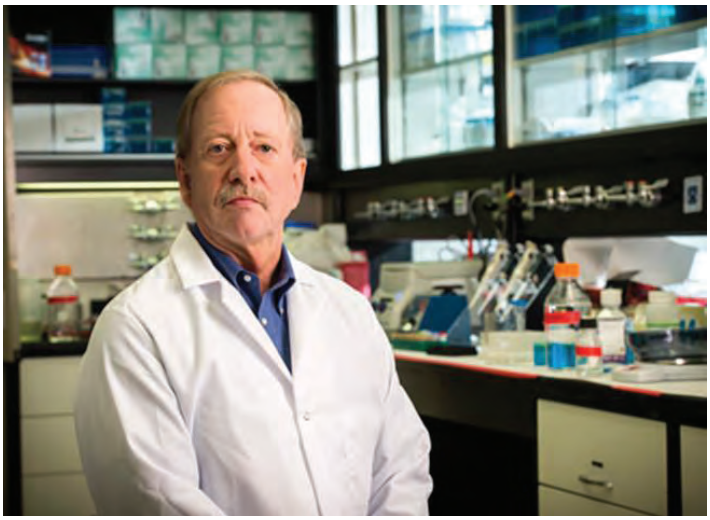
Of the initial thirty-four participants, five went on to experience a psychotic episode and twenty-nine did not. Remarkably, the computer analysis had predicted the correct outcome for each participant. The speech-analysis program, at the core of which is a complex algorithm, honed in on a few key features in the speech of the five participants who eventually developed psychosis: breaks in the flow of meaning from one sentence to the next, use of unusually short phrases, and a lack of elaboration. For example, consider the following passage spoken by a participant who later developed psychosis: "I was always into video games. I mean, I don't feel the urge to do that with this, but it would be fun. You know, so the one block thing is okay. I kind of lied though and I'm nervous about going back." These breaks in and of themselves are not highly specific to the development of psychosis, though the combination of brief syntax with semantic incoherence is a strong predictor of psychosis.

To determine whether this automated speech-analysis software is consistent and applicable to a wide variety of potential patients, further studies with a larger group of individuals are required. However, the present study does open the possibility of using these new technologies to aid clinicians in prognosis and track treatment response. Speech analyses have the significant advantages of being non-invasive, inexpensive, portable, and fast. With further development, they have the potential to become powerful tools that can complement clinical interviews and ratings. Beyond diagnosis, if speech analyses are able to identify individuals at risk for developing psychosis before symptoms start, targeted preventive measures may become possible. Ultimately, this would mean a chance to delay the onset of symptoms or reduce their severity.

"Scientists Are Wrong Most of the Time" An Interview with Dr. Steven Austad

Susmita Murthy

Every successful scientific researcher was always interested in science, right? Not Dr. Steven Austad, one of UAB's foremost experts in biological research. Dr. Austad has been involved in scientific research for over thirty-five years, yet as an undergraduate student he was not at all interested in pursuing a research career. In fact, his first bachelor's degree is not even in a laboratory-based science major, but in English Literature! So, how did an English major become not only interested in science, but an accomplished researcher? Dr. Austad described to me how his experiences training animals in Hollywood for the film industry first sparked his interest in biology and animal research: "It is hard to be around animals 10, 12, 14 hours a day watching their behavior without getting very interested; why do they do this and not that? What causes them to do this and not that?" This curiosity about animal behavior led Dr. Austad to get another bachelor's degree, this time in biology. He went on to complete his doctorate and postdoctoral training, focusing on combat behavior in animals and on group-living birds, respectively.



Dr. Steven Austad is a distinguished professor of biology and the chair of the Department of Biology at the University of Alabama at Birmingham.

Today, Dr. Austad's research has transitioned from the field into the laboratory as he has begun to explore the biology of aging, which he describes as one of the "most interesting biological puzzles." His studies start from the viewpoint that aging represents the failure of an organism to repair itself—essentially, a breakdown over time of the processes that naturally correct for accumulated damages and rebuild each cell of the body as all its components are continually recycled. In his laboratory at UAB, Dr. Austad studies several organisms as models for this process. One of these is a species of clam that lives for over five hundred years and is believed to contain a molecule that prevents its proteins from folding incorrectly. Practically, Dr. Austad explains, "Diseases like Alzheimer's and Parkinson's are caused by protein misfolding, and this is a potential clue to preventing them. We could find something in a clam that could be the treatment for Alzheimer's disease, and that's pretty exciting!" Dr. Austad also studies hydra that have the potential not to age at all—some species will age when they are stressed by environmental conditions, while others will never age. Hence, Dr. Austad's hydra research focuses on differences among the genomes and patterns of gene expression of different individual hydra, to explore the molecular basis of this conditional aging within a single species. Exotic animal research is certainly fascinating in its own right, but Dr. Austad is also confident that it can be extremely important for insights into our own health: "This research can be translated into something that is very beneficial for human health. I'm trying to figure out ways to improve human health by studying aging in animals."

Despite the fact that his research is novel and interesting even to many non-scientists or non-biologists, Dr. Austad does admit that "the day-to-day life [of a research scientist] is very tedious." So what keeps him motivated to continue pursuing a career in scientific research? Dr. Austad gives the most credit for his motivation to the subset of biology that his career is rooted in—aging research holds his interest mainly because every answer he gets leads to more questions, thereby allowing him to always discover new things. Dr. Austad holds hope that he will discover something that no other person has, which he finds to be an exhilarating prospect. He feels that this kind of potential for innovation and discovery through science should be emphasized to undergraduates and can help to get more of them involved in research. In fact, he believes that undergraduate students are inherently curious, and thus are naturally drawn to the opportunity to make a novel discovery. Unfortunately, he thinks, the majority of undergraduate students simply don't realize just how exciting scientific research can be—they only see the short-term, tedious day-to-day laboratory grind and aren't open to the long-term possibilities and rewards of a career in science. What would Dr. Austad tell these disillusioned undergraduates? "No hypothesis survives the first experiment. Scientists are wrong most of the time, but when we are right it is extremely satisfying."

EXOPLANETS: Our Second Chance?

By: NEHA UDAYAKUMAR & NATASHA CHAWLA

"If we find ourselves with a desire that nothing in this world can satisfy, the most probable explanation is that we were made for another world."

- C.S. Lewis

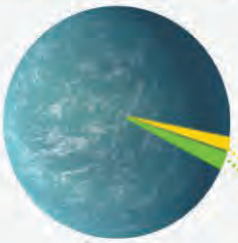
WHAT IS AN EXOPLANET?

A *planet* outside of our solar system

WHAT IS A PLANET?

According to the International Astronomical Union (IAU):

A planet is a celestial body that:
- orbits a star
- has enough gravity to settle it into a round shape
- is large enough to clear objects out of its orbital neighborhood



Since 1992, there have been nearly *2000* exoplanets discovered.*

51 Pegasi b was the first exoplanet to be discovered that orbits a main sequence star.

31 are potentially habitable

10 terran (earth size)
$0.5M_E - 5M_E$ or $0.8R_E - 1.5R_E$
(M_E = mass of the earth; R_E = radius of the earth)

21 superterran (larger than earth)
$5M_E - 10M_E$ or $1.5R_E - 2.5R_E$

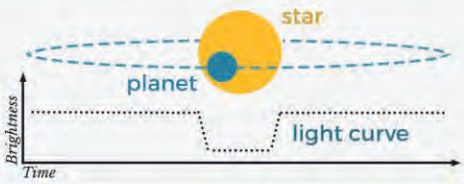
* as of October 2015

EXOPLANET DISCOVERY

TRANSIT METHOD

Over 1,000 exoplanets have been discovered by the Kepler telescope using this method.

When a planet passes in front of a star, it blocks a small portion of the star's light, which reduces its apparent brightness. An instrument detects the brightness of a star, and this brightness is used to estimate the planet's orbital period and size



EXCEPTIONAL EXOPLANETS

OLDEST EXOPLANET
"Methuselah" PSR B1620-26b

13 billion years old! Formed less than a billion years after the galaxy was made. (Planet earth is only *4.5 billion* years old.)

HOTTEST EXOPLANET
HD-149026b

Has a surface temperature of around 3,700°F or 2,000°C!

LIGHTEST EXOPLANET
TrES-4

Almost twice the size of Jupiter, but it weighs as little as a cork! (Also called the *puffy planet* due to its extremely low density)

DENSEST EXOPLANET
55 Cancri e

Only *twice* the size of Earth but 8 times more *massive* and twice as *dense*

DARKEST EXOPLANET
TrES-2b

Only reflects about 1% of light. It is *impossible* to see since it appears only as a pitch black ball of gas.

DAREDEVIL EXOPLANET
Kepler-78b

Orbits *extremely* close to its star, with only 550,000 miles in between! (Mercury orbits about 28.5 *million* miles away from the Sun).

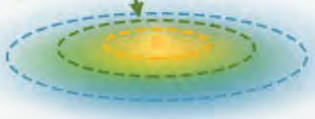
POTENTIAL HABITABILITY

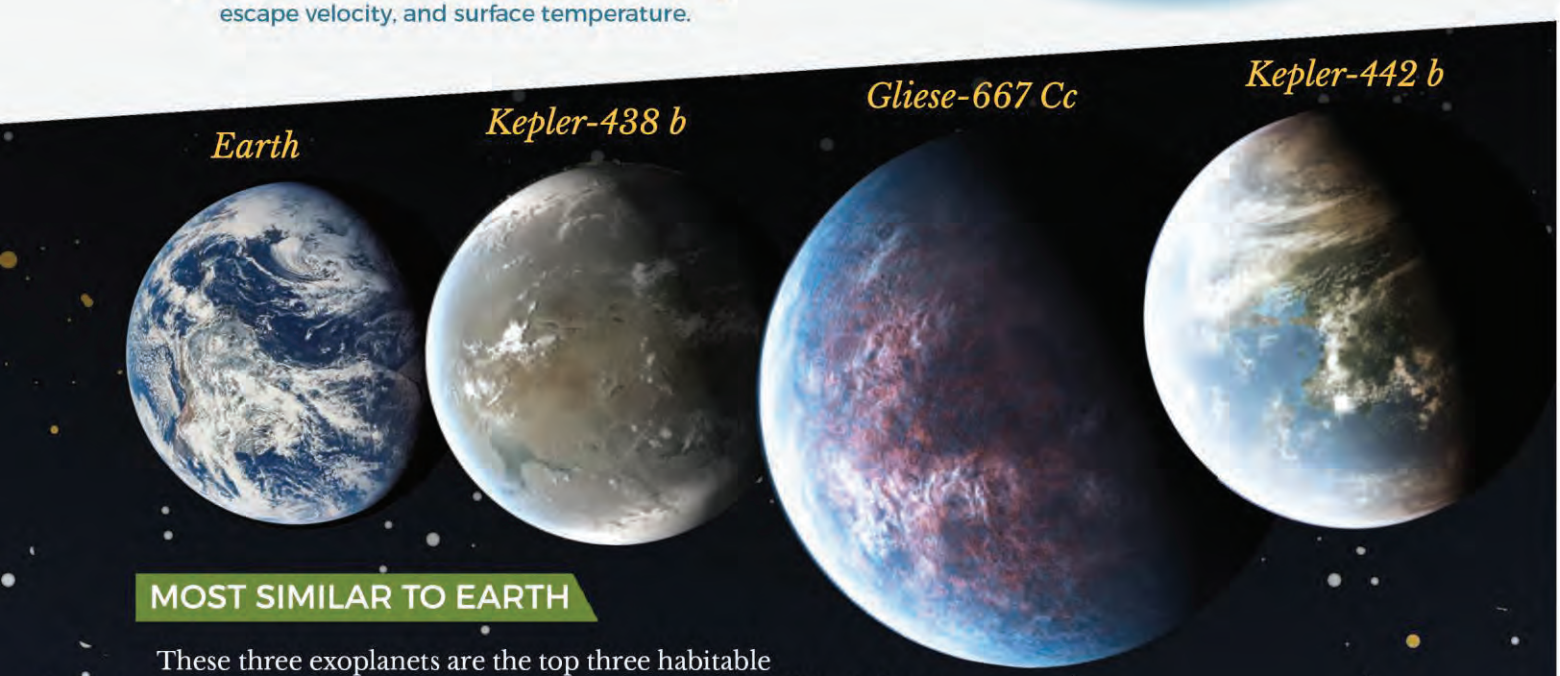
Exoplanets are ranked by how physically similar they are to Earth using the **Earth Similarity Index (ESI)**. The ESI scale goes from *zero* (no similarity to Earth) to *one* (identical to Earth).

$$ESI = \prod_{i=1}^{n} \left(1 - \left|\frac{x_i - x_{i_0}}{x_i + x_{i_0}}\right|\right)^{\frac{w_i}{n}}$$

ESI accounts for the planet's radius, bulk density, escape velocity, and surface temperature.

A vital factor considered when determining an exoplanet's habitability is its ability to support liquid water. A planet has to be a certain distance from its star to have ideal temperatures in order for liquid water to exist. This range of distance is called the **"GOLDILOCKS ZONE"** (the temperature is not too hot or too cold.)





MOST SIMILAR TO EARTH

These three exoplanets are the top three habitable exoplanets discovered so far. They are all in the Goldilocks zone.

ESI	0.88	0.84	0.84
Light Years from Earth	470	22	1200
Days in One Year	35	28	112
Radius Compared to Earth	1.12 times larger	1.8 times larger	1.34 times larger
Average Temperature (°C)	0 - 60 °C	27 °C	-40 °C
Stellar Flux (radiant energy emitted from sun per unit time)	1.38× greater	0.875× greater	0.66× greater
Star system	Single	Triple	Single
Parent Star	Red dwarf, smaller and cooler than Sun	M class dwarf	K-type orange dwarf

TRANSPORT METHODS

THE SOLAR SAIL

The solar sail spacecraft operates by harnessing the sun's energy for propulsion. Photons traveling as a packet of light have energy and momentum, so when they hit the solar sail, their momentum is transferred to the sail. This continuous acceleration allows a spacecraft to reach very high speeds over time!

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The Unexpected Connection Between Internet Security and the Riemann Hypothesis

David Chasteen-Boyd

Introduction to prime numbers

Think back to elementary school during which you learned about a seemingly useless mathematical relic called prime numbers. Your teacher told you in class one day that they are special numbers, divisible only by themselves and one. You also learned about prime factorization, or factor trees, in which you kept dividing a number until it could be divided no further. Then you were given a worksheet, which you scrambled to finish so you could go to recess, and promptly forgot about prime numbers until it was time to take that standardized test at the end of the school year.

As it turns out, prime numbers are incredibly important in math and even in fields as far-reaching as quantum physics and Internet security. They are often referred to as the building blocks of all numbers. Composite numbers, integers that are not prime, are made up of prime numbers combined together in some way. One way of forming composite numbers is by reversing the process of prime factorization: instead of dividing a composite number into its prime number components, you can multiply prime numbers together to form a composite number. Prime numbers have been studied for thousands of years. The Greek mathematician Euclid showed that every number is either prime or the product of prime numbers and proved that there are infinitely many prime numbers. Other theories of prime numbers include the twin primes conjecture, which states that there are infinitely many "pairs" of prime numbers that are only two units away from each other (the larger prime minus the smaller prime equals 2, such as with 41 and 43). However, despite thousands of years of study and great interest, there are still many unanswered questions in analytic number theory, which is the study of the properties of integers and prime numbers. One of the most significant of these questions is the Riemann hypothesis.

At its core, the Riemann hypothesis seeks to find and study the pattern and distribution of prime numbers as they get larger and larger. Consider the pattern in the first four prime numbers: 2, 3, 5, and 7. At first glance, it seems like all of the odd numbers less than 10 are prime, but then comes 9. Still, it seems like most odd numbers are prime. The next few are 11, 13, 17, 19, and 23. That set initially increases by increments of 2 or 4 but then suddenly jumps up by 6. The next set of primes is 29, 31, 37, 41, 43, and 47. The spacing between them seems to be increasing, until you reach the primes 71 and 73. So, what exactly is the pattern? Are the primes getting farther apart or closer together with increasing magnitude? Or is the average distance between them staying the same?

The Prime Number Theorem, which Riemann first tried to prove when he was proposing his hypothesis, states that the average distance between the primes in the first N integers after 0 can be approximated using the following formula

$$D = \ln(N)$$

where ln is the natural logarithm (the inverse of e^x). However, it is important to remember that this average distance is an approximate value: the natural log function only approximates the location of primes over a large interval, not in small regions. The natural log function is analogous to a forest; even though you may know where the forest is, it is still hard to find the specific tree that you are looking for. Furthermore, the actual distance between two consecutive prime numbers is difficult or impossible to predict. Since the Prime Number Theorem was proven, many increasingly accurate approximations for the distribution of prime numbers have been discovered[1]. However, no function yet exists that reveals exactly where each prime is located on the number line.

What is the Riemann hypothesis?

The Riemann hypothesis itself states that the zeros of a particular function, known as the Riemann zeta function, all lie along a specific line in what is known as the complex plane. The zeros of this function act the same as the roots of a quadratic equation – plugging them into the function as its values causes the function to equal zero. However, determining these values is difficult due to the fact that the Riemann zeta function is a complex-valued function. In this case, complex means that it contains complex numbers, numbers which have both a real and imaginary component and have the form $a + bi$ where i is the square root of negative one. Because of this property, the function's domain is plotted on the complex plane, in which the horizontal axis represents real numbers and the vertical axis represents imaginary numbers. Because the zeta function is a complex-valued function, its roots must also be plotted in the complex plane.

There are two types of roots of the Riemann zeta function. The first type is called the trivial zeros, which are all of the negative even integers. The second type, the nontrivial zeros, which are more complicated than the trivial zeros, is the type that the Riemann hypothesis seeks to define. Riemann showed in his 1859 paper[2] that all of the nontrivial solutions to the Riemann zeta function lie in a region of the complex plane known as the critical strip – the region between 0 and 1 on the real axis that extends infinitely upwards in the imaginary direction (Figure 1). Riemann proposed that all of the nontrivial zeros of the function might lie on an even more specific region of the complex plane: the vertical line whose values all have a real part equal to ½; this statement is the Riemann hypothesis. Interestingly, this vertical line is located in the center of the critical strip.

The form of the zeros to the Riemann zeta function implies much about the distribution of the prime numbers along the number line. So if the Riemann hypothesis is proven correct in that all of the solutions to the Riemann zeta function do have the form ½ + bi, we will gain insight into the locations of the prime numbers and how much they deviate from the functions that the Prime Number Theorem presents.

Why should non-mathematicians care?

Though few people other than mathematicians may find it interesting to discuss the location and distribution of the primes, the Riemann hypothesis is relevant to other fields as well. Recent research has suggested that the locations of the zeros of the zeta function might have implications in quantum physics. A relatively recent field of research in quantum physics, quantum chaos, studies quantum systems that behave like classical chaotic systems (like a double pendulum or a billiard ball on a nonrectangular table). The defining equations of quantum chaotic systems belong to a class of equations known as trace equations.

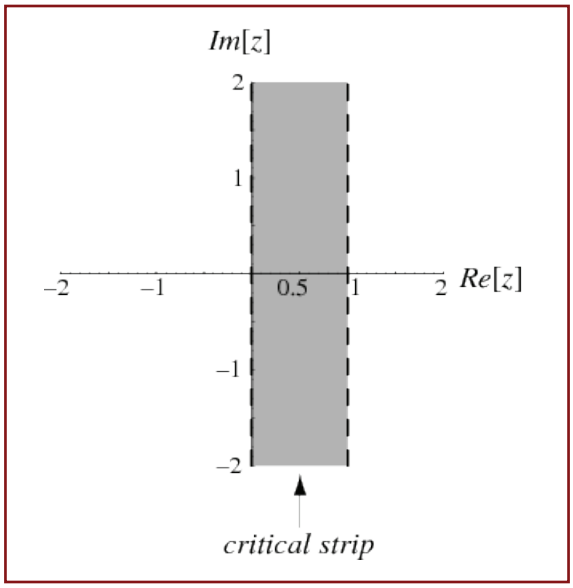

Figure 1 | The complex plane, showing the critical strip[3].

As it turns out, the Riemann zeta function can also be written as a trace equation. It is therefore possible to create a quantum chaotic system whose behavior is defined by the Riemann zeta function. Mathematicians and physicists are hopeful that studying this system could lead to a better understanding of the zeta function, and vice versa. However, this application pales in comparison to the effect that the Riemann hypothesis could have on a field of global importance: Internet security and encryption.

Creating a factor tree involves a lot of tedious labor: going one by one through the number line to find a factor of the number, and repeating this process for each factor until only prime numbers are left. Imagine trying to factor a number that is near 10^{600}, instead of one that is only in the tens or hundreds. For comparison, the total number of atoms in the universe is approximately 10^{80} [4]. This number would take a lifetime, if not longer, to factor, and computers are not much more efficient than humans at large number factorization. Modern encryption methods take advantage of this weakness.

The basic mechanism through which modern encryption methods work hinges on the fact that computers take a long time to factor large numbers. One type of encryption, developed by RSA Security, LLC, uses public key encryption[5]. There are two main components to this type of encryption: the encryption key, which is made public, and the decryption key, which is kept secret. The decryption key is composed of two large prime numbers that are near 10^{300} digits long. The encryption key is the product of those two numbers, which is near 10^{600} digits long. The encryption key allows those who would like to send messages to encrypt them so that they cannot be read or manipulated by outside parties. The only parties that can decrypt the coded message and read it properly are those that have access to the decryption key. Because of the time required to factor such a large number,

and because of other mathematical manipulations performed on the keys in order to obfuscate the original numbers, it is almost impossible to obtain the decryption key unless it is given to you.

The ethics of Riemann hypothesis research

Because of the implications that the Riemann hypothesis could have on our understanding of the distribution of prime numbers, especially large prime numbers, it is possible that a proof for the Riemann hypothesis could lead to quicker and easier methods of finding large primes. This could in turn lead to the breakdown of modern encryption methods because choosing numbers to use as potential factors of the encryption key would be more efficient. Basically, if we already know where the primes are, we only have to pick those as factors instead of picking every number. Many processes that are carried out online and intended to be secure, such as banking transactions and military and government communications, could potentially no longer be sufficiently protected. Despite this security threat, a solution to the Riemann hypothesis could also provide beneficial applications. Its potential to improve our understanding of quantum physics could lead to improved and innovative technologies. Furthermore, recent research has shown that the zeta function has a strong connection to quantum mechanics, and many mathematicians are optimistic that a novel approach combining physics and math could be the motivation that researchers in this field need to finally find a solution.

The potential negative consequences of finding a solution to the Riemann hypothesis create an ethical dilemma for researchers to consider. Mathematicians and scientists dedicate their lives to the pursuit of knowledge, often just for knowledge's sake. However, is it morally right to pursue something that could have such a devastating global impact? Is there a certain point at which the potential benefits of new knowledge are outweighed by the damage it could cause? It seems that a proof for the Riemann hypothesis is still, at the very least, a few years away; therefore, a global internet security issue should not exist anytime in the near future. Perhaps this potential for harm also presents a new impetus for computer scientists to develop new, more secure encryption algorithms that do not depend on prime numbers, thereby encouraging further innovation and progress.

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Scientists Worldwide: An International Perspective on an American Research Lab Setting

Jessica Maya

Science is an international subject: it transcends languages and cultures alike. In the United States alone, groundbreaking research is being done in every scientific field, from cancer to aging to biomedical engineering. Many young scientists come from around the world to perform research under the guidance of mentors in America. A recent study shows that foreign students account for the majority of enrollments in U.S. graduate programs in many of the STEM fields[1]. At UAB alone, there are more than 665 international students in both the undergraduate and graduate programs[2]. Because of this, it is imperative to hear from these voices to see where our strengths are as a leading nation in the STEM fields, as well as to acknowledge our weaknesses. Three international graduate students were interviewed about their experiences at UAB and in their home countries.

One M.D. from Hungary, Andras Rab, has been working in research labs since his days in medical school back home. For several years, he gained experience working in translational research by analyzing patient samples for diagnostic purposes. Once he began his residency in Hungary, he had the opportunity to work in a research lab in the Department of Cell Biology at UAB. When asked about the different mentors he has had across the board, he says his experience depended more on each mentors' personality rather than what country they were from. He says, "I believe the mentor should be your teacher in research and also in life." When asked why he came to America to perform research, he explains that funding and grant support were very limited in Hungary, which significantly decreased the effectiveness of his research. His biggest challenge coming to America was the language barrier, forcing him to learn both the everyday language as well as scientific jargon.

Another student here at UAB has worked in many labs, including academics and industry, in both India and the U.S.A. He likes what he has experienced so far at UAB and says, "You get the freedom to think like an independent researcher and work on your hypothesis." He thinks the mentors here provide more freedom of thought compared to other mentors and has observed a high level of work ethic at UAB and in America in general. When asked what made him want to perform research in America, he responds with his interest in the cutting edge technologies and his zeal to find something new. While he says that it is always a challenge for a foreigner to blend in with the current settings in the lab, he thinks he is fortunate to have exceptional colleagues who have supported him throughout his experience.

Anukul Shenoy, another Ph.D. student from India who received his masters at the University of Mumbai, has done research in the clinical and experimental field for the past few years. He is interested in immunology and microbiology and is now doing his doctoral project on pneumococcus pathogenesis and the host-pathogen interaction associated with pneumococcal infections. He contrasts the different academic environments of India and America: "In my opinion, [America is] such a dynamic environment [that] allows for broadening of horizons and understanding of different topics out of the field of syllabus which otherwise wouldn't be possible with the rigid atmosphere [in India]." To further explain this point, he suggests that his relationships with mentors in India were associated with a level of formalism that limited his experience as a mentee. Mentors could only be contacted by message or calls during office hours. Here, he says he has been very fortunate with his mentor, who makes it a point to extend his mentorship beyond the scope of the lab and research work. "Discussions, criticisms, praise and complaints are welcome equally," he says. "I would like to take this opportunity to thank Dr. Carlos J. Orihuela for his help, patience, and belief in my abilities and to make me a better person capable of making sound decisions." Coming to America was a decision he made due to the limited available options and lack of leading labs working in bacterial pathogenesis. His adjustment to UAB and the U.S. in general was not so difficult, he says, because he loved collaborating and making friends. He still finds it hard to address his elders and teachers less formally or by their first names, but he is slowly adopting the practice and is adjusting well to his new environment and peers.

These three scientists have made and will continue to make discoveries in their respective fields. Understanding both the good and the bad that we as a country, and as a university, have to offer to make our community great is crucial. It is because of them and their colleagues that we at UAB can say that our STEM field is ever growing.

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A New Theory of Consciousness

Josh Purvis

The debate over the source of human consciousness has persisted for thousands of years, dating back to Plato and Aristotle. While we have made huge advancements in neuroscience in recent years, our own subjective experience of the world remains one of the biggest mysteries in the universe. Today's theories don't resemble Plato's or Rene Descartes' conceptions of the mind and soul as entities separate from the physical body, because it has become clear that consciousness depends on the brain. Still, it remains difficult to say what exactly constitutes consciousness, and we are far from understanding the neural mechanisms by which it is produced. One pitfall has been the search, ultimately unsuccessful despite the considerable efforts of many scientists, for a single "consciousness center" in the brain. The failure to find such a locus has led many contemporary scientists to maintain that consciousness is an integrated process involving many, if not all, areas of the brain. One such proposal is called the Integrated Information Theory (IIT), which was advanced by Tononi and Edelman[1] as a "theoretical framework for understanding consciousness." The objective of this framework is to precisely define consciousness, then to characterize it using mathematics and use this information to account for current knowledge of the brain[2].

Tononi argues that consciousness has two basic properties: it is informative, and it is integrated[3]. Barrs[4] attributed the same properties to consciousness. Information is defined as the ability to reduce uncertainty among a number of possible alternatives[5]. For example, when you are in a pitch black room, your brain processes all the available visual information and can determine that the room is dark by eliminating all other possibilities. It is clear that the more possible states a system possesses, the more informative it can be. Second, consciousness is integrated. This means that the informative system with many possible states is unified—that is, it is not divisible into independent components. In other words, a conscious system according to IIT cannot have any self-sufficient elements. Similarly, it is not enough for a system to have either a large number of possible states or a large amount of integration. A highly conscious system will have both[5].

Integrated Information Theory initially became popular because it offered a quantitative value (Φ, or phi) that purportedly represented a system's capacity for consciousness that could theoretically be applied to any sufficiently-known information-processing system. For this reason, IIT is sometimes known as "the mathematical theory of consciousness." While the math behind Φ's calculation is quite dense, the underlying concept of what Φ represents can be broken down. Basically, Φ is the amount of integrated information (information that is indivisible into smaller components) that a system possesses. Φ is measured by splitting the entity being evaluated for consciousness into parts A and B, then perturbing A in all possible ways to see how many states it produces in B, and vice versa. A system that has a high value of Φ is highly conscious according to the theory, but to obtain a large Φ requires that the system be highly interconnected[2]. All elements should be connected to a different subset of elements, and each element should be able to interact with other elements. If a system satisfies these requirements, then congratulations: it has a high Φ value and could be at least minimally conscious! Because IIT is concerned with consciousness as an abstract state of an arbitrary information-processing system, your smartphone, the internet, and even some cars could be at least minimally conscious according to IIT.

Of course, a successful theory must both explain the known facts and make accurate predictions. Thus, the final step in Tononi's theory is to apply the fundamental properties of consciousness—information and integration—to what is known about the brain. One of the questions Tononi confronts is why lesions of certain brain areas affect consciousness while lesions in other areas have no apparent effect. For example, lesions to the thalamocortical system can severely disrupt consciousness, yet lesions in the cerebellum rarely have the same effect. Given that the cerebellum contains more neurons than the cerebral cortex, one might wonder why it would seem not to play a large role in consciousness. Tononi argues that it's because the cerebellum can be easily separated into nearly independent modules, which would drastically lower the Φ value. The thalamocortical system, however, is not easily subdivided and, as such, would have a higher Φ value[5]. In practice, however, no one has yet been able to determine a Φ value for a brain region—only for model systems. Advances in the field of connectomics will hopefully lead to calculation of Φ values for real brain regions.

Another limitation of IIT is that it does not address what David Chalmers calls the "hard problem of consciousness"[6]. IIT is potentially useful for its ability to offer an objective, mathematical model of consciousness in terms of information processing, but it cannot tell us why the activity within our brains "feels" like anything—why we have internal experience at all, and why it is the way it is. The hard problem of consciousness is thought by some to be a fundamentally-insurmountable barrier, and so far no theory of consciousness has satisfied the hard problem while also accounting for known facts and making accurate predictions. Whether IIT, or any other scientific theory, can ever account for the hard problem of consciousness remains to be seen. Perhaps this

fundamental part of our own experience will always remain mysterious to us.

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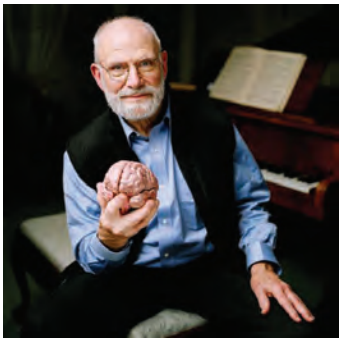
A Review of *An Anthropologist on Mars* by Oliver Sacks

Amy Stewart

An important and challenging aspect of being a scientist is communicating information in a way that is both accurate and engaging, and furthermore disseminating the work to a widespread audience. Oliver Sacks is one of the few scientists who has accomplished this with his chosen subject matter, the brain: he skyrocketed to fame in 1973 with his book *Awakenings* and has remained a household name with his myriad of publications and various film adaptations. Framing a discussion of the scientific backgrounds of neurological conditions within the context of case studies, Sacks ingeniously weaves together humanity and science in his novels, culminating in a thoughtful and compelling read. Written at the midpoint of his authorial career, *An Anthropologist on Mars* marks a maturation and expansion of the overarching themes in his narratives that he began to explore ten years prior in his most well-known book, *The Man Who Mistook His Wife for a Hat. An Anthropologist on Mars* details the experiences of seven individuals with neurological disorders ranging from cerebral achromatopsia to Tourette's syndrome to autism, supplementing descriptions of these disorders, fascinating in their own right, with stories of the manifestation of creativity borne out of these conditions.



Left: Oliver Sacks, author of An Anthropologist on Mars[2]. *Right: Six Oliver Sacks books arranged in a collage, including* An Anthropologist on Mars[3].

It is clear from the first chapter that *An Anthropologist on Mars* will not read like a dry, erudite textbook; nor will it be exhibitionist, exploiting its subjects as oddities to be gawked at by onlookers. The novel opens with the story of an elderly man, Mr. I., who has been rendered completely devoid of color vision after a car accident and is seeking Sacks's help. His sudden loss of color perception, devastating under any circumstance, is made all the more poignant because Mr. I. is a painter. Sacks goes on to recount Mr. I.'s symptoms anecdotally, much more effective than any mere list, and then the methods he used to confer the diagnosis of cerebral achromatopsia. A large portion of the chapter characterizes Mr. I.'s struggle to find pleasure in his daily life and to reconcile his artistic expression in his new sickening and disorienting black-and-white world. Intertwined cleverly within these anecdotes are introductions to the neurological basis of color vision, the history of cerebral achromatopsia, and landmark experiments in the field. The rest of the book follows this same general format, with each chapter a self-contained narrative. Thus, the reader is able to learn about a surgeon with Tourette's who can perform hours-long surgeries, an artist with an eidetic memory obsessed with painting his childhood town in Italy, a prodigious autistic boy able to draw incredibly detailed sketches of buildings and landscapes from memory, and an autistic professor with a Ph.D. in animal science who feels a connection with animals that she lacks with humans.

Though each story can stand independently, there is an interesting progression from the first to the last chapter. The first few chapters involve individuals whose conditions have been thrust upon them, like by a car accident or a tumor. They have learned not only to adapt to their condition, but to thrive. In fact, the colorless world that Mr. I. first considered so hideous eventually came to be viewed as pure and uncluttered with irrelevant color; he rejects any suggestions of regaining his color vision. His art gains a complexity and depth mirroring his own transformation. This sense of unity

between the individual and the disorder is echoed and amplified by the other characters in the book whose conditions are not caused by external factors. Dr. Bennett, the surgeon with Tourette's, views his disorder as an integral part of his personality. Temple Grandin, the autistic professor said in one of her lectures, "If I could snap my fingers and be nonautistic, I would not – because then I wouldn't be me. Autism is part of who I am." Sacks's writing conveys a deep respect and even admiration for his subjects and their extraordinary abilities. It also provides an immersive experience replete with the struggles and hardships juxtaposed against the resultant beauty and creativity accompanying and sometimes counterintuitive to the various neurological perturbations experienced. In fact, the only flaw in Sacks's writing is that he at times tends to be too compassionate and empathetic. In the case of the autistic boy, Stephen, who sketched buildings like the Notre Dame, Chrysler Building, and St. Basil's from memory with painstaking intricacy, he imbues Stephen and his art with emotions and motives that perhaps are just not there, or at the very least, impossible to quantify.

Still, Oliver Sacks's works continue to be relevant today precisely because of his inclusion of the human element in science. *An Anthropologist on Mars* will not become antiquated because it relies on background information and underlying questions central to each disorder that will hopefully spur the reader to seek more recent research if interested. There has been a resurgence of science in popular culture that has expanded beyond the written word: there are shows like *Through the Wormhole* with Morgan Freeman, movies like *Interstellar*, podcasts like *StarTalk* by Neil deGrasse Tyson, and an abundance of TED Talks on a wide variety of subjects. Though the medium through which popular science is primarily spread has changed over the decades, it is clear that hard science fields like physics, astronomy, and chemistry, in addition to biology and psychology, thrive in the general public's eye when given the Oliver Sacks treatment and integrated into a human story, for it is human stories that remain timeless even as their scientific underpinnings are constantly shifting and evolving.

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Obsessiv-Compulsive Disorder: A Distorted Reality

Charles Keith

Stressed. Constantly worrying. Fidgeting. Obsessing. These are just a few of the many constant symptoms affecting millions of people around the world. In the United States, approximately 40 million adults (aged 18 or older) are affected by some sort of anxiety disorder[1]. This equates to approximately 18% of the American population.

Anxiety disorders develop from a myriad of risk factors. Your genes and your brain chemistry, as well as any traumatic events that have occurred in your life, play a large role in the development of anxiety disorders. One anxiety disorder in particular is known as obsessive-compulsive disorder (OCD). Characterized by a persistent need to check things, perform routines, or rituals, or by repetitive (often negative) thoughts, OCD can certainly disrupt a person's life[2]. For those with OCD, every day is a constant struggle of dealing with intrusive and compulsive thoughts. Although the symptoms seem rare, OCD is a rather common condition. Approximately 2.5% of the world's population has the disorder[3]. This is such a large portion of the population that you probably already know someone who suffers from OCD.

People who have OCD often have compulsive responses to intruding thoughts. Those who have OCD can show a variety of symptoms and can obsess over an array of items, topics, and ideals. Some people might have a repetitive hand washing ritual that they must perform due to a constant worry of having dirty hands. Others might exhibit symptoms of OCD by exceedingly taking on the responsibilities of those close to them. They may project their fear by constantly concerning themselves for the well-being of those who they care most about[3].

The causes of OCD can vary. Many studies suggest that OCD is an onset of genetic disorders and it is likely that OCD could be a result of epigenetic alterations. Research says that OCD is mainly a problem in the communication of signals in the brain. Furthermore, the neurotransmitter, serotonin, may play a large role in this miscommunication. In a study conducted by Duke University, it was found that having larger amounts of methylated serotonin transporter DNA resulted in greater reactivity of the amygdala[4]. The amygdala is a part of the brain that plays a large role in decision-making as well as emotional reactions and memory. The study suggests that "increased promoter methylation of the serotonin transporter gene" is a good predictor for amygdala reactivity and decreased mRNA expression[4]. This indication is important because the serotonin

transporter plays a large factor in anxiety disorders, often being blocked pharmacologically to treat such disorders[4]. Also, it is important to note that any DNA methylation that occurs as a result of stress or some environmental factor could lead to the onset of OCD. Therefore, learning more about epigenetic alterations of DNA through DNA methylation is crucial in understanding OCD and anxiety disorders alike.

Unfortunately, OCD can lead to a sense of shame for those that are affected. As with some disorders not well understood by the public, OCD carries a plethora of social stigmas. Those who have OCD often do not seek treatment and attempt to hide their symptoms. However, this leads to the possibility of worsening symptoms. Ociskova *et al*. investigated the effect of stigmatization on the severity of an individual's disorders. They found that mental illness stigma tended to increase the anxiety of patients with OCD[5]. They also discovered that an increased presence of OCD was associated with an increased probability of social isolation from family members and others.

Treatments for OCD vary. One modern treatment consists of serotonergic antidepressants, which are composed of clomipramine and selective serotonin reuptake inhibitors. Current research shows that intravenous clomipramine has proven more effective than its oral counterpart[6]. Other studies have demonstrated the effectiveness of cognitive-behavioral therapy (CBT) as a means of treatment. CBT proved to be more effective in instances in which the administration of serotonin reuptake inhibitors was not an effective means of treatment.

OCD is a very critical and crippling disorder that affects 3.3 million Americans. Further studies are crucial to understanding OCD and other anxiety disorders[7]. Although treatment is available, the stigma of having an anxiety disorder can worsen matters for an individual with OCD, or even cause them to not seek treatment. Understanding OCD as a real disorder and helping the millions of individuals suffering from it can hopefully eradicate the social stigma surrounding the disease. While you may not be able to see the direct results of OCD displayed by affected individuals, this doesn't mean that they are not suffering.

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Science Meets Business: Turning an Idea into Reality

Emily Haley

Earlier this year, I had the pleasure of meeting Forrest Satterfield, a junior biomedical engineering major here at UAB who has a passion for innovation and problem solving. During his time at UAB, Forrest has established himself as an entrepreneur by founding and leading Satterfield Technologies, the first startup company of the UAB Collat School of Business's Innovation Lab.

Although Forrest wanted to be an entrepreneur from a young age, the idea for his company emerged during his freshman year at UAB. The original concept for the product consisted of a series of three actuators, or small motors with built-in computers, that would power prosthetics and orthotic braces. However, Forrest's company and target product has evolved in tandem; the current model involves using a laser scanner and 3D printer to produce custom prosthetics and orthotics with modular sensors and interchangeable actuators. These components will be combined in a mix-and-match fashion based on the patients' needs and budgets, a model that will prioritize affordability without sacrificing quality.

As an engineering student and entrepreneur, Forrest has a unique perspective on the design process and its integration in business. When asked about how the scientific process has contributed to his work, Forrest explains that the scientific method has been mainly involved in the business aspects of his project, like testing a certain product component or business model. He will predict a particular outcome, rigorously test the product or idea, evaluate the results and proceed with building, or redesign and retest if needed. For the engineering aspects, most of the research involved has been data sourcing to learn all available information that

could aid in developing ideas for the new design.

Stressing the importance of multidisciplinary study, Forrest readily acknowledges that his knowledge of business has been invaluable to the development and success of his engineering project. Throughout this endeavor, Forrest has discussed his project with UAB faculty members, as well as local businesses, businessmen, and engineers; he has been able to use their different viewpoints as a system of checks and balances to manage the strengths and weaknesses of his project. Engineers, he says, tend to focus on the idea and exploring the full possibilities of the technology, while the business viewpoint reminds him that one of the most important goals is to produce a product that meets the wants and needs of the consumer. Forrest points out that while it has always been important for researchers to have a rudimentary understanding of the business perspective, it is becoming even more advantageous to be able to communicate in the business vernacular and present statistical evidence so that the product will be accepted by the targeted consumers. He credits the business certificate program at the Mervyn Sterne Library for providing him with these skills and resources, and he strongly recommends the course to other researchers who are considering distributing their products to the public.

In addition to his work as an entrepreneur, Forrest serves as a University Innovation Fellow, a title that comes with the responsibility to "increase student innovation and entrepreneurship." To this end, Forrest has spearheaded the development of the UAB Makerspace, which is designed to provide important tools to students participating in innovative design projects by "catalyzing cross-disciplinary collaboration, facilitating connecting students with university resources and faculty, and enabling students to quickly prototype and test their ideas." The Makerspace, located in Sterne Library, held its Grand Opening on February 22, 2016, and has five 3D printers and four HP sprouts, as well as circuit scribe kits, laptops, and more. Students can use these tools for virtual reality, software design, circuit building and design, as well as 3D printing anything from flower pots and figurines, to anatomical models and senior engineering design projects. Forrest is currently open to collaboration with interested students and other members of the community and would be happy to provide more information about his experiences and current project by email at **fvsatt7@uab.edu**.

Forrest also acknowledges the great difficulty and importance that is associated with identifying mentors to provide advice in entrepreneurial endeavors. In fact, the hardest part of Forrest's experience was finding mentors to help guide him throughout his project. To find mentors, he researched professors who were involved with or interested in work similar to his start-up. After identifying these professors, he persistently, but respectfully, contacted them by email. In doing so, he learned to be genuine in explaining his goals while remaining open to criticisms and changing his ideas to get mentors to commit to his project. In this spirit, he says, "I love to help people achieve their potential...helping people to reach their goals appeals to me."

Forrest concludes with a word of advice to our readers: "There is no such thing as success the first time. It is probability, not luck, that puts a person in the right place at the right time. So reach out to everyone and remember that it is better to receive no response than to have lost the opportunity to try."

Forrest Satterfield (center) with his fellow student workers in the UAB Makerspace.

The Neutrino: A Particle Ahead of Its Time

Emily Jennings

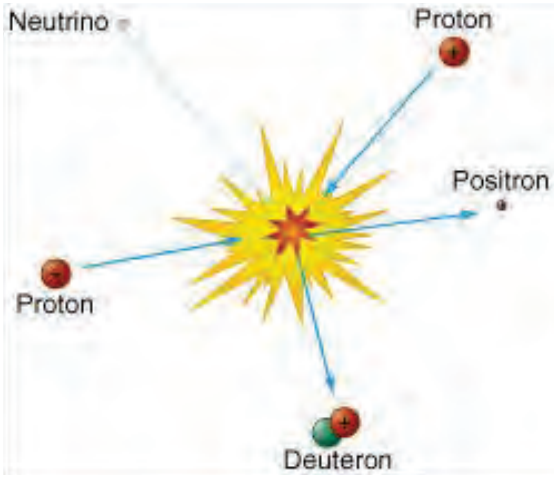
Throughout school and in any physics class, students are told that nothing travels faster than the speed of light. However, that statement might no longer be true because of the neutrino—the particle that may be able to transcend dimensions that light cannot. The concept of the neutrino is significant to our understanding of the universe because the neutrino can be used to determine how fast the universe is expanding, as well as its ultimate destiny[1].

Everyone has heard of electrons, protons, and neutrons, but what exactly is a neutrino? A neutrino is an exponentially small particle with no electrical charge. To put the remarkably small size of a neutrino into perspective, consider that neutrinos are thought to be a million times smaller than electrons, which have a mass of 9.11×10^{-31} kilograms[2]. Neutrinos are likely the most abundant particles in the universe and may be more common than photons, the basic unit of light. Because neutrinos are so common, their mass, which remains unknown, is thought to have an effect on the gravity of the universe[1]. Neutrinos can pass through almost anything, and they do so constantly. In fact, about 400 billion neutrinos from the sun alone pass through each person on Earth each second. According to physicist Frank Close, "One neutrino can fly through a light year of lead without hitting anything"[1]. Physicists also suspect that neutrinos can move through dimensions forbidden to light[2].

If neutrinos are so small and pass through almost everything known to us, as well as through potential dimensions unknown to us, how do we know that neutrinos exist? Where do neutrinos come from? These questions are best answered through a consideration of the history of the neutrino's discovery. In 1930, Wolfgang Pauli (the father of the Pauli Exclusion Principle) proposed the existence of the neutrino to explain the conservation of energy in beta radioactive decay. Beta radioactive decay occurs when protons change into neutrons, such as when protons fuse near the center of the sun. In 1930, Pauli speculated that when neutrons form from the fusion of protons, the extra energy is carried away by light-weight, electrically neutral particles. At the time, Pauli did not believe what he had proposed was true; he said, "I have done a terrible thing, I have postulated a particle that cannot be detected"[3].

In 1933, an Italian physicist named Enrico Fermi named Pauli's mystery particle the "neutrino" and created a quantitative theory for weak particle interactions involving the neutrino. About 20 years later, in 1956, two American physicists, Frederick Reines and Clyde Cowan, reported that they had detected neutrinos using a fission reactor as a neutrino source and a well-shielded scintillator detector[4]. Neutrinos had finally been detected, but much remains to be discovered about these tiny particles. The next piece to the long-standing neutrino puzzle came from Takaaki Kajita and Arthur McDonald, who were awarded last year's Nobel Prize in Physics for their discovery of neutrino oscillations[3].

One question about neutrinos that has puzzled scientists is why the Earth receives so few neutrinos. Scientists have calculated the theoretical number of neutrinos produced by the fusion reactions that power the sun, but when the number of neutrinos Earth actually receives was measured, almost two-thirds of the calculated amount were missing[3]. The idea of neutrino oscillations was proposed in 1998 by Kajita at the Super-Kamiokande neutrino detector in Japan to explain the discrepancy in the number of neutrinos measured on Earth.



Neutrinos are emitted when protons are converted into neutrons during beta radioactive decay[6].

In quantum mechanics, particles exhibit properties of both point particles and waves, and neutrinos are no exception. When three different neutrinos (each with a different mass) are traveling through space with waves of different frequencies, they are thought to each be a different type of neutrino, and are thus each a different "flavor." These three different flavors are the three different types of neutrinos: muon neutrinos, electron neutrinos, and tau neutrinos[5]. Physicists describe waves in terms of both amplitudes and phase, and when multiple waves add together, their phases are altered. When the waves that make a neutrino add together, the phases do not cancel to zero. Since the flavor of a neutrino depends on its phase, the flavor can change over time. This phenomenon is called a neutrino oscillation, the metamorphosis of a neutrino of one flavor to a neutrino of a different flavor.

Kajita and McDonald proved that neutrino oscillations are not just theoretical. Kajita, working at the Super-Kamiokande neutrino detector just outside of Tokyo, Japan, discovered

that the detector caught some muon neutrinos coming from the atmosphere above and some from the other side of the planet after the particles had traveled through the Earth. Because the Earth does not present any considerable obstacle to neutrinos, equal numbers of neutrinos should have come through the Earth and directly from the sun. However, Kajita found that the muon neutrinos that came straight down to Super-Kamiokande were more numerous than those first passing through the globe. The only way to explain this observation is if the muon neutrinos traveling through the earth morphed into another type of neutrino, in this case, the tau neutrino. At the Sudbury Neutrino Observatory in Ontario, Canada, McDonald encountered a similar oddity. The lab was measuring the electron neutrinos that come directly from the sun, but the captured number of electron neutrinos was only one-third of the expected number. When the labs pieced together their numbers, they discovered that the theoretically calculated number of neutrinos had in fact reached Earth, confirming that neutrinos do morph into other types of neutrinos. Thus, neutrino oscillations are real!

This discovery and conclusion have led to another ground-breaking conclusion in particle physics: the Standard Model, the theory of how the universe is fundamentally organized and works, is not actually fully developed because it requires that neutrinos be massless[3,5]. However, in order for neutrino oscillations to be possible, neutrinos must have mass[3]! These conclusions have posed many other questions for particle physicists. What is the mass of a neutrino? Why are neutrinos so light-weight? Finally, what do these conclusions suggest about the potential dimensions alluded to earlier?

The existence of a neutrino with mass could suggest that those other dimensions forbidden to light are real and are waiting to be discovered. If it can be demonstrated that a neutrino can travel faster than the speed of light by taking a shortcut through one of the forbidden dimensions, then we can extend Einstein's theory of relativity to those dimensions light is forbidden from entering. The implications of such an application could radically alter our understanding of time and space relative to life as we know it.

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The Effects of an American Diet on Health

Courtney Walker

Though the potential for weight gain and cardiovascular disease are commonly known results of an unhealthy diet, the general public often does not realize that their diet can also adversely affect other aspects of health—even mental health. Additionally, the extent to which the American diet affects society as a whole brings this issue to a greater public importance. The *Dietary Guidelines for Americans* states that the average American diet consists of excess sodium, saturated fat, refined grains, and calories from solid fats and added sugars[1]. Furthermore, the guidelines state that Americans eat less vegetables, fruits, whole grains, dairy products, and oils than recommended. Almost 35% of adults in the U.S. are obese[2], and it is estimated that this statistic will increase to almost 50% within 15 years[3].

Some of the blame has been placed on fast food chains, which tend to serve foods high in calories, fats, sugars, and sodium, and low in vitamins and minerals[4]. While these restaurants may contribute to the poor diet of the average American, they are not the sole cause. Stepping into any American grocery store will make it abundantly clear why the American diet is severely lacking. Nutritious foods, like fresh fruits, vegetables, and lean meats, are often more expensive than packaged foods. Packaged foods tend to contain higher amounts of sodium, refined grains, sugar, and unhealthy oils than recommended by the *Dietary Guidelines for Americans*[1]. There are also more options when it comes to packaged foods: whole aisles are dedicated to chips alone, while healthier options are limited to a smaller section of the store.

Poor diet is predominantly associated with weight gain and obesity; however, the harmful effects do not end there. Animal and human studies indicate that an unhealthy diet can contribute to the development of many diseases, like cardiovascular disease and cancer, and can even affect the brain. In animal models, animals are often fed some type of high-fat, high-sugar diet, referred to as a Western Diet or high-fat sucrose (HFS) diet, to simulate the diet that a typical

A typical example of a grocery store aisle[5].

American consumes. For human studies, the participants often fill out questionnaires about their eating habits and are then placed in groups based on their answers.

As expected, studies in mice and rats show that those following a Western diet tend to gain more weight than those eating standard chow[6,7], although the extent of the difference between groups varies between studies; however, this variance could be caused by biological differences between mice and rats. Additionally, these studies administered diets with different fat and sugar compositions and provided varying access to running wheels for exercise.

In addition to weight gain, a Western diet also results in impaired cardiac function in mice, as indicated by changes in contraction and relaxation of the heart[6]. A Western diet has also been shown to elevate fasting insulin levels in rats and lower insulin sensitivity[7]. This result suggests that the rats on a Western diet were developing insulin resistance, which is a precursor to type two diabetes. Although these studies were conducted using rodents rather than humans, it is still important to consider the findings, since cardiovascular disease is the leading cause of death in the United States[8] and 9% of the population has diabetes[9].

Another health concern that is associated with diet is asthma. About 8% of U.S. adults have asthma, and in 2013, over 3,600 people died from asthma[10]. A study by Brigham and colleagues[11] shows that the Western diet worsens the severity of asthma, but does not have a role in causing it.

Interestingly, studies have shown that unhealthy diets affect the brain as well as the body. Diets high in saturated fats and refined carbohydrates are associated with greater incidences of depression, depressive symptoms, and anxiety[12,13]. In older adults, an unhealthy diet is associated with a smaller left hippocampus, a brain structure associated with learning, memory, and mood regulation, and is thought to play a role in depression[14]. A study in rats showed that the longer a rat consumed a diet high in fat and sugar, the greater the effect on hippocampal functioning and brain plasticity, resulting in impaired learning and memory[15]. This study showed that even short-term consumption of such a diet results in cognitive impairment in rats.

Further evidence of the impact of diet on the brain is provided by studies of diet-induced obesity and Parkinson's disease. Parkinson's disease is characterized by clumps of a protein called α-synuclein in the brain. Rotermund *et al.*[16] have shown that in mice, diet-induced obesity can increase the risk of developing these protein clumps. The study used a mouse model of Parkinson's disease, in which the mice were genetically predisposed to develop α-synuclein clumps. The mice with diet-induced obesity exhibited accelerated age of onset of protein clumps in the brainstem, onset of lethal locomotor symptoms, and onset of neurodegeneration[16].

Clearly, diet is incredibly important and can affect more than just physical size and weight – it can even affect mental health. Eating a diet high in fat and sugar, which many Americans do, can contribute to cardiac dysfunction, decrease insulin sensitivity, and worsen symptoms of asthma. Additionally, such a diet is associated with greater incidence of depression and depressive symptoms, impaired learning and memory, and greater risk of developing α-synuclein clumps, which are a hallmark of Parkinson's disease. In a day and age in which time is becoming increasingly precious, it is important to acknowledge that what is convenient is not always what is best, and that dietary choices have far-reaching effects on health. Making small changes, like choosing whole grain bread over white bread, is the first step toward lowering your risk of various health problems and leading a healthier, happier life.

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An Overview of Neuroepigenetics in Learning and Memory

Daniel Gilliam

The remarkable ability of cells in the brain to store and process information has fascinated scientists for centuries. To accomplish this feat, neurons must have mechanisms to generate both transient and persistent changes in response to incoming information. One mechanism proposed to mediate both long and short term changes in neurons is epigenetics.

Cells use epigenetics to modify and regulate their genome without altering the underlying DNA sequence. The prefix "epi," meaning above, illustrates that epigenetic processes do not alter the information contained in DNA. Instead, they alter how and whether this information is used, often by regulating the three-dimensional structure of chromatin and the ability of proteins to bind at specific sites. Traditionally, epigenetic modifications were considered permanent, heritable markers that determine the sets of genes active within a given cell and its progeny. Because coordinated programs of gene expression underlie differentiation, epigenetics is known to play a crucial role in development. However, in the past two decades, investigators have discovered that the molecular machinery of epigenetics has been co-opted by the nervous system to mediate both dynamic and persistent changes in gene expression that underlie learning and memory, a process sometimes referred to as neuroepigenetics[1].

Two main epigenetic mechanisms have been implicated in learning and memory: DNA methylation and histone modification[2,3]. DNA methylation involves the addition of a methyl group (-CH_3) to cytosine residues within DNA. Typically, only cytosine residues adjacent to guanine are methylated, and such pairs are identified as "CpG" sequences (the "p" refers to the phosphate group in the intervening covalent bond). The distribution of these CpG pairs in the genome is much lower than would be predicted according to random chance. Although approximately 70% of these pairs are methylated, those in regions involved in regulating transcription are largely unmethylated. DNA methylation usually represses transcription, especially when it occurs within the promoter region. This repression is attributed to changes in interactions between proteins and DNA; some proteins have methyl binding domains and will only recognize a sequence if it is methylated[1].

A crucial discovery implicating epigenetics in learning and memory was the observation that neuronal activity can induce changes in DNA methylation[4]. As one neuron communicates with another, signaling cascades are activated in the receiving neuron which can ultimately lead to alterations in the epigenetic status of particular genes. Because many different genes can be loosely defined as memory-permitting or memory-suppressing, transient changes in gene methylation provide a potential mechanism for temporary recruitment or suppression of genes to facilitate memory formation. For example, in the mouse hippocampus during fear learning the memory promoting gene *Reelin* is rapidly demethylated and transcriptionally activated. Similarly, the memory suppressing gene *PP1* is rapidly methylated and deactivated[4].

A few hours after fear conditioning, the methylation status of these genes in hippocampal neurons returns to its original level[4]. Such observations suggest the existence of both active methylation and demethylation mechanisms. DNA methylation is known to be catalyzed by DNA methyltransferases (DNMTs), but the mechanisms of demethylation are less clear. Some have proposed that demethylation takes advantage of mechanisms that otherwise function in cells to repair DNA base pair mismatches[1]. Figure 1B shows a proposed mechanism for demethylation of

DNA by such a pathway. Some studies have suggested that intermediates in this pathway do occur in the central nervous system and may serve as precursors for DNA demethylation[5, 6]. Whatever the mechanisms, it seems that active demethylation can play a role in a neuron's return to basal gene expression after a learning event[4].

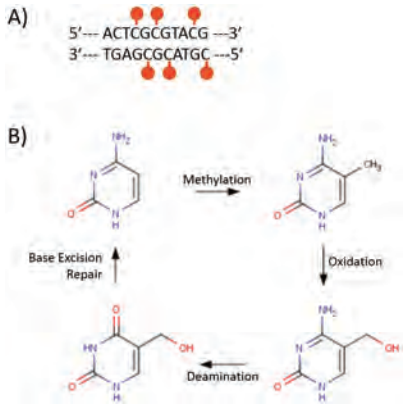

Figure 1 | Proposed mechanism of DNA methylation and demethylation. A) Within the genome, the majority of CpG pairs are methylated by addition of –CH_3 to cytosine. B) DNA methylation involves conversion of unmethylated cytosine to 5-methyl-cytosine (5mC). A proposed method for DNA demethylation involves sequential oxidation and deamination of 5mC, followed by base excision repair[1].

A second epigenetic mechanism implicated in learning and memory involves modification of histones, the proteins that DNA wraps around for organization and packaging within the nucleus[3, 7]. The basic organizational unit of the genome is a nucleosome, which is a short segment of DNA wound around a complex of eight histone subunits. Each of these histones has a tail region which can be extensively modified to alter the interactions between histones and DNA and affect the three dimensional structure of chromatin, which in turn can affect transcription. Modifying histones can affect transcription by altering the structure of chromatin and by facilitating interactions with accessory proteins that recognize modified histones. Thus, histone modifications generally represent a more complex and diverse mechanism to affect gene transcription than DNA methylation. In fact, one effect of DNA methylation can be to recruit proteins to mediate histone modification[1].

Some major examples of histone modifications are methylation, phosphorylation, acetylation, and ubiquitination, which involve addition of the relevant group to specific amino acids on the histone tails[1, 8]. These modifications play an essential role in regulating the activity of genes by recruiting regulatory proteins and altering the accessibility of DNA. Histone acetylation and phosphorylation generally promote gene activation, the effects of histone methylation are bidirectional and complex, and the effects of ubiquitination remain unclear[1]. Distinct classes of enzymes are known to mediate each of these modifications. For example, histone acetylation and deacetylation are catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively[1].

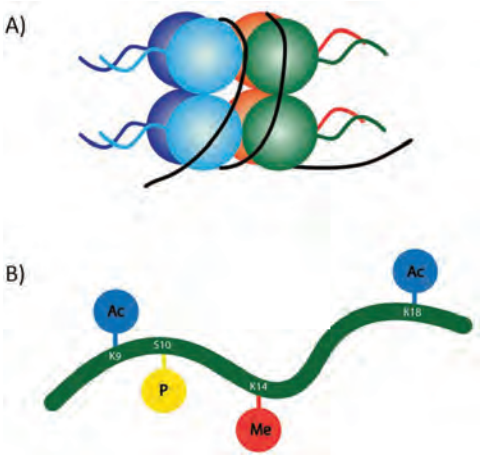

Figure 2 | Nucleosomes and histone tail modifications. A) A nucleosome consists of DNA associated with eight histone proteins, each with a tail domain. B) Along each histone tail, a number of different moieties can be attached to specific amino acids. Acetyl groups (Ac) and methyl groups (Me) are added to lysine (K), and phosphates are added to serine (S)[1, 7].

The histone modification most studied in learning and memory is histone acetylation[3]. Compounds that inhibit histone deacetylase enzymes (HDACs) have been shown to enhance certain forms of long term memory formation and synaptic plasticity *in vivo*[9]. Despite our knowledge of the role of histone acetylation in learning and memory generally, the specific catalog of genes known to be affected by histone acetylation during learning is relatively sparse. However, some specific cases are well-studied: for example, increased promoter acetylation and expression of brain derived neurotrophic factor (BDNF) in rat prefrontal cortex has been associated with fear conditioning training and extinction[10, 11].

Histone subunit exchange is a separate type of histone modification beyond addition or removal of functional groups to the tail domains. A full histone complex usually contains two each of histone H2A, H2B, H3, and H4. Beyond these four, there are other isoforms that can be inserted into a nucleosome via a process known as histone subunit exchange[1]. Only recently has evidence emerged that histone subunit exchange is involved in learning and memory. Last year a group from Dr. David Sweatt's laboratory at UAB provided the first evidence of dynamic histone subunit exchange as a novel epigenetic mechanism in learning and memory[12]. They report evidence that the histone variant H2A.z is actively exchanged in the hippocampus and the cortex during fear memory consolidation. Incorporation of H2A.z into a nucleosome is generally associated with absence of DNA methylation and gene activation. Learning-induced recruitment or exclusion of H2A.z from nucleosomes near

the transcription start sites of learning and memory genes appears to negatively regulate memory consolidation[12].

A role for dynamic epigenetic regulation in at least some forms of learning and memory has been clearly demonstrated. In essence, these mechanisms enable neurons to transiently activate or repress transcription of ensembles of genes that facilitate learning and memory. Epigenetics is a particularly interesting subject for scientists studying learning and memory because it has the potential to underlie both long term and transient changes in gene expression. Additionally, epigenetic dysregulation has been implicated in a wide variety of neurological disorders[1, 7]. Neuroepigenetics promises to be an exciting and productive area of inquiry as we continue to unravel the complex biological underpinnings of learning and memory.

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"How to Succeed in Science..." A Review of *The Race for the Double Helix* (1987)

Marina Triplett

To what lengths is a scientist willing to go to become successful? For anyone who has ever had experience with scientific research, it becomes evident that science is a commitment that requires a novel approach to problem solving, long hours in the lab, and some amount of good fortune. However, these factors alone do not guarantee that a scientist's research will "make it" in the competitive environment of academia. In the dog-eat-dog world of science, time is key. Today's scientists must acquire funding, perform the necessary experiments, and publish their work before their competitors, lest their careers perish. This concept is not necessarily novel. History has shown that the first to publish receives the recognition—it's the same reason why Charles Darwin is a household name and Alfred Russell Wallace has been overshadowed, despite both developing similar theories of evolution around the same time. To succeed in science, a scientist must be both competitive and willing to make his or her research a priority.

One film that successfully depicts the competitive nature of scientific research and the sacrifices that scientists make for their careers is *Life Story: The Race for the Double Helix*, a 1987 made-for-television film that dramatizes the discovery of the structure of DNA by the legendary duo James Watson and Francis Crick in 1953. The film stars Jeff Goldblum as Watson and Tim Pigott-Smith as Crick, who are working at Cambridge to understand the structure of DNA before their competitors, Maurice Wilkins (Alan Howard) and Rosalind Franklin (Juliet Stevenson) of Kings College London, can determine the structure themselves. The film won a BAFTA TV Award in 1988 for Best Single Drama.

The opening scene introduces James Watson, a young and ambitious molecular biologist working as a postdoctoral fellow in Copenhagen. He dreams of fame and recognition, and he believes that DNA is his ticket to scientific notoriety. After accepting a research position at Cambridge, Watson is introduced to Francis Crick, a graduate student working on a hemoglobin project who would rather be working on DNA. The two connect immediately when Crick states that

he believes that the genetic code is contained in DNA, not protein—a theory with which Watson also staunchly agrees. The two decide to team up to create a three-dimensional model of DNA, which they believe must have some type of helical structure. Meanwhile, X-ray crystallographer Rosalind Franklin is hard at work producing X-ray diffraction images of A form and B form DNA, but faces difficulty in being taken seriously as a scientist by her male colleagues, including Maurice Wilkins, whom she fears is trying to take credit for her work. Franklin eventually decides to quit and continue her work on the A form of DNA, while ruefully conceding to having Wilkins work on the B form. Wilkins, realizing that the B form image may be the key to unlocking the helical structure of DNA, decides to show Watson and Crick the image generated by Franklin. The pair have a "Eureka" moment upon seeing this image, realizing that DNA must have a double helical structure. The two construct an accurate representation of the structure of DNA—and the rest is history. Watson, Crick, and Wilkins are awarded the 1962 Nobel Prize in Physiology or Medicine. However, Rosalind Franklin dies of ovarian cancer before the award is given and is not recognized, as the rules of the Nobel Committee state that an award cannot be given posthumously.

One of the primary themes of the film is the competitive nature of science as a profession and how competition can raise questions about ethics in scientific research. Throughout the film, Watson and Crick work feverishly to construct their model of DNA, as they know that there are other scientists around the world who are working towards the same goal. When Nobel laureate Linus Pauling releases a paper regarding the structure of DNA, Watson and Crick fear that their dreams of recognition and fame are over. However, they are relieved when they realize that Pauling's structure is incorrect and that they still have time to work on their model. The competition between Watson and Crick and Linus Pauling portrayed in the film calls into question the motives of scientists. Is the main goal, as a scientist, to make a scientific discovery in order to contribute to a body of knowledge, or do scientists work simply for the prospect of recognition, accolades, and acclaim? Although the answer probably varies from scientist to scientist, there is no denying that scientists dedicate their lives to their work because they truly believe that their work is worthwhile. The film also depicts competition between Watson and Crick and Wilkins and Franklin, as the pairs are working on similar research at different universities. There is also rivalry between Wilkins and Franklin once Wilkins realizes that Franklin's X-ray diffraction images could be of importance; he fears that she alone will be given credit for her work, even though he was under the impression that they were working collaboratively. The film calls into question the theme of scientific integrity. There can often be gray areas with respect to crediting others for their work, and it is necessary for scientists to give credit to whom credit is due. At what point does "referencing" another scientist's work transition into "stealing" another person's work? It has been argued for years that Rosalind Franklin did not receive the credit she deserved for her X-ray diffraction images and that Watson, Crick, and Wilkins used her work without her permission. Although we can never be certain what actually took place, it is important for scientists and their collaborators to be clear on the rules of ethical conduct with regards to the publication of their research.

The film also serves as a commentary on the lonely life of a scientist, an idea emphasized by both Franklin and the male characters of the movie. Throughout the course of the film, Watson is constantly searching for a girl, but is reminded by Crick that he still "has time" for such matters later and should focus more on his work. When asked by a friend if she has ever wanted to get married, Franklin rejects the idea, stating that she would "rather do one thing well than two things badly." This attitude is one on which many women in science still agree. There is no denying that as a scientist, one must occasionally make sacrifices when it comes to leisure time or family time if he or she wants to be successful and productive in the lab. Many believe that splitting time between science and family is impossible if one does not want either or both to suffer. Science typically requires long or irregular hours in lab, which can often make married life or family life challenging. How can one expect to have time for a family while still being able to dedicate time to a truly successful research career in the competitive field of science? Although women arguably have more trouble with this issue, it can still be difficult for both male and female scientists to find a successful work-life balance. Like in daily lab work, time management is key to achieving this balance.

Overall, *Race for the Double Helix* gives a fairly accurate depiction of the professional and personal struggles of a scientist. A scientist must be both ambitious and ethical and be hardworking while also not allowing the work to consume his or her entire life. While these contradictions may seem to leave scientists at an impasse, the important thing to remember is that above all, successful scientists generally have a passion for research. A career in research may require a monumental amount of dedication, but many scientists are readily willing to make this compromise because they cannot imagine themselves doing anything else. Sometimes it is the potential for scientific discovery, the vast number of modern problems not yet answered by science, or the competitive atmosphere of academia that pushes inquisitive and ambitious individuals into the fields of science, regardless of the difficult research career that may follow.

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A Newly Found Relationship between Heat and Sudden Infant Death Syndrome

Aashka Patel

For adults, sleep is a mundane activity, and very few sleep-related concerns exist for adults aside from the fear of not getting enough of it. For children under the age of one, however, sleep sometimes becomes permanent. Sudden Infant Death Syndrome (SIDS) is defined as the sudden death of an infant without an explainable cause, usually occurring during sleep[1]. Recently, many studies of this phenomenon have been conducted in the hopes that better preventions might be found.

Currently, there are three major physical risk factors associated with SIDS: brain abnormalities, low birth weight, and respiratory infection[2]. Infants with improper development of brain regions associated with breathing, heart rate, and arousal have a greater risk of SIDS. Premature birth (thus, low birth weight) leads to immature autonomic processing, which impairs the infant's regulation of heart rate and breathing pattern. Furthermore, babies who suffer respiratory illnesses also have greater trouble breathing and a greater chance of SIDS. Various environmental factors also appear to contribute to the prevalence of SIDS. For example, babies sleeping on their sides, on soft surfaces, or with their parents have an increased risk of SIDS because of ineffective breathing, most likely due to restricted airways.

Recent research has identified another important environmental factor in SIDS: heat[3]. Back to Sleep, a popular campaign that encourages parents to make sure infants sleep on their backs instead of their stomachs to prevent the constriction of airways, could also provide benefits concerning the newly-discovered risk factor of heat. Infants lack the thermoregulatory systems that adults have, and instead can regulate their body temperature through the face. Sleeping on the stomach interferes with this regulation, and thus provides another reason why sleeping on their backs is beneficial for infants.

In Canada, a 30-year study was conducted to observe whether increased environmental temperatures resulted in an increase in SIDS[4]. A total of 196 cases of SIDS were analyzed by comparing the prevalence of SIDS during days of extreme temperatures versus those near average temperature for a given month. The results showed a positive correlation between temperature and SIDS in 3–12 month-old infants specifically. On days when temperatures were greater than 29 °C, there was a 2.78 times greater chance of sudden infant death than on 20 °C days. The connection between SIDS and heat has also been studied using stress gene expression[5]. Cultured fibroblasts and connective tissue cells were taken in autopsies of SIDS-related deaths, and the expression of four stress responsive genes was measured. High temperatures increased expression of all four genes. The genes *HSPAA1B* and *HSPD1*, which become active during elevated stress responses, had the highest levels of expression compared to the control gene.

A decrease in the prevalence of SIDS from 153 to 51.6 deaths per 100,000 live births has occurred since 1980[6], which may be largely due to the Back to Sleep campaign's promotion of awareness of SIDS and safer sleeping positions. Of course, no parents should have to worry about their children not waking up. Thankfully, many scientists continue to work towards identifying new causes and preventive measures for SIDS, offering the hope that its incidence will only continue to decrease.

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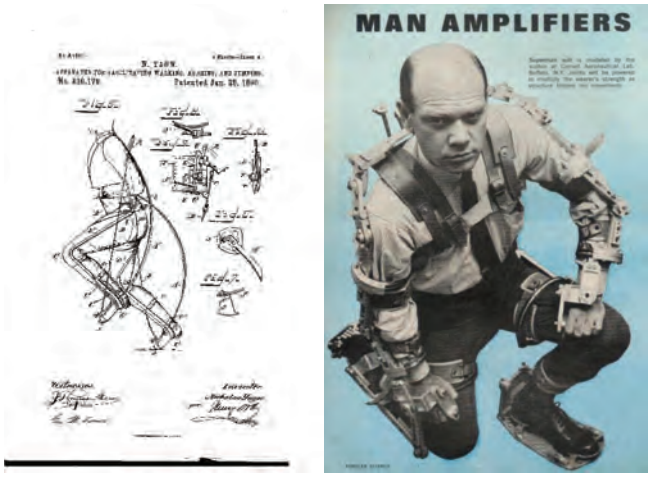
Exosuits: Past, Present, and Future

Alexander Chang

When a condition affects mobility, a person will often use a technological invention to improve their quality of life and regain a portion of their lost mobility. One of the most common forms of technological aid is prosthetics. The earliest use of prosthetics can be seen in ancient Egypt around 950–710 B.C. where two prosthetic toes were discovered at the necropolis of Thebes-West[1]. Even to this day, prosthetics are used as assistive devices; however, other conditions that affect mobility have led to the invention and use of modern biomedical technologies. Spinal cord injury, arthritis, back disorders, cerebral palsy, neuromuscular disorders, fibromyalgia, and other physiological problems also affect a person's ability to move, something that cannot always be adjusted with prosthetics alone[2].

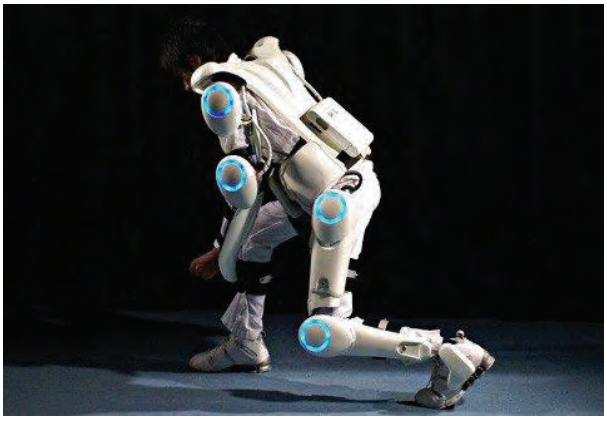
For those with conditions that affect mobility such as paraplegia or paralysis of the legs and lower body, previous limitations in biomedical advancement presented little hope of recovering the ability to walk. Other alternatives, such as epidural electrical stimulation or stem cell implantation, offer hope, but none of these biomedical advancements are close to bringing mobility up to a satisfactory level. Aside from curing the condition or injury, one of the few existing alternatives to regaining a large portion of a person's mobility is the use of an exosuit.



First patented exoskeleton design[3] (left) and Cornell's "Man Amplifier"[5] (right).

Exosuits are not a new idea to the public: exosuits have even established a place in popular culture, a point made evident by movies like *Aliens* (1986), *The Avengers* (2012), and *Elysium* (2013). Although present-day exosuits are nowhere near as advanced as those seen in movies, actual exoskeletons are slowly making their way into the present-day public market. In January 28, 1890, Nicholas Yagn patented the first exoskeleton-like device that could facilitate walking, running, and jumping[3]. Seventy-four years later, Niel J. Mizen created an exoskeleton named "The Man Amplifier" in a Cornell Aeronautical Laboratory that marked the advent of exoskeletons from ideas to reality[4]. These were just rough designs that were clunky, not easy to take off or operate, and expensive, thereby making the innovation not fit to be released to industry or the public.

One of the first exoskeletons that showed actual potential for the public market was proposed by Yoshiyuki Sankai of Cybernics in 1992[6]. HAL or hybrid assistive limb has two different movement settings built in based off of years of research: cybernic voluntary control and cybernic autonomous control. Under cybernic voluntary control, HAL provides "physical support/actions according to the operator's voluntary intention caused by the bioelectrical signals including muscle activity"[6]. When a bioelectrical signal is detected, the suit will calculate the necessary power assist torque required from the suit to supplement the joint torque of the wearer in completing an action such as walking or standing. This setting can assist those with disabilities in their limbs, but with persons that have a gait disorder, this setting cannot be used because the bioelectrical signals that induce a broken walking pattern are not factored into a power assist torque calculation. Additionally, persons with gait disorders often have injured spinal cords that are unable to sufficiently send signals from the brain to other parts of the body, signals which, if successfully transmitted, would prompt movement assistance from the device. HAL's cybernic autonomous control monitors center of gravity shifts that usually occur before a person starts executing an action. For example, when walking, the center of gravity shifts to one leg prior to the initiation of the action. The lab coined this technology "intention-based walking support." Currently, the hybrid assistive limb exoskeleton suit can be purchased for $14,000–$19,000[7].



Cyberdyne's HAL EU Model and Full Body Type[8]

Aside from the HAL series created by Sankai and Cybernics, there have been a series of other companies and universities developing similar exoskeletons. Vanderbilt University created their own exoskeleton called Indego for rehabilitation purposes and as an alternative mode of mobility assist[9]. Another type was created by Ekso Bionics for survivors of stroke, spinal cord injury, and other forms of lower extremity complications[10]. All of these exoskeletons were created for the purpose of helping those with conditions that affect mobility, but Berkley decided to take exoskeletons a step further and created BLEEX, Berkley lower extremity exoskeleton, in order to enhance normal human capabilities. In 2005–2006, BLEEX was able to carry a significant load and support its weight while walking energetically autonomous, a first for human exosuits[11]. With the potential to enhance humans past physiological bounds, it is no surprise that the military is attempting to integrate the technology for combat purposes.

The Hardman I Prototype[12]

In August 30, 1971, General Electric released a report, *Final Report on Hardman I Prototype For Machine Augmentation of Human Strength and Endurance*, that contained information on an exosuit that the company was developing for the Naval Air Systems Command at the Army Mobility Equipment Research and Development Center in Virginia[13]. As the first exosuit designed for military purposes, it was extremely heavy and bulky at 1,500 pounds and three feet wide[13]. Many issues were present, but the concept itself was significant because the exoskeleton could hold up to 1,500 pounds in addition to its own body weight[13]. Thirty-nine years later, another company, Raytheon, released a press announcement and demonstration of an exosuit that was developed for the US Defense Advanced Research Projects Agency (DARPA)[14]. In 2001, DARPA commissioned five exoskeleton designs for the Human Performance Augmentation program, but only two made it past the drawing board: Raytheon's XOS exoskeleton line and Lockheed Martin's Human Universal Load Carrier (HULC). It is worth noting that HULC was designed using BLEEX as a base[14].

Raytheon X02[14] *(left) and Lockheed Martin's Exosuit*[15] *(right).*

From medicine to military, exoskeletons are being developed by separate research and engineering groups, but the key issues behind advancement are the same. One key limitation of current exoskeleton technology involves issues with torque and power[16]. The unnatural shape of the current models negatively affects the efficiency of muscle actuators. Muscle actuators are "contractile and linear motion engines operated by gas pressure"[17]. A cylindrical membrane has gas pumped into it or sucked out of it in order to extend or contract the pseudo-muscle. An alternative to muscle actuators are electroactive polymers that can be crafted into synthetic muscle fibers that may offer advantages for smoother, more controlled actions, noise-free operation, and a more anthropomorphic appearance[16]. Another key issue is the limitations associated with the flow of information between the device and the user's nervous system. Currently, exoskeletons only predict intentions in order to process movement or just enhance motions given, but a truly autonomous exoskeleton will require sensors to be placed inside the user's body to give the device better access to the motor cortex and other tissues involved with movement. Ultimately, this fully autonomous exoskeleton would allow the wearer to control the suit with just thoughts[16]. Solving these issues, as well as complications presented by mechanical interface and construction costs, might allow future exoskeletons to revolutionize the medical technology industry, and perhaps even the apparel industry.

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Experience in the Study of Dystonia

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In the spring of 2015, I had the wonderful opportunity to join the lab of Dr. David Standaert and participate in research involving dystonia, a mysterious movement disorder characterized by involuntary muscle contractions and tremors. My connection to the field of movement disorders stems from much more personal roots besides my interest in the underlying mechanisms of science. During my freshman year of high school, my father was diagnosed with Parkinson's Disease. I remember my confusion and personal struggle to understand the debilitating disease that had taken over my father's body. As I matured, my eagerness to be fully educated and understand the means underlying movement disorders grew along with me. My father has been participating in the Parkinson's Research study at UAB under Dr. David Standaert for a couple of years. After each appointment, my father would return with a stack of research articles explaining the latest findings of his disease that I would enjoy reading, amazed by the different terminology and techniques that I never knew existed. When time came around to find a research mentor, I had no hesitation in delving into studying movement disorders under a more fundamental level in the Standaert lab.

As it was my first time in a laboratory-based research setting, I was quite nervous and unfamiliar with many of the skills and techniques required. Nevertheless, thanks to the help of my mentor, I was trained to acquire all that I needed to know in order to be successful in the lab. Dystonia, which leads to abnormal postures or repetitive and slow movements, is one of the most common movement disorders in the US. What differentiates dystonia from Parkinson's Disease is that the combination of symptoms targets a wide range of ages, from infancy to late adulthood. Dystonia cannot be cured and may last either for a few years or throughout a lifetime. Depending upon the type of dystonia, symptoms may include foot cramps, rapid eye movements, muscle tremors, as well as some difficulty in speech. The cause for dystonia is still a mystery but it is suspected to stem from damage in the basal ganglia or other regions of the brain involved in motor control. Currently, there are no medications that can slow dystonia's progression or prevent it; however, there are a few treatments that can alleviate some of the symptoms, such as anticholinergic agents, dopaminergic agents, injections of botulinum toxin (Botox) within affected muscles, and deep brain stimulation. Dystonia is currently an active area of investigation, with new therapies continually being developed in the hope to find a better treatment.

My study focuses on DYT1 early onset torsion dystonia, which is a rare form of dominantly-inherited generalized dystonia, which affects most of the body. Specifically, I work with mice called DYT1 deltaGAG knock-in mice (DYT1 KI), which carry the same genetic anomaly as human DYT1 carriers. This mutation involves the *TOR1A* gene, which codes for torsinA. Studies in our lab and some others have found that there is an abnormality in neurotransmitter release within the striatum, a brain area involved in motor control. Specifically, dopamine release is reduced in these mice, while cholinergic function is elevated. Thus, DYT1 mice are studied to investigate whether these neurotransmitter systems can be modulated in order

to reverse the symptoms of dystonia. In the clinic, dystonia is usually treated with anticholinergic drugs. These nonselective muscarinic receptor antagonists, such as trihexyphenidyl and benztropine, function to block the receptors for the neurotransmitter acetylcholine. Anticholinergic drugs can be quite effective but come along with a number of side effects such as sleepiness.

Working in the Standaert lab, I have been able to test anticholinergic drugs, specifically trihexyphenidyl, on DYT1 KI mice in order to see how they affect both acetylcholine and dopamine levels in the brain. Our analysis focuses on the striatum because many patients with striatal defects develop dystonia and striatal direct brain stimulation is an effective therapy. The major technique we use to study neurotransmitter activity in the striatum is *in vivo* microdialysis, which involves the insertion of a probe into tissue in order to sample the extracellular cerebral fluid (ECF). This technique can be used to measure the amount of neurotransmitters in blood and tissue while infusing substances into the brain or spinal cord. Various samples at different phases through the period of *in vivo* microdialysis are taken and analyzed using high-pressure liquid chromatography (HPLC). HPLC allows us to quantify the concentrations of acetylcholine and dopamine in our ECF samples, thus providing the outcome measure for our tests of anticholinergic drugs. Although this study can be expanded, this approach gives me a starting point to achieving my future goals in finding effective treatments for dystonia.

During the last few months of my research experience, I have watched myself grow and have been astonished at the skills and information I have been able to learn. Specific skills my project has required include preparing tissue samples for analysis, performing behavioral tests, *in vivo* micro dialysis, genotyping using PCR, brain slicing, and many other "wet lab" techniques. I have also learned the importance of trial and error and how not every day in the laboratory will be a day of successful results. It is important to be patient when conducting research since there are various factors that can pose threats to the ideas we construct. For example, there have been numerous times in which the analysis of my data post microdialysis yields disappointingly low HPLC results.

Prior to experiencing what research really is, I had no idea about the amount of time and effort it really takes to conduct an experiment that can potentially be worthwhile. It has definitely been quite an exciting learning experience to acquire such advanced lab techniques and understand their use and contribution in science today. In addition, it is an honor to be surrounded by "cream of the crop" mentors and graduate students that have allowed me to feel comfortable as I work inside and outside of the lab. I am very excited to see the future growth and development of my project as I grow in the lab field in the remainder of my undergraduate career.

An Adaptive Kernel-Growing Median Filter for High Noise Images

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ABSTRACT

A novel adaptive median filter is presented that can restore images corrupted by salt and pepper noise levels greater than 90%. The algorithm operates by adapting to the amount of available visual data in the image by iteratively increasing the size of the median kernel. The algorithm then detects the edges and reruns the adaptive median filtering process on just those edge pixels to improve edge consistency. Lastly, post-processing is done on the image using the Perona-Malik diffusion process for smoothing and an Unsharpen filter to improve contrast. The results of our algorithm show root-mean-square error improvement of the reconstruction compared to the state-of-the-art filter for image reconstruction.

KEYWORDS: adaptive-median filter, impulse noise, kernel, reconstruction

INTRODUCTION

Noise is one of the main problems faced in any kind of signal or image reconstruction process. One very common type of noise that arises in natural images is impulse noise, commonly referred to as "salt and pepper" noise[1]. Impulse noise manifests as image pixels (picture elements) that spike in color or intensity relative to the surrounding pixels; i.e. if part of an image is mostly green pixels, a random bright white pixel would be considered impulse noise. Impulse noise at lower levels can be filtered out rather trivially by a simple median filter, but at ultra-high levels, i.e. greater than 80% of the image is affected by noise, the image reconstruction process is significantly harder. Furthermore, since color images consist of 3 channels (red, green and blue) the noise also exists in all 3 domains, and, as such, it must be filtered out on each domain, which adds to the complexity of the problem. When images are strongly corrupted, (i.e. a radio-

astronomical image that has been subjected to atmospheric noise), only a small portion of their pixels are truly correct, which means the amount of accurate data that can be used to reconstruct the corrupted noise pixels is sparse and thus not completely reliable. Various methods have been proposed to handle this problem, though median filters and interpolation methods have traditionally been some of the main techniques used due to their robust nature[2, 3]. Linear filters make use of 2-D convolution in which *n*×*n* mask of pixels (called the kernel) is swept across the image and the central location in the kernel corresponds to the specific pixel that is being filtered. An illustration of this concept can be seen below in Figure 1.

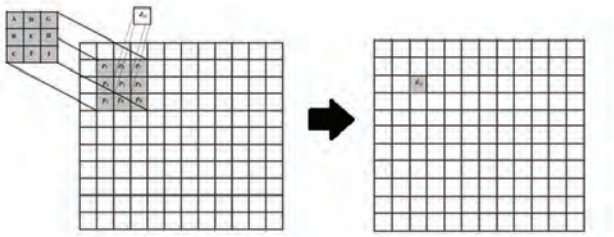

Figure 1| A 3 × 3 filter with output pixel $Z_{i,j}$

Each pixel location where the kernel intersects the image is the product of the kernel value at that location with the corresponding actual image pixel value. This gives *n*×*n* different products, and the summation of all these products is used to replace the pixel in the image that corresponds with the central pixel in the kernel, thus performing the filtering on that pixel. This concept is mathematically shown in (1).

$$Z_{i,j} = A(P_1) + B(P_2) + C(P_3) + D(P_4) + E(P_5) + F(P_6) + G(P_7) + H(P_8) + I(P_9) \quad (1)$$

The median filter is slightly different, in that the central pixel is replaced with the median of the pixels in the kernel instead of the weighted sum. Recently novel additions to the median filter have been implemented that employ a variety of concepts, such as adaptiveness, fuzzy logic, or dynamic programming[4, 5]. This paper will present the novel application of a new type of adaptive-median filter that has been shown to robustly reconstruct high-noise images to a very high level of accuracy. This paper will then compare the results of the novel adaptive-median filter to existing interpolation methods.

METHODS AND IMPLEMENTATION

Several images were obtained in order for the algorithm to be tested. Each image was corrupted to have impulse noise levels of 90%, 95% and 98% by randomly setting that percentage of the image's pixels to have either the minimum or maximum possible intensity value. Each image was then separately processed. First, noise pixels were located in the image by identifying pixels of minimum or maximum intensity, and then set to zero. The adaptive median filter ran specifically on these zero-valued pixels for each of the 3 pages of the image. The kernel size for the initial iteration of the adaptive median filter was set to 3 × 3. If a pixel was still found to have a value of zero after the first iteration, its position would be saved and then another iteration of the median filter would be run, but this time the size of the kernel would be increased to 5 × 5. Likewise, this process of increasing the length and width of the kernel by 2 pixels was done until all of the zero-valued pixels were fully restored.

Upon achieving a reconstruction of the image, the object and feature edge pixel locations were detected using a standard deviation filter that was run on each page of the image. This filter calculates the standard deviation of all pixels in the kernel, and then replaces the central value of the kernel with that standard deviation value. This can be seen in (2), where x_i corresponds to each element in the filtering kernel and μ corresponds to the average of all the pixels' intensities. *M* corresponds to the number of elements in the kernel; so for a 3 × 3 kernel, *M* would be equal to 9.

$$Standard\ Deviation = \sqrt{\frac{1}{M}\sum_{i=1}^{M}(x_i - \mu)^2} \quad (2)$$

This is useful since image regions that contain edges will have high local standard deviations. Upon detecting the edges by thresholding the image intensity, the width of these edges was then increased using a single iteration of binary morphological dilation followed by morphological closing. After performing these morphological operations the location of each edge pixel was saved. The same adaptive-median filtering process previously mentioned was then run specifically on these edge pixels.

After fully reconstructing the edge pixels over the course of multiple iterations of the adaptive-median filter, the image was then processed to improve the appearance. This "post-processing" step first required the use of Perona-Malik anisotropic diffusion on the image. Perona-Malik anisotropic diffusion is the process of selectively blurring an image at different regions, such as edges, based on a specified "conductance function" that has several parameters[6]. This anisotropic diffusion is based on the heat equation with a variable conductance function, denoted as *c(x,y,t)*, which can be seen below in (3).

$$\frac{\partial I}{\partial t} = c(x, y, t)\Delta I + \nabla c \cdot \nabla I \quad (3)$$

The conductance function chosen specifically for this algorithm can be seen below in (4).

$$c||\nabla I|| = e^{-\left(\frac{||\nabla I||}{K}\right)^2} \quad (4)$$

For the Perona-Malik diffusion, the conductance parameter *K* was set to 1000. Upon completion of the Perona-Malik diffusion process, the image contrast was then sharpened using MATLAB's built-in unsharpen filter in which the standard deviation of the Gaussian low-pass filter was by default set to 1, and the contrast threshold was set to 0.

Lastly, each fully processed and reconstructed image was

compared to the original uncorrupted image to see if the algorithm could accurately recreate the images for each noise level. A function for root-mean-square error that relates how close the reconstructed and original images truly are was defined as follows, and was then calculated for the red, green and blue image channels.

$$Error_{RMS} = \sqrt{\frac{1}{MN}\sum_{i=0}^{M-1}\sum_{j=0}^{N-1}(A_{i,j} - B_{i,j})^2} \quad (5)$$

$A_{i,j}$ and $B_{i,j}$ represent the pixels in the i^{th} row, j^{th} column of the original image and reconstructed image, respectively.

For comparison, a triangulation based interpolation image reconstruction method was then applied to the same images with the same noise levels, since current literature has made extensive use of this technique due to its perceived speed improvement[3]. This comparison was done using MATLAB's built-in scattered interpolation function, and the root-mean-square error between the output reconstructed images and the originals was again calculated. The root-mean-square error of our adaptive-median filter algorithm was then compared to this error to see if our adaptive-median filter provided any advantage to the existing interpolation reconstruction methods.

MATERIALS

For the experiment, MATLAB R2015a in combination with Microsoft Excel were used for the computation. The MIT SUN database was used to obtain the test images[7]. All computations were carried out on a desktop computer with an AMD A10 quad core processor and 8.0 GB of RAM.

RESULTS

Images were chosen from the MIT SUN database for testing our algorithm[7]. For each test image and each noise level, the noisy image, the reconstructed image obtained from our adaptive-median filter, and the comparison reconstructed image obtained from standard interpolation can be seen in Figures 2 through 4. It is worth noting that for illustrative purposes four noises levels (30%, 90%, 95%, and 98%) are shown in Figures 2 through 4, but only the last three levels (90%, 95%, and 98%) were analyzed for results. Graphs displaying the RMS error, as a function of raw pixel intensity, for each image and noise level, that compare our novel adaptive-median filtering algorithm to the existing interpolation method are shown in Figures 5 through 7. A table showing the percentage of pixels restored after each iteration of the adaptive-median filtering algorithm, for each noise level and image, can be seen in Table 1.

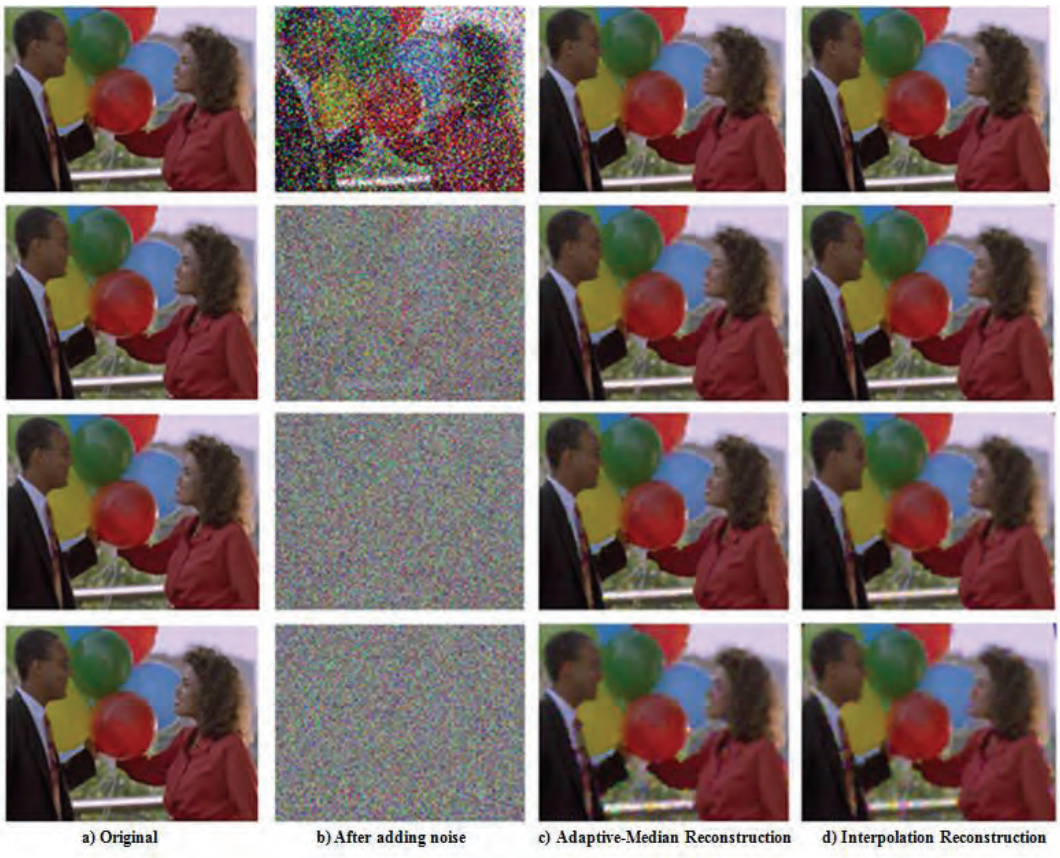

Figure 2| The reconstructed "balloons" image comparison. The noise levels in column b are 30%, 90%, 95%, and 98%, from top to bottom.

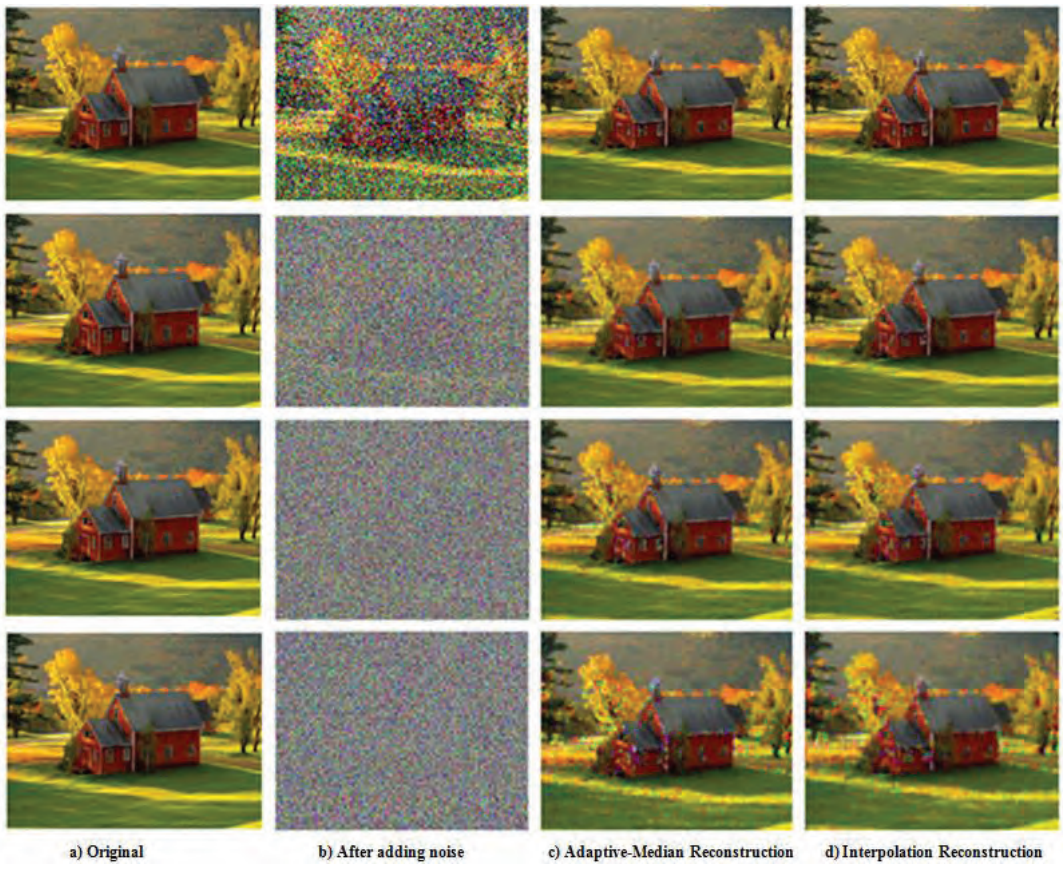

Figure 3| The reconstructed "farm" image comparison. The noise levels in column b are 30%, 90%, 95%, and 98%, from top to bottom.

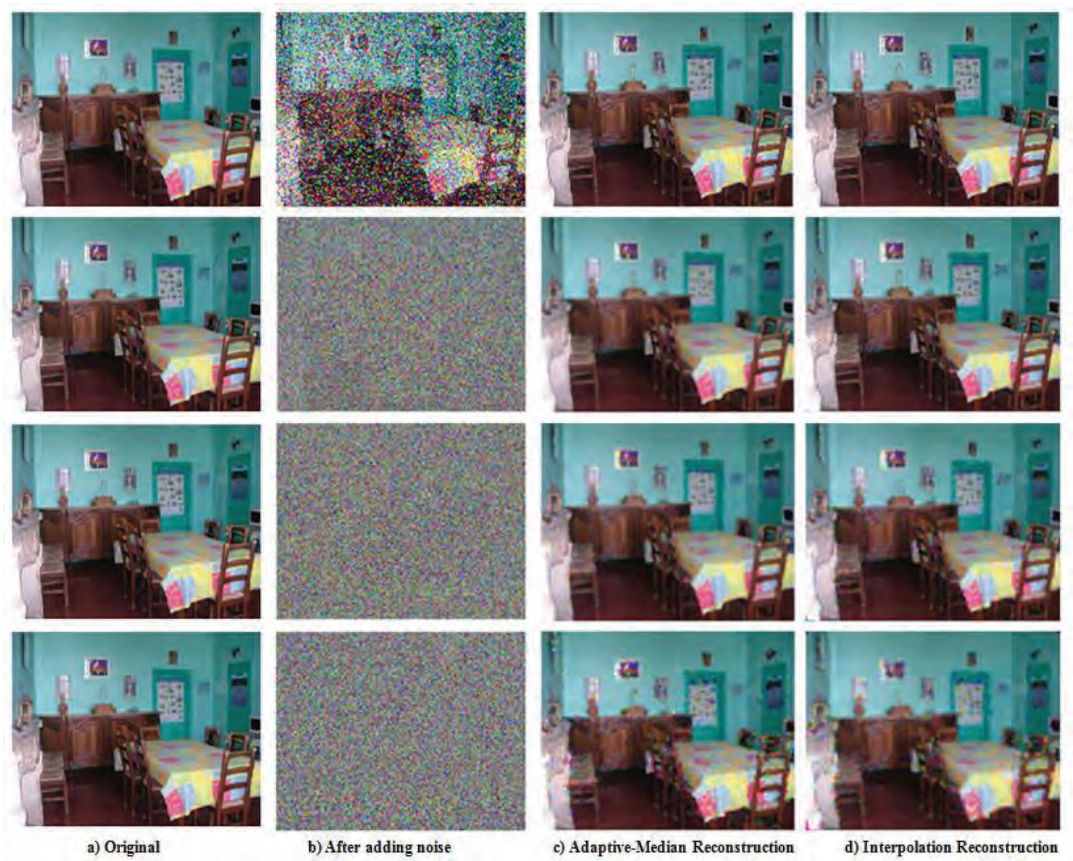

Figure 4| The reconstructed "room" image comparison. The noise levels in column b are 30%, 90%, 95%, and 98%, from top to bottom.

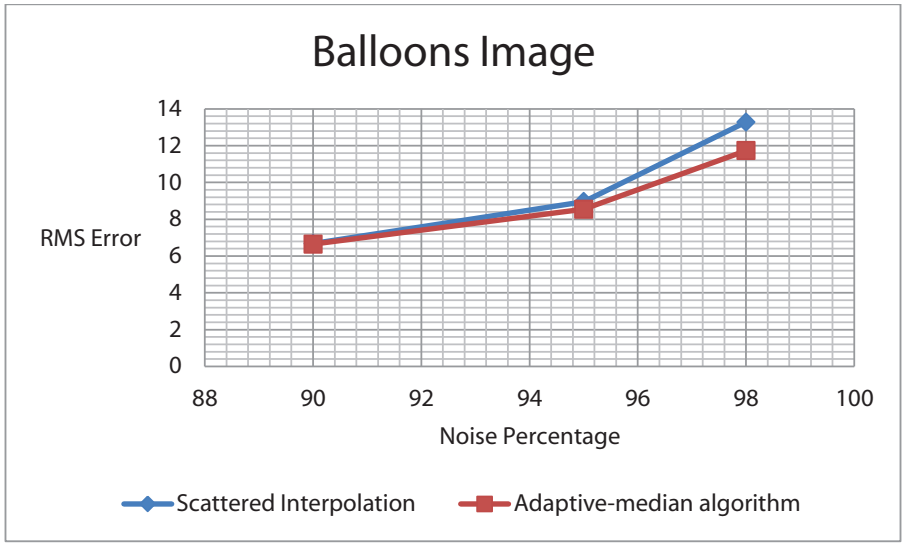

Figure 5| Performance curve for the proposed algorithm and standard interpolation method for the balloons image.

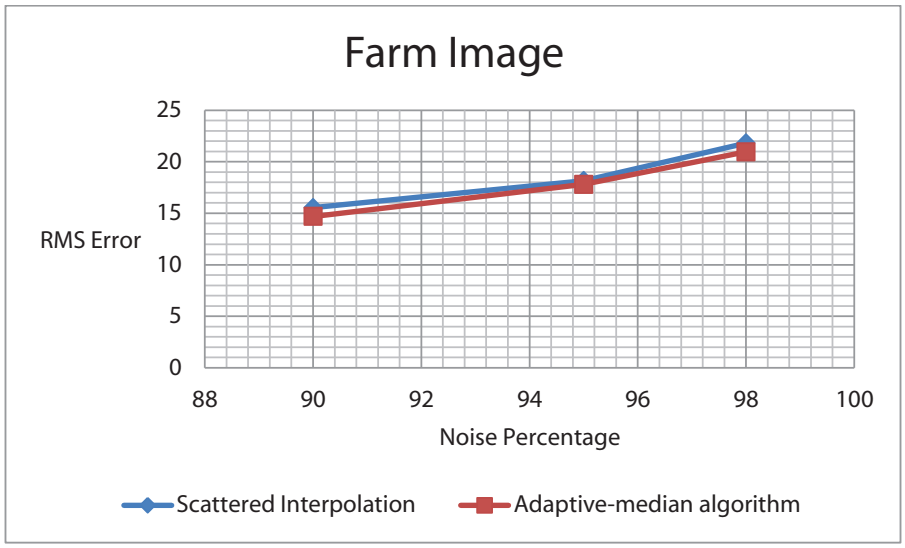

Figure 6| Performance curve for the proposed algorithm and standard interpolation method for the farm image.

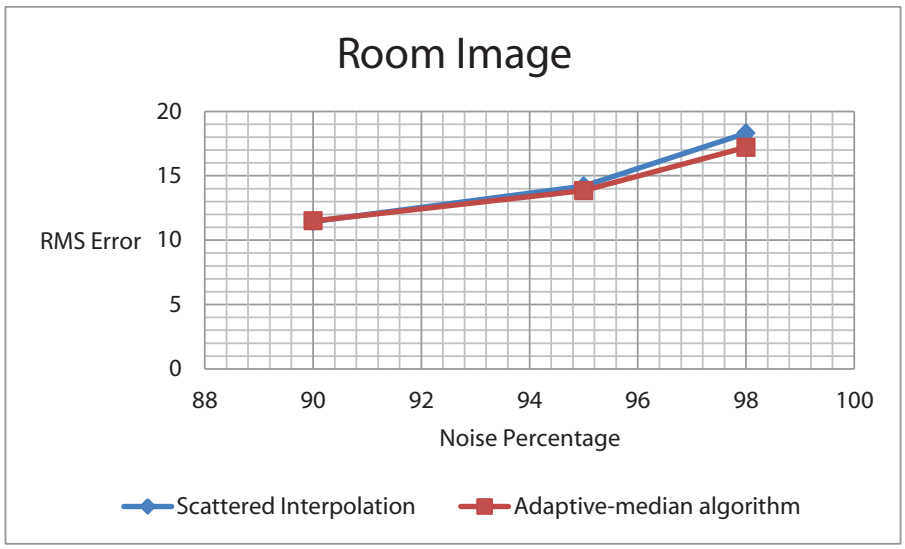

Figure 7| Performance curve for the proposed algorithm and standard interpolation method for the room image.

Iteration	Balloons			Farm			Room		
	90% noise	95% noise	98% noise	90% noise	95% noise	98% noise	90% noise	95% noise	98% noise
0	9.965	4.974	1.991	9.724	4.8356	1.959	9.948	4.996	1.987
1	60.96	36.768	16.506	59.8252	35.8247	16.286	60.896	36.929	16.478
2	99.260	91.534	62.510	99.1118	90.5767	61.692	99.310	91.763	62.219
3	100	99.960	96.514	100	99.9476	96.150	100	99.976	96.314
4	100	100	100	100	100	100	100	100	100

Table 1| Percentage of pixels restored for each iteration of the proposed algorithm for each image and noise level.

DISCUSSION AND FUTURE DIRECTIONS

Looking in Figures 2 through 4, it can be seen that for higher levels of noise, specifically the last two rows, our proposed algorithm provides an image that contains less visual artifacts when compared to the existing interpolation method. The interpolation method erroneously creates many false spots of color in the corners, as can be seen in Figure 2, column d. Our proposed algorithm also was better at maintaining contrast in the image, since the interpolation method blurred many of the key image details, such as the tablecloth in Figure 4, column d. It can also be seen from Figures 5 through 7 that our error curve (red) is slightly lower than the interpolation-based method's error curve (blue), thus showing that our algorithm successfully achieved lower RMS error. Our method provides a noticeable improvement on existing reconstruction methods for images, and computationally, ran just as fast as the existing methods; in some cases, ran noticeably faster, and thus has broad applications for this field. Future work will focus on trying to improve object edge textures, since these edges were the image regions that presented the most difficulties in accurate restoration.

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SHORT REPORT

The Effect of Behavioral Interventions on Body Weight in Children: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: The Centers for Disease Control and Prevention has acknowledged the growing epidemic of childhood obesity and, with it, the likelihood of an increasing percentage of obese adults in the coming years. This problem raises alarm because more people will be susceptible to heart disease, type 2 diabetes and other weight-related illnesses. Thus far, effective interventions have proven elusive.

Methods: We conducted a meta-analysis of pediatric obesity behavioral interventions to examine the effect for improving weight/body composition. We reviewed 14 studies (total N = 3,363) that examined the effects of behavioral therapies on weight change (reduced weight gain while growing or reduced body fat percentage) in children. The studies included are randomized control trials (RCTs) from a prior systematic review that documented weight change as a primary or secondary outcome and were published between January 2007 and July 2009. Using standard meta-analytic methods, we quantified effects to determine whether behavioral therapies are effective in improving body weight/body composition in children.

Results: The overall standardized mean difference of -0.23 with a 95% Confidence Interval of -0.38 to -0.07 indicates that behavioral therapies do have a significant effect as an intervention for improving weight outcomes in children. There was significant heterogeneity present (I^2 = 61%, p = 0.0003).

Conclusions: Behavioral interventions may be superior to other types of obesity interventions in children. Larger studies are warranted. These results may be utilized to treat patients, create policies and better inform the public. Future studies might increase intervention duration, which might improve maintenance of effects post-intervention.

Keywords: pediatric obesity, behavioral intervention, weight maintenance, meta-analysis

INTRODUCTION

Permanent weight loss is not an easy process. Most ongoing weight loss interventions are temporary and not effective. This is evident based on the growing obese population. The Centers for Disease Control and Prevention (CDC) states that childhood obesity has more than doubled in children in the last 30 years[1]. Childhood obesity increases the likelihood that a person will be obese later in life, thus contributing to growth of the obese population[2–4].

Obesity has become a major public health concern, and new studies are constantly being published about its prevention and treatment. The most common forms of treatment are diet, exercise, prescription drugs, surgery and behavioral therapies[2]. For this study, behavioral therapies are categorized as those that do not involve surgery or medication and utilize any form of a "lifestyle" education program, cognitive behavioral therapy, parent/guardian involvement, or the creation of support systems. In children, behavioral therapies and lifestyle interventions have shown the best results for weight maintenance[3].

A number of studies have reported effects of behavioral therapies for weight maintenance in children, but there have been few meta-analyses quantifying the degree of effects compared to other therapies. After categorizing studies as behavioral, we attempted to analyze whether behavioral therapies have a significant effect on pediatric weight gain and to quantify the effects of this form of intervention versus other forms of treatment. All of the studies evaluated are randomized control trials, which can provide the best measurement of effectiveness of an intervention.

METHODS

The initial step was to collect all the literature that reported behavioral therapy interventions for weight gain prevention in children. These were collected from a previous systematic review[5].

The following criteria were used in the present review: (1) the collected information was from a human study, (2) the study design used was a RCT, (3) sample sizes were at least 30 before randomization, (4) the intervention lasted 8 weeks or longer, (5) the primary or secondary outcome of the study was weight loss or prevention of weight gain, (6) the article could be acquired in English and (7) the study was published between January 1, 2007 and July 1, 2009. Additionally, studies included had to have utilized a behavioral therapy

intervention. Of the 45 pediatric studies, 15 were categorized as behavioral therapy interventions, 3 of which did not contain the necessary data to be included in the meta-analysis. We attempted to contact the authors for the 3 studies; two responses came after the allotted time, and were later added to the analysis. Of the 14[6–19] studies included, 5 of the studies had 2 intervention arms[11, 12, 14, 20].

For all the studies the raw outcome data extracted was in one of three forms:

- Body Mass Index (BMI)
- Body Weight in kilograms
- z-BMI score (age- and sex-adjusted values of BMI)

To analyze the data, the mean weight or BMI change within all arms of each study was required, along with the standard deviation or standard error. The methods used were adopted from the Cochrane Collaboration Handbook[19]. The mean difference and standard deviation for each study were either calculated manually or using Review Manager (RevMan) Software, version 5.3, from the Cochrane Collaboration website[20]. The RevMan software was also then used to create a forest plot with the included study values. After the initial analysis of data, a subgroup analysis was used to further summarize the data based on three age range subgroups: pre-pubescent (≤10 years), early puberty (10–12 years), and post-pubertal (≥12 years). In addition, a funnel plot was generated to evaluate the presence of selective bias reporting among the studies (a bias towards studies showing a positive effect).

Finally, a Risk of Bias Assessment was tabulated to gauge different areas of potential bias in each study. The Risk of Bias Assessment was conducted by two authors (KTW and AC) for each individual study. The resulting information was discussed (with KAK) to reach a consensus, and the final results were then tabulated.

RESULTS

The initial systematic review yielded 45 pediatric obesity studies; the selection process is shown in Figure 1. Among the included studies overall, there was a statistically significant effect for behavioral interventions on pediatric weight/body composition. The standardized mean difference (SMD) and confidence interval (CI) are highlighted in Figure 2. Three studies, of the 19 comparisons evaluated, showed results that favored the control versus the treatment arm, meaning there was a negative effect of treatment. The heterogeneity of outcomes among the included studies is significant, p = 0.0003 and I^2 = 61%. Figure 2 also displays the SMD of each study and its weight on the overall outcome.

As a post hoc data analysis, a subgroup comparison based on three age ranges was conducted: pre-pubescent (<10 years), early puberty (10–12 years), and post-pubertal (>12 years). For the pre-pubescent (n = 291) group of studies[8, 10, 12, 15] the SMD was -0.05, 95% CI = -0.37 to 0.27; heterogeneity of 65%, p = 0.009. In the early puberty (n = 1,793) group, the SMD was -0.37, 95% CI = -0.65 to -0.08; heterogeneity of 69%, p = 0.003. For the post-pubertal (n = 1,049) group[7, 13, 16, 17] SMD was -0.32, 95% CI of -0.67 to 0.03; heterogeneity = 55%, p = 0.07. The funnel plot in Figure 3 indicates that for the studies included in this meta-analysis, there was no evidence of reporting bias. The Risk of Bias Assessment pictured in Figure 4 evaluates potential sources of bias in individual studies as well as the potential risks of bias in the collective group of studies. The most common potential source of bias was the Lack of Attention Placebo in most of the included studies.

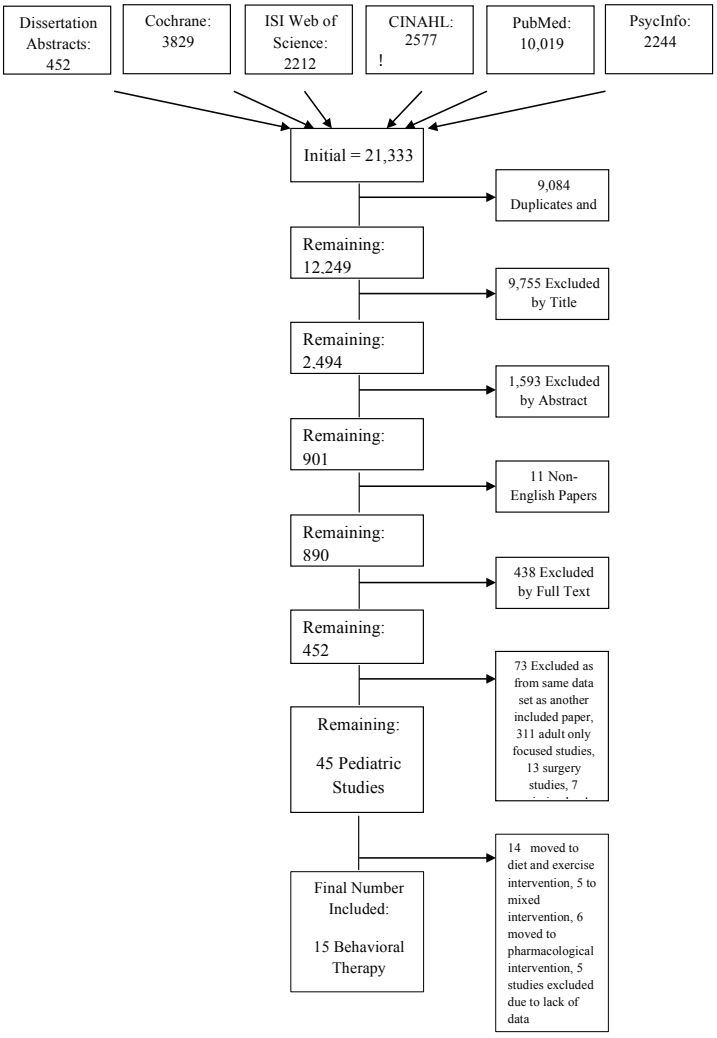


Figure 1| PRISMA diagram of literature search and selection process

DISCUSSION

Our results indicate that behavioral therapies generally have significantly favorable effects on body weight in children. In the post hoc subgroup analysis, by age range/pubertal status, the effect of behavioral therapies on body weight was not significant for the pre-pubescent and post-pubertal age groups. For the early puberty age group, the effect of behavioral therapies did have a significant effect. For the three subgroups, the SMD of effects appear largest in the early puberty age group (10–12 years).

Study or Subgroup	Std. Mean Difference	SE	Experimental Total	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Doyle 2008	-0.4472	0.2265	40	40	5.9%	-0.45 [-0.89, -0.00]
Epstein 2008	-0.163	0.245	35	32	5.4%	-0.16 [-0.64, 0.32]
Estabrooks (FC+IVR)	-0.05259	0.209573	62	36	6.3%	-0.05 [-0.46, 0.36]
Estabrooks 2009 Class	0.341398	0.215101	56	36	6.2%	0.34 [-0.08, 0.76]
Foster 2008	-0.0195	0.0695	749	600	10.6%	-0.02 [-0.16, 0.12]
Fullerton 2007	-0.75441	0.282388	40	20	4.6%	-0.75 [-1.31, -0.20]
Golley 2007 P	-0.0439	0.2874	37	18	4.5%	-0.04 [-0.61, 0.52]
Golley 2007 P+DA	-0.2578	0.2872	38	18	4.5%	-0.26 [-0.82, 0.31]
Janicke 2008 FB	-0.7209	0.3616	29	11	3.3%	-0.72 [-1.43, -0.01]
Janicke 2008 PO	-0.4437	0.367	31	10	3.3%	-0.44 [-1.16, 0.28]
Kalavainen 2007	-0.7526	0.2479	35	35	5.3%	-0.75 [-1.24, -0.27]
Kitzman-Ulrich 2009 Psych	-0.1	0.5969	13	4	1.5%	-0.10 [-1.27, 1.07]
Kitzman-Ulrich 2009 Psych+LS	0	0.5669	14	4	1.6%	0.00 [-1.11, 1.11]
McCallum 2007	0.0631	0.1619	80	73	7.7%	0.06 [-0.25, 0.38]
Shelton 2007	0.6793	0.3291	28	15	3.8%	0.68 [0.03, 1.32]
Sichieri 2008	-0.0805	0.0658	434	493	10.7%	-0.08 [-0.21, 0.05]
Tsiros 2008	-0.8997	0.308	25	22	4.1%	-0.90 [-1.50, -0.30]
Wilfley 2007 BSM	-0.609	0.2494	51	25	5.3%	-0.61 [-1.10, -0.12]
Wilfley 2007 SFM	-0.6192	0.2538	50	24	5.2%	-0.62 [-1.12, -0.12]
Total (95% CI)			**1847**	**1516**	**100.0%**	**-0.23 [-0.38, -0.07]**

Heterogeneity: Tau² = 0.05; Chi² = 46.02, df = 18 (P = 0.0003); I² = 61%
Test for overall effect: Z = 2.92 (P = 0.004)

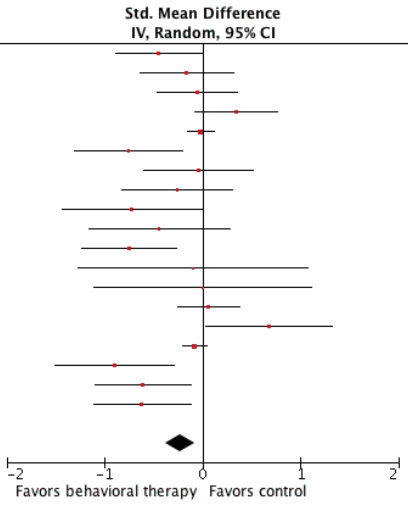


Figure 2| Forest Plot of Behavioral Therapy Effects on Pediatric Obesity

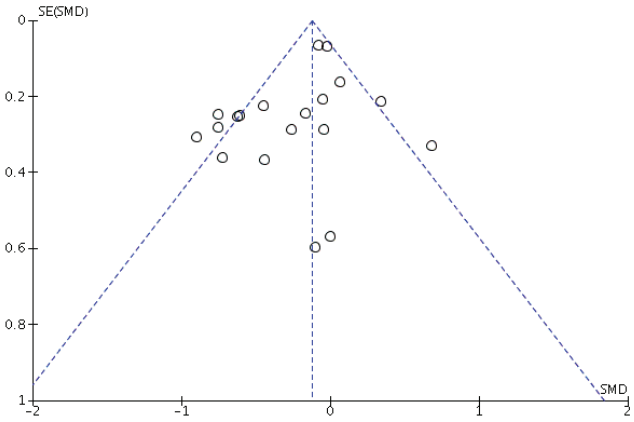
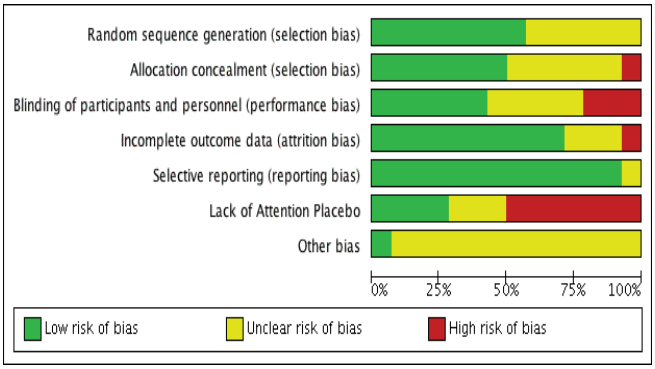


Figure 3| Funnel Plot Summary of Reporting Bias



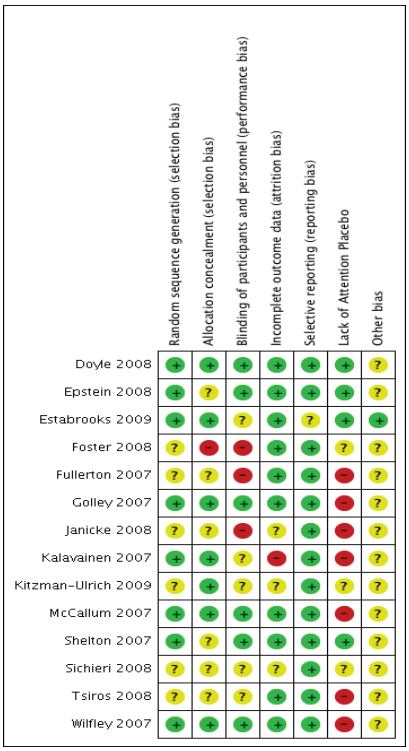


Figure 4| Study-level Risk of Bias (top) and Risk of Bias of all included studies (bottom)

This meta-analysis contained 14 recently published studies ranging in intervention duration from three months to two years. All included studies were randomized control trials, which help to minimize confounding effects. However, this meta-analysis was limited by the small number of studies and the high heterogeneity of observed effects. Future studies should include attention to placebo in their study designs to further minimize the potential for confounds. Additionally, given that other evaluations have indicated that lifestyle interventions are the best intervention for long-term weight maintenance in children[6], a standardized definition of behavioral therapy is desirable because it would allow better classification and comparison of studies.

In light of the continuing increase of pediatric obesity, the results of this study could be used in support of policies geared toward behavioral therapy-based interventions. However, studies have noted the complexity of designing lifestyle change programs for children because their environments are not isolated[19]. The best option for implementing such programs is to include parents in creating goals for their children and incorporating professionals from nutrition, health, and psychological backgrounds in the treatment

team[19]. The more educated the population becomes on ways to reduce weight gain or elicit weight loss in children, the more effective interventions can become in the future.

ACKNOWLEDGMENTS

This research was partially supported by the UAB PARAdiGM Program (NIH/NHLBI R25HL120883-KTW), NIH GM K12GM088010 (PLC), and NIDDK R01DK078826 (DBA). Dr. David Allison was the primary investigator for the systematic review from which included papers were identified. We are grateful to Dr. Allison for his mentorship and use of the data.

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Eye Lens Protein Composition in Aging Cataractous ICR/f Rats

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ABSTRACT

Although cataracts are the most prevalent cause of blindness in the world, the only effective treatment currently available is surgical lens replacement, an invasive and expensive procedure.[1] The probability of developing cataracts is a function of increasing age. Thus, as an increased proportion of people live longer, the need for a less invasive, more accessible treatment, including prevention, becomes more important.

Understanding the mechanism of cataract formation through the use of basic research approaches can potentially lead to alternative treatment. The ICR/f rat in this investigation is a spontaneous, hereditary model of cataract disease and is one of the few effective animal models available for the study of senile cataractogenesis. Because of mutations in chromosomes 8 and 15, all ICR/f rats will develop cataracts at approximately 75 to 80 days of age.[1]

The goal of this study is to examine changes that occur in the proteins in the eye lens of these rats with aging. The hypothesis is that changes in the solubility of lens proteins occur with aging and these alterations are associated with the process of cataract development.

The soluble protein in the lens ocular tissue collected from ICR/f rats of varying ages were analyzed by SDS-PAGE gels in order to characterize them by molecular weight. A method was developed to study the remaining insoluble lens protein fraction in conjunction with the soluble extractions.

The results from this investigation suggest that the once soluble lens proteins appear to become insoluble during the aging process. As the proteins precipitate out of solution in the lens, the refractive index decreases and the lens becomes opaque, the characteristic feature of cataracts. This method provides biochemical information about cataract development and can be used to evaluate the efficacy of potential cataract treatments.

Keywords: cataracts, aging, lens, vision, protein

INTRODUCTION

Although cataracts are the most prevalent cause of blindness in the world, the only effective treatment currently available is surgical lens replacement, an invasive and expensive procedure.[1] As with any other surgery, the risk of complications such as lens capsular tear or endophthalmitis, although rare, can accompany a cataract surgery.[2] In addition, a lack of trained professionals to complete this procedure or even the lack of the infrastructure necessary to accommodate for surgery can prevent patients from regaining their vision in third-world countries. The probability of developing cataracts is a function of increasing age. Hence, as an increased proportion of people live longer, the need for a less invasive, more accessible treatment, including prevention, becomes more important.

Understanding the mechanism of cataract formation through the use of basic research approaches can potentially lead to alternative treatment. The goal of this study is to examine changes that occur in the proteins in the eye lens of these rats with aging. The hypothesis is that changes in the solubility of lens proteins occur with aging and these alterations are associated with the process of cataract development.

Lens Structure

The lens is not innervated, lacks a blood supply, and does not come into contact with other tissues.[3] Instead, the aqueous humor provides the lens with nutrients and removes waste products through ion channels.[2] The lens is located between the cornea and retina and consists of structures that enhance its transparency and refractive index.[3] An example of these structures is the lens fiber, an anucleated, elongated epithelial cell. Lens fibers scatter little to no light because they have few organelles which allows for the transparency of the lens. However, they do not undergo apoptosis. [3] The eye lens continues to grow with age as fiber cells are constantly produced. Of the proteins present in the fiber cells, 90% of them are crystallin proteins, which will be discussed in detail in a later section. However, differentiated fiber cells cannot synthesize proteins, so the nucleus contains proteins largely from the period of embryogenesis.[4]

Filaments are also present in the lens. Some serve structural purposes but the unique beaded filament is somehow involved with maintaining lens transparency. This special filament may control distribution or orientation of the crystallin proteins to facilitate lens transparency.[3]

Energy and Metabolism

The lens capsule surrounds the eye lens and is essential to the metabolism of the lens. The lens capsule is a semi-elastic basement membrane and serves as a barrier between the lens and other tissues. Nutrients and other essential molecules pass through the lens capsule, but larger entities such as bacteria and viruses are inhibited from reaching the lens. Most of the metabolic activity occurs in epithelium which is attached to the lens capsule.[2]

Glycolysis is the main form of energy production because

very little oxygen is available to the lens.[5] The oxygen that is available is present in the aqueous and vitreous humors around the lens. However, the aerobic respiration pathway yields plenty of ATP in addition to the ATP produced through the less efficient but more common anaerobic pathway in the lens.[3]

Lens Protein

Crystallins are synthesized along with the lens fibers.[3] Most proteins in the ocular lens are low molecular weight, crystallin proteins.[6] Crystallin proteins are common to all vertebrates and are highly homologous in mammals.[4] The more water-soluble crystallin proteins present, the greater the refractive index of the lens.[3] A refractive index gradient is present in the lens since most of the protein is concentrated in the nucleus, the center of the lens. In contrast, little to no protein is present in the periphery, or cortex, of the lens.[2] Hence, the transparency of the lens depends on the fragile balance of the fractions of water and soluble protein; a deviation in concentration of either factor can impact the clarity of the lens.[3]

Furthermore, the high refractive index remains consistent throughout the lens because of the even distribution of lens protein. Transparency is achieved because of the close packing of the soluble and small crystallin proteins. Larger proteins tend to be less water-soluble; thus they cause more light to scatter, compromising lens transparency. Although the exact structure of the crystallin proteins is not known, the protein is believed to possess a tight spherical shape. The filaments in the cytoskeleton are believed to assist in the maintenance of the packing and distribution of the proteins.[3]

Of the three types of crystallins present in the lens, α-crystallins, the most common lens protein (40%), is the largest. The β-crystallin is moderate in size and γ-crystallins have the lowest molecular weight.[6] The molecular weight of a substance is the mass of the substance present in one mole and is generally expressed in Daltons (Da). Although the monomeric weight is 20 kDa, α-crystallin is usually found in complexes with itself, and the molecular weights of these complexes are most commonly within 300 to 1,000 kDa. However, the molecular weight of the native structure varies because of environmental factors; thus, the exact molecular weight is very difficult to accurately measure.[4] Methods have been developed to maximize the accuracy of these measurements, but the molecular weight of the native structure is not relevant or necessary for this investigation since the denatured protein was studied.

Since α-crystallins are the most abundant crystallin protein in the lens, the introduction will focus on elaborating on the types and known functions of this protein. αA and αB crystallins are the two subclasses of α-crystallins, and in humans, they have a 57% amino acid sequence homology. In mammals, these subclasses are generally present in a 3:1 ratio of αA to αB-crystallins. αA is found primarily in the eye lens and can act as a molecular chaperone. In contrast, αB is commonly found throughout the body and is classified to be a ubiquitous chaperone protein.[4]

Yet, the function of alpha crystallin is not fully understood, especially in tissues outside the lens. Prior to 1985, the αA-crystallin was believed to be exclusive to lenticular tissue, but αA-crystallins have been found in the spleen and thymus. αB-crystallins are found throughout the body. Increased amounts of αB-crystallins have been linked to many neurological diseases. Two genes code for the two classes of α-crystallins. The αA gene is on chromosome 21 and the αB gene is on chromosome 11 in humans.[7]

The lens cannot synthesize protein, so the proteins contained in the lens last a lifetime. However, the long lifespan of the lens proteins leaves them vulnerable to chemical and physical modifications.[5] These modifications and their relationship to cataracts are addressed in the following section.

Cataracts and PTMS

Older lens proteins are more susceptible to PTMs (post-translational modifications) such as deamination and aggregation. Deamidation is the most common cause of insolubilization in proteins. PTMs of the crystallins have been linked to nuclear hardening which leads to low antioxidant concentrations.[2] The role of antioxidants in cataract formation will be elaborated upon in the remainder of this section. Older proteins can denature or unfold. As mentioned previously, α-crystallins are small heat shock proteins that can act as a chaperone protein which maintains the solubility of any damaged proteins in the lens. But with age, α-crystallins are not as effective, so proteins begin to aggregate causing cloudiness in the eye. The increased protein aggregation can compromise the transparency of the lens leading to cataracts.[4]

In addition to aggregation, proteins can fragment with age. Full length proteins are common in the lens of younger rats, but protein fragments are ubiquitous in older rats.[8] Mutations in the alpha-crystallin protein have also been linked to cataract formation in humans and animals.[4] Currently, the opacification of the lens cannot be reversed; the lens can only be replaced with a prosthetic lens.[1]

Additionally, environmental factors can influence the transparency of the lens. Increasing exposure to ionizing and UV radiation can hasten the onset of cataract disease. Ultraviolet radiation leads to the production of reactive oxygen species (ROS). ROS cause protein denaturation which increases the risk for cataracts.[1] The antioxidants ascorbic acid and glutathione (GSH) along with the UV filter 3-hydroxykynurenine are naturally produced in the epithelium to protect the lens. GSH is most prevalent in the lens and helps prevent post-translational modifications of the

crystallin proteins. As the concentration of GSH in the lens nucleus decreases, the rate of PTMs increases. However, antioxidants in the lens rely on water-soluble transport. The rates of transport have been shown to decline with age which can hasten the onset of cataracts due to the lack of protection from oxidation.[2]

Thus, antioxidants are currently being researched as a potential therapeutic solution to cataract formation.[1] However, others factors have been linked to cataracts as well. Elevated calcium and sodium levels have been noted in naturally occurring cataracts, and increasing these levels have induced cataracts in experimental lenses.[3] An increased level of calcium is thought to facilitate the degradation of crystallins through the protease calpain.[2]

Furthermore, the loss of the compound lanosterol has been linked to the onset of cataracts in patients who lack the enzyme Lanosterol Synthase (LSS) which catalyzes the reaction that yields lanosterol. Lanosterol is an amphipathic intermediate in the *de novo* synthesis of cholesterol and is able to solubilize insoluble proteins. In a recent study, cataractous rabbit lenses were incubated in lanosterol resulting in clearer lenses. Further investigations are being conducted to evaluate lanosterol as a possible cataract treatment.[9]

Animal Models

Hereditary animal models without congenital onset are the closest representation of senile cataractogenesis, the focus of this study. The inheritance of cataracts in these rats is not fully understood. Inducible rats are often used to study the risk of cataract onset for conditions not directly associated with aging. For example, the inducible rats can be exposed to chemicals such as galactose and streptozotocin to study the effects of cataract formation in diabetics.[1]

The ICR/f rat in this investigation is a spontaneous, hereditary model of cataract disease and is one of the few effective animal models available for the study of senile cataractogenesis. Because of mutations in chromosomes 8 and 15, all ICR/f rats will develop cataracts at approximately 75 to 80 days of age. The rapid lens discoloration is believed to be caused by increased oxidation of the lens during early development in the rats. No statistically significant differences in cataract development have been noted between males and females of the same age under identical conditions.[10]

However, these rats develop cataracts much earlier in their life span, relative to senile cataractogenesis in humans, but no animal model is a perfect representation of human senile cataracts.[10,2] Moreover, the lens shape of rodents and humans vary because human lenses accommodate, but rodent lenses do not accommodate. In species that can accommodate, the lens adopts a flattened shape. In contrast, species that cannot accommodate have spherically shaped lenses.[3, 11] Despite some of the differences between human and rodent lenses, the ICR/f rat is a good model for this study. Rodents are a common model for this type of research. All of the ICR/f rats develop cataracts in a similar time frame which allows for a controlled study comparing lenses from rats of different ages. This particular strain was used in this investigation because the ICR/f rat colony has been maintained by this facility for several years. Thus, the care, breeding, and tissue collection protocols for these animals are not only standardized but are specific and effective for this colony. The formation of cataracts from the initial stages to the latter stages cannot be studied effectively with human lenses due to the paucity of human cataractous lenses.[2] Lenses from each stage of cataract development in the ICR/f rats were procured and analyzed.

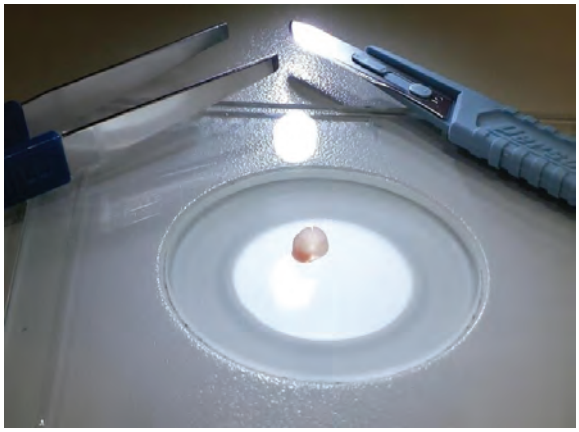

Figure 1| An eye from an ICR/f rat. The lens is isolated prior to experimentation (See "Experimental" section for more information.

EXPERIMENTAL

Homogenization and Extraction

The rat eyes were extracted prior to the start of the study immediately following euthanasia and were stored at -80 °C. Since only the lens was needed, each lens was removed from the eyeball using a scalpel and forceps under a microscope. The lens resides in a capsule within the eye. Each lens was then pulverized in the presence of liquid nitrogen using a glass homogenizer. The pulverized material was then transferred into a centrifugation tube.

Four aqueous extractions resulted from this process. An aliquot of 200 µL of Tris-HCl buffer (50 mM, pH 7.4) was added to the homogenized lens and the solution was centrifuged for 5 minutes at 16,000 rpm. Then, the supernatant was removed and 200 µL of Tris-HCl buffer was added again to the precipitate with vigorous mixing. The samples were centrifuged under the same condition once again. In order to solubilize non-aqueous proteins, the last two extractions were conducted using 6 M tris-urea solution (200 µL per extraction). The urea-protein solution was stored on ice for 15 minutes prior to centrifugation. These four supernates were stored at

-80 °C and were further studied in protein analyses. A solid "pellet" consisting of water-insoluble protein remained and was set aside.

Bradford Assay

A 20% Bradford Solution was prepared using BioRad Bradford Reagent. Five BSA Standards of 0, 4, 8, 12, and 16 µg mL^{-1} were analyzed in the spectrophotometer at 595 nm. The absorbance resulting from these standards were used to construct a linear calibration curve. The four extractions from each of the lens were all analyzed through the Bradford Assay. Each absorbance was converted to a corresponding protein concentration using the calibration curve. The results from the assay provide an estimate of the concentration of protein (µg mL^{-1}) present in each of the samples which was used to determine the amount of sample loaded into protein gels for further analysis.

The Bradford Assay was conducted for several lenses aged 40 days and 166 days. Eleven lenses were analyzed. The average lens protein concentration (µg mL^{-1}) for the two age groups is shown in the charts below.

Table 1| Average protein concentration for younger lenses (40 days old). The table below includes the average protein concentration values for the 4 extractions of 40 day lenses determined by interpolation into the BSA standard curve.

40 day Lens Extraction	Average Protein Concentration (µg mL^{-1})
Extraction 1 (Tris)	116.5
Extraction 2 (Tris)	10.63
Extraction 3 (Tris-urea)	4.97
Extraction 4 (Tris-urea)	3.75
Average Total Protein Concentration: 135.86 µg mL^{-1}	

Table 2| Average protein concentration for older lenses (166 days old). The table below also includes the average protein concentration values for the 4 extractions of 166 day lenses determined by interpolation into the BSA standard curve.

166 day Lens Extraction	Average Protein Concentration (µg mL^{-1})
Extraction 1 (Tris)	22.94
Extraction 2 (Tris)	11.47
Extraction 3 (Tris-urea)	9.15
Extraction 4 (Tris-urea)	5.58
Average Total Protein Concentration: 49.14 µg mL^{-1}	

When the results for older and younger lenses were compared, the total protein concentration is approximately two-fold higher in the younger rats than the older rats. The statistical significance of the data was evaluated (see "Statistical Analysis" subsection below). Based on the results from the two-tailed t-test ($\alpha = 0.05$), there is a statistically significant difference between the total protein concentrations of younger and older lenses.

According to the Bradford Assay, the older lenses contain significantly less lens protein. Protein cannot simply disappear. This "missing" protein had to go somewhere and was most likely present in the insoluble extraction. After lens homogenization, a white, solid pellet remains at the bottom of the test tube – this is the insoluble fraction. In the figure below, the vials containing the insoluble pellets for an older lens from a rat of 166 days and a younger lens from a rat of 40 days are shown below. A large pellet is prominent for the older lens, but the insoluble pellet is extremely difficult to see for the younger lens.

Therefore, it is possible that a larger fraction of the total lens protein precipitates out of solution as the cataract develops with age. One of the Bradford Assay's limitations is that all samples have to be in solution, so the concentration of the insoluble protein could not be measured by this assay or most other protein quantification methods.

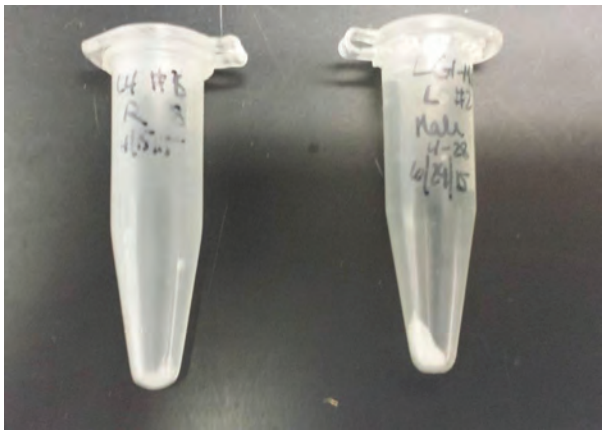

Figure 2| Vials containing the insoluble pellets for an older lens from a rat of 166 days and a younger lens from a rat of 40 days. A large pellet is prominent for the older lens, but the insoluble pellet is extremely difficult to see for the younger lens.

Statistical Analysis

A Grubbs' Outlier test was conducted to identify outliers in the data set. An F-test determined that a t-test assuming equal variances should be completed as opposed to a t-test assuming unequal variances. A two-tailed t-test ($\alpha = 0.05$) was then completed. Microsoft Excel was used for all tests and calculations.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Since the Bradford Assay could not be used, the auxiliary hypothesis was tested by analyzing the insoluble and soluble protein extractions through SDS-PAGE. Several trials were initially run to develop a procedure that allowed for the insoluble protein to be analyzed as the solid pellet had never been analyzed. Although the entire insoluble pellet did not go into solution, the solubilized proteins from the pellet were suitable for analysis by SDS-PAGE. The process of determining an effective protocol is further described at the end of this section.

Separating Gel

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) gels of 1.5 mm thickness were poured by hand. Tris-HCl (1.5 M, pH 8.8), 30% acrylamide/0.8% bisacrylamide, deionized water, TEMED, and ammonium persulfate were used to prepare the resolving gel. All of the lens samples were analyzed in 12.5% acrylamide gels unless otherwise noted. Other acrylamide percentages were tested with the lens protein samples, and 12.5% acrylamide gels yielded the best separation.

Once the unpolymerized gel was loaded, water saturated isobutanol (50% isobutanol and 50% water) was pipetted over the gel to flatten the gel, prevent evaporation and desiccation of the gel, and remove any air pockets present in the gel. Polymerization of the gel required a minimum of 30 minutes at room temperature. For overnight storage of the gel, the apparatus was wrapped in plastic saran wrap and stored at 4 °C.

Stacking Gel and Sample Preparation

After the separating gel polymerized, the stacking gel and samples were prepared. A solution of BioRad SDS Laemmli Buffer and mercaptoethanol was prepared and mixed with each of the samples. These solutions were then boiled in a hot water bath for 5 minutes.

A 3.9% acrylamide stacking gel was prepared and loaded over the separating gel once the isobutanol was removed. The stacking gel is composed of Tris-HCl (0.5 M, pH 6.8), 30% acrylamide/0.8% bisacrylamide, deionized water, TEMED, and ammonium persulfate. The volume of each solution varied based on the number of gels. A comb was placed between the plates as the stacking gel was loaded to create wells to load the sample after polymerization.

After the 30 to 45 minutes had elapsed for polymerization of the stacking gel, the casting set-up was dismantled and the gels were prepared for the next step. The apparatus was then placed in a buffer tank and the samples were loaded with a micropipette into the wells. The tank was filled with BioRad Tris-Gly-SDS running buffer. The lid was connected to the electrodes and the gel was run for approximately 45 to 60 minutes at 200 V depending on the gel.

Once the gels were removed from the electrodes and glass plates, they were placed in a 40% methanol and 7% acetic acid fixing solution. The trays were then washed with water before staining with approximately 20 mL of Invitrogen stain. The gels were de-stained with 7% acetic acid.

Procedure Development

A procedure was developed for analysis of the insoluble fractions. SDS was included in the sample preparation prior to gel loading to solubilize the insoluble proteins in the presence of heat. When insoluble proteins are heated in SDS, some of the insoluble proteins can solubilize. The amount of protein that becomes soluble depends on the nature of the proteins in the sample. Different volumes of the insoluble fraction were loaded on to the gel to determine the best loading volume. An example of one of the gels used to evaluate the solubilization method is shown below. This gel contains a lens from a 166 day old female.

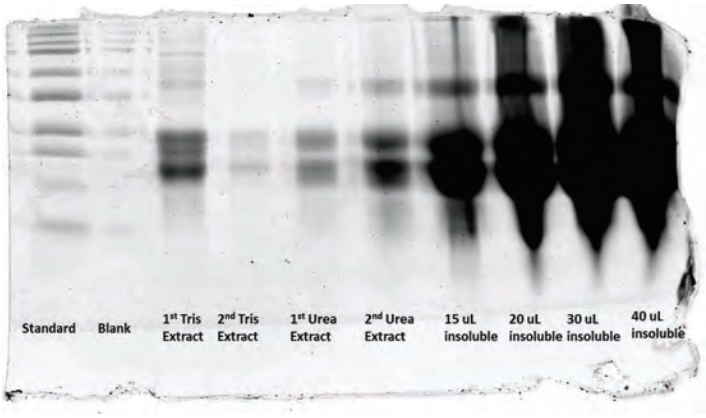

Figure 3| Loading volume test. The gel above contains a lens from a 166 day old female. Different volumes of the insoluble fraction were loaded on to the gel to determine the best loading volume.

This gel shows that the procedure used to prepare the insoluble fraction solubilized a sufficient portion of the insoluble protein analysis by SDS-PAGE. Volumes of 15, 20, 30, and 40 microliters of the insoluble fractions were loaded. All of these volumes resulted in overloaded lanes, so the succeeding gels were loaded with a lower volume of insoluble protein. Another gel with the lens from a 166 day old female was analyzed and is shown below.

Band separation in this gel is clearer, so three corresponding groups of bands can be seen for all of the extractions. Similar loading volumes were used for all subsequent gels.

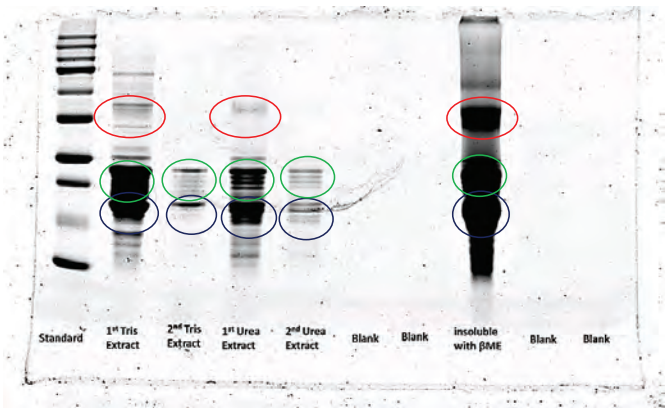

Figure 4| Gel with protein from a 166 day female. The gel above contains lens protein from another 166 day old female but this gel was run after a general procedure had been established. The band groups containing similar sized proteins are circled in matching colors for each of the extractions.

During sample preparation, β-Mercaptoethanol (BME) is usually mixed with the Laemmli Sample Buffer, a reagent that is added to all samples loaded onto the gel. BME breaks disulfide bonds between protein chains unfolding the proteins further. In order to gain a greater understanding of the nature of the proteins present in the insoluble fraction, the insoluble fractions from young and old lenses were analyzed without BME ("SDS Only") and compared with the same sample treated with BME (see Figure 5 in the Results section).

RESULTS AND DISCUSSION

The results of the Bradford Assay comparing the average total lens protein concentrations of relatively younger and older rats suggest there is a statistically significant difference in the amount of protein in the lenses of the two age groups. A two-tailed t-test ($\alpha = 0.05$) was conducted to evaluate the assay results. The p-value was calculated to be 0.021. These results prompted the study of the solid pellet that remains after the lenses are homogenized. (See Experimental section for more information.) A procedure to study the protein in the insoluble fractions through SDS-PAGE was developed. The results from the resulting experiments are shown below.

As described in the Experimental section, the band separation is more discernible in the gel presented in Figure 4. Three corresponding groups of bands are visible in each of the lanes.

These groups are marked in red, green, and blue circles. The 2nd Tris buffer and Tris-urea buffer extractions do not contain as much protein as the other extractions since most of the proteins soluble in each of the buffers were extracted in the first extraction. Thus, the bands are fainter for the second extractions, and the highest molecular weight band (red circle) is extremely faint and not visible for these two extractions.

The results from the gel suggest that the proteins present in the insoluble fraction are the same proteins in the other aqueous extractions since they all have bands in the same molecular weight region of the gel. The main difference among the extractions is the intensity of the bands. Some of the extractions contain more protein. For example, the darkest bands are from the insoluble extraction, so the most protein in the 166 day old female's lens is concentrated in the insoluble fraction. The 1st Tris extraction contains less protein than the insoluble fraction but more protein than the other extractions. The following lists the data in descending order (highest protein content to least): the insoluble fraction, 1st Tris buffer extraction, 1st Tris-urea buffer extraction, 2nd Tris buffer extraction, and 2nd Tris-urea buffer extraction.

Furthermore, the group marked by the green circle contains bands in the 20 kDa range. α-crystallin proteins, the most common protein in the eye lens, has a molecular weight of 20 kDa[6], so these bands on the gel are most likely to be α-crystallin. Since the insoluble fraction contains bands in the same region, it is possible that the insoluble extraction contains α-crystallin proteins that have become insoluble. α-crystallin is usually soluble, so its presence in the insoluble fraction is unexpected (see Introduction).

To better understand the proteins in the insoluble fraction, the insoluble fractions from young and old lenses were analyzed without BME ("SDS Only") and compared with the same sample treated with BME (see Figure 5). Insoluble protein from a young rat (40 days) and an old rat (166 days) were analyzed to study the potential differences correlated with age.

The bands resulting from the samples treated with BME are generally of lower molecular weight than the samples treated with SDS only in the Sample Buffer because the BME further fragments the proteins by breaking the disulfide bonds. The samples treated with only the SDS in the Laemmli Sample Buffer are unfolded by the SDS, but they are not as fragmented. Therefore, the insoluble proteins unexposed to BME provide more information about the structure of the proteins as there are more variations in size and an increased number of high molecular weight proteins.

Although most bands are still present in the 20 kDa region (green circle), the samples not treated with BME show extensive "streaking" in the lane indicating that a variety of proteins with different molecular weights are present in the sample. Since the lens cannot synthesize proteins, these unidentified proteins have to be proteins already present in the lens. α-crystallin proteins are the most common protein in the lens, so these proteins could potentially be α-crystallin polymers of varying sizes. However, this assertion cannot be confirmed by SDS-PAGE. Mass spectrometry (MS) could provide more information about the insoluble protein.

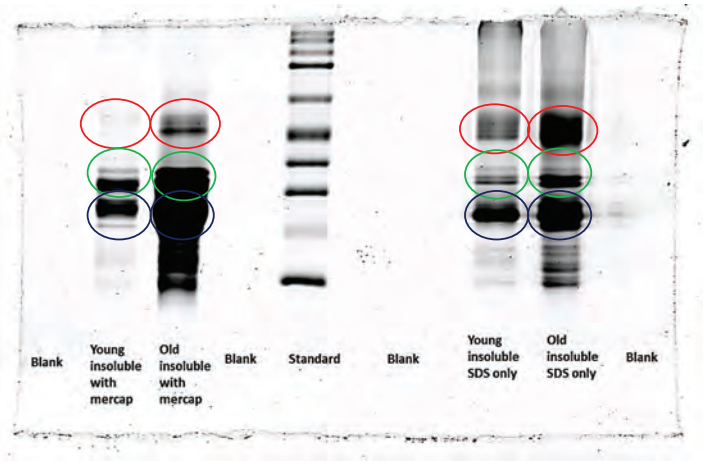

Figure 5| Insoluble protein gel analysis (with and without β-Mercaptoethanol or BME). To better understand the proteins in the insoluble fraction, the insoluble fractions from young and old lenses were analyzed without BME ("SDS Only") and compared with the same sample treated with BME. The "young" lens is from a rat of 40 days of age and the "old" lens is from a rat of 166 days of age.

In addition to the differences between the lens protein in the presence of BME and the protein in the absence of BME, disparities in the concentration of the insoluble protein are apparent when the young and old lenses are compared. The younger lens appears to have less insoluble protein. At 40 days of age, the rats do not typically have visible cataracts, but rats that are 166 days old have a fully developed, stage 4 cataract. The increased precipitation of lens protein in older rats may be correlated to the phenotypic appearance of the clouding in the lens. α-crystallins may polymerize in response to a signal or change within in the lens that occurs in these rats as they age.

Consequently, this hypothesis based on the SDS-PAGE and Bradford Assay results was tested by running SDS-PAGE gels for lenses of varying ages:

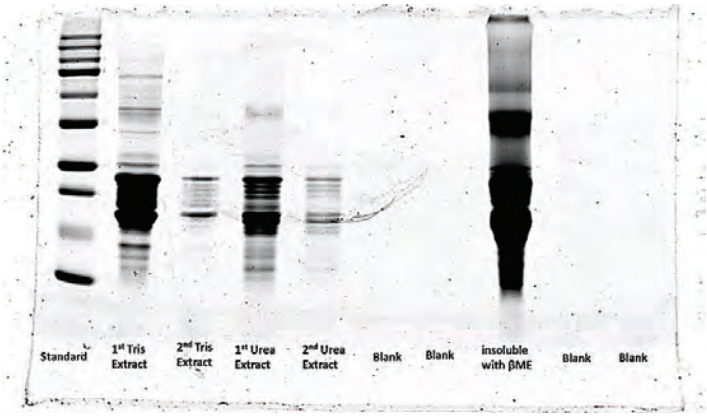

Figure 6| Gel with 166 day female. The aqueous and insoluble fractions of a lens from a 166 day old female were analyzed by SDS-PAGE. This gel is the same gel as the one in Figure 4 and is juxtaposed with the other gels for convenience.

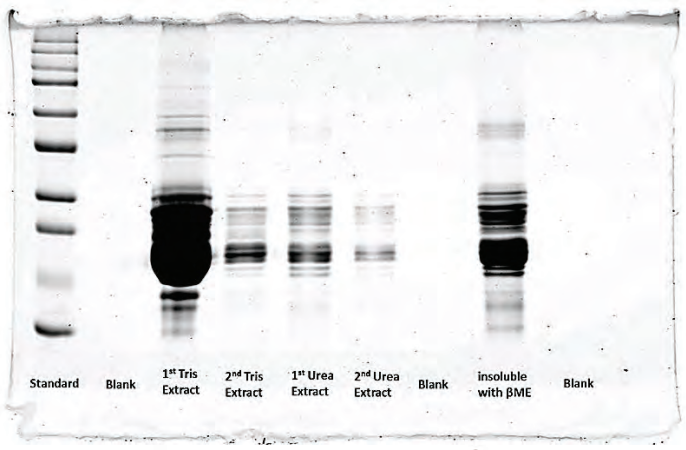

Figure 7| Gel with 66 day female lens. The aqueous and insoluble fractions of a lens from a 66 day old female were analyzed by SDS-PAGE.

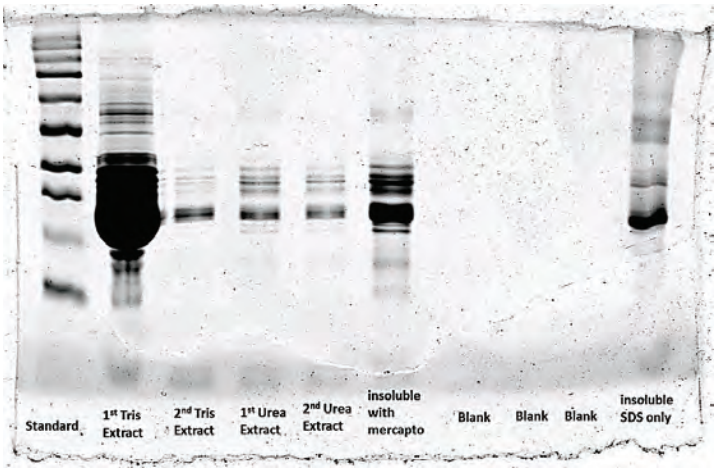

Figure 8| Gel with 46 day female lens. The aqueous and insoluble fractions of a lens from a 46 day old female were analyzed by SDS-PAGE.

Three lenses for each age group were analyzed. The loading volume and all other experimental aspects were controlled; the only difference among the gels is the age of the lens analyzed. A gel from one of three trials for each age is shown in Figures 6–8 above. Most of the protein in the youngest lens (46 days old, Figure 8) is concentrated in the first aqueous extraction suggesting that the majority of the proteins are soluble. A much smaller proportion of the protein is present in the insoluble fraction, as expected, since a 46 day old rat generally does not exhibit visible lens clouding. Similar to the 46 day old rat lens, the 66 day old rat lens contains predominantly soluble protein but the band for the 1st Tris buffer extraction is not as intense as that of the 46 day lens. However, the insoluble extraction appears to be more intense for the 66 day lens.

In contrast, the 166 day old lens (Figure 6) has a strikingly lower amount of protein in the aqueous extractions. The 1st Tris buffer extraction band is intense but not nearly as dark as those of the younger lenses. The decrease in protein in

the aqueous extractions is compensated by the increase in protein in the insoluble extraction. The band for the insoluble extraction of the 166 day lens is as dark as the band for the 1st aqueous extraction of the 46 day lens.

For easier comparison of older and younger lenses, the following gel juxtaposes the major lens extractions for a 166 day and 40 day old rat:

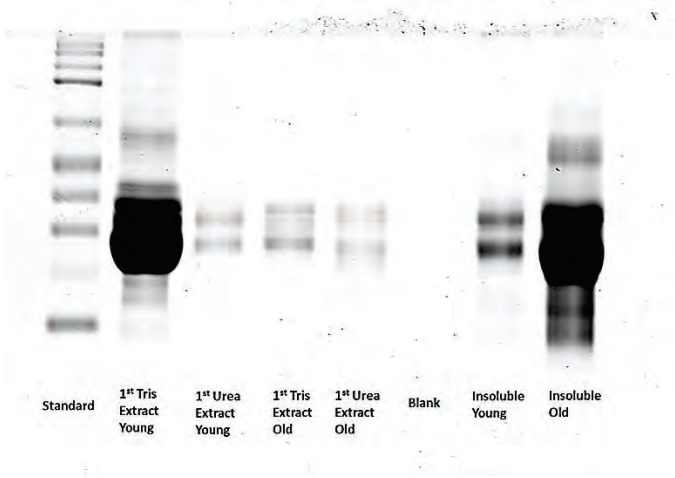

Figure 9| "Summary Gel." The gel above juxtaposes the protein from a young and old lens (40 days and 166 days of age, respectively). Qualitatively, the 1st Tris buffer extraction for the young lens and the insoluble extraction for the old lens have similar concentrations. Similarly, the insoluble extraction for the young lens and the 1st Tris buffer extraction for the old lens have similar concentrations.

As seen with Figures 6–9, the 1st Tris buffer extraction for the young lens and the insoluble extraction for the old lens have similar concentrations. Similarly, the insoluble extraction for the young lens and the 1st Tris buffer extraction for the old lens have similar concentrations. Because the 166 day lens belonged to an older rat, the cataract was fully developed (Stage 4), so more of the proteins in the lens were insoluble. All of the lanes appear to have protein bands in the three regions highlighted in Figures 4 and 5 suggesting that all of the lens extractions contain the same proteins but in different forms and varying distribution during cataract development. The results seem to indicate that once soluble lens proteins are prompted to become insoluble during the aging process. As the proteins precipitate out of solution in the lens, the refractive index decreases and the lens becomes opaque, the characteristic feature of cataracts.

In addition to the time points compared above, two older lenses were compared. A 187 day male lens was analyzed and compared with the 166 day female lens from Figure 6. A 166 day old male or a 187 day old female was not available, so the lenses from two different genders had to be compared.

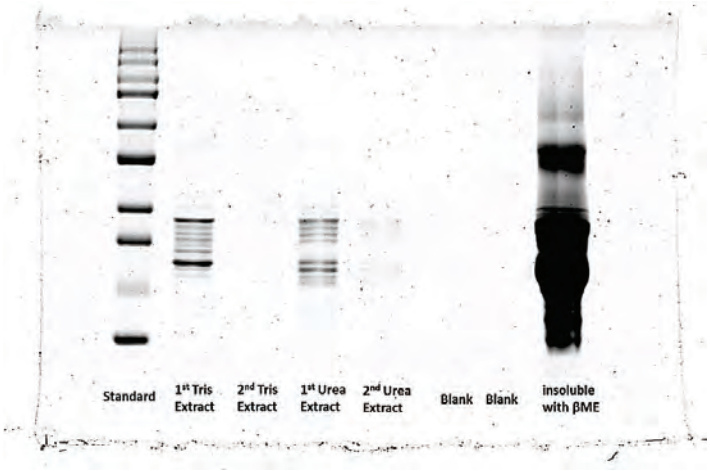

Figure 10| Gel containing lens protein from a 187 day old male. The aqueous and insoluble fractions of a lens from a 187 day old male were analyzed by SDS-PAGE.

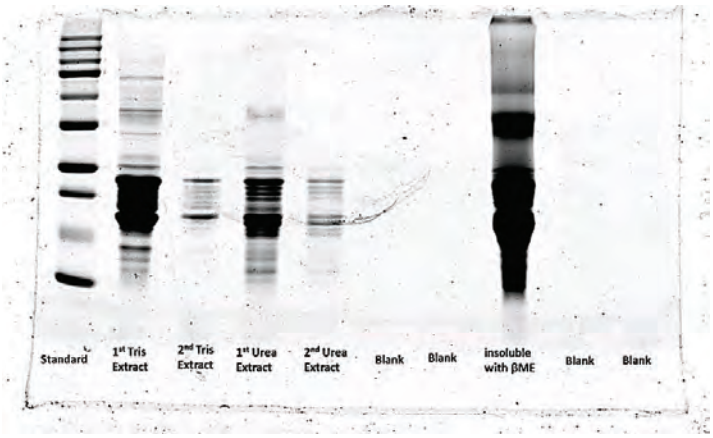

Figure 11| Gel containing 166 day female lens. The aqueous and insoluble fractions of a lens from a 166 day old female were analyzed by SDS-PAGE. This gel is the same gel as the ones in Figures 4 and 6 and is juxtaposed with the gel containing the 187 day male lens for convenience.

Based on Figures 10 and 11, protein aggregation continues to occur with age, even when the cataract is considered to have reached the latest stage in the clinical classification scheme. More protein is present in the insoluble fraction for the 187 day old since the band is much darker than that of the 166 day old rat. The aqueous fraction bands are extremely faint for the 187 day lens.

Sex-based Differences and Experimental Limitations

Although the SDS-PAGE results seem promising, a major limitation is that only three time points were studied (46, 66, and 166 day old rats). Ideally, the lenses from rats of 30 days to 80 days should be studied for a total of six time points. An increased number of trials would also improve the accuracy of the results. In this investigation, three trials were conducted for each of the time points, but for the 66 day lenses, two of the rats were female and one was male. There was not sufficient time to analyze a third 66 day old female rat.

Based on the lenses that were studied, a difference between male and female lenses of the same age was not observed. However, only one male lens with a comparable female lens was studied (both lenses were from 66 day old animals). The resulting gels are shown in Figures 12 and 13.

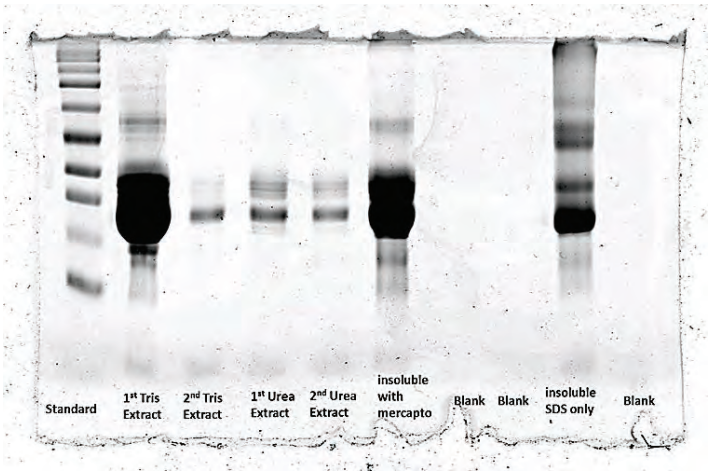

Figure 12| Gender comparison, male lens. The aqueous and insoluble fractions of a lens from a 66 day old male were analyzed by SDS-PAGE.

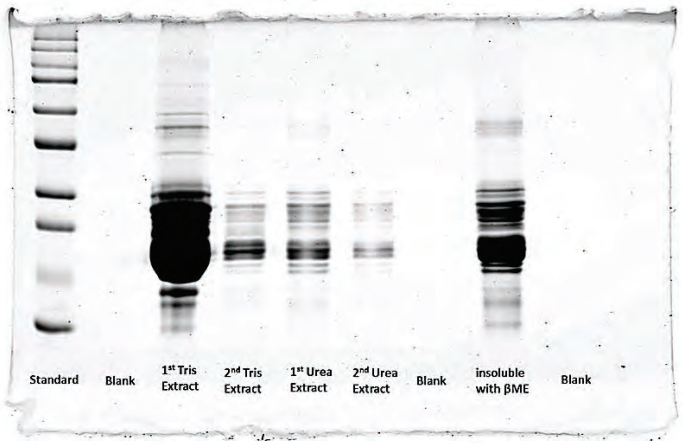

Figure 13| Gender comparison, female lens. The aqueous and insoluble fractions of a lens from a 66 day old female were analyzed by SDS-PAGE. This gel is the same gel as the one in Figure 7 and is juxtaposed with the gel with the 66 day male lens for convenience.

A difference between the two genders is not apparent, but only one male lens was analyzed. More male lenses at this time point and at other time points should be analyzed in order to determine if a sex-based difference in lens protein distribution exists. Nevertheless, some variation can be seen between the two gels. This variation is also present among gels of the same age and even two different gels containing lens protein from the same lens. Thus, comparing lenses of similar ages is difficult because the differences in the bands are too minute. Qualitative analysis of gels from lenses of significantly different ages is easier because the disparities in the bands are very noticeable and cannot be explained by variation inherent to the gels. Therefore, quantitative analysis would be a likely solution to this limitation.

Future Studies

As mentioned previously, MS can be used to identify and quantify the proteins present in each of the aqueous extractions. MS will be able to verify the protein identifications that were tentatively suggested based on current literature and the results from the SDS-PAGE gels. An untargeted mass spectrometry analysis could also reveal the entire protein composition of the insoluble and aqueous lens fractions.

Furthermore, densitometry is a method that can quantify relative protein content of the aqueous and insoluble fractions by analyzing the intensities of the corresponding bands on the gel. Quantitative analysis would help distinguish normal variation from actual differences in the bands of two gels. In addition, this method would also allow for the comparison and validation of data by statistical analyses such as the t-test.

Dark bands are convenient for the qualitative analysis whereas lighter bands are necessary for quantitative analysis by densitometry. In the gels shown in this paper, some of the lanes are often overloaded, usually the insoluble fraction or 1st Tris buffer extraction depending on the age of the lens. The loading volume could have been reduced to prevent overloading, but because of the disproportionate protein distribution among the extractions, the already faint bands would no longer be visible with a decrease in loading volume. Hence, qualitative analysis would not be possible for these gels. However, for densitometry, a lower loading volume would be required because the instrument measures the intensity of the bands and cannot distinguish between very high intensity readings. Although some bands will be too faint to see with the naked eye, the instrument would still be able to detect those bands.

On the other hand, the same protein concentration could be loaded onto the gel to ensure all the bands are visible, but the protein concentration cannot be determined for the insoluble fraction. Also, the purpose of this experiment was to compare relative protein content among the aqueous and insoluble fractions within in the same lens in addition to comparing the results of different lenses. For these reasons, a consistent loading volume for all the protein samples was selected.

A two-dimensional gel analysis in which the SDS-PAGE gel and the isoelectric strip could be run at once to separate the proteins based on molecular weight and isoelectric point could also be conducted. The 2-D gel will provide an additional perspective to the study.

Although no control group was used due to a limitation in resources in this investigation, white Wistar rat lenses should also be analyzed at identical time points. The control group would indicate the natural change in lens protein composition that occurs with aging. The addition of the control group would result in a more thorough analysis and, therefore, will provide a better understanding of the unique changes in the lens that leads to cataracts.

Applications in the Medical Field

To further understand cataract development, the phenotypic appearance of cataracts can be related to the results from the biochemical approach this technique provides. The rat's lenses can be analyzed by slit-lamp prior to ocular tissue extraction. Furthermore, this technique can be used to evaluate the effectiveness of a potential cataract treatment in addition to following the progression of cataracts with age. Untreated cataractous lenses can be compared to treated cataractous lenses by comparing the relative protein contents of the aqueous and insoluble fractions.

CONCLUSION

A difference in total protein concentration between a young and an old lens prompted the study of the previously unanalyzed insoluble fraction. When 46, 66, and 166 day female lenses were compared by SDS-PAGE, once soluble lens proteins appear to become insoluble during the aging process. As the proteins precipitate out of solution in the lens, the refractive index decreases and the lens becomes opaque, the characteristic feature of cataracts. Furthermore, based on the comparison of a 187 day lens and a 166 day lens, the aqueous lens protein continues to precipitate even when the cataract is considered to have reached the latest stage in the clinical classification scheme.

No difference could be distinguished between male and female rats of the same age, but this conclusion is based on a statistically insignificant number of trials. More trials could not be conducted in this investigation because of constraints in time and resources. Nevertheless, current literature suggests that there is no sex-based difference in cataract formation.[10]

However, this study did not address the composition of the proteins in each of the fractions made from the lenses at different ages. Besides 2-D gel electrophoresis, further studies should use mass spectrometry to identify and quantify the protein in the different lens fractions.

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Investigating the Regulation of Alpha-Synuclein Clearance by Alpha-Galactosidase-A in Parkinson Disease

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ABSTRACT

Parkinson Disease (PD), the second most common neurodegenerative disease, affects 1% of Americans over the age of 65. PD pathology is characterized by dopaminergic neuron loss and accumulation of insoluble alpha-synuclein (αsyn) aggregates. Pre-clinical studies suggest that the aberrant accumulation αsyn contributes to PD pathogenesis. The autophagy lysosomal pathway (ALP) is a metabolism pathway capable of high capacity clearance of αsyn. Thus targeting the clearance of αsyn, in particular by enhancing ALP function, could be a valuable treatment option for PD. We have shown previously that brains of mice deficient in the lysosomal enzyme alpha-Galactosidase A (αGalA) exhibit the pathological accumulation of αsyn concomitant with ALP dysfunction. ***These findings led us to hypothesize that increasing αGalA activity would enhance αsyn clearance.*** To test this hypothesis, we examined if increasing αGalA activity using recombinant αGalA (Fabrazyme, Genzyme Corp.) accelerates the clearance of conditionally over-expressed αsyn in M17 human neuroblastoma cells. Fabrazyme treatment increased the level and activity of αGalA in M17 cells and promoted the clearance of over-expressed αsyn. These data suggest the utility of αGalA activity as a therapeutic target for promoting the clearance of αsyn in a preclinical model of PD.

Keywords: Alpha-Galactosidase A, Alpha-Synuclein, Parkinson Disease, Autophagy Lysosomal Pathway

INTRODUCTION

Parkinson Disease (PD), the second most common neurodegenerative disease, affects 1% of Americans over 65 and this number is expected to rise[1]. This disease not only affects patients and their families, but also has a profound economic impact. Expenditures on PD medical care in the U.S. exceed 8 billion dollars annually; additional economic impact comes from lost employment and other non-medical factors associated with the disease[1]. PD is characterized by classic motor symptoms such as resting tremor, rigidity, and bradykinesia that result from the progressive degeneration of dopaminergic neurons in the substantia nigra. PD is also characterized by non-motor symptoms such as depression, personality changes, and cognitive impairment[2]. Approved therapies for PD treat symptoms but do not prevent or delay disease progression. Faced with these challenges, the discovery of novel therapeutic targets is critical for the future treatment of PD.

PD is a member of the class of disorders called synucleinopathies, characterized by the inclusion of accumulated insoluble αsyn aggregates known as Lewy bodies[3]. The function of αsyn is not well known, but it is believed to play a role in regulating vesicular neurotransmitter release[4]. Normally, αsyn is a soluble monomer, but *in vitro* studies show it is able to undergo a process of folding and oligomerization, yielding insoluble fibrils similar to what are seen in PD brain[5]. The aberrant accumulation of monomeric αsyn is believed to promote its misfolding into insoluble species, the accumulation of which is evident in synucleinopathies like PD. Studies of mutations in αsyn genes, such as *SNCA* triplication, in familial PD provide support for the pathogenic role of αsyn in PD[6]. Further evidence supporting a role for αsyn in PD pathogenesis is supported by the Unified Lewy Body Staging system, in which the incidence of αsyn-containing Lewy bodies progressing from lower to higher brain regions correlates with the progression of PD symptoms[2]. While the mechanisms by which αsyn regulates PD pathogenesis are poorly understood, its neurotoxic potential has been shown in numerous *in vitro* and *in vivo* studies[7-11]. Therefore, therapeutics aimed at promoting the high-capacity clearance of αsyn, could be valuable as a method to reduce αsyn pathology in PD[12].

The ALP is an intracellular degradation pathway that maintains energy balance and organellar quality control and is responsible for the high capacity clearance of αsyn[13]. Notably, function of the ALP has been shown to decline with normal brain aging and is disrupted in PD brain[14-19]. Experimental inhibition of the ALP has been shown to increase αsyn accumulation while its experimental induction has been shown to attenuate αsyn accumulation[11,19-26]. Together these studies suggest that ALP dysfunction may contribute to αsyn-associated pathogenesis in PD and that the ALP could be targeted in the development of PD therapeutics.

The glycosphingolipid metabolism pathway involves the action of several lysosomal enzymes and has been shown previously to be disrupted in PD[17,27]. Alpha Galactosidase A (αGalA) is a soluble lysosomal enzyme in this pathway. Its deficiency, caused by many different mutations in the human *GLA* gene that encode αGalA, results in Fabry disease, a

lipidosis type of lysosomal storage disorder[28]. Our lab has shown that brains of mice deficient in αGalA exhibit the pathologic accumulation of αsyn concomitant with alterations to ALP markers suggesting that αGalA regulates αsyn metabolism, potentially through disruption of ALP function[20]. These findings led us to propose αGalA as a candidate therapeutic target for promoting the effective clearance of αsyn.

In the present study, we tested whether increasing αGalA activity in a neuronal cell line increases the clearance of over-expressed αsyn, to determine if αGalA is a useful therapeutic target for PD. Using this model system, we evaluated an *in vitro* cell model of the PD synuclein pathology disease state. We also investigated whether increasing αGalA activity promotes the clearance of over-expressed αsyn.

MATERIALS AND METHODS

Cell culture maintenance and lysate collection

We obtained an M17 cell line with conditional tetracycline inducible (tet-on) expression of the gene encoding human wild type αsyn as a generous gift from Dr. Talene Yacoubian (UAB Dept. of Neurology) and as previously reported[29]. M17 human neuroblastoma cells were maintained in MEM media with 10% fetal bovine serum and 500 μg ml^{-1} G418 (for selection pressure) and allowed to grow between passages to 70–80% confluency. Cells were treated with the tetracycline derivative, Doxycycline (Dox; 10 μg ml^{-1}) for up to 7 days to drive the conditional over-expression of αsyn. To assess clearance of over-expressed αsyn, Dox was removed from the media and the cells were allowed to continue to grow for up to 7 days in its absence.

αGalA increase by Fabrazyme

Our lab has recently obtained the pharmaceutical Fabrazyme (Fz; Genzyme Corp.), a type of recombinant enzyme replacement therapy (ERT) that is approved for human use in the treatment of Fabry disease[30]. Fz was administered in culture media at concentrations ranging from 1–100 μg ml^{-1} for 24–72 hours. Following Fz treatment, culture media was removed and cells were washed with 1X PBS to remove extracellular Fz. Whole cell lysates were then prepared in a 25 mM HEPES lysis buffer containing 1% SDS, 1% Tritox X-100, 1% protease inhibitor cocktail (Sigma P8340), and 1% each of two different phosphatase inhibitors (Sigma P5726 and P0044).

Detection of αGalA activity

Whole cell lysates were assessed for αGalA activity using a fluorometric activity assay as previously described[31]. Active αGalA enzyme cleaves the assay substrate, 4-Methylumbelliferone α-D-galactopyranoside (Sigma M7633) to 4-Methylumbelliferone (Sigma M1381). N-acetyl-D-galactosamine (Sigma A2795) was used to inhibit the non-specific activity of αGalactosidase B (N-acetyl-α-D-Galactosaminidase), which also cleaves the 4-Methylumbelliferone α-D-galactopyranoside substrate[32]. Fluorescence of the cleavage product 4-Methylumbelliferone was measured at 460 nm. Activity is reported as units of nmol converted substrate/mg protein in sample/hour. Proteins were quantified using the bicinchoninic acid (BCA) protein assay as previously reported[25-26].

Detection of αGalA and αsyn protein levels

Western blot of whole cell lysates was used to detect the levels of proteins of interest using methods as previously described[23,25-26]. Antibodies used include Santa Cruz sc-7011C-20 clone and BD Biosciences 610787 to detect total αsyn monomer (17 kDa), Abcam ab168381 to detect αsyn phosphorylated at serine 129 (P129S- αsyn) (17 kDa)[33], and Genetex GTX101178 N1C2 to detect αGalA active species (46 kDa)[34]. Side by side comparisons of Santa Cruz and BD Biosciences antibodies for total αsyn indicated no difference in detection of 17 kDa monomer (data not shown). Gels were run with 20–25 μg protein per lane with equal loading across each gel using an N of 3–4 per data point. Blots were probed for antibodies of interest then stripped using Restore™ Western Blot Stripping Buffer (Thermo Scientific 21059) and re-probed for either actin (Sigma 1978; 42 kDa) or GAPDH (Cell Signaling 2118; 37 kDa) loading controls. Films were scanned and quantified using UN-SCAN-IT™ software (Orem, UT).

STATISTICS

Statistical analyses were completed using either Student's t-test for comparisons between two treatment groups, or one-way analysis of variance (ANOVA) for comparisons between multiple treatment groups. Post hoc analysis was performed using Bonferroni's test with significance set *a priori* at $p < 0.05$.

RESULTS

First, we established a working *in vitro* model of regulated, conditional over-expression of αsyn in which we could monitor its clearance over time. We chose the M17 human neuroblastoma cell line, an immortalized cell line with neuronal processes, commonly accepted as an *in vitro* model for neurons[29]. Treatment with Dox significantly increased levels of total αsyn 17 kDa monomer after 3–7 days (Day 3; Day 7) (Fig. 1). We also probed for P129S-αsyn (17 kDa), a pathologic species of αsyn known to accumulate in PD brain and localize to Lewy bodies, and found that it was significantly increased after 7 days of Dox treatment (Fig. 1). The increases in both species were still present at 1 day (Day -1) after Dox removal (Fig. 1). Between 1 and 3 days after Dox removal (Day -1; Day -3) the levels of both total and phosphorylated αsyn began to decline, and by 7 days after Dox removal (Day -7) they returned to approximately basal levels (Fig. 1).

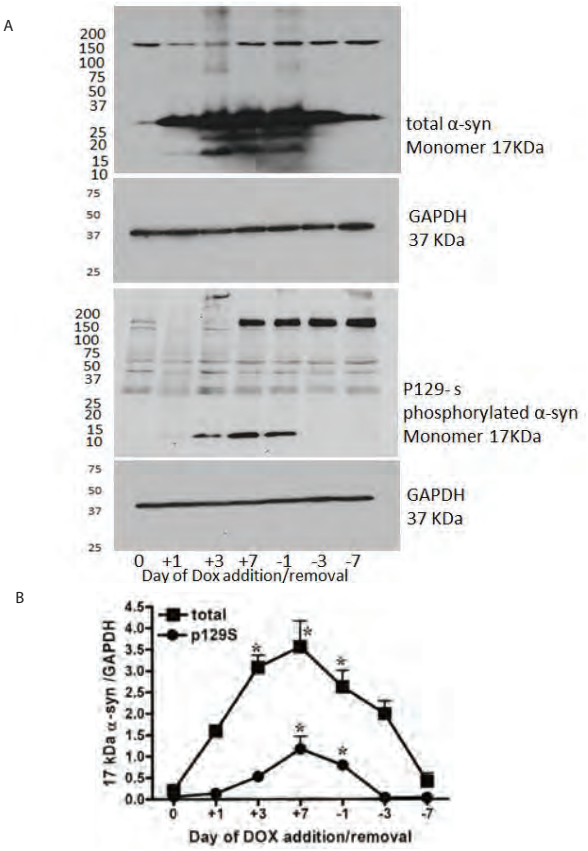

*Fig 1| Relationship of αGalA activity and αsyn protein quantity. (a, b) Western blot indicates conditional over-expression and clearance of human WT αsyn in M17 cell lysates following treatment with 10 μg ml^{-1} doxycycline (DOX) for 7 days, followed by DOX removal for 7 days, measured by 17 kDa species for total αsyn (BD "syn-1") and p129S-αsyn. Asterisks indicate days of peak levels for each αsyn species. GAPDH (37 kDa) served as loading control. All data are expressed as mean ± SD, n = 3 replicates for each data point. *p < 0.05, 1-way ANOVA and Bonferroni's post hoc test.*

After establishing the M17 cell model of transient, over-expression of αsyn, we treated cells with Fz to confirm the pharmacological increase of αGalA *in vitro*. We first performed a Fz dose-response for αGalA levels and activity following its addition to M17 cell culture media for 24 hr. Treatment of M17 cells with Fz significantly increased αGalA levels and activity (Fig. 2). Concentrations of Fz ranging from 1–100 μg ml^{-1} significantly increased levels of protein, as measured by western blot analysis (Fig. 2B) and activity as measured by fluorimetric activity assay (Fig. 2C) compared to cells treated with vehicle control. The increase in activity was concentration dependent with significant increases observed with concentrations ranging from 3–100 μg ml^{-1}, and the greatest increase in activity observed at 100 μg ml^{-1} (Fig. 2C).

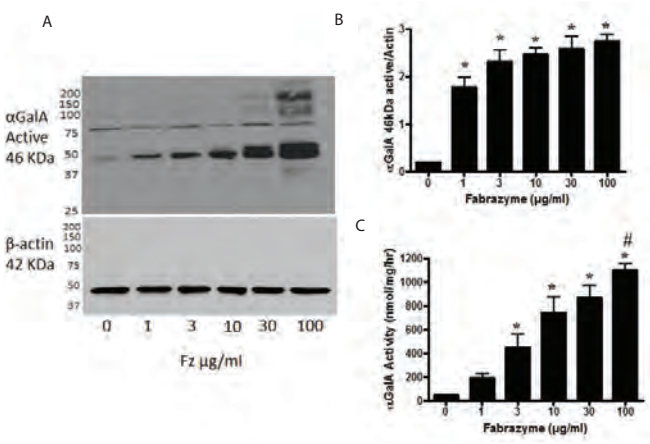

*Fig 2| Fabrazyme increases αGalA. (a) Western blot analysis of whole cell lysates shows an increase of αGalA 46 kDa active species in M17 cells following administration of Fabrazyme (Fz) at 1, 3, 10, 30, or 100 μg ml^{-1} compared to vehicle control. (b) The 46 kDa active species was quantified relative to actin loading control. The assay for αGalA enzymatic activity (nm/mg/hr, expressed as % CTL was increased in αGalA (c) following Fz administration. All data are expressed as mean ± SD, n = 4 replicates for each data point. * p < 0.05, 1-way ANOVA and Bonferroni's post hoc test (difference from control, 0). # maximal increase in activity.*

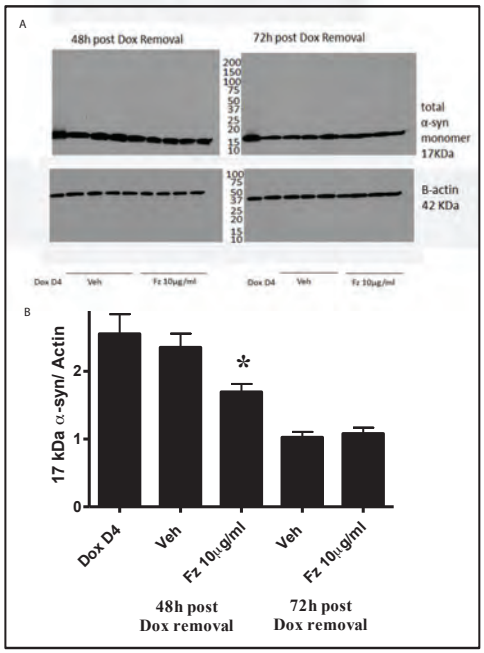

*Fig 3: Increasing αGalA activity increases clearance of over-expressed αsyn. (a) Western blot analysis of αsyn in M17 whole cell lysates shows increased clearance of αsyn 17 kDa monomer after 4 days of Dox induced over-expressed with administration of Fz (10 μg ml^{-1}) for 48 and 72 hours at the removal of Dox compared to vehicle control. (b) Monomer was quantified relative to actin loading control. All data are expressed as mean ± SD, n = 4 replicates for each data point. *p < 0.05, t-test and Bonferroni's post hoc test (difference from control, VEH).*

Our next goal was to determine if increasing αGalA activity regulated the clearance of over-expressed αsyn. Over-expression of αsyn was induced by four days of Dox treatment and αsyn levels were evaluated for 72 hours after removal of Dox in the presence or absence of Fz. Treatment with Fz in culture media significantly decreased levels of total αsyn monomer in M17 cells compared to vehicle controls at 48, but not 72 hours after Dox removal (Fig. 3).

DISCUSSION

PD has a drastic impact on the health of millions of Americans and creates a major financial burden on the healthcare system. Reports of ALP dysfunction in PD along with the discovery of the pathogenic accumulation of αsyn in αGalA deficient mouse brains led us to investigate the therapeutic potential of increasing αGalA in a preclinical model of αsyn accumulation in PD. We established an *in vitro* model of transient αsyn over-expression and pharmacological enhancement of αGalA to provide proof-of-principle validation of αGalA as a therapeutic target for αsyn pathology in PD. We found that increasing αGalA activity by Fz accelerates the clearance of over-expressed αsyn in our M17 model of transient αsyn over-expression.

We confirmed that the addition of Dox to culture media for 7 days induced a significant, but transient increase in αsyn, making it an effective model for studying the relationship between increasing αGalA activity and the clearance of αsyn. Using an *in vitro* system with an immortalized cell line limits the extent to which these experimental results can be generalized to the brain, but future studies in our laboratory will investigate the same "clearance" effect of αGalA on αsyn in our *in vitro* primary neuron culture model and an *in vivo* mouse model of αsyn over-expression.

The concentration-dependent increase in αGalA activity resulting from Fz treatment are believed to be internalized, not just found in the culture media, as cells were washed with PBS prior to lysis. Preliminary immunocytochemistry data from our lab indicates the localization of increased αGalA by Fz to lysosomes (data not shown), suggesting that Fz is indeed internalized and reaching the lysosome. Although we found significant effects of Fz treatment at concentrations ≥ 1 μg ml^{-1}, Fz is known to be internalized in cells via the mannose 6-phosphate receptor pathway at concentrations up to 1 μg ml^{-1} [35]. This suggests that higher concentrations may be internalized via different mechanism(s). As such, ongoing studies in our laboratory are focused on the therapeutic potential of Fz concentrations ≤ 1 μg ml^{-1}, in addition to confirming the mechanisms by which low and high concentrations of Fz are internalized in M17 cells and its lysosomal localization subsequent to internalization.

Our *in vitro* model system was used to evaluate the effect of increasing αGalA on the clearance of over-expressed αsyn. Fz significantly decreased total levels of over-expressed total αsyn when it was added for 48 hr after Dox removal, supporting our hypothesis that increasing αGalA activity accelerates the clearance of over-expressed αsyn. Further studies are necessary to elucidate the mechanism(s) by which αGalA regulates αsyn, including the functional examination of ALP-specific markers. One possible mechanism by which αGalA may promote the clearance of αsyn is by regulating levels/activity of cathepsin D, a lysosomal aspartic acid protease that has been shown to proteolytically degrade αsyn[36]. This and other mechanisms of ALP function will be explored in future studies. The current study focused on clearance of soluble αsyn monomers. Future studies will also determine if increasing αGalA activity promotes the clearance of existing insoluble αsyn aggregates, and/or prevents them from accumulating in the first place.

Induction of autophagy as a therapy for PD has had few successes in clinical trials and has known side effects[37-39]. In addition, the induction of autophagy concurrent with the inhibition of lysosomal function has been used as a therapy for killing cancer cells, suggesting it may further increase the stress to neurons in PD if autophagy is induced without proper lysosome function[40]. This suggests that inducing autophagy in PD under conditions of lysosomal dysfunction would not only be ineffective, but could in fact exacerbate neurodegeneration. Evidence for the involvement of the ALP in PD pathology is ample. Cathepsin D (CD) deficiency decreases both lysosome and proteasome function and leads to αsyn accumulation[22]. *ATP13A2* gene mutations in autosomal dominant PD cause multiple impairments of lysosome function including substrate degradation and autophagosome processing[19]. Inhibition of lysosomal acidification by bafilomycin A1 increases toxicity of αsyn aggregates[24], knock-down of *ATP6V0C* H+ pump increases accumulation of αsyn[26], and pathogenic lysosome depletion has been shown in PD[16]. Lastly, glucocerebrosidase (GBA) activity and αsyn accumulation interact bidirectionally in synucleinopathies including Gaucher Disease and PD[17]. Our data suggest that αGalA, another lysosomal enzyme in the glycosphingolipid metabolism pathway with GBA, is also capable of affecting αsyn levels through a yet unknown mechanism. Our lab is focused on the continued investigation of αGalA and lysosome function as therapeutic targets for attenuating αsyn-associated pathogenesis in PD.

CONCLUSION

We established an *in vitro* model of inducible αsyn over-expression to determine if pharmacologically increasing the activity of αGalA affects its clearance. Fabrazyme increases levels and activity of αGalA in M17 human neuroblastoma cells and promotes the clearance of over-expressed αsyn in these cells. ***These data provide support for the continued investigation of αGalA as a therapeutic target for PD.***

ACKNOWLEDGMENTS

This research was partially supported by the SIBS-UPS Undergraduate Research Program funded by the UAB Department of Pathology and by Grant: P30 DK079626/DK/NIDDK NIH (Diabetes Research Center) Bio-Analytical Redox Biology (BARB) Core.

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Retinitis Pigmentosa: A Brief Review of the Genetic and Clinical Aspects of the Disease

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ABSTRACT

Retinitis Pigmentosa (RP) is a heterogeneous set of inherited retinal diseases that affects 1 in 3,000–7,000 people worldwide. Typical onset is from 10–30 years old and most forms are progressive, often leading to blindness. Defects in more than 200 genes have been identified that cause RP. The disease is characterized as a progressive rod-cone dystrophy that presents with night blindness, loss of peripheral vision, waxy pallor of the optic disc, pigmentary changes, and a reduced visual field. There are different modes of transmission of RP: autosomal dominant (ADRP), autosomal recessive (arRP), X-linked (XLRP) and mitochondrial. The genetics behind the different forms of RP and the degree of severity vary, although some overlap, thus contributing to the difficulty of differential diagnosis. RP can manifest either as a non-syndromic disease, or as part of a syndrome, such as in Usher's syndrome (hearing and vision loss) and Bardet-Biedl syndrome (a ciliopathy). The purpose of this review is to summarize the major genetic and molecular findings, as well as the diseases, associated with RP. Due to space limitations, this review is not fully comprehensive.

Keywords: Retinitis pigmentosa, non-syndromic retinitis pigmentosa, rod-cone dystrophy, rhodopsin

INTRODUCTION

Retinitis pigmentosa (RP) is a heterogeneous set of inherited retinal diseases. RP affects 1 in 3,000–7,000 individuals worldwide and is characterized by a primary loss of night and peripheral vision as a result of rod photoreceptor cell degeneration[1,2]. Central vision is often lost as the cone photoreceptor cells degenerate, consequently causing progressive degeneration of the macula and fovea as well[1]. This degeneration can be a result of the disease itself or secondary to the rod cell degeneration[1]. RP is also characterized by atrophy in the pigment epithelium and outer retina, RPE cell migration into the retina, reduced visual field, waxy pallor of the optic disc, abnormal or absent a- and b-waves on an electroretinogram (ERG), and an abnormal fundus with bone cell spicule deposits as a result of cell apoptosis[3].

RP can occur either as part of a syndrome or in a non-syndromic fashion. There are several forms of this disease due to mutations in different genes and to different mutations within the same gene with each varying in age of onset,

severity, and mode of inheritance[4]. Autosomal Dominant RP (ADRP) is usually the mildest form of the disease, with patients maintaining relatively good visual acuity until the sixth decade[3]. Autosomal Recessive RP (arRP) is the most prevalent form of the disease, accounting for 50–60% of cases[5]. RP can also occur in an isolated form in which the patient has no family history of the disease, but even in these cases, parental contributions of defective genes can be established. There are also rarer forms of the disease such as mitochondrial, digenic, and X-linked (XLRP). XLRP has the most severe phenotype, accounting for 10–15% of RP cases[1]. Visual acuity is usually severely impaired by the fourth decade[1]. Onset of RP usually occurs in adolescence or young adulthood, but in cases of congenital RP, patients can be diagnosed with Leber Congenital Amaurosis (LCA), a largely autosomal recessively inherited retinal dystrophy that is usually diagnosed prenatally[6]. LCA is one of the most frequent causes of childhood blindness, accounting for 10–20% of cases[6]. It also accounts for approximately 5% of RP cases[6].

There are at least 56 genes that have been confirmed to be associated with non-syndromic RP, with more than 3,100 associated mutations[4]. With recently developed technology, for example, next generation sequencing (NGS), 30–80% of mutations in patients can now be identified[2]. NGS has led to the discovery of more mutant genes and has become a novel technique for possible molecular diagnosis of the disease[2].

Autosomal Dominant Retinitis Pigmentosa (ADRP)

To date, 23 genes have been found to be associated with ADRP[2]. Molecular diagnosis is becoming a more feasible idea with the recent development of a DNA array that is able to detect up to 385 mutations in 16 of the known causative ADRP genes[1]. The following genes are those that are responsible for the highest percentages of RP cases (see Table 1).

RHO

The *RHO* gene encodes the opsin protein, the protein portion of a molecule that, with its chromophore, 11-*cis* retinal, is responsible for initiating the phototransduction cascade within the rod photoreceptor cells, an enzymatic cascade that is crucial for vision. Mutations in this protein are responsible for 26.5% of all RP cases and 30–40% of ADRP cases[1]. More than 100 mutations have been associated with this gene, with the more detrimental mutations resulting in protein mis-folding, mis-trafficking, 11-*cis* retinal binding impairment and/or G-protein coupling/activation impairment[1]. There are two classes of mutations: Class I and Class II[7]. Class I mutations are naturally occurring mutations that have similar expression levels, 11-*cis* retinal relationship, and plasma membrane association to the wild-type (WT) rhodopsin[7]. Most of these mutations are found at the C-terminal end of the protein, with the entire class being responsible for 15% of ADRP mutations[7]. Class II mutations, in contrast, exhibit lowered expression levels, impaired 11-*cis* retinal binding and plasma membrane association, although the primary issue with this class is protein mis-folding[7]. This class is also responsible for 85% of ADRP mutations[7].

Peripherin-2/*RDS*

The peripherin-2 gene (*PRPH2*), also called *RDS* for the mouse mutant retinal degeneration slow (RD2), encodes a 39-kDa intermembrane glycoprotein which localizes to the outer segment (OS) discs of rod and cone cells and is important in disc morphogenesis and stabilization[1]. Sub-retinal injection of the *PRPH2* transgene through adeno-associated virus (AAV) into the *Prph2*$^{RD2/RD2}$ mouse model resulted in restoration of the structural integrity of the photoreceptor layer, stabilization of OS generation, new disc formation and improved ERG a- and b-waves, which were originally undetectable by the second month of life in this model[8]. Mutations in this gene account for about 5–9.5% of ADRP cases[1].

RP1

The *RP1* gene codes for the 240-kDa retinal-photoreceptor specific ciliary protein, Retinitis Pigmentosa 1[1,9]. Mutations in this gene account for 5–10% of all ADRP cases, including 4% of cases in the U.S.[1] These mutations cause both dominant and recessive RP[1]. The RP1 protein is expressed in both rod and cone photoreceptor cells in which it localizes to the axoneme of the connecting cilium (CC) and OS in mice. RP1 affects photosensitivity of the cell, assembly and stabilization of microtubules[10]. Mouse models, the *rp1-/-* for example, have shown that upon disruption of RP1, OS mis-alignment and dysplasia occur[10]. Mutations in this gene result in variable onset and severity of the disease, most likely due to genetic modifiers or environmental influences[1]. In general, however, heterozygous inheritance does result in a milder phenotype and later onset, as compared to homozygous inheritance, which results in earlier onset and a more severe phenotype[10].

Autosomal Recessive Retinitis Pigmentosa (arRP)

To date, 37 genes have been found to be associated with arRP[2]. Molecular tests for mutation screening are now able to detect 594 mutations in at least 20 of the associated genes[1]. *RPE65*, *USH2A* and *PDE6* genes can now be fully sequenced at the DNA level[1]. Most mutations are rare and cause 1% or fewer of cases[1]. The following mutations account for a more prevalent percentage of cases (see Table 1).

RPE65

This gene codes for an isomerohydrolase that is found in the retinal pigment epithelium (RPE)[2]. Isomerohydrolase is thought to play a role in the regeneration of 11-*cis* retinal from all-*trans* retinal when all-*trans* retinyl ester is converted to 11-*cis* retinol, which is a critical step in this enzymatic conversion[11].

This process is important because the protein portion of rhodopsin needs to be able to bind to its chromophore, 11-*cis* retinal, in order to form the conformation necessary for the conduction of the phototransduction cascade[11]. There are over 60 mutations associated with this gene[1]. There are a good number of relevant animal models, which has resulted in the disease mechanism of this mutation being better understood[1]. For example, *RPE65*-deficient mice are found to have a build-up of retinyl esters and other intermediates of the visual cycle in the RPE[12]. Sub-retinal injection of a human *RPE65* cDNA cassette using recombinant adeno-associated virus (rAAV) into *RPE65-/-* purebred Briard dogs also showed a restoration of visual function in the treated retina of these animals[1].

PDE6

The heterotrimeric phosphodiesterase 6 (PDE6) complex regulates the intracellular levels of cGMP in the OS of the rod and cone cells by hydrolyzing it in response to the G-protein, Transducin, activation initiated by rhodopsin[1]. There are four subunits: two catalytic subunits, alpha and beta, and two inhibitory gamma subunits. Defects in all three genes have been identified that cause arRP[1]. Low levels of the alpha and beta subunits have a possible link to rod and cone cell degeneration[1]. PDE6 inactivation is also associated with an excessive influx of Ca^{2+}; this is thought to be a possible cause of rod cell apoptosis, but the use of Ca^{2+} blockers to reduce this effect in the *PDE6b*^{rd1}/*PDE6b*^{rd1} mouse and the Irish setter dog models have yielded inconsistent results[1]. Interestingly, the protein responsible for synthesizing cGMP, guanylate cyclase (GC1), does not cause any forms of RP, except recessive Leber Congenital Amaurosis (LCA-1) and cone-rod dystrophy (CORD6)[13].

Mutations in these two genes are the second most identifiable causes of arRP, with mutations in *USH2A* being the first[1]. Complex mutations in the PDE6 alpha and beta subunit genes are also responsible for 8% of all diagnosed cases of arRP[1].

X-linked Retinitis Pigmentosa (XLRP)

Ten to fifteen percent of patients with RP are diagnosed with XLRP, presenting with a severe phenotype early in the disease[1]. This condition can also occur in females, although these cases are usually milder compared to the condition in male populations[1]. These cases are usually due to a non-random or skewed inactivation of an X chromosome[1]. Six gene loci have been located, although only three genes to date have been found to be associated with XLRP[1]. Oral facial digital syndrome type 1 (OFD1) gene mutations are X-linked dominant and result in primary cilia dysfunction and embryonic lethality in males[1,14].

RPGR/RP3

The Retinitis Pigmentosa GTPase Regulator (*RPGR*) gene undergoes complex splicing to yield multiple isoforms that localize to the rod outer segment (ROS) and are essential for cell viability[15]. There are two isoforms that are the main focus of study since they are the most widely expressed: the RPGR^{ORF15}, primarily associated with primary cilia, localizing to the OS and connecting cilium (CC) of photoreceptors, and RPGR$^{1-19}$, which localizes to the endoplasmic reticulum[15]. Mutations on the C-terminus of RPGR^{ORF15} are the most relevant as they cause XLRP3, the most severe form of RP[16]. XLRP3 also accounts for 14% of all RP cases[16]. Discovering more about the localization and physiological effects of the isoforms are critical for determining more about the disease mechanism[16]. Mutations in this gene are the principal cause of XLRP, accounting for mutations in 70% of patients[16].

RP2

The *RP2* gene product is predicted to be homologous with human cofactor C, which is involved in the terminal step of beta tubulin folding; mutations result in an accumulation of misfolded proteins[1]. In patients, missense mutations and other mutations that can lead to truncated proteins have been found throughout the coding region. *RP2* and *RPGR* together are responsible for over 80% of XLRP clinical cases which together with their associated pathology makes them good candidates for small molecule or gene therapy[1].

Table 1| Genes, gene products and functions of important proteins that cause non-syndromic RP.
Abbreviations: RP, retinitis pigmentosa. ADRP, autosomal dominant retinitis pigmentosa. arRP, autosomal recessive retinitis pigmentosa. XLRP, X-linked retinitis pigmentosa.

Types of RP	Gene	Gene Product	Percentage of cases	Function	References
ADRP	RHO	Rhodopsin	30-40%	Phototransduction	1
	PRPH2	Peripherin-2	5-9.5%	Photoreceptor outer segment structure	1
	RP1	Retinitis Pigmentosa 1	5-10%	Photosensitivity, assembly and stabilization of microtubules	1, 10
arRP	RPE65	Retinal pigment epithelium-specific 65kDa protein	2%	Production of 11-*cis*-Vitamin A	4
	PDE6	Phosphodiesterase 6	8%	cGMP hydrolysis	1
XLRP	RPGR	Retinitis pigmentosa GTPase regulator	70%	Cell viability	1,4
	RP2	Retinitis Pigmentosa 2	10%	Involved in beta-tubulin folding	1,4

Syndromic RP

RP can also occur concomitantly with systemic disease, with the symptoms, age of onset, and severity varying with the particular disease. The two of focus in this review are

Usher's syndrome and Bardet-Biedl syndrome (BBS), two diseases under the classification of a group of diseases called ciliopathies. One note to make, however, is that although Usher's syndrome can be considered a ciliopathy based on the fact that its interactome is linked with the proteins of other ciliopathies, it is not exclusively a ciliopathy[17]. This group of diseases is characterized by dysfunction in relation to the primary cilia, with most of the diseases presenting with system-wide pathology in addition to RP. Usher's syndrome and BBS are the most common of the ciliopathies with over 1,200 mutations associated with these diseases[2].

Bardet-Biedl Syndrome (BBS)

Bardet-Biedl Syndrome (BBS) is a rare disease that affects 1:100,000–1:160,000 worldwide[18,19]. It is an autosomal recessive disease, although one case of triallelic (three genes involved) inheritance has been reported[18,20]. BBS is one of the major causes of syndromic retinal dystrophy, accounting for over 90% of cases[21]. BBS can be characterized by cone-rod, rod-cone and/or choroid dystrophy, although rod-cone dystrophy in the form of RP is the most common and often used as a means of diagnosis[18,21]. Truncal obesity, intellectual disability, post-axial polydactyl and renal abnormalities are several other signs of the disease[18]. The renal abnormalities cause the most complications with the disease, increasing the morbidity of the disease as well as the mortality rate[18]. Night blindness often occurs around 7–8 years of age causing the patient to generally be classified as legally blind after 15.5 (mean) years with the disease[18]. Overall, the phenotype and onset of the disease are highly variable[21].

BBS Genetics

The gene products associated with the BBS genes are proteins that localize to the ciliary axoneme and basal body, involved in cilia biogenesis and maintenance of ciliary function[18] (see Table 2). There are 17 genes that are associated with the disease, although those only account for approximately 80% of diagnoses, indicating that there are more genes and/or mutations to be identified to account for the other 20%[4,18]. Pathogenic variants in the BBS genes 1–14 are known to be associated with the disease, whereas pathogenic variants in BBS genes 15–19 are suspected to be associated with the disease[18]. BBS1, 2, 4, 5, 7, 8 and 9 make up what is called the BBSome, a protein complex thought to be involved in ciliary targeting[18]. There is also a chaperonin complex associated with the BBSome made up of BBS6, 10 and 12[18].

Animal Models

There are quite a few animal models that are used to study this syndrome, but one difficulty is generating or finding a model that accurately models the human phenotype. One animal model that has shown promise is the *Bbs1*^{M390R} mouse model, which specifically models the common human mutation[20]. There has been a genotype-phenotype correlation established with this model, with a milder phenotype being associated with this mutation[20]. The model exhibits retinal degeneration, male infertility, and obesity due to hyperphagia, similarly to what is exhibited in human patients[20]. Animal models have also given some insight into possible mechanisms underlying the disease. For example, the mouse model that is null for *BBS2, 3,* and *4* expressed a resistance to leptin activity, which may reveal an underlying mechanism for the obesity that is characteristic of BBS patients[20].

Table 2| List of the gene product and function, if known, of BBS genes.

BBS, Bardet-Beidl syndrome.

	Gene	Gene Product	Gene Function	References
BBS	BBS1	BBS1 protein	Unknown function; possible ciliary function	4, 18
	BBS2	BBS2 protein	Unknown function; possible ciliary function	4, 18
	BBS3/ ARL6	ADP-ribosylation like factor 6	Unknown function; possible ciliary function	4, 18
	BBS4	BBS4 protein	Similar to O-linked N-acetylglucosamine transferases; possible ciliary function	4, 18
	BBS5	Flagellar apparatus-basal body protein DKFZp7621194	High conservation in flagella and cilia; possible ciliary function	4, 18
	BBS6/ MKKS	McKusick-Kaufman Syndrome protein	Protein sequence is similar to chaperonins; ciliary function	4, 18
	BBS7	BBS7 protein	Unknown function	4
	BBS8/TTC8	Tetratricopeptide repeat domain 8	Localizes to ciliary structures; interacts with PCM1, a protein involved in ciliogenesis	4, 18
	BBS9	Parathyroid hormone-responsive B1 protein	Unknown function	4, 18
	BBS10	BBS10 chaperonin	Possible role in planer cell polarity	4, 18
	BBS11	TRIM32	Tripartite motif-containing protein 32	4, 18
	BBS12	BBS12 protein	Type II chaperonin family	4, 18
	BBS13/ MKS1	Meckel syndrome type 1 protein	Component of flagellar basal body	4, 18
	BBS14/ CEP290	Centrosomal protein 290 kDa	Associates with microtubule proteins in centrosomes and cilia	4, 18
	BBS15/ WDPCP	WD repeat-containing planar cell polarity effector	Involved in planar cell polarity in embryogenesis; may affect ciliogenesis	4, 18
	BBS16/ SDCCAG8	Serologically-defined colon cancer antigen 8	Localizes to centrioles	4, 18
	BBS17/ LZTFL1	Leucine zipper transcription factor like-1	Negative regulator of BBSome; affects ciliary trafficking	4, 18
	BBS18/ BBIP1	BBSome interacting protein 1	Member of BBSome complex	4, 18
	BBS19/ IFT27	Intraflagellar transport 27 *Chlamydomonas* homolog	Associated with intraflagellular transport in green algae	4, 18

Diagnosis

The criteria for diagnosis is that at least four of the main manifestations, some of which are mentioned above, along with two secondary manifestations are identified in the patients[18, 20]. Secondary manifestations can include Diabetes Mellitus (DM), speech disorders, congenital heart disease, dental anomalies and/or ataxia[18]. Differential diagnosis is often an issue with this syndrome as many of the manifestations can arise singularly and do not necessarily indicate BBS[18]. There are no treatments for this disease, only management of symptoms and the associated complications, such as monitoring blood sugar for DM management[18].

Usher's Syndrome (US)

Usher's syndrome is an autosomal recessive disease that is the most frequent cause of deaf-blindness, accounting for over 50% of those with inherited deaf-blindness diseases[22]. This disease affects 3–6.2 per 100,000 individuals worldwide[22]. There are three subtypes of the disease, each varying in severity and onset, and characterized by congenital deafness, retinal degeneration, and varying degrees of vestibular function[22]. The first subtype is the most devastating, characterized by severe to profound congenital deafness, followed by pre-pubertal retinal degeneration and no vestibular function[22,23]. Another subset of this type, Usher 1B, is caused by a mutation in the *MYO7A* gene[23]. This form accounts for approximately 50% of Usher 1 cases and also has bi-allelic mutations associated with Usher type 2[23,24]. The product encoded by this gene is also required for normal localization and function of RPE65[25].

The second sub-type is characterized by moderate to severe congenital hearing loss, pre- or post-pubertal RP onset, and, unlike the other two sub-types, these patients still have vestibular function present[22,26]. This subtype accounts for over half of all Usher cases[22].

Sub-type 3 is the rarest, characterized by progressive hearing loss, either pre- or post-lingual, variable onset of RP and variable vestibular function[22,27].

Genetics

To date, there are ten genes associated with Usher's syndrome[22]. There are six genes associated with Usher 1: *MYO7A* (USH1B), *USH1C*, *CDH23* (USH1D), *PCDH15* (USH1F), *USH1G*, and *CIB2* (USH1J)[22] (see Table 3). There are three genes associated with Usher 2: *USH2A*, *GPR98* (USH2C), and *DFNB31* (USH2D)[22]. There are three other genes proposed to be associated with the disease as well, but have yet to be confirmed[22]. Defects in the *USH2A* gene are responsible for the majority of Usher 2 cases and have been associated with recessive non-syndromic RP[22]. Defects in this gene are also responsible for some cases of atypical Usher syndrome, those cases that do not fall into the sub-types and categories listed above[22]. *CLRN1* is the only gene associated with Usher 3 syndrome[22]. These genes are a part of what is called the Usher-interactome, a complex of proteins known to participate in common pathways in the retina and inner ear[22]. Most of the Usher 1 and 2 genes are a part of this complex, involved in localization of proteins in the stereocilia, hair bundle of inner ear cells, and periciliary areas of photoreceptors[22]. In the ear, these proteins are involved in the development and cohesion of the hair bundle cells in the cochlea and vestibular organ[22]. In the retina, these proteins act as support in the membrane junction between the inner segment (IS) and the CC[22]. They also play a role in the control of vesicle docking and cargo handover in the periciliary ridge.

	Gene	Gene Product	Gene Function	References
US Type 1	MYO7A (USH1B)	Myosin VIIA	Actin-based motor protein	4, 23
	USH1C	Harmonin	PDZ-domain protein	4, 23
	CDH23 (USH1D)	Cadherin23	Adhesion protein	4, 23
	PCDH15 (USH1F)	Protocadherin15	Adhesion protein	4, 23
	USH1G/ SANS	Human homolog of mouse scaffold protein containing Ankyrin repeats and SAM domain	Scaffold	4, 23
	CIB2 (USH1J)	Calcium and integrin binding family member 2	Calcium and integrin binding	4
US Type 2	USH2A	Usherin	Transmembrane linkage	4, 23
	GPR98 (USH2C)	VLGR1	G-protein coupled Receptor	4, 23
	DFNB31 (USH2D)	Whirlin	PDZ-domain protein	4, 23
US Type 3	CLRN 1 (USH3A)	Clarin 1	Possible role in hair and photoreceptor cell synapses	4

Table 3| List of the gene product and function of US genes. US, Usher's syndrome.

Animal Models

Some animal models of Usher Syndrome are available, for example the *Shaker1* mouse model which expresses the phenotype of vestibular dysfunction[28]. However, an issue with mouse models is that they do not adequately express the retinal degeneration that is characteristic of this syndrome[28]. Fish models somewhat compensate for this by not only expressing vestibular dysfunction, but also expressing retinal degeneration and diminished ERG amplitudes, indicative of a defect in photoreceptor response[28]. *Orbiter*, a fish model, has aided in the discovery that the *pcdh15b* gene is important in OS organization and retinal function[28]. Though fish models express these phenotypes and are able to teach us more about the syndrome, the scope to which fish models can be utilized is limited due to uncertainty of how representative they are of the human model since the genes may not be well conserved[28].

Diagnosis

Diagnosis is difficult with this disease, specifically differential diagnosis, because many of the symptoms can be misconstrued to be other ciliopathies, such as BBS[27]. Genes like *USH2A* and *CLRN1* mutations are not only involved in the development of Usher's syndrome, but have also been associated with isolated cases of arRP, further contributing to the difficulty of differential diagnosis[27]. Genetic testing for diagnosis is not applicable since the heterogeneity of the disease prevents genotype-phenotype correlations from being made[27]. The proteins also have multiple functions and are even involved in non-syndromic cases of hearing loss and RP as well[27]. There is, however, novel technology being developed for clinical practice; for example, there is now a DNA microchip available that is able to identify 30–50% of mutations in those affected by the disease[27].

There is currently no treatment available, only management of symptoms[27]. Hearing aids and cochlear implantation are a few

of the methods utilized to manage the progressive hearing loss[27]. There are currently no cures for RP, and techniques such as gene therapy, damaged cell replacement and vitamin supplementation to stop or slow degeneration and apoptosis are proposed ideas[27]. The lack of animal models, pre-clinical studies and human trials has prevented these methods from being utilized clinically[27].

Gaps and Future Directions

Even though there has been great progress in discovering more about this disease, several gaps still exist in the available literature. Although new technology, such as NGS, is making it more effective to discover new genes and their function, determining what they contribute to the molecular and clinical manifestations of the disease is a challenge that cannot be quickly overcome. Deciphering the genotype-phenotype correlations is one of the major issues, since one gene mutation can lead to multiple diseases, as is the case for mutations in the *RHO* gene, which can result in ADRP, arRP or Congenital (stationary) Night Blindness (CNB)[1]. Other genes can exhibit intra- and inter-familial phenotypic variability and incomplete penetrance, further contributing to the difficulty in discerning the underlying cause of the disease[2]. Phenocopies (non-genetic causes of an RP phenotype) represent another complication in the characterization and management of the disease.

In terms of clinically applying the growing pool of information on this disease, there are issues with translating what is discovered in the lab to a format that can be efficiently and accurately interpreted by physicians. Because of the genotype-phenotype correlation discrepancies, there also can be conflicting results between what is diagnosed clinically and molecularly. Certain tests have been proposed to resolve these issues, such as functional assays, which could aid in determining the pathogenic effects of some of the genes, but as of now, it is too in-depth of a tool for clinical practice since there has been no database developed to filter and organize the results[1].

Despite these obstacles, research is progressing at a fast pace in terms of discovering more about this disease, and this knowledge is being translated to the clinics, albeit at a slower pace. For example, one of the first successful gene therapy treatments in clinical trials is for LCA. CRISPR-Cas9, a genome editing tool, has been shown to be able to disrupt and/or correct harmful mutations, and deliver functional transgenes[29]. Because of the promising results from the past 2 years of study, clinical trials will begin in 2017 for patients with LCA[30]. Overcoming the existing challenges will lead to large gains not only in the molecular mechanisms of RP, but in clinical practice as well. Screening, diagnosis, and treatment of individuals will drastically improve, shaping a better outlook not only for RP, but other inherited retinal diseases as well.

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Tannic Acid-Containing Nanothin Coatings Dampen Innate Immune-Derived Pro-Inflammatory Chemokine Synthesis

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ABSTRACT

Type 1 Diabetes (T1D) is a chronic pro-inflammatory autoimmune disease consisting of reactive oxygen species (ROS), pro-inflammatory cytokines, and islet-infiltrating leukocytes involved in pancreatic β-cell lysis. One promising treatment for T1D is islet transplantation; however, its clinical application is constrained due to limited islet availability, adverse effects of immunosuppressants on islet function, and declining graft survival. Islet encapsulation may provide an immunoprotective barrier to help preserve islet function and prevent immune-mediated rejection after transplantation into T1D patients. We previously demonstrated that a novel cytoprotective nanothin coating for islet encapsulation consisting of tannic acid (TA), an immunomodulatory antioxidant, and poly N-vinylpyrrolidone (PVPON), was efficacious in dampening diabetogenic CD4 T cell and macrophage responses involved in transplant rejection. Therefore, we hypothesized that in addition to suppressing pro-inflammatory cytokine synthesis, TA/PVPON would similarly blunt the production of pro-inflammatory chemokines involved in recruiting immune cells to the site of islet engraftment. Our results provide further support that TA/PVPON-containing encapsulated islets are effective in suppressing pro-inflammatory CCL5 and CXCL10 chemokine synthesis. The use of novel TA/PVPON nanothin coatings may potentially decrease immune-mediated responses and enhance islet allo- and xenograft acceptance to restore euglycemia in T1D patients.

Key words: Type 1 Diabetes, chemokines, tannic acid, immunosuppressant, islet transplantation

INTRODUCTION

Type 1 Diabetes (T1D) is a T cell-mediated autoimmune disease characterized by the targeted lysis of insulin-producing pancreatic β-cells. Patients with T1D are genetically predisposed to autoimmunity, but mounting evidence suggests an environmental trigger is required to instigate the development of autoimmunity[1]. As a result of insulin loss, T1D patients are unable to adequately maintain normal blood glucose levels; thus, patients regulate blood glucose levels via daily insulin injections or an insulin pump. Numerous complications arise as a result of insulin deficiency, including cardiovascular disease, nephropathy, and retinopathy. Another viable alternative to insulin injection is pancreatic islet transplantation, a process that involves the isolation of islets from cadaveric donors and the transplantation of those islets into T1D patients[2]. One of the main advantages of islet transplantation is that the pancreatic β-cell is finely tuned to properly regulate glucose levels within the body, and episodes of hyperglycemia (elevated blood

glucose) and hypoglycemia (low blood glucose) are less frequent. Wide fluctuations in blood glucose can significantly alter metabolism and contribute to life-threatening diabetic complications.

The landmark Edmonton protocol demonstrated great promise that islet isolation from multiple cadaveric donors and transplantation into the portal vein with a glucocorticoid-free immunosuppressive cocktail could restore euglycemia in Type 1 diabetic patients without the use of exogenous insulin therapy[2]. Transplant recipients require immunosuppressants that include injections of daclizumab for a period of 8 weeks and sirolimus (daily) to protect the donor's islets from being rejected[2]. Unfortunately, immunosuppressants can weaken the immune system and compromise islet function; therefore, attempts to transplant islets without immunosuppressive therapies and/or develop novel immunotherapies with low toxicity are highly desired.

Prior studies have utilized various methods of islet encapsulation for immunoprotection including macroencapsulation with alginate or polyethylene glycol (PEG), polymembrane pouches within an oxygenated chamber, and microencapsulation in high viscous alginate droplets[3]. However, there are significant drawbacks as macro- and microencapsulation with alginate requires extreme purity since microbial contaminants in alginate can inadvertently activate the immune system and cause cell death[4]. Also, PEG hydrogels for islet encapsulation are large, degradable, and present a barrier for rapid transport of oxygen, nutrients, and therapeutic factors[5]. Therefore, a flexible and immunosuppressive barrier is needed to perform the task of protecting living islets while maintaining their functionality within the body.

One effective method of islet encapsulation utilizes the Layer-by-Layer (LbL) protocol of coating islets with nano-thin layers of water soluble polymers in aqueous solutions[4]. The LbL method of applying a thin coating on cells allows them to respond to stimuli, contains a conformal and adjustable coating, and can be efficiently conjugated with specific inhibitors, proteins, and antibodies to modulate specific biochemical pathways. We have utilized this LbL method with biomaterials of tannic acid (TA) and PVPON (poly N-vinylpyrrolidone). TA is an antioxidant that scavenges free radicals and inhibits free radical-induced oxidation[4]. PVPON is a hydrophilic, non-toxic polymer which can function as an efficient scaffold. These features of TA/PVPON are important in hindering the production of reactive oxygen species (ROS) that are responsible for signaling and activating other immune cells. Our previous results demonstrated that TA/PVPON encapsulation of pancreatic islets was non-toxic and maintained islet function and stability up to 7 days *in vitro*[6]. More importantly, we were also able to demonstrate that TA/PVPON exhibited an immunosuppressive effect on activated macrophages and autoreactive T cells by dampening the synthesis of innate immune-derived pro-inflammatory cytokines and adaptive immune T cell effector responses involved in islet rejection[4,7].

Chemokines play an integral role in the pathogenesis of Type 1 Diabetes and islet transplant rejection. They are a specific type of protein that drives cellular chemotaxis, which induces pro-inflammatory cell migration to a specific region in the body. This use of chemotaxis can directly drive immune cells to the site of newly transplanted islets and cause pancreatic β-cell necrosis. There are many chemokines associated with T1D, but one widely known chemokine is CXCL10, which has been identified as a dominant chemokine involved in murine models of T1D and human T1D[8]. RANTES, also known as CCL5, plays a key role in T cell proliferation and activation of T cells in inflammatory sites of T1D patients. Zhernakova *et al.*[9] demonstrated that single-nucleotide polymorphisms (SNPs) in RANTES result in lower RANTES production in T1D. Results show that T1D carriers of RANTES SNPs exhibited lower levels of RANTES compared to non-carriers and suggest that CCL5 chemokine play a role in the parthenogenesis of T1D[9]. Other chemokines such as CCL2, CCL3, and CCL4 are presented at lower levels than CCL5 and CXL10. However, they also play a crucial role in recruiting immune cells through chemotaxis.

Despite the immunotherapeutic potential of TA/PVPON encapsulation material for islet transplants, little is known regarding the effects on chemokine production. In an attempt to identify how TA/PVPON may regulate chemokine expression in macrophages, the NOD (Non-Obese Diabetic) mouse model of spontaneous T1D was utilized to generate bone marrow-derived macrophages[6]. Classically-activated macrophages, termed M1, are pro-inflammatory and possess specialized abilities to kill intracellular microbes and destroy β-cells[10]. These macrophages become activated in response to microbial products such as lipopolysaccharide (LPS) or viral double-stranded RNA upon engagement with extracellular or intracellular specialized toll-like receptors (TLR). Upon activation, pro-inflammatory cytokines specific for M1 activation such as TNF-α will be secreted; ROS and macrophage activation markers CD80 and CD86 will also be expressed on the cell surface. In contrast, M2, or alternatively-activated macrophages, are involved in wound healing, allergic responses, and immune suppression. They will synthesize a different cytokine and chemokine profile, produce Arginase-1, and increase expression of scavenger receptors such as CD206[10]. Our results provide further evidence that the antioxidant and immunosuppressive properties of TA/PVPON can elicit a decrease in pro-inflammatory chemokine production, decrease pro-inflammatory M1 macrophages phenotypes, and potentially protect encapsulated islets from transplant rejection.

MATERIALS AND METHODS

Mice

NOD.ShiLtJ mice were bred and housed at the Research Support Building of the University of Alabama at Birmingham under pathogen-free conditions and observing IACUC-approved mouse protocols[11]. Male mice (age 7-9 weeks) were used in all experiments.

Tannic Acid and poly (n-vinylpyrrolidone) capsules (TA/PVPON)

TA/PVPON capsules were synthesized as previously described[4]. The three capsules provided were named according to their size: capsules 2-5, 4-5, and 4-5.5. Hollow hydrogen-bonded capsules were prepared by coating 4.0 ± 0.1 μm and 2.0 ± 0.1 μm silica particles in diameter with tannic acid and PVPON through a layer-by-layer method[4,7]. Number 5 and number 5.5 represents either tannic acid or PVPON on the outermost layer, respectively. Therefore, the numbers 2-5 represents a 2 μm silica particle with tannic acid on the outermost layer, while 4-5.5 represents a 4 μm silica particle with PVPON on the outermost layer.

Differentiation and stimulation of bone marrow-derived macrophages (BM-MΦ)

Bone marrow hematopoietic stem cells were isolated from femurs and tibias of NOD mice and differentiated into macrophages using L-929 conditioned macrophage media as previously described[12]. Stem cells were plated in 24-well plates (1 mL per well), petri dishes (15 mL per petri), and chamber slides (350 μL per chamber) at a concentration of 1.0×10^6 cells mL^{-1}. After 7 days, differentiated macrophages were treated with macrophage media depleted of L-929 conditioned media for 24 hours prior to stimulation. Cells were then stimulated with 25 μg mL^{-1} of the TLR3 ligand, poly(I:C), (low-molecular-weight double-stranded RNA synthetic analog InvivoGen) and treated with 1.0×10^7 counts mL^{-1} of tannic acid and poly (N-vinylpyrrolidone) capsules at various time intervals[11].

ELISA and Quantitative RT-PCR

Chemokine expression was measured in the supernatant of untreated, poly(I:C)-, and TA/PVPON-treated macrophages. CCL2, CCL3, CCL4, CCL5, CCL17, CXCL10 was detected with a DuoSet ELISA kit (R&D Systems) according to the manufacturer's instructions. ELISA plates were read on a Synergy 2 microplate reader (BioTek) using Gen5 software.

RNA was isolated from poly(I:C)-stimulated and TA/PVPON-treated BM-MΦ using TRIzol (Invitrogen) and cDNA prepared by SuperScript III (Invitrogen) according to the manufacturer's protocol. The generated cDNA was amplified on a Roche LightCycler 480 instrument by quantitative PCR using the following TaqMan gene expression assays (Applied Biosystems): *Emr1* (Mm00802529), *Ccl2* (Mm00441242), *Ccl3* (Mm00441259), *Ccl4* (Mm00443111), *Ccl5* (Mm01302428), *Ccl17* (Mm00516136), *Cxcl10* (Mm00445235). The relative gene expression levels were calculated with $2^{-\Delta\Delta Ct}$ method, and *Emr1* was used as a housekeeping control gene for normalization[11]. The unstimulated samples were used as calibrator controls and set as 1.

Flow Cytometry

Prior to staining, Fcγ receptors of bone marrow-derived macrophages were blocked with Fc block (BD Biosciences) for 10 minutes at 4 °C and then incubated with fluorochrome-conjugated antibodies specific for F4/80, CD80, CD86, TNF-α, and Arg-1 (BD Biosciences, eBiosciences) for 30 minutes at 4 °C. Cells were collected on the Attune NxT Flow Cytometry (ThermoFisher) and analyzed with FlowJo (10.0.8r1) software (Tree Star, Inc.).

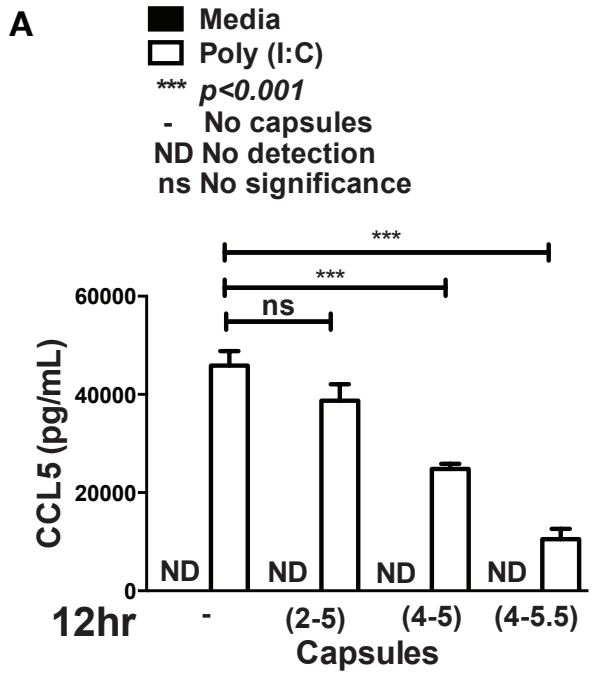
Statistical analysis

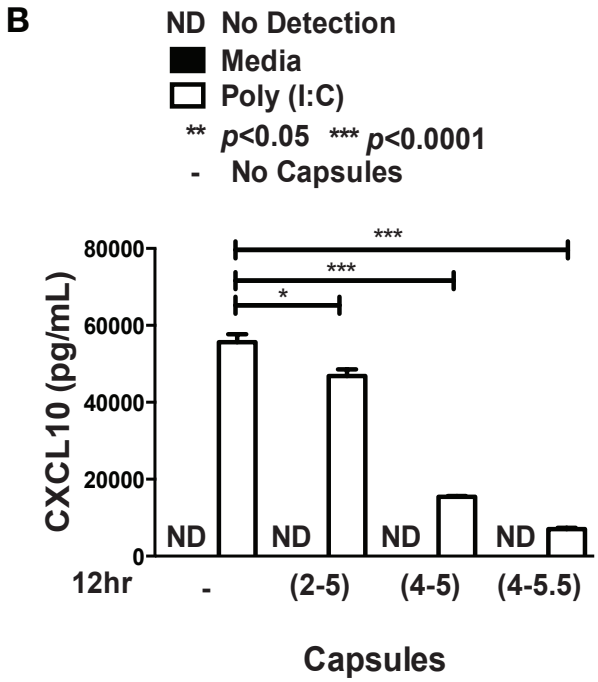
Data were analyzed using GraphPad Prism Version 5.0 statistical software. Determination of the difference between mean values for each experimental group was assessed using the 2-tailed *t*-test, with $p < 0.05$ considered significant. All experiments were performed at least three separate times with data obtained in triplicate wells in each experiment.

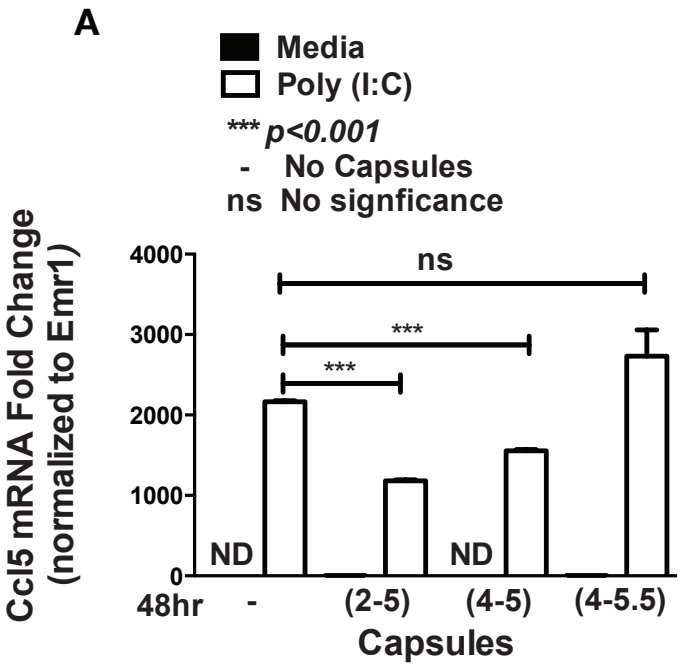
RESULTS

Pro-inflammatory chemokine expression was dampened in the presence of TA/PVPON

To determine the immunosuppressive role of TA/PVPON in regulating chemokine expression of bone marrow-derived macrophages, the levels of mRNA accumulation and protein expression were measured by quantitative RT-PCR (qRT-PCR) and ELISA, respectively. We determined that the synthesis of CCL5 and CXCL10 protein and mRNA chemokine expression were lowered when p(I:C)-stimulated samples were treated with TA/PVPON capsules. At 48-hour, *Ccl5* mRNA levels via qRT-PCR were significantly reduced 1.8-fold with capsules 2-5 and 1.4-fold with 4-5, but no difference was observed with capsule 4-5.5 (Figure 1A). Similarly, in Figure 1B, mRNA levels at 48-hour for *Cxcl10* displayed significant reduction of 2.6-, 3.6-, and 1.6-fold with 2-5, 4-5, and 4-5.5, respectively. To corroborate the decrease in chemokine mRNA levels, CCL5 and CXCL10 protein levels were suppressed consistently over a period of 96 hours *(data not shown)* by TA/PVPON capsules. At the 72-hour time point, CCL5 was reduced with capsule 2-5 at 1.8-fold while capsules 4-5 and 4-5.5 decreased CCL5 levels by 2-fold (Figure 2A). CXCL10 protein levels followed similar results with a 1.5-, 1.6-, and 1.4-fold reduction with capsules 2-5, 4-5, and 4-5.5, respectively (Figure 2B). Analyses of CCL2, CCL3, CCL4, and CCL17 mRNA and chemokine levels did not differ when p(I:C)-stimulated macrophages were treated with 2-5, 4-5, and 4-5.5 capsules *(data not shown)*.







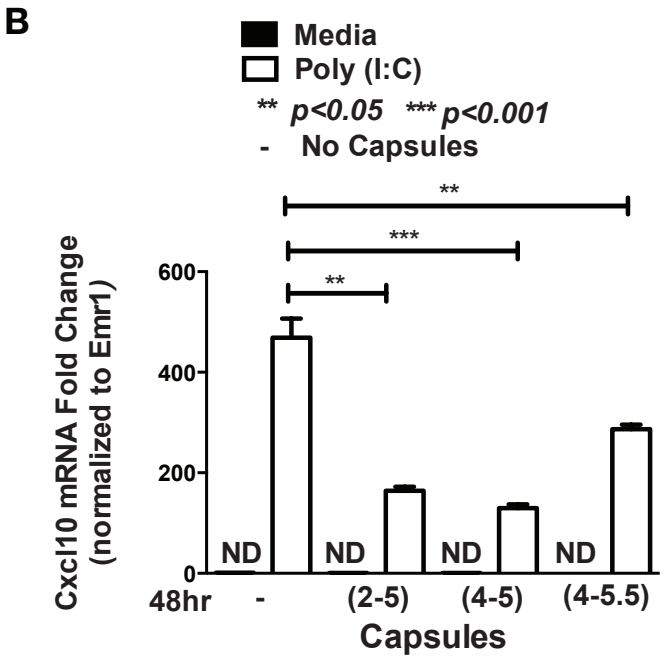
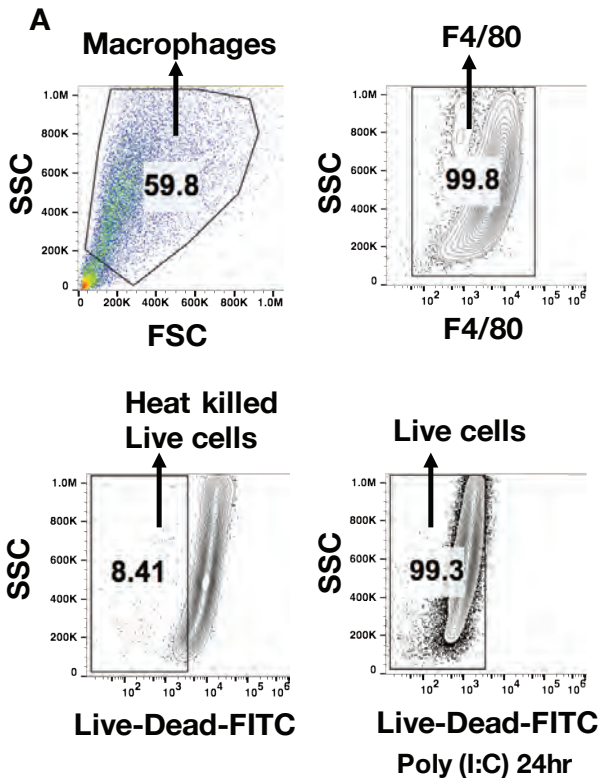


Figure 1| Ccl5 and Cxcl10 mRNA is reduced in poly(I:C)-stimulated macrophages co-treated with TA/PVPON capsules. Ccl5 *(A) and* Cxcl10 *(B) mRNA accumulation from TA/PVPON (at 1.0 × 10⁷ cells mL⁻¹)-treated bone marrow-derived macrophages was examined by qRT-PCR after 48-hour stimulation with 25 µg mL⁻¹ of poly(I:C). Results were normalized to no antigen/no capsules control group. Graphed data represent 3 independent experiments done in triplicates. With the standard deviation calculated as ns, not significant; ND, not detected.*

Figure 2| TA/PVPON capsules are efficient in decreasing CCL5 and CXCL10 chemokine expression. Supernatant of TA/PVPON (at 1.0 × 10⁷ cells mL⁻¹) treated bone marrow-derived macrophages stimulated at 25 µg mL⁻¹ of poly(I:C) was examined via ELISA. CCL5 (A) and CXCL10 (B) chemokine expression at 72-hour were normalized to the no antigen/no capsules control group. Graphed data represent 3 independent experiments done in triplicates. Statistics was performed with a two-tailed Student's t-test *and displayed as* ns, *not significant; ND, not detected.*

M1 activation markers and pro-inflammatory cytokine were reduced while M2 activation marker was elevated in the presence of TA/PVPON

To determine if bone marrow-derived macrophages exhibit reduced activation markers in the presence of TA/PVPON capsules, flow cytometric analysis of CD40, CD80, TNF-α, and Arg-1 expression levels with p(I:C)-stimulated macrophages was examined at the 24-hour time point. Shown in Figure 3 are gating strategies for the various macrophage markers. From the contour FACS plots, the y-axis represents the expression of the macrophage specific marker, F4/80, and the x-axis displays M1 and M2 macrophage activation markers, CD80, CD86, TNF-α, and Arg-1. In the upper right hand quadrant for each plot, double positive cells expressing F4/80 and cell surface activation markers shown via percentage and geometric mean fluorescence intensity (gMFI) is shown. The levels of CD80, an extracellular M1 macrophage activation marker, exhibited a reduction after co-treatment with capsules 4-5 and 4-5.5 of 2.5- and 1.3-fold via gMFI, respectively (Figure 4). Similarly, with CD86, another M1 macrophage co-stimulatory molecule, the gMFI and percentage was reduced by 2.3- and 1.3-fold for capsules 4-5 and 4-5.5, respectively (Figure 5). In Figure 6 the level of TNF-α, an intracellular M1 pro-inflammatory cytokine, was also lowered in gMFI and percentage for all capsules by 1.4-fold (2-5), 3-fold (4-5), and 1.8-fold (4-5.5). In contrast, Arg-1, an M2 intracellular activation marker, was increased slightly via gMFI when treated with capsule 2-5, but showed no elevation with capsules 4-5 and 4-5.5 (Figure 7).



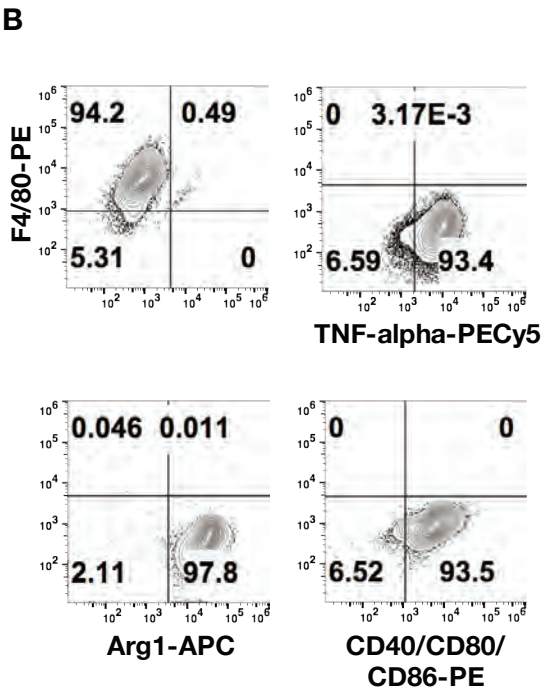


Figure 3| Gating strategies for F4/80 and macrophage activation markers via flow cytometry. A) Macrophages were gated using forward scatter side scatter profiles. Macrophages were heat-killed and gated via side scatter profile using a fixable live/dead APC stain (Invitrogen). F4/80 macrophage marker was gated via side scatter. Sample showing live cells gating was taken from poly(I:C) stimulated macrophages at 24-hour time point. B) Single color controls for macrophage markers. Each marker was stained with specific fluorochromes, PE, PeCy5, and APC, as labeled. F4/80 positive cells are located in the upper left-hand quadrant. The various markers are presented on the x-axis with bottom right quadrant representing positivity for particular marker.

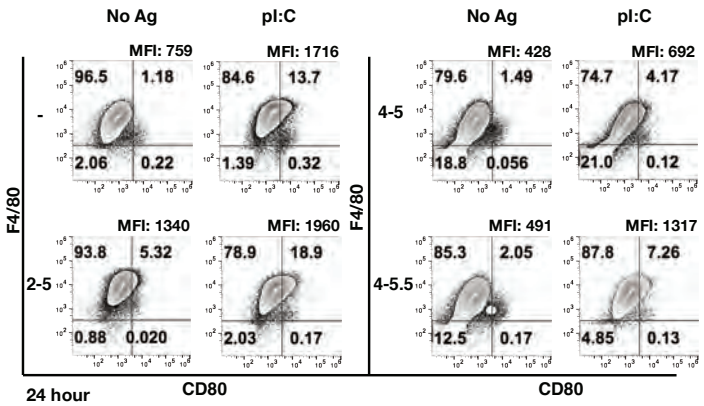


Figure 4| Macrophage activation marker, CD80, showed reduction in the presence of capsules. Flow cytometric analysis of CD80 by F4/80, a macrophage marker, is shown for 24 hours with TA/PVON (at 1.0 × 10^7 cells mL^{-1}) treated bone marrow-derived macrophages stimulated with 25 μg mL^{-1} of poly(I:C). CD80 was gated from live, F4/80 positive population of macrophages. FACS plots data represent 3 independent experiments done in triplicates.

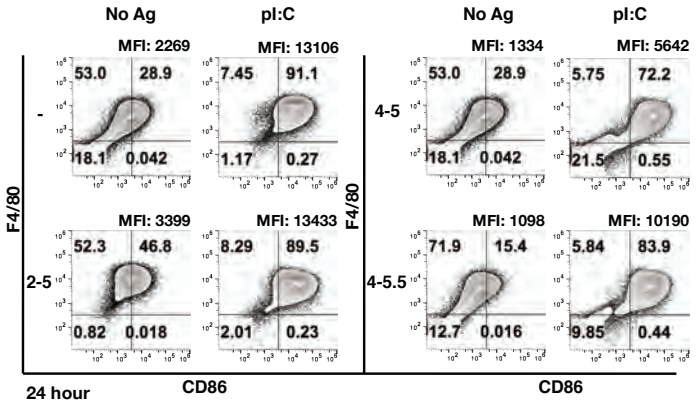


Figure 5: CD86, M1 macrophage activation marker, is suppressed in the presence of TA/PVPON. TA/PVON (at 1.0 × 10^7 cells mL^{-1}) treated bone marrow-derived macrophages stimulated with 25 µg mL^{-1} of poly(I:C) was examined using flow cytometry. Costimulatory marker CD86 was gated from the live, F4/80 positive population. Graphed data represent 3 independent experiments done in triplicates.

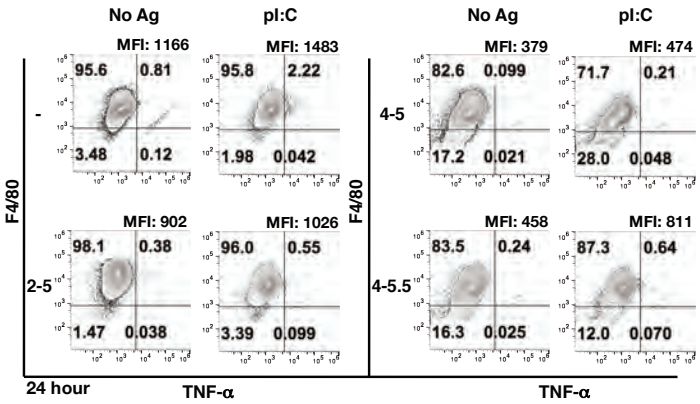


Figure 6: TA/PVPON capsules attenuate intracellular pro-inflammatory cytokine TNF-α expression. FACS plots were obtained at the 24-hour time point for TNF-α by F4/80 with 25 µg mL^{-1} poly(I:C) stimulated bone marrow-derived macrophages introduced to capsules (at 1.0 × 10^7 cells mL^{-1}). TNF-α was gated from live, F4/80 positive population. Graphed data represent 3 independent experiments done in triplicates.

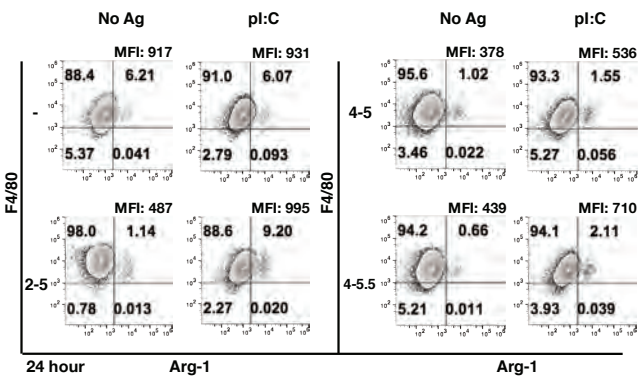


Figure 7: Arg-1 expression is elevated with the treatment of capsule 2-5. Flow cytometry analysis of Arg-1 by F4/80, macrophage marker, at 24-hour time point. TA/PVON (at 1.0 × 10^7 cells mL^{-1}) treated bone marrow-derived macrophages stimulated with 25 µg mL^{-1} of poly(I:C) were examined. Arg-1 was gated from live, F4/80 positive population of macrophages. Graphed data represent 3 independent experiments done in triplicates.

DISCUSSION

We have reported the importance of TA/PVPON capsules in dampening chemokine expression by ELISA and quantitative RT-PCR. With capsule treatments, both CXCL10 and CCL5 chemokines displayed decreased expression over a 96-hour time point. In addition, both chemokines presented significant decreases in mRNA accumulation at the transcriptional level. CXCL10 and CCL5 are important pro-inflammatory chemokines shown to correlate with T1D in humans and in mice. In the serum of T1D patients, elevated CXCL10 levels were expressed suggesting that CXCL10 is a probable marker for predicting T1D[8]. For CCL5, elevated levels of CCL5 in mice splenocytes have shown to relate to the disease onset. As shown in Figure 1, the result displays capsule 2-5 exhibiting the most reduction in *Ccl5* and *Cxcl10* mRNA, followed by 4-5 and 4-5.5. From the data, we can conclude that thinner capsules containing tannic acid on their outer layer provide more immunosuppressive properties at the transcriptional level while thicker capsules provide more suppression at the translational level. In addition, it may be that thicker capsules, such as capsule 4-5.5, present their antioxidant properties much slower and more gradually than thinner capsules due to the PVPON being on the outermost layer. In contrast, at the protein level, Figure 2 displays capsule 4-5.5 exhibiting more reduction in CCL5 and CXCL10. From the results we can postulate that having PVPON on the outermost layer may delay the antioxidant properties of TA and provide a protective layer for the tannic acid layer to function properly over a longer span of time. Both layer size and composition of TA/PVPON capsules can contribute to the effectiveness of these capsules in suppressing the immune response. At both protein and mRNA levels, reduction in CXCL10 and CCL5 chemokines with the presence of TA/PVPON support the hypothesis that these capsules are immunosuppressive.

In addition to suppressing chemokine expression, macrophage activation markers CD80, CD86, and pro-inflammatory cytokine, TNF-α, were both dampened by TA/PVPON (Figures 4 and 5). Recall that CD80, CD86, and TNF-α are indicative of an M1 macrophage phenotype. Due to this fact, the expected result would be to observe the minimal CD80, CD86, and TNF-α expression. As predicted, decreased levels of CD86, CD80, and TNF-α suggest that TA/PVPON capsules 4-5 and 4-5.5 are reducing the level of M1 (pro-inflammatory macrophages) activation. In addition, little to no reduction in CD80 and CD86 with capsule 2-5 may arise from the properties of the capsule. It may be that the thickness of capsule 2-5 is insufficient in dampening the activation markers presented by M1 macrophages. However, because CD80 and CD86 are extracellular M1 macrophage activation markers while TNF-α is an intracellular M1 activation marker, the reduction in these markers provide evidence that capsules 4-5 and 4-5.5 are effective in dampening M1 macrophage differentiation. With M2 macrophage activation, we would expect an increase in Arg-1 marker for all three capsules; however, only capsule 2-5 resulted in an increase at the 24-hour time point. These observations may suggest that with thinner capsules consisting of TA on the outermost layer, macrophages take on a more protective characteristic that is not seen in capsules 4-5 or 4-5.5. Overall, the decrease in CD80, CD86, and TNF-α does support the overarching hypothesis that thicker TA/PVPON capsules can dampen pro-inflammatory M1 macrophage differentiation and activation while supporting M2 macrophage differentiation with thinner capsules.

In our future studies, we want to test the *in vitro* and *in vivo* capability of TA/PVPON capsules through islet encapsulation and islet transplantation in NOD mice to determine if there is a decrease in chemokine expression as well as macrophage activation markers. We also want to observe pro-inflammatory chemokines such as CXCL10, CCL2, CCL3, CCL4, CCL5 and CCL17 that are involved in the inflammation process *in vivo*. We want to determine if there is any correlation in data between *in vitro* and *in vivo* experiments. With the findings from this experiment, further progress can be made in developing a novel material that can encapsulate and protect pancreatic β-cell islets from being targeted by the immune system. Furthermore, the success of micro islet transplantation via stem cells or pancreatic cells can be sustained by the progress made in TA/PVPON research.

ACKNOWLEDGMENTS

This work was supported by an NIH/NIDDK R01 award (DK099550), an American Diabetes Association Career Development Award (7-12-CD-11) (HMT), the NIH NIAID (5T32AI007051-35), and the Immunologic Diseases and Basic Immunology T32 training grant (LEP). The following core facilities of UAB were used to generate data for the manuscript: Animal Resources Program (G20RR025858, Sam Cartner, DVM, PhD), the Comprehensive Arthritis, Musculoskeletal, and Autoimmunity Center: Analytic and Preparative Cytometry Facility (P30 AR48311, John D. Mountz, MD, PhD), the Comprehensive Arthritis, Musculoskeletal, and Autoimmunity Center: Epitope Recognition Immunoreagent Core (P30 AR48311, Mary Ann Accavitti-Loper, PhD).

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2016-2017 *Inquiro* Submission Guidelines

Any UAB undergraduate student participating in scientific research and/or any undergraduate student participating in research at UAB is invited to submit a manuscript to be considered for publication in the 2016 – 2017 issue of *Inquiro*. Papers will be subject to anonymous review by faculty and students.

The deadline for submission is October 17, 2016; however, students participating in summer research at UAB or at another institution are encouraged to submit by September 9, 2016.

The journal accepts submissions in the following categories:

Short reports: A short report should give a concise overview of an original research project. Its content is comparable to that of science posters. The suggested length is 1,000 – 2,000 words.

Long papers: A long paper should give a substantial description of an original research project. It should include detailed discussions of the methods utilized and the results obtained. The suggested length is 2,500 – 4,000 words.

Research narratives: A research narrative describes an author's personal experiences in research using an editorial or narrative style. The suggested length is 600 – 800 words.

Theses: A thesis is similar to a long paper and should therefore be of similar length and content. The suggested length is 2,500 – 4,000 words. Students participating in undergraduate programs that require a thesis are encouraged to submit their work.

Literature Reviews: A literature review should give detailed information on the methodology of a scientific topic as well as the current scientific findings associated with the topic. Unlike a research paper, a literature review does not report novel information obtained from research performed by the author(s). The suggested length is 2,500 – 4,000 words.

The editorial board is also open to considering other forms of scientific writing for publication. Authors who wish to publish work that does not fit in one of the previously mentioned categories are strongly encouraged to contact the editors before submitting to the journal.

Initial submissions should follow these guidelines:

1. All submissions should be submitted as Microsoft Word documents, double spaced and formatted in 12 pt Times New Roman font. Pages should be numbered with the name of the primary author appearing in a header on every page.

2. Research papers should be written in third person. Research narratives should be written in first person.

3. Research papers should include a title, the full name(s) and affiliation(s) of the author(s), and the following sections: Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusion, and References. Please consult the Manuscript Guidelines for Authors on our website for more specific instructions on each section.

4. Figures, tables and graphs should be submitted in their original formats in the highest resolution possible. They may be submitted as separate files or embedded in the text of the document in the locations in which the author would like them to appear in a published version. If submitted separately, please indicate in the manuscript where the figures should appear.

All manuscripts should be submitted through email, and authors should complete the submission form linked on the Submit page on our website. Include the article title as well as your name, university, major, and class standing in the body of the email.

Before submitting, please ensure that your mentor has read and approved of your manuscript. Your mentor will automatically receive an email requesting his or her approval of your submission. The manuscript guidelines document linked on our website should also be reviewed before submission.

Please send any questions or comments to inquiro@uab.edu. Students who wish to join the *Inquiro* staff may also contact the editors at this address.

For more information or to view previous publications, visit our website at uab.edu/inquiro.

Chief Editors

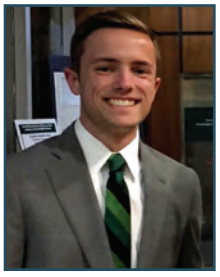
John Decker

John Decker is a 2016 graduate of UAB and the University Honors Program with a B.S. in Neuroscience. His undergraduate research at UAB included three years of work relating to the neural control of eye movements, conducted under the supervision of Dr. Paul Gamlin in the Department of Ophthalmology. Outside of school, John enjoys literature, fencing, the outdoors, and aviation. In the fall of 2016, he will begin the Ph.D. program in Biomedical Engineering at Duke University.

Maggie Collier

Maggie Collier is a junior Biomedical Engineering and Electrical Engineering student and a member of the Science and Technology Honors Program. Under the mentorship of Dr. Ho-Wook Jun, Maggie researches the use of a nanomatrix coating applied to the surface of microcoils used in coil embolization, a common brain aneurysm treatment. She is also a part of a team of undergraduate and graduate students working to a build a 3D bioprinter for Dr. Zhang, Chair of the Department of Biomedical Engineering. After graduation, Maggie intends to pursue a research career in biotechnology while continuing to participate in various forms of scientific communication, particularly in scientific writing.

Assistant Editor



Josh Purvis

Josh is a sophomore neuroscience major. He is involved with two projects currently. First, Josh performs neuroimaging analysis to look for structural changes in the brain after Constraint-Induced Movement Therapy in MS and TBI patients. The other project is using Constraint-Induced Biofeedback Therapy to help restore upper motor function to a person with tetraplegia. His future plans are to get a Ph.D. in either behavioral or cognitive neuroscience and one day to become a professor at a major research university.

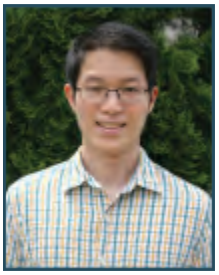
Editorial Board

Muna Al-Safarjalani

Muna Al-Safarjalani is a junior in the Honors College pursuing a major in Chemistry with a minor in French. For the past two years, Muna has researched under the mentorship of Dr. Andrei Stanishevsky. Her undergraduate research concerns the electrospinning and centrifugal spinning of biopolymer nanofibers and the consequential characteristics that affect their biocompatibility as systems for drug delivery and tissue healing. Muna has also conducted research on the local and national economic repercussions of the American prison system. Upon graduation, she hopes to complete a Pharm.D./Ph.D program.

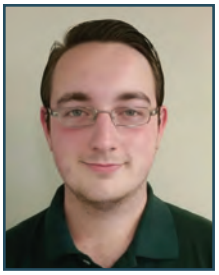
Hriday Bhambhvani

Hriday is a rising senior pursuing a dual degree in neuroscience and mathematics. He works in the lab of Dr. James Meador-Woodruff, where he studies cytoskeletal dynamics in schizophrenia. Eventually, Hriday hopes to work as a physician-scientist and study the molecular basis of neuropsychiatric illness.



Alexander Chang

Alexander is a junior pursuing a B.S. in Molecular Biology and a minor in chemistry. After graduating, he plans on attending medical school. He has worked in Dr. Shahid Mukhtar's lab researching transcription factors associated with *Ralstonia solanacearum*, a plant pathogenic bacterium categorized under bioterrorism and in Dr. Lubin's lab researching the epigenetics of OGT. During his free time, he enjoys playing cello, discovering music, and cooking food.

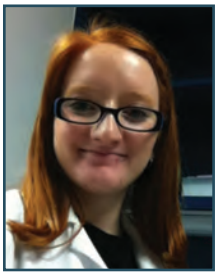


David Chasteen-Boyd

David Chasteen-Boyd is a junior majoring in biomedical engineering, mechanical engineering, and math. He currently does research in Dr. Ho-Wook Jun's lab on the use of an implantable matrix composed of peptide amphiphile nanofibers and hydroxyapatite crystals that mimics the extracellular matrix of bone to speed growth and repair of fractures and bone defects. He also works on the feasibility of incorporating pH-sensitive liposomes as a sustained-release drug delivery system into many of the different projects that his research lab is working on. After graduating, David plans on entering an M.D./Ph.D. program and working on the development of prosthetic limbs.

Daniel Gilliam

Daniel is a senior in the Science and Technology Honors Program and the Early Medical School Acceptance Program double majoring in Neuroscience and Chemistry. He works in Dr. David Sweatt's lab investigating the epigenetic processes underlying learning and memory. In his spare time he enjoys reading, hiking, and playing piano and cello. After graduation Daniel will be pursuing a Ph.D. in Neuroscience at Harvard.



Emily Haley

Emily is a junior neuroscience major. She is studying an enzyme (alpha Galactosidase) in Parkinson Disease that may help with clearance of the pathogenic alpha-synuclein protein aggregates. She works with cell culture and mouse models. After earning her neuroscience B.S. in December she plans to pursue a neuroscience Ph.D. and continue studying neurodegenerative disorders.

Emily Jennings

Emily Jennings is a junior majoring in Neuroscience and minoring in Chemistry and Biology. Under the mentorship of Drs. Thomas van Groen and Inga Kadish in the Department of Cell, Developmental and integrative Biology, Emily has helped research a way to reduce the cognitive deficits associated with Alzheimer's Disease using a transgenic mouse model and the ANK6 dipeptide. Emily has also worked with aging and longevity. In her spare time, Emily enjoys traveling, reading, and keeping up with soccer (the real football).

Charles Keith

Charlie Keith is a sophomore in the Science and Technology Honors Program. He is majoring in Anthropology with minors in Biology and Peace, Justice, and Ecology. He is working in Dr. Morris' lab learning different bioinformatics packages to sequence genomes and analyze microbial communities. He enjoys all kinds of music and plays the guitar and the piano in his free time.

Jessica Maya

Jessica is a Junior majoring in Molecular Biology and is interested in research dealing with genetics, genomics, and bioinformatics. She is currently collaborating with Dr. Eric Sorscher and Dr. John Hartman in the UAB Cystic Fibrosis Research Center, looking at the structure and function of the gene product responsible for cystic fibrosis. They are working at a molecular level to discover new approaches for therapy for this disease. She hopes to pursue a Ph.D. in the future.

Susmita Murthy

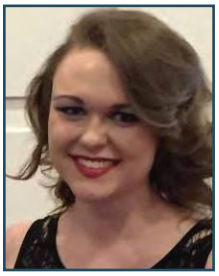
Susmita Murthy is a junior double-majoring in History and Biology. She plans on attending medical school after the completion of her undergraduate degree. She has done research in the lab of Dr. Michael Miller in the Department of Cell Biology studying the effects of Amyotrophic Lateral Sclerosis in *C. Elegans*.

Aashka Patel

Aashka Patel is a junior in the Early Medical School Acceptance Program and University Honors Program. She is pursuing a major in Neuroscience and a minor in Chemistry and Philosophy. Her research focuses mainly on frontotemporal dementia in Dr. Erik Roberson's lab in the Department of Neurobiology. In addition to being a member on the *Inquiro* board, Aashka serves as a UAB Ambassador. In her free time, she enjoys reading and spending time with her family and friends.

Amy Stewart

Amy Stewart is a senior Neuroscience major in the University Honors Program. She is researching the antidepressant effects of ketamine in hippocampus in Dr. Lori McMahon's lab and will be attending Duke University in the fall to pursue a Ph.D. in Cell and Molecular Biology. In her free time, she enjoys reading and playing the flute.



Marina Triplett

Marina Triplett is a junior in the Science and Technology Honors and Chemistry Scholars programs. She is pursuing a degree in Chemistry with minors in Biology and Spanish. She conducts research in the Department of Pharmacology and Toxicology in the lab of Dr. Mary-Ann Bjornsti, where she is studying the relationship between DNA supercoiling and yeast cell toxicity. After completing her undergraduate degree, she plans to earn a Ph.D. in Biochemistry or Genetics and pursue a career in scientific research. In her spare time, she enjoys watching classic films and attending musicals and classic rock concerts.

Neha Udayakumar

Neha Udayakumar is a junior in the Science and Technology Honors and BioScholars programs. Her research in the Department of Biology investigates the potential for combinatorial treatments with dietary compounds for adjuvant treatment of breast cancer. Aside from academics, she is a freelance graphic designer and has an interest in medical illustration. She plans to pursue a career as a physician in the future.

Courtney Walker

Courtney Walker is a junior neuroscience major in the Science and Technology Honors Program. She conducts research in Dr. Rosalinda Roberts's lab on the role of the substantia nigra in schizophrenia. In the future, she will pursue a Ph.D. in neuroscience and a career in research on mental illnesses. In her spare time, she enjoys playing the piano.

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acknowledgments

Without the help and support of UAB faculty and staff, the vision of Inquiro *could not have been made a reality. Many thanks to the following individuals:*

2015 Faculty Reviewers:
Dr. Pat Bucy
Dr. Jeremy Day
Dr. Suzanne Judd
Dr. Steven Pittler
Dr. Jack Rogers
Dr. Laura Volpicelli-Daley

Dr. Mike Sloane and the University Honors Program
Dr. Diane Tucker and the Science and Technology Honors Program
Ryan McAnulty, UAB Printing Services (production and printing)

A special thanks to Dr. Suzanne Austin, Senior Vice Provost, Libba Vaughn, Director of Service Learning and Undergraduate Research, and Gareth Jones, Program Administrator for Service Learning and Undergraduate Research.

The production and publication of this journal was made possible through the funding supplied by the Office for Student and the Faculty Success of the University of Alabama at Birmingham.

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Volume 9 • 2015

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