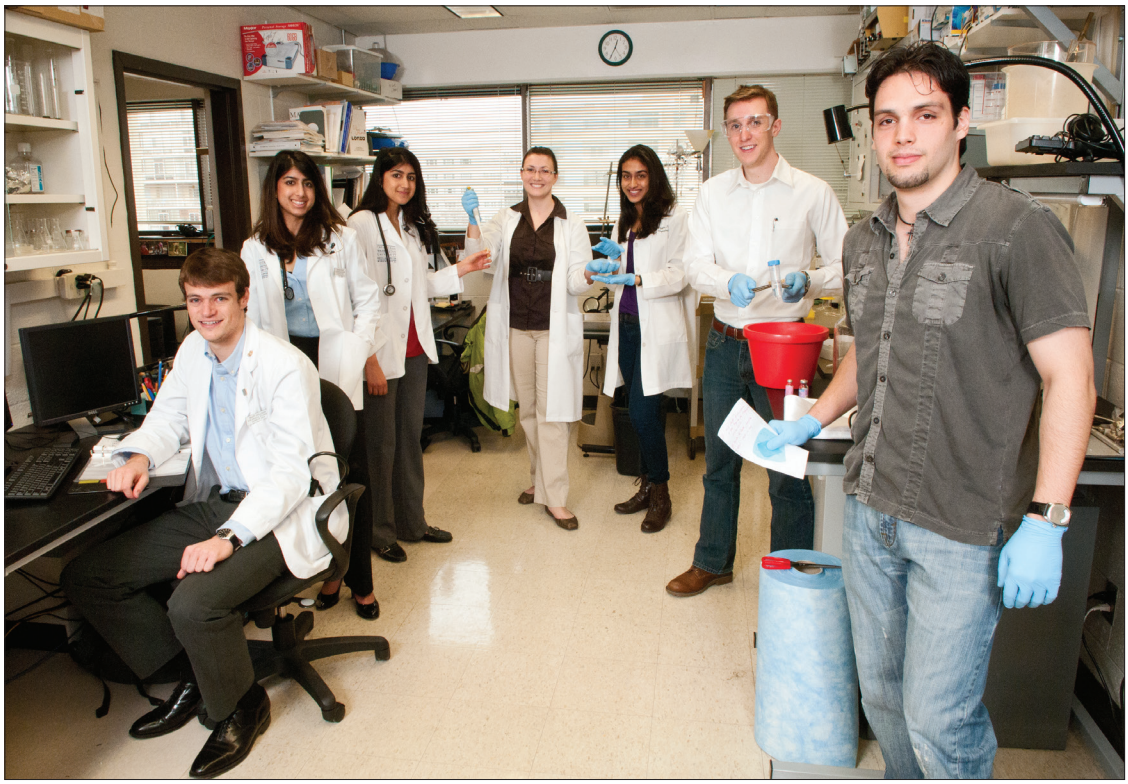
Fusion

The student-run scientific journal of the George Washington University School of Medicine and Health Sciences

The William H. Beaumont Medical Research Honor Society Vol. VI, Spring 2012

Welcome to Fusion

Dear GW Community and Fellow Research Enthusiasts,



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From left: Matt Holzner, MSII; Neena Passi, MSII; Monica Passi, MSI; Kerri Vincenti, MSII; Yasmin Akbari, MSII; Justin Ertle, MSII; and Thomas Zaikos, MSII.

As part of our commitment to fostering student interest in research, the William Beaumont Medical Research Honors Society at GW's School of Medicine and Health Sciences (SMHS) is proud to introduce the 2012 edition of Fusion — a student-run research journal that serves as a forum for students to share their research experiences with the GW medical community.

This year's edition focuses on the theme of putting basic science research into clinical practice. Medicine is an incredibly dynamic and rapidly evolving field. At the same time, basic science is identifying new molecular pathways, genetic polymorphisms in disease, and targeted pharmaceutical therapies at an impressive rate. While these developments are aimed at advancing the medical field, the integration of these innovations into clinical practice also poses a unique challenge for today's health care professionals.

Never before has the collaboration and coordination of researchers and health care practitioners been more important. As future medical professionals it is imperative that we seek to continue to integrate these dynamic aspects into our own practice. However, learning these skills most often requires us to immerse ourselves in enriching experiences outside the classroom, and it is these endeavors that *Fusion* highlights. This publication features a "fusion" of clinical, basic science, and public health research, reflecting the experiences of a number of enthusiastic and dedicated members of the SMHS student body. We hope that as you enjoy reading the following articles you are inspired to seek out opportunities that will help strengthen your own skills as future health care professionals.

Lastly, we would like to thank all of our authors and artists who help to make *Fusion* a great success each year. We also extend our many thanks to our faculty advisor, Vincent A. Chiappinelli, Ph.D., interim associate vice president for Health Affairs and associate dean of SMHS, and the Office of the Dean for their generous support, and to SMHS Office of Communications and Thomas Kohout and Michael Leong for making *Fusion* a reality.

Your editors,
Justin, Kerri, Matt, Monica, Neena, Thomas, and Yasmin

Fusion

is a publication of the George Washington University School of Medicine and Health Sciences William H. Beaumont Medical Research Honor Society.

This research journal is published by students in collaboration with the Office of the Dean and the School of Medicine and Health Sciences Office of Communications and Marketing.

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Letters

Following the Route to Discovery

Jeffrey S. Akman, M.D. '81, GME '85 Interim Vice President for Health Affairs, Dean, School of Medicine and Health Sciences

I am thrilled to congratulate the students of the William H. Beaumont Medical Research Honor Society for their dedication to research and their willingness to take on the challenge of producing the sixth edition of their scientific journal, *Fusion*. This publication is an example of how our students frequently go beyond their didactic and clinical requirements to delve into finding the root causes of disease and discovering new therapies. The research published on these pages is a culmination of a wide-range of research interests, a lot of hard work, and desire to push the needle forward to ensure that health professionals have the information they need to make informed decisions.

As an institution, GW is committed to research and the School of Medicine and Health Sciences is a leader in those efforts. I am extremely proud of our faculty for fostering our student's desire to learn, as well as for identifying opportunities for continued research in an environment where it is not always easy to attain financial support. As our health care system evolves to meet the needs of the future, research and discovery will continue to grow in importance. The lessons medical students learn today will lay the foundation for successful medical careers in the years to come.

GW's Beaumont Society was established at the School of Medicine in 1935 to encourage student research. The Society promotes the development of research knowledge, skills, and experiences while gaining an appreciation of the influence of research on the current and future practice of medicine.

Once again, congratulations to the Beaumont Society and its faculty advisors on a fantastic publication and I wish you the best in your future scientific endeavors.

Enthusiasm and Endurance are the Keys to Academic Achievement

David Leitenberg, M.D., Ph.D., associate professor, Microbiology, Immunology and Tropical Medicine; Pediatrics; Pathology

By the time they reach medical school, student have already achieved a significant level of academic success and have developed unique interests that set them apart as individuals. It is critical that as students progress through medical school and post-graduate training there be opportunities to preserve and foster the development of these and additional interests. One way this is done is by encouraging the pursuit of individual research experiences, be they basic, clinical, or translational.

There are many ways to achieve a meaningful research experience, from a two-month summer experience to a decade of Ph.D. and post-doctoral training. In reflecting on my own career path as an M.D.-Ph.D. student, it is clear that the most important feature was not to be the student who got the highest test scores (that was never true), but to simply have the endurance and enthusiasm to persevere through an extended period of training, and to tolerate the inevitable failures and critical reviews inherent in research.

My primary motivation was not dependent upon whether a given project was clinically relevant. The potential clinical impact of basic science research, as well as clinical research, is difficult if not impossible to predict. I believe that the most important motivation for those pursuing research is an inherent curiosity and enthusiasm for learning how things work or determining how to make things work better.

Students come to medical school with that curiosity and enthusiasm to learn about how the complexities of biological systems are related to clinical care. Unfortunately, it's often lost, partly because of the way we teach in the pre-clinical years and also because as students become more exposed to clinical decision making it becomes self-evident that a lot of the biomedical science knowledge that they committed to memory has relatively little relevance to patient care. Because of this disconnect, one may justifiably ask why there is such an emphasis on basic biology in medical school. I think the answer partly lies in the ability of scientific teaching to foster critical thinking and intellectual curiosity. As students go on in clinical practice, whether this is combined with a research interest or not, it is important that they are intellectually equipped to manage the more atypical patient presentations and critically challenge standard ways of treating patients.

It is here that those students involved in research and who have contributed to *Fusion* have a special role. You can serve as role models for your peers by demonstrating a commitment to intellectual curiosity. And perhaps, with enthusiasm and perseverance, you will develop the skills and opportunity to devise new approaches to clinical care and challenge some of the more counter-productive aspects of the current practice of medicine.

The Use of Ayahuasca Among the Kichwa of Ecuador: The Potential Dangers of Mixing Western Pharmacotherapy and Traditional Shamanistic Medicine

Alissa McInerney, MSII

Ayahuasca [aja'waska] is a hallucinogenic beverage that is made from two indigenous plants from the Northwest Amazon region, especially by the Kichwa tribe of Ecuador. Shamans in these regions have used the elixir for centuries, claiming it has diagnostic and curing effects, in addition to its psychedelics effects believed to serve as a route to the supernatural realm. Shamans of the community are very knowledgeable in its usage, as well as other Amazonian medicinal plants; however, until recently, Western medicine was not readily available. With the recent construction of a road connecting the community to other small communities and the river launch point to the city, there are concerns of the potential blending of modern pharmacotherapy with traditional medicinal plants such as Ayahuasca.[2]

Studies of this brew have isolated its active substances and derivates, two of which are N,N-dimethyltryptamine (DMT) and a plant alkaloid from the β-carbolines. DMT is a natural psychoactive indolealkylamine, a non-selective serotonin 5HT agonist. It has a high affinity for serotonin 5HT1A receptors, while the active metabolite bufotenine has higher affinity for 5HT2A. The active metabolite occurs as a result of metabolism by the cytochrome P450 system CYP2D6. DMT is mainly inactivated through the deamination pathway mediated by monoamine oxidase-A (MAO-A). β-carboline alkaloid is a well-known potent MAO-A inhibitor. Thus, when dually ingested in the Ayahuasca drink, a β-carboline such as harmaline inhibits the breakdown of DMT and increases the synaptic levels of the active metabolite, bufotenine.[3]

After spending several days with the very small and isolated Kichwa community in the Napo region of Ecuador, I realize how mixing traditional shamanistic medicine and western pharmacotherapy could lead to potentially fatal consequences. The improved access to Western medical care for the Kichwa people and the influx of Western tourists, students, and volunteers, poses a great risk of mixing the use of Ayahuasca with their current pharmacotherapy. Common drug interactions of concern are selective serotonin reuptake inhibitors (SSRIs) among others. This class of drugs is commonly prescribed in the western world and when taken with Ayahuasca can cause a toxic increase in serotonin causing serotonin syndrome.[3] This adverse drug reaction can have mild to life threatening symptoms caused by increased serotonergic activity. The classic clinical description includes changes in mental status, autonomic hyperactivity, and neuromuscular abnormalities. However, symptoms can range from diarrhea, tremor, myoclonus, dilated pupils, hyperthermia, and increased blood pressure and heart rate that may eventually lead to metabolic acidosis and shock. It is often difficult to diagnose because of the range of severity of the symptoms.[1]

Members of the Kichwa community of Rio Blanco in a traditional performance.

After speaking with a Kichwa shaman, it was very clear that he was unaware of any interactions or adverse reactions that could occur with Ayahuasca or any other medicinal plant therapy because he was never exposed to such Western pharmacotherapy before. Therefore, it is in my opinion that shamans of the indigenous communities in the Napo region of the Amazon receive some education about pharmacotherapy of the modern world and about fusing the indigenous shamanistic medicine with modern western medicine. It is also important that travelers and physicians treating those who may be traveling to indigenous communities be made aware of the potential risks including drug-drug interactions before taking Ayahuasca or other medicinal plants.

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The Importance of Pediatrics in Disaster Planning

Ireal Johnson, MSII

During the 2010 Earthquake in Haiti, children under the age of 12 accounted for 65.9 percent of all deaths; they were eleven times as likely to die due to sustained injuries, and twice as likely to die due to illness compared to adults.[1] There is a disparity in options for treating children that becomes clearer in disasters that largely affect the pediatric population, necessitating a re-evaluation and restructuring of current strategies in pediatric emergency management.

Once a disaster involving children has occurred, health care professionals should determine and manage surge capacity by using an effective pediatric triage system.

Emergency management is the discipline of preparing for and avoiding risks that have large catastrophic consequences. It involves a coordinated effort from multiple professional fields with the main goal of public safety followed by the protection of resources and property. There are four phases that work in sequence to reach this goal: mitigation, preparedness, response and recovery. Many resources are available that outline health care planning and mitigation in response to a disaster event. However, the majority of these plans focus on care for adults and wrongly assume that the same strategies will work for children. In a study of pediatric surgeons, 77 percent felt, "definitely responsible" to help during a disaster, yet only 24 percent reported feeling "definitely prepared."[2] Many emergency physicians have also felt less prepared and more uncomfortable with pediatric emergencies versus emergencies in adults.

Children are more vulnerable during disasters because they have important biological differences that make them more susceptible to insults like disease, physical injury, and dehydration.[3] Children also present special challenges during disasters, such as separation from caregivers and inability to relay important medical information. Reunification may be more difficult to achieve for younger children since many are unable to identify themselves, their parents or their caregivers. Additionally, many hospitals are not adequately equipped to handle the large variations in sizes that children go through as they develop. This equates to sub-standard quality of treatment, especially for smaller children, since they are the least compatible with readily available adult-sized equipment.

There are some things that can be done to help address these problems. In preparation for a disaster, health care professionals should discuss emergency plans with patients and their families and suggest assembling a disaster tool kit.[4] Health care professionals should be involved with organizations that perform drills in order to increase and test their disaster response ability. They should also try to advocate for pediatric-specific protocols in disaster planning. This will educate all types of health professionals on the basics of treating children and increase awareness on the differences between pediatric and adult medicine. Once a disaster involving children has occurred, health care professionals should determine and manage surge capacity by using an effective pediatric triage system. Determining the level of care each patient needs improves allocation of resources; with more attention and resources being spent on acutely injured and treatable victims.[4] Professionals should also understand the established communication system in order to increase the effectiveness of their efforts. Improved communication would also assist in reunification between separated children and families. Patients and their families should get adequate support on how to cope after a disaster. It is important for experienced professionals to work with children to guide and provide developmentally appropriate ways of dealing with their feelings.[2]

There is a widespread lack of confidence and comfort in treating ill or injured children among health care professionals. It is important to address the discomfort and disparities in treating injured children as they make up a quarter of the U.S. population. Ideally, these suggestions will increase awareness and improve pediatric disaster care. Future studies should determine the necessary steps to implement pediatric specific procedures and policies in disaster preparedness and planning.

For more information about pediatric emergency management, visit *www.pedsem.com.*

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Radiosensitization of Melanoma Cell Lines Using Molecular Mutant B-Raf Drug Inhibitor GSK2118436 Treatment

Bradley T. Anderson, MSII
Advisor: Erik P. Sulman, M.D., Ph.D., The University of Texas MD Anderson Cancer Center

Melanoma is an aggressive form of cancer that has an increasing incidence in the United States.[1] Patients with metastatic melanoma commonly develop brain metastases.[2] An activating valine-glutamate substitution at position 600 of the B-RAF protein has been implicated in approximately 50 percent of melanoma cancers.[3] The B-RAF protein is a serine/threonine protein kinase of the RAS-RAF-MEK-MAPK signaling pathway involved in growth and proliferation. GSK2118436 is a molecular inhibitor that selectively binds and blocks the activity of mutant B-RAF proteins. Early clinical trials show that GSK2118436 may control and reduce brain metastases from melanoma.[4] Primary and secondary drug resistance has been observed in clinical trials of B-RAF drug inhibitors such as GSK2118436.[5] The effects of combinatorial treatment of melanoma brain metastases with GSK2118436 and ionizing radiation therapy are currently unknown. The combined therapy could be toxic, neutral, additive or synergistic. By determining if GSK2118436 can increase the sensitivity of radiation resistant melanoma cells to ionizing radiation treatment, this study assesses the capability of GSK2118436 to synergistically kill or radiosensitize human BRAF mutant melanoma cell lines.

Clonogenic survival assays were performed to assess in vitro cell survival following increasing doses of radiation (0–8 Gray or J/Kg). This assay establishes the relative sensitivity to radiation of the following BRAF mutant cell lines harvested from human melanoma tumors: A375, WM2664, WM35, and SKMEL5. Radiosensitization of cell lines was determined by comparison of survival with and without the B-RAF inhibitor, GSK2118436. Molecular inhibition of mutant B-RAF was confirmed by immunoblot analysis.

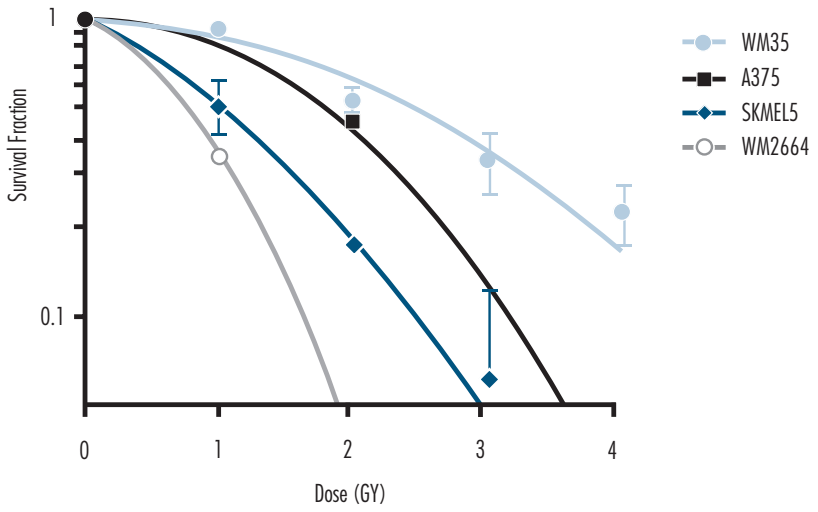

FIGURE 1: Melanoma Cell Survival Curves. *Survival curves for A375, WM35, WM2664, and SKMEL5 performed at 0-8 Gy of ionizing radiation showing the relative radiosensitivity of human melanoma cancer cell lines. The highest survival fraction at 2 Grays (SF2) was 0.91 for WM35. The lowest SF2 was 0.48 for WM2664.*

BRAF mutant melanoma cell lines showed varying radiosensitivities to radiation treatment. Figure 1 shows the results of the radiation clonogenic assay establishing a radiosensitivity baseline. The radiosensitivity was determined with the cell lines listed in order of decreasing radiosensitivity: WM2664 > SKMEL5 > A375 > WM35. WM2664 was the most sensitive to radiation with the highest survival fraction after 2 Gray (SF2). WM35 was the most resistant to radiation with the lowest SF2 value. A375 and WM35 were selected as candidates for examining radiosensitivity effects of combinatorial GSK2118436 and radiation therapy. The results of this combinatorial therapy are shown in Figure 2. The survival fraction decreased significantly with low dose GSK2118436 combination in the radiation resistant cells (WM35). A375 are semi-radiation sensitive cells and the response to B-RAF inhibitor was not as dramatic as seen in the radioresistant line WM35.

The B-RAF inhibitor GSK2118436 increases radiation sensitivity in the radiation resistant, mutant BRAF melanoma cancer cell lines. Our results confirm that melanoma cell lines demonstrate variable sensitivity to radiation therapy. Cell lines that were more radioresistant were more effectively radiosensitized by inhibition of mutant B-RAF by GSK2118436. Further

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validation in vivo is ongoing. The data thus far suggest a role for the use of GSK2118436 in combined treatment with radiation for patients with B-RAF mutated melanoma brain metastases.

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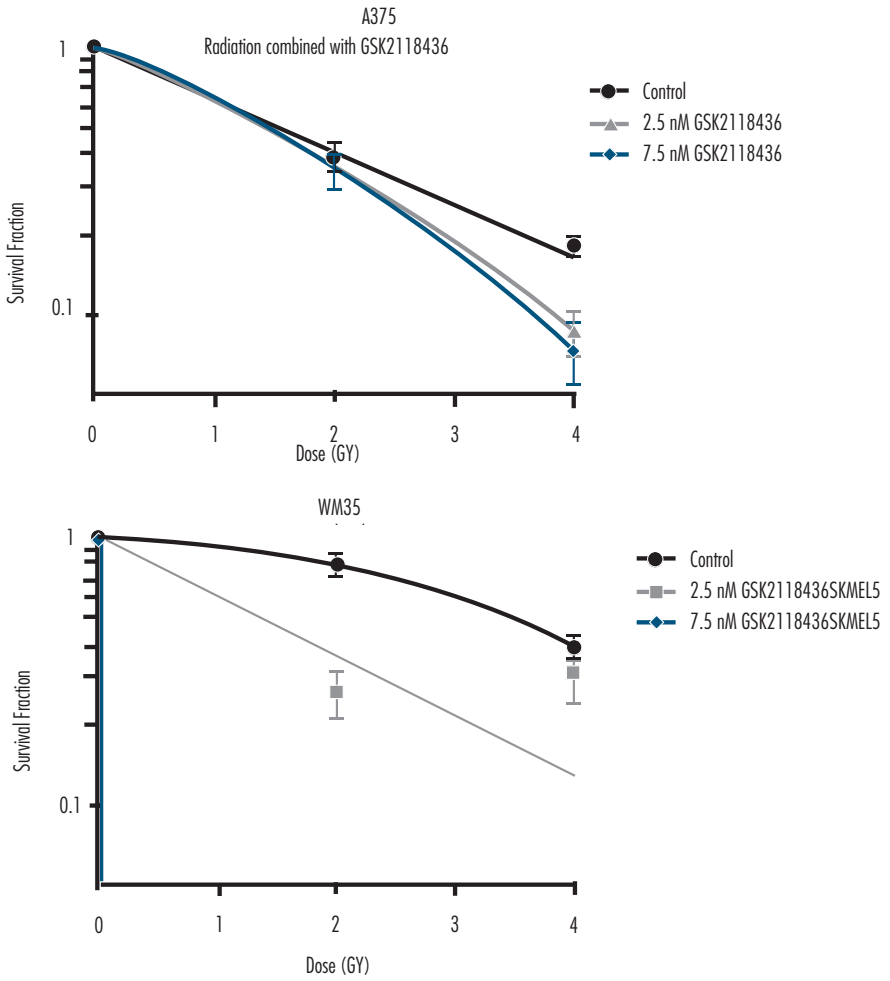

FIGURE 2: Radiation Therapy Combined with GSK2118436. *Survival curves at 0-4GY of ionizing radiation (IR) pretreated two hours before radiation with low (2.5nM) and high (7.5nM) doses of GSK2118436. Both survival curves show increased melanoma turmor cell death.*

Cold Plasma Therapy as a Novel Chemotherapeutic Strategy

Jacob Kirsch, MSII
Advisor: Anthony Sandler, M.D. Children's National Medical Center

Plasma, the fourth state of matter, is composed of partially ionized gas containing electrons, excited molecules, free radicals and ions.[1] Historically, plasma could only be produced at high temperatures or in vacuums, however, recent advances in plasma physics have led to the production of plasma at room temperature.[2,3] Non-thermal atmospheric plasma, or "Cold Plasma" is produced by applying a high voltage electric field to a plume of compressed gas. While theoretically any gas or mixture of gasses can be used, our group used helium due to the advantages of it being monatomic and chemically inert. Cold plasma treatment (CPT) can potentially be used in cancer therapy by generating reactive ion species that may induce tumor cell apoptosis. Our initial studies demonstrated efficacy in killing tumor cells in both in vitro and in vivo mouse models of neuroblastoma. We therefore sought to advance these findings by evaluating the effects of cold plasma in human neuroblastoma and normal fibroblast cell lines.

Cold plasma treatment (CPT) can potentially be used in cancer therapy by generating reactive ion species that may induce tumor cell apoptosis.

In this study, we attempted to

elucidate the chemotherapeutic effects of CPT by evaluating its effects on IMR-32 human neuroblastoma cells and human fibroblasts (ATCC 895. sk). A constant dose of CPT was administered for 30, 60, and 120 seconds, respectively. Cell apoptosis was analyzed by flow cytometry at four different time points following treatment with CPT: immediately, 24 hours, 48 hours, and one week. Normal human fibroblasts were chosen to serve as a negative control for comparison. Staining for apoptosis (annexin V-FITC) as well as cell death (7-AAD) allowed us to differentiate between necrotic cells, which died through mechanisms unrelated to CPT, and cells that were induced to undergo apoptosis as a direct result of CPT. The potential utility of CPT as a chemotherapeutic agent is dependent on its ability to produce the latter.

Our study showed that CPT of neuroblastoma cells induced a three to six-fold increase in apoptosis compared to controls. CPT induced a dose-response effect in the tumor cells, decreasing cell viability by up to 89 percent 24 hours after treatment (data not shown) and 98 percent 48 hours after treatment (Figure 1). Conversely, CPT was largely ineffective in eliciting an effect on human fibroblasts; these cells maintained their viability compared to untreated controls both 24 hours and 48 hours post-treatment (Figure 2). This effectively demonstrated that CPT can induce apoptosis of human neuroblastoma cells, without affecting normal human fibroblasts. Cold plasma therefore represents a potential emerging adjunct in cancer therapy, as demonstrated by its selective and effective in vitro targeting of human neuroblastoma. The mechanism of this tumor selectivity is currently still under investigation, however it is believed to be mediated by the generation of reactive oxygen species.

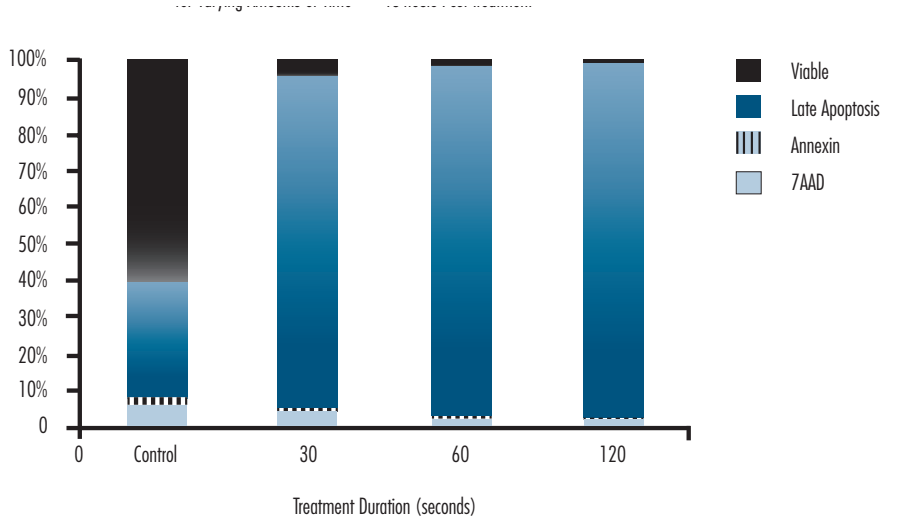

FIGURE 1: Annexin and 7AAD staining in IMR-32 cells 48 hours after treatment with cold plasma for varying amounts of time.

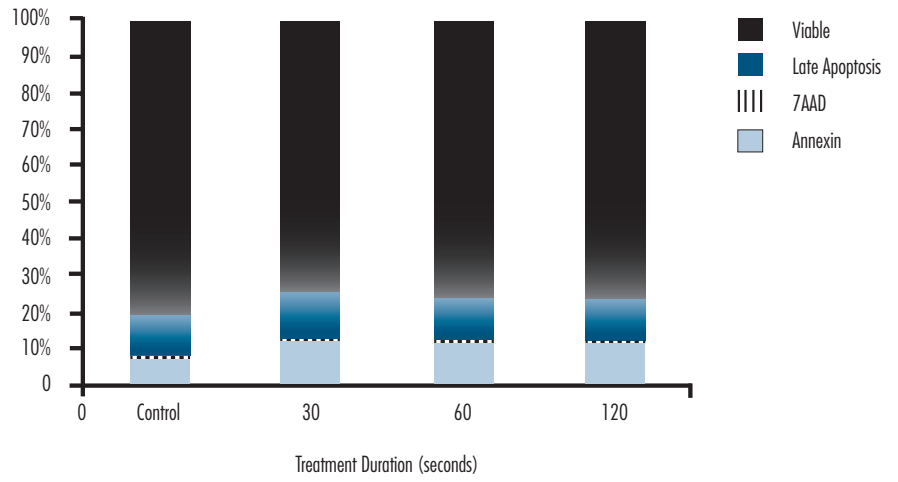

FIGURE 2: Annexin and 7AAD staining in Fibroblasts 48 hours after treatment with cold plasma for varying amounts of time

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Characterization of the Role of Lman1 in Retinal Degeneration

Sana Idrees, MSII
Advisors: Hong Hao, Janina Gregorski, and Anand Swaroop, National Eye Institute
Bin Zhang, Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic Foundation

The process of vision begins at the photoreceptors in the retina, which provide nearly 30 percent of the sensory input to the brain. Neurodegenerative blinding diseases are due to genetic defects that cause abnormal differentiation or homeostatis of retinal photoreceptors, and such defects are the primary cause of visual impairment in diseases of the retina. The neural retina leucine zipper (NRL) transcription factor is a key regulator of rod photoreceptor differentiation and homeostasis. NRL functions as a molecular switch to produce rod photoreceptors from post-mitotic retinal precursor cells.[1] *Lman1* encodes ERGIC-53, a protein mediating the transfer of a subpopulation of glycoproteins from the ER to the Golgi complex.[2] ERGIC-53 has a secondary function in glycoprotein quality control and it is ubiquitously expressed.[3] We identified the *Lman1* gene as a direct transcriptional target of NRL. To identify the potential role of *Lman1* in retinal degeneration, we investigated its regulation and function in rod photoreceptors using *Lman1* knockout mice.

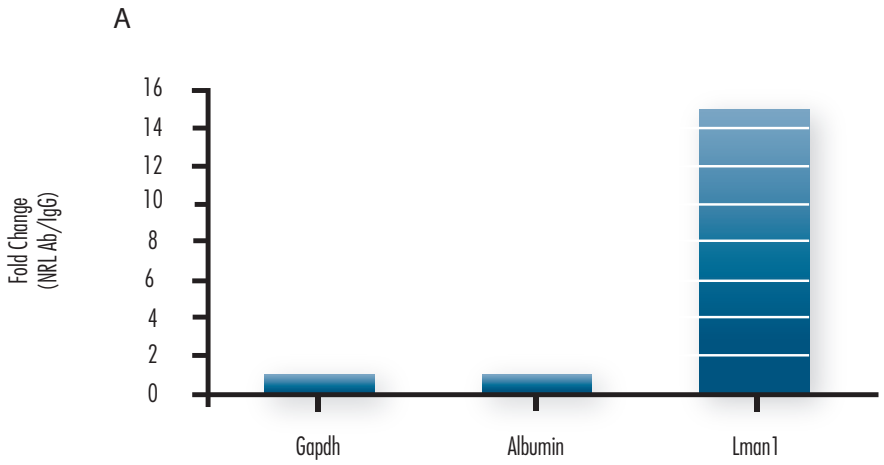

FIGURE 1: Comparison of NRL antibody ChIP DNA to IgG control ChIP DNA showed a 15-fold difference in the expression of Lman1.

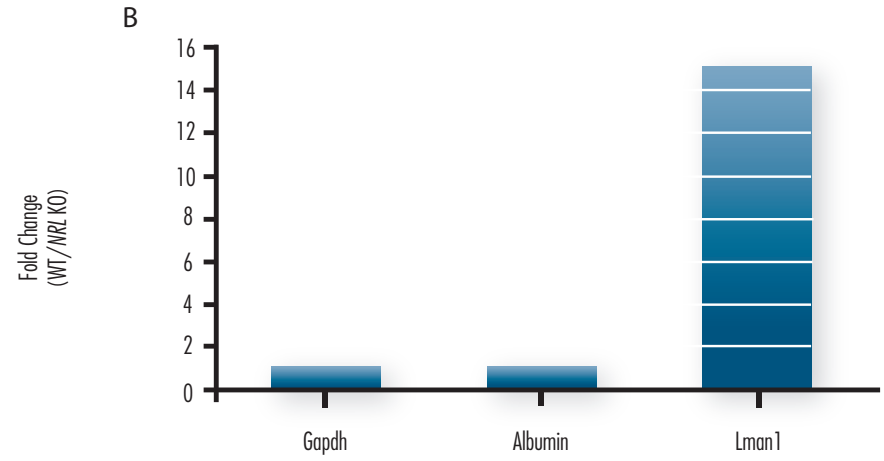

FIGURE 2: A 15-fold difference in Lman1 expression was observed between WT and NRL knock out mouse retinas.

Chromatin immunoprecipitation followed by real-time quantitative polymerase chain reaction (ChIP-qPCR) and enhancer analysis of the *Lman1* enhancer element were used to validate the interaction between NRL and *Lman1* promotor sequences. ChIP-qPCR and enhancer analysis detected the *Lman1* promotor as a direct target of NRL.

Physiologic relevance of *Lman1* in the retina was tested using immunohistochemistry (IHC) in *Lman1* knockout mice. Retinas were collected from *Lman1* -/- mice at 2 months and 6 months of age. The retinas were sectioned, and IHC was performed against the Golgi markers GM130 and Grasp65, glial marker GFAP, and the rod photoreceptor visual pigment rhodopsin.

IHC showed reduction in Grasp65 and rhodopsin in 2 month and 6 month old *Lman1* -/- mice compared to WT, whereas no siginificant difference in GFAP was observed. IHC against GM130 also showed significant reduction in 2 month old *Lman1* mice compared to WT.

Our results suggest that *Lman1* has an important role in rod photoreceptor development and homeostasis. Function tests will be conducted to evaluate the retina of *Lman1* -/- mice through light damage studies and electroretinography analyses.

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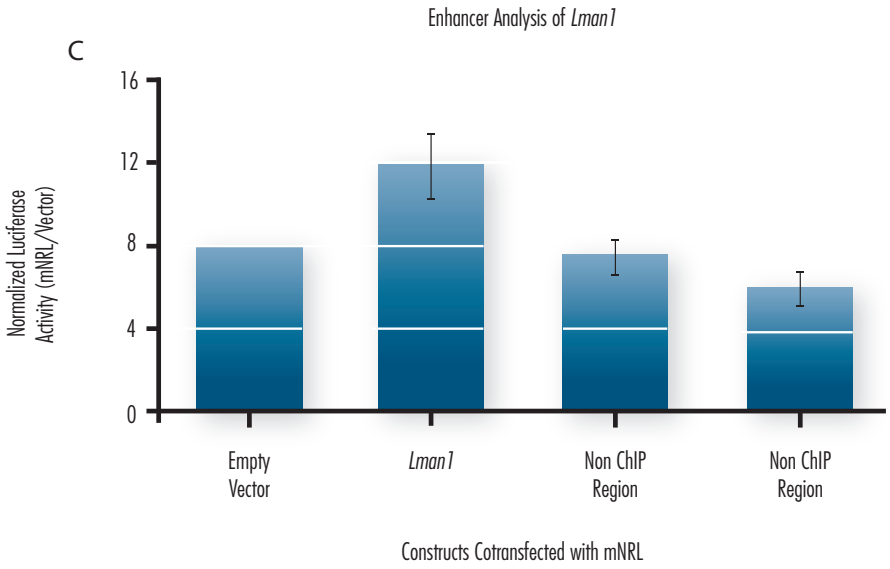

FIGURE 3: There was a significant difference in the normalized luciferase activity in HEK293T cells cotransfected with a Lman1 enhancer construct and mouse NRL (mNRL) compared to cells cotransfected with a control vector and mNRL. Non ChIP regions (controls) showed no significant luciferase reporter activity.

Developing a 3D Glandular *In Vitro* Model from Human Airway Epithelial Basal Cells

Maureen Banigan, MSII
Advisor: Xiaofang Wu, M.D., Children's National Medical Center

Submucosal glands — invaginations of the surface epithelium in cartilaginous mammalian airways — are a major source of mucus production in the upper respiratory tract. Submucosal glandular (SMG) hyperplasia is a primary histopathologic feature in obstructive respiratory pathologies — such as cystic fibrosis and chronic rhinosinusitis — associated with mucus hypersecretion and obstruction of the sinonasal tract.[1,2] While the morphogenesis of SMGs during fetal development is well described,[3] the underlying mechanisms that lead to invagination of the epithelium and differentiation of epithelial cells to glandular cells with ductal tubules and acini are unknown. Glandular hyperplasia in respiratory tract mucosa is markedly understudied, reflecting the lack of an in vitro cell model system whereby respiratory tract epithelial cells differentiate into glandular cells.

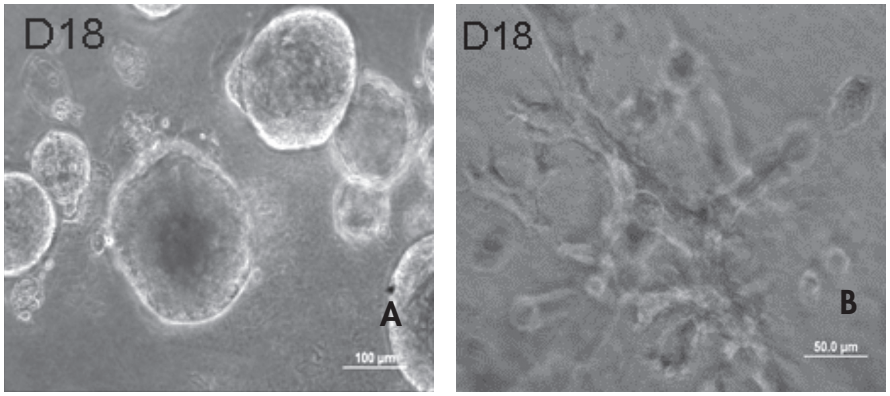

FIGURE1: Brightfield pictures of HNE cells on D18. (A) HNE cells cultured on growth-factor reduced Matrigel proliferate into glandular-like acini. (B) HNE cells form tubule structures in collagen I matrix.

The current paradigm in lung biology is that there are progenitor cells in respiratory tract epithelium poised to facilitate epithelial repair and remodeling. These cells are capable of undergoing epithelial invagination into the underlying basement matrix that results in the formation of SMG with ductal tubules and acini. The SMG progenitor cells in both mice and humans have been identified as basal cells (BCs) within the surface airway epithelium of the lower respiratory tract.[4,5] Predictably, the progenitor cells in nasal and sinus epithelium are also basal cells, but limited studies exist on BCs isolated from the upper respiratory tract. We hypothesize that BCs differentiate to form glands in a three-dimensional extracellular matrix

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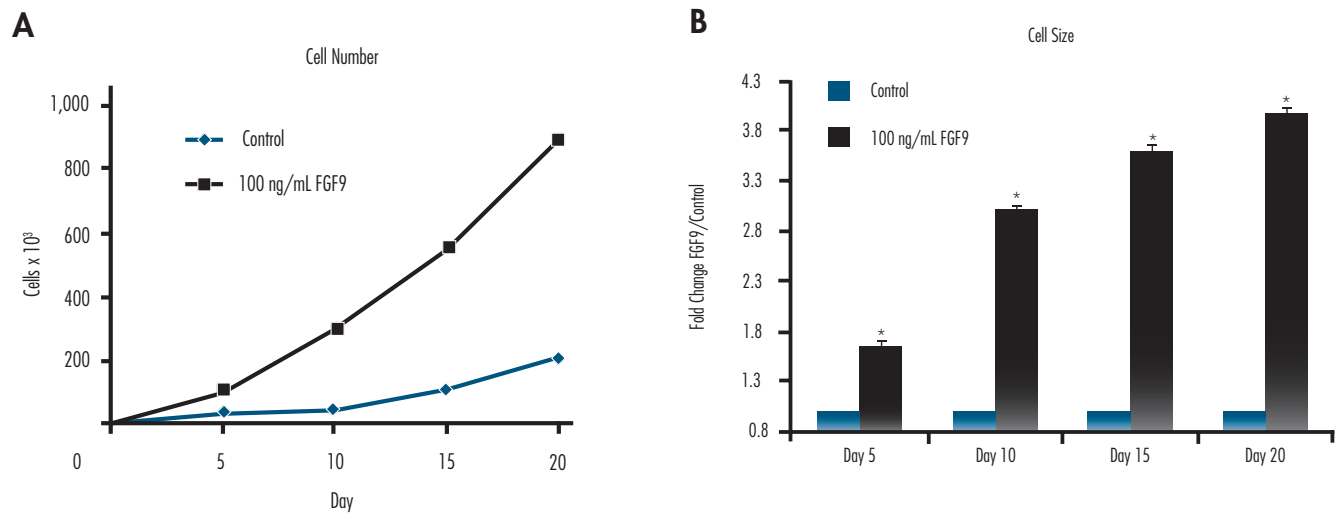

FIGURE: (A) Growth kinetics of HNE cells with and without exposure to 100-ng/mL FGF9. The results represent the mean values from three different experiments. (B) Ten brightfield images of HNE acini were taken every five days until day 20. Acinar areas were measured in 25 individual acini by Image J (NIH). Fold changes of HNE acinar average area after exposure to FGF-9 is shown. Values are expressed as means of acinar sizes + standard error (SE) of three experiments. *indicates significant difference at p<.05

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and are triggered by inflammatory/immune response mediators and growth factors to produce glandular hyperplasia in disease states. To test this hypothesis, we developed an in vitro 3D system wherein BCs from primary epithelial cells differentiate into glandular structures when grown on different basement membrane matrices.

Fluorescent activated Cell Sorting (FACS) analysis of purified human nasal epithelial (HNE) cells and human bronchial epithelial (HBE) cells derived from human airway epithelium indicated that 98 percent of cells were basal cells and immunofluorescence (IF) staining for basal cell markers supported this finding. We found that the morphological differentiation of BCs in in vitro culture is dependent upon extracellular matrix conditions. While HSE BCs differentiate into glandular acini when overlaid on Matrigel®, they differentiate into glandular tubules when embedded in collagen (Figure 1). Both glandular acini and glandular tubules formed by HNE cells in vitro expressed in vivo markers of mucous and serous glandular cells. Exposure of HNE basal cells to EGF, FGF7, and FGF9 significantly enhanced their proliferation rate, suggesting that growth factors may play an important role as positive regulators of the morphogenesis and differentiation of submucosal glands in respiratory tract mucosa (Figure 2). Although statistically significant, the increased HNE acinar size seen after exposure to inflammatory mediators (CXCL5, CXCL13, and GM-CSF) was small.

Our developed in vitro glandular acinar model is a useful model for studying the effects of growth factors and other mediators on HSE and HNE glandular proliferation and differentiation. The use of basal cells, which can be cultured in a chemically defined medium, permits analysis in a controlled model system of the activity of growth factors and inflammatory mediators added to the culture. Further investigation into the role of immune/inflammatory response mediators and growth factors in de novo gland development will allow identification and eventual circumvention of the mechanisms that lead to SMG hyperplasia with subsequent mucus hypersecretion.

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Diagnosis of Coronary Artery Disease by Deep Sequencing of Blood

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Cardiovascular disease is the leading cause of death in the United States.[1] Coronary artery disease (CAD), in which atherosclerotic plaque builds up and obstructs the arteries supplying the heart, affects over 16.5 million people in the United States.[2] Nearly 500,000 people in the U.S. are newly diagnosed with CAD each year.[2]

The current gold standard for diagnosing CAD is coronary angiography using coronary artery catheterization. However, only 60 percent of patients undergoing diagnostic coronary catheterization have obstructive CAD (≥50 percent stenosis of left main coronary artery or ≥70 percent stenosis of major epicardial vessel), while 39 percent of patients undergoing elective coronary catheterization have no CAD (<20 percent stenosis in all vessels).[3] Given the risks associated with coronary catheterization and the expense of this procedure, it would be valuable to develop an alternative highly predictive test for CAD.[3,4]

There is evidence that specific changes in gene expression accompany CAD.[2,5] Several studies have identified differentially expressed gene transcripts in blood samples associated with CAD, raising the possibility that detecting changes in transcript expression in patient blood samples may be a feasible means of predicting the presence of CAD.[2,5] Therefore, transcript expression profiles of differentially expressed genes are the potential basis of a blood-based diagnostic test for CAD.

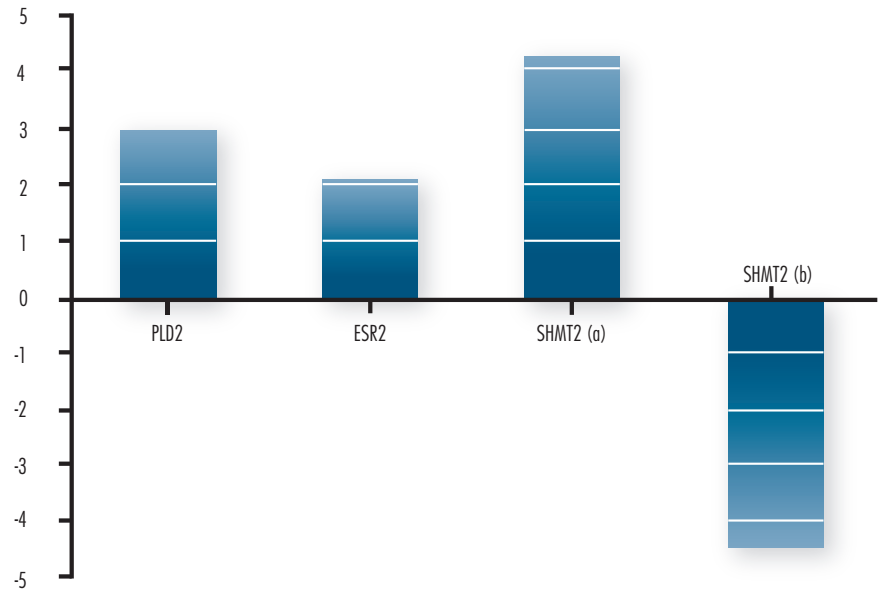

FIGURE: FOLD ELEVATION OF SELECTED GENE TRANSCRIPTS IN CAD: Fold elevation of selected gene transcripts in patients with CAD (>20 percent stenosis) relative to the transcript expression level of the same gene in patients without CAD (0 percent stenosis). ($p < 0.01$)

PLD2: Phospholipase D2
ESR2: Estrogen Receptor β
SHMT2: Serine Hydroxymethyltransferase 2 (a/b = splice isoforms)

To investigate the possibility of developing a test for CAD based on differential transcript expression, we used next-generation sequencing of blood sample RNA (RNA-seq) to identify Transcripts Associated with CAD (TRACs). While previous studies have used microarray-based methods to identify differential gene expression in CAD, we used RNA-seq because it provides the advantages of directly quantifying all known transcripts and allowing analysis of splicing and allele usage.[5]

Here, we show that RNA sequencing of blood samples identifies TRACs in our patient population. Blood samples were collected from patients without previously diagnosed CAD about to undergo non-emergency cardiac catheterization. As a midpoint analysis, from our coronary catheterization cohort we identified 10 patients with significant CAD (>20 percent stenosis of one or more arteries) and 10 patients without CAD (0 percent stenosis). These patients were matched for age and gender; no significant differences existed between groups for risk factors such as blood pressure, BMI, or diabetes. RNA was extracted from these blood samples and sequenced on the Helicos sequencing platform.

These preliminary RNA-seq digital gene expression data identified 151 gene transcripts differentially expressed in patients with stenosis (> 1.5 fold change) passing a t-test corrected for

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multiple testing ($p < 0.01$). These 151 TRACs included 17 of 23 transcripts associated with CAD identified in a 2008 microarray-based study.[5] The fold elevation of Phospholipase D2, Estrogen Receptor ß, and Serine Hydroxymethyltransferase gene transcripts in CAD are highlighted in the Figure. Of particular interest is the greater than 4-fold increase in expression of the SHMT2(a) isoform, and the greater than 4-fold decrease in the SHMT2(b) isoform, illustrating the ability of this method to assess expression of splice variants.

[T]ranscript expression profiles of differentially expressed genes are the potential basis of a blood-based diagnostic test for coronary artery disease.

These preliminary results demonstrate identification of 151 gene transcripts differentially expressed in patients with CAD using RNA sequencing. After analysis of all enrolled patients is complete, the next planned step in this ongoing study is to use these TRACs to train a Support Vector Machine classification algorithm to identify CAD in new patient samples. This expression profile algorithm will be used to develop a blood-based rapid PCR array or ELISA test to provide a rapid, minimally invasive CAD detection test. The possibility of preventing needless catheterizations with this blood test has the potential to improve patient outcomes while increasing time and cost efficiency of CAD diagnosis.

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Identifying CSF Biomarkers of Oxidative Stress in Patients with Multiple Sclerosis

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Multiple Sclerosis (MS) is an immune-mediated disorder of the central nervous system (CNS) that leads to inflammation, demyelination, and axonal degeneration. Although the cause of MS remains unknown, it is believed that reactive oxygen species (ROS) released from macrophages and microglial cells in the CNS react with myelin to result in the pathology seen in MS. Specifically, ROS react with myelin fatty acid side chains resulting in cell damage and a breakdown in Blood Brain Barrier (BBB) integrity.[1] Two end-products generated from the lipid peroxidation of myelin are 4-Hydroxynonenal (4-HNE) and 8-Isoprostane (8-IsoP), and are therefore expected to be increased in the CNS of MS patients.[2] Uric acid is an antioxidant that would be expected to increase in the serum of MS patients as a host defense mechanism against oxidative stress.

	CIS	NIND	OIND	PPMS	RRMS	SPMS	ALL
Number (n)	10	38	20	38	74	12	192
Female, n	6	30	8	19	42	6	111
Median Age (years)	39.5 (20-57)	48 (27-65)	39 (40-62)	52 (20-67)	38.5 (18-68)	53 (32-67)	45.5 (18-68)

TABLE: Demographics and clinical characteristics of patients and controls.

The goal of this study was to identify biomarkers of oxidative stress in the cerebrospinal fluid (CSF) of MS patients. The discovery of a novel biomarker whose levels correlate with the amount of oxidative damage in the CSF would offer a quantifiable entity that could be used as a diagnostic tool and as a measure of disease progression. This study investigates 4-HNE and 8-IsoP as potential CSF markers.

Enzyme-linked Immunosorbent Assays (ELISA) were optimized to quantify the concentrations of 4-HNE histidine adducts and free 8-IsoP in the CSF of a blinded cohort of subjects. Serum uric acid levels were also obtained as a measure of antioxidant activity. A total of 124 patients with MS, 10 patients with a Clinically Isolated Syndrome (CIS) not yet classified as MS, 38 patients with Noninflammatory Neurologic Diseases (NIND), and 20 patients with Other Inflammatory Neurologic Diseases (OIND) were included. Patients were classified as

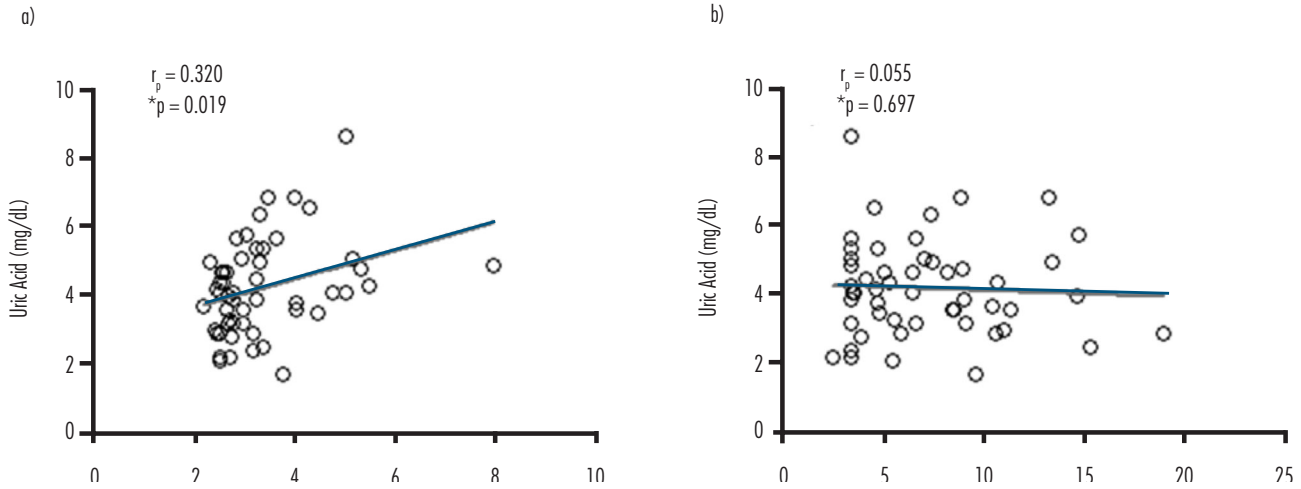

FIGURE 1: Serum levels of uric acid were obtained and compared with CSF levels of 8-IsoP and 4-HNE. Uric acid showed a significant correlation with 4-HNE levels (p=0.019; a), but did not correlate with 8-IsoP levels (p=0.697; b) (n=52).

having Primary Progressive MS (PP-MS) (n=38), Relapsing Remitting MS (RR-MS) (n=74), or Secondary Progressive MS (SP-MS) (n=12).

4-HNE was found to be significantly elevated in the CSF of MS patients compared to controls ($p<0.05$), while 8-IsoP showed only a modest elevation. The increase in both species supports the proposed role of oxidative stress in the pathogenesis of MS. In addition, serum levels of uric acid correlated with CSF levels of 4-HNE but not with 8-IsoP (Figure 1). The correlation between 4-HNE and uric acid suggests that the two may be associated with MS pathology; specifically, oxidative stress causes an increase in uric acid concentration as an antioxidant defense mechanism.

Among MS subtypes, only PP-MS and RR-MS showed a significant increase in CSF levels of 4-HNE compared to controls, the former being most statistically significant (Figure 2). PP-MS is characterized by a gradual decline in disability with no distinct periods of remission, while RR-MS shows periods of partial remission.[3] Hence, 4-HNE levels should indeed be highest in these patient groups

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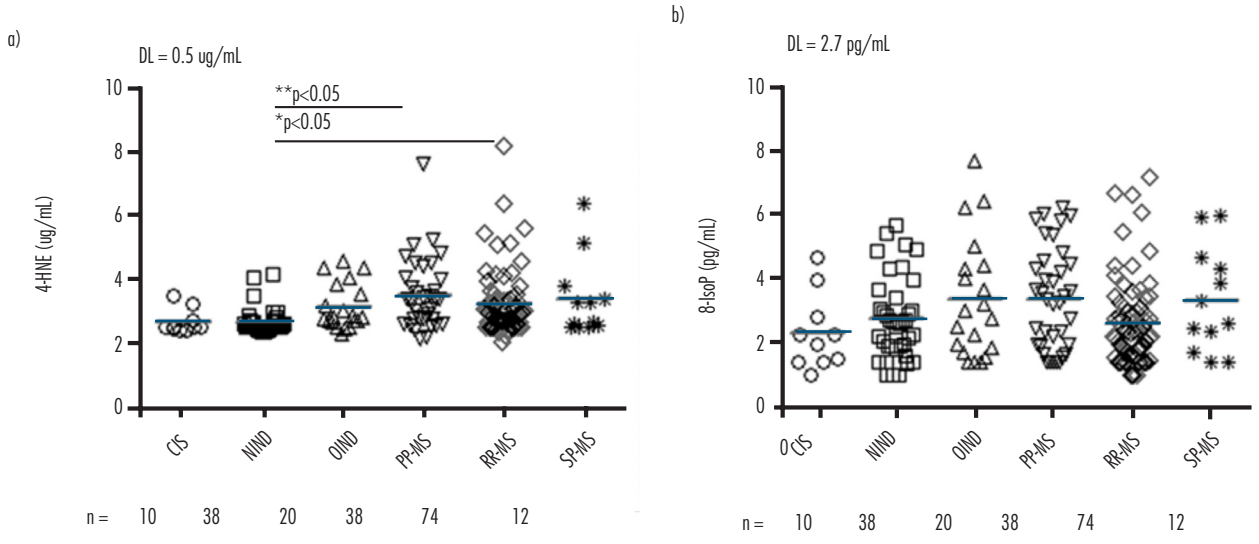

FIGURE 2: The absorbance of each patient sample was measured and analyzed using standard curves to determine 4-HNE and 8-IsoP concentrations. Among MS subtypes, PP-MS and RR-MS patients had significantly higher CSF levels of 4-HNE compared to NIND controls ($p<0.05$; a), whereas SP-MS did not reach statistical significance. PP-MS patients had the highest mean concentration of 8-IsoP, but no group showed a statistically significant difference in 8-IsoP concentrations compared to controls ($p>0.05$; b).

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compared to SP-MS patients who are in a state of chronic remission.

CSF levels of 4-HNE were independent of both patient age and gender, while 8-IsoP levels were independent of gender but increased with age. This implicates 4-HNE, but not 8-IsoP, as a marker for patients of all demographics.

A future study already in progress involves the development of ELISA assays to measure additional products of oxidative stress. CSF levels of these products will be analyzed and compared to the levels of 4-HNE, 8-IsoP, and uric acid.

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A Variant in the Supervillin Gene is Associated with Lean Muscle and Adiposity Phenotypes

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Supervillin is a 205-kD F-actin binding protein encoded by the SVIL gene that functions as a linkage protein between the cellular actin cytoskeleton and plasma membrane.[1] The protein localizes within lipid-raft domains of the plasma membrane, and potentially functions in cellular adhesion and motility. While SVIL is expressed in numerous tissues, expression is highest in striated, cardiac, and smooth muscle.[2]

Archivillin is a 297-kD, alternatively spliced isoform of SVIL that is specific to skeletal muscle tissue and myogenic cell lines. Archivillin also binds directly to F-actin and is associated with the cytoskeletal protein, dystrophin, as well as the adhesion proteins, caveolin-3, and vinculin. It has been proposed that archvillin may play a role in early myogenesis and muscle cell differentiation. Additionally, it may contribute to the integrity of the myotendinous junction, which serves to distribute longitudinal forces developed during muscle contraction.[3]

The role of the SVIL gene in skeletal muscle has been further explored through its relationship to the androgen receptor (AR). AR activation and its association with increased muscle mass has been previously elucidated. The SVIL gene has been found to act as a co-regulator of the AR in skeletal muscle, increasing transactivation. This result indicates that SVIL may play an important role in muscle development and repair. Additionally, previous studies have suggested the potential for cross-talk between the AR and vitamin D receptor (VDR) mediated by co-regulators of the AR as a mechanism for increasing VDR transactivation. Consequently, the SVIL gene may play a role in VDR functioning and associated bone health.[4,5]

Our study sought to further describe the relationship between the SVIL gene and phenotypes associated with lean muscle, bone, and adipose tissue by examining single nucleotide polymorphisms (SNPs) that may have a functional relationship to the SVIL gene.

We studied 155 ethnically diverse, healthy volunteers (average age 22.05± 4.6 yrs females and 23.31 ± 5.61 yrs males) recruited from the Bone and Muscle Study performed at the University of Massachusetts. We genotyped two SNPs found in SVIL (rs36027845 and rs10437410), and examined associations between these SNPs and measures of lean muscle tissue, bone mineral density, and adiposity. Mean quantitative muscle,

Significant Results

SNP	Phenotype (units)	Sex	P-value	N: adjusted mean +
SVIL rs36027845	% Tissue fat	M	0.0190	AA (N=31; 20.2 ± 1.1) AG/GG (N=35; 23.9 ± 1.1)
	Total fat (g)	M	0.0175	AA (N=31; 16455 ± 846) AG/GG (N=35; 19358 ± 794)
	Total lean (g)	M	0.0157	AA (N=31; 59894 ± 819) AG/GG (N=35; 57034 ± 769)
	Arms lean (g)	M	0.0216	AA (N=31; 7193 ± 140) AG/GG (N=35; 6755 ± 131)

TABLE 1: In males, a single copy of the G allele for a SNP located near the SVIL gene (rs36027845) was associated with decreased total lean muscle and total arm lean muscle mass, as well as increased total percent tissue fat and total fat. No significant results were observed in females.

bone, and adipose measurements were compared in relation to SNP genotypes using analysis of covariance (ANCOVA) methods.

The SVIL gene and associated supervillin protein play a functional role in maintaining the integrity of the cytoskeleton of the cell. Previous studies have also implicated the SVIL gene as an important co-regulator of the androgen receptor in skeletal muscle, and it has been suggested that SVIL may increase transactivation of the vitamin D receptor.[4,5] Associations between the SVIL gene and phenotypic variations in muscle, bone, and adipose tissue have not been previously studied.

The present study reports significant associations between a variant in the SVIL gene (rs36027845) and decreased total lean muscle mass and arm lean muscle mass, as well as increased percent tissue fat and total body fat in males. Pupasuite 3.1, a web-based SNP analysis tool that is used to identify potential functional properties of a variant, suggests this SNP affects transcription factor binding. Our findings were sexually dimorphic as no significant findings were established in females. There were no significant findings between the genetic variants in the SVIL gene of interest and phenotypes associated with bone.

These results are consistent with previous studies that have investigated the role of the SVIL gene and the associated supervillin protein in muscle development, and provide further evidence that the SVIL gene may play an important role in early myogenesis and regulation of skeletal muscle development via co-regulation of the androgen receptor. An inverse relationship between skeletal muscle and adipose tissue was observed in males with the genetic variant, indicating that the polymorphism may also be associated with an increased risk for adiposity. Additional study is needed to further elucidate the function of the SVIL gene in skeletal muscle growth and development as well as identify implications in disease processes of the musculoskeletal system.

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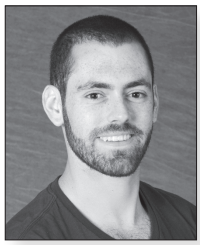
The Relationship Between Single Nucleotide Polymorphisms (SNPs) (rs1366594, rs2941740, rs87938) and Phenotypic Predictors of Bone and Muscle Health



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Osteoporotic fractures cause injuries that result in significant morbidity and mortality. Substantial effort has been made to identify persons at risk for osteoporotic fracture so that preventative measures can be initiated in a timely fashion. To date, one of the best predictors of fracture is bone mineral density (BMD).[1] If it were possible to identify persons at high genetic risk for developing low bone density, preventative strategies might be employed as early as childhood.

Rivadeneira et al recently performed a meta-analysis of five genome-wide association studies of femoral neck and lumbar spine BMD in 19,195 subjects of Northern European descent. They identified 20 single nucleotide polymorphisms (SNPs) reaching genome-wide significance that mapped to genes related to bone metabolism in signaling pathways.[2] However, bone structure is not determined by signaling pathways alone. Past studies indicate that bone and muscle are interconnected tissues and that the mechanical forces of muscles play a major role in determining bone quality.[3,4]

We sought to determine whether the SNPs identified by Rivadeneira et al found to be associated with higher bone density (an element of bone quality) would be predictive of additional measures of musculoskeletal health such as skeletal muscle and bone volume in a population of young, healthy adults.

The study population included 753 college-age subjects (449 female; 304 male; average age 24 yrs) recruited to undergo a 12-week strength-training program. Measurements of upper arm muscle volume, strength, and cortical bone volume were collected prior to and following resistance training. The cohort was genotyped for three SNPs (rs1366594, rs2941740, rs87938) demonstrated to be associated with fracture risk in genome-wide association studies. Hardy-Weinberg equilibrium was tested in each SNP using a chi-square test. Associations between mean phenotypes

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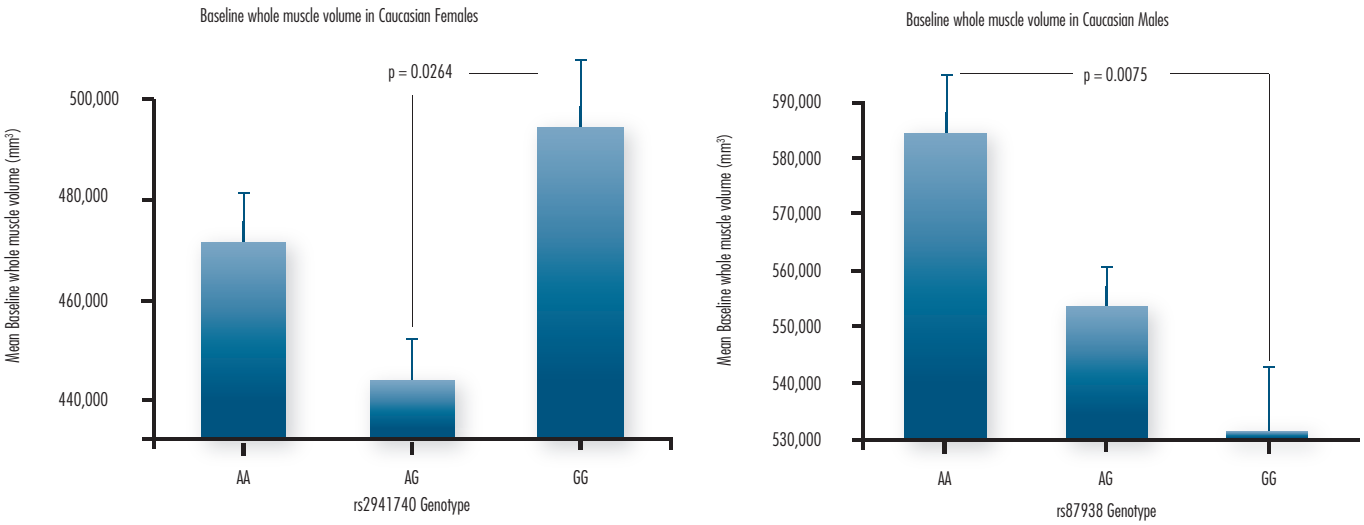

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and genotypes were tested using an ANCOVA with age and baseline weight as covariates (cortical bone adjusted for age only). For those associations showing a significant F-test, pair-wise post hoc comparisons were performed and resulting p-values adjusted for multiple comparisons using the Sidak method.

Our results demonstrated that females that were homozygous for the recessive allele (n=46) for the rs2941740 SNP demonstrated significantly larger pre-exercise upper-arm skeletal muscle volume compared to females heterozygous for that SNP (p=0.026). Males with two copies of the dominant A allele for rs87938 demonstrated significantly larger pre-exercise upper-arm skeletal muscle volume compared to males with two copies of the rare G allele (p=0.008). No significant association was found for the rs1366594 SNP in males or females for the measured phenotypes.

Our data suggest that SNPs previously shown to be associated with BMD/fracture risk (rs2941740 and rs87938) are also associated with upper arm muscle volume. These findings are consistent with the mechanostat hypothesis, which posits that there should be a close relationship between bone strength (or weakness) and muscle size/force. According to the mechanostat hypothesis, healthy bones dynamically adapt to skeletal loads, the majority of which come from muscular forces. Although moderate-to-vigorous physical activity has been reported to be associated with a hip fracture risk reduction of 45 percent in men and 38 percent in women, the exact contribution of physical activity and muscle strength on BMD and fracture risk remains poorly delineated.[5] Further study of the interplay of genetic influences on bone and muscle health may lead to a more robust ability to predict fracture risk.

Although moderate-to-vigorous physical activity has been reported to be associated with a hip fracture risk reduction of 45 percent in men and 38 percent in women, the exact contribution of physical activity and muscle strength on BMD and fracture risk remains poorly delineated.[5]

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Genetic Variation in NADSYN1 (rs12785878) Predicts Skeletal Muscle Response to Resistance Training and Vitamin D Levels in Men

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Adequate vitamin D levels are essential for bone health and muscle function and therefore physical performance.[1] Low circulating levels of vitamin D have been correlated with fracture risk and frailty.[2] Recent genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) in the vitamin D binding protein that influence circulating levels of vitamin D and might be correlated with deficient states.[3,4] Identification of genetic variants that influence vitamin D homeostasis may lead to early identification of individuals at risk for vitamin D deficiency.

The goal of this study was to further delineate the influence of four loci [GC (rs2282679), CYP2R1 (rs2060793), and NADSYN1 (rs12785878, rs3829251)] that have been strongly associated with circulating vitamin D levels. We strove to explore whether these loci might influence changes in skeletal muscle as a result of resistance training. NADSYN1 is a novel locus for association with vitamin D status. There is evidence that CYP2R1 is a key enzyme underlying 25-hydroxylation of vitamin D in the liver, and so is a crucial first step in vitamin D metabolism. GC encodes vitamin D binding protein and is strongly associated with circulating concentrations, which affects the delivery of circulating vitamin D.[3,4]

The population used for this study was derived from a large multicenter study (FMS) designed to identify genetic variants that influence muscle size and strength and individual variability in response to resistance training. Our cohort included 753 college-aged subjects (449 female, 304 male) who underwent 12 weeks of supervised resistance training on the nondominant arm. At the beginning and upon completion of the exercise protocol, arm muscle volume was measured via MRI and strength measurements (one repetition max [1RM] and maximal voluntary contraction [MVC]) were taken. The study participants were genotyped for four genetic variants (rs2282679, rs2060793, rs12785878, and rs3829251) previously demonstrated to be associated with circulating levels of vitamin D in a cohort of 4501 persons of European ancestry.[3,4] Mean phenotypes were compared among genotypes using ANCOVA with age and baseline weight as covariates (bone phenotype adjusted for age only). For co-dominant associations showing a significant F-test, pair-wise post-hoc comparisons were performed and resulting p-values adjusted for multiple comparisons using the Sidak method.

The identification of genetic variants that influence vitamin D homeostasis may lead to early detection of individuals at risk for vitamin D deficiency.

Our goal was to determine whether

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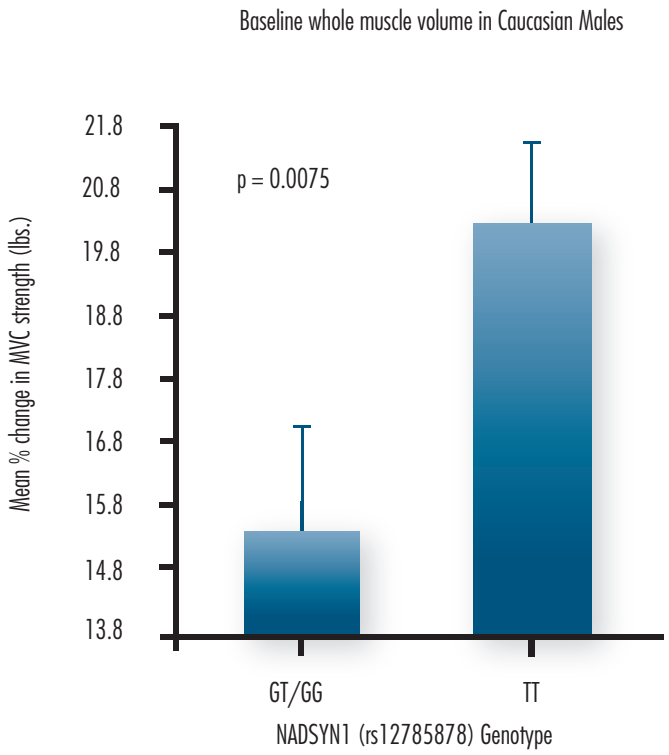
FIGURE 1: Significant associations of the rs12785878 genotype and percent change in MVC strength in Caucasian males.



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SNPs that have exhibited an association with circulating vitamin D levels are also associated with skeletal muscle size and strength, and their response to resistance training. We found that males with two copies of the common allele for rs12785878 demonstrated a significantly larger increase in skeletal muscle volume (p=0.035) with resistance training and a greater percent change in MVC (p=0.026). No associations with any of our measured phenotypes were demonstrated with the other three SNPs.

Our results demonstrate that certain SNPs that are associated with higher levels of circulating vitamin D are also associated with changes in skeletal muscle size and strength in males. We found that males in the FMS cohort with the common allele of rs12785878 demonstrated a significantly greater increase in skeletal muscle strength following resistance training. This SNP is located in the promoter of the DHCR7 gene that encodes the enzyme 7-dehydrocholesterol (7-DHC) reductase. This enzyme converts 7-DHC to cholesterol, thereby removing the substrate from the synthetic pathway of vitamin D3, a precursor of 25-hydroxyvitamin D3.[4] The effect of this enzyme on vitamin D status requires further investigation. Our results suggest novel roles for this gene and potential directions for future research. The identification of genetic variants that influence vitamin D homeostasis may lead to early detection of individuals at risk for vitamin D deficiency. This may lead to early screening and subsequent prevention of various bone diseases such as osteoporosis and fractures in males.

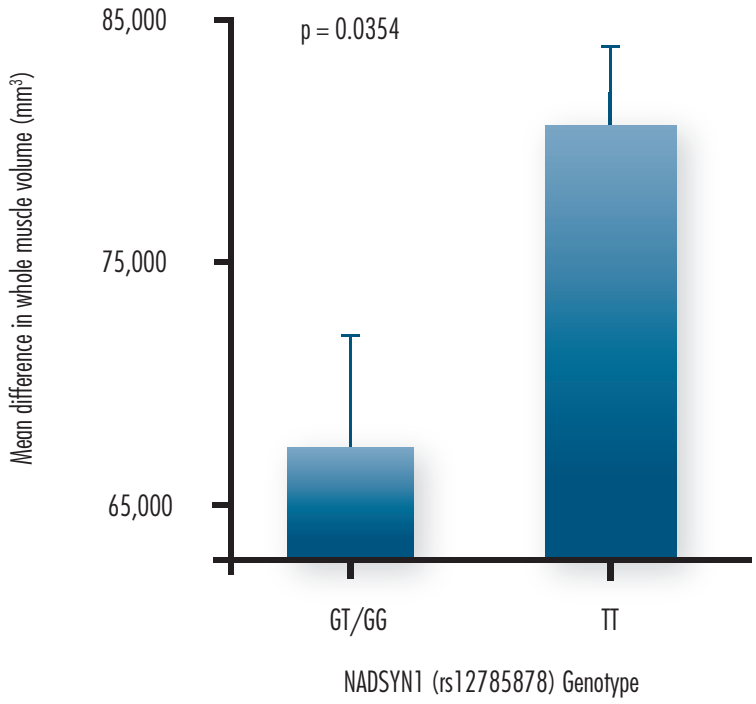

FIGURE 2: Significant associations of the rs12785878 genotype and difference in whole muscle volume in Caucasian males.

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Treatment of Kyphoscoliosis in Proteus Syndrome: Case Report and Review

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Proteus syndrome (PS) is a rare disorder involving a hamartomatous overgrowth with less than 100 confirmed cases.[1] Bone and connective tissue are the most common tissue types that undergo overgrowth in Proteus syndrome with local gigantism, hemihypertrophy, exostoses, macrodactyly, skull anomalies, limb-length discrepancy, kyphosis, and scoliosis constituting common manifestations, orthopaedic management is crucial.[1,2] There are only six reports in the orthopedic literature that deal directly with treatment of scoliosis or kyphosis in PS. This limited literature has demonstrated difficulty in attaining lasting curve stabilization, unpredictable tissue reactions to surgery, and complicating blood loss and thromboses.[2–5]

Here, we present a case of severe kyphoscoliosis associated with PS in order to address the safety and efficacy of surgically correcting a spinal deformity in PS. A male patient was followed from the age of eleven to the age of fifteen and received surgical treatment for progression of severe kyphoscoliosis associated with PS with posterior spinal instrumentation and fusion, a process in which the joints of the spine are removed, rods are fixed to the spine, bone graft is placed, and the spine heals as one solid fusion mass so that curved growth is arrested.

Our patient was satisfactorily corrected from scoliotic curves of 65° to 17°at C6-T5, 88° to 39° at T5-T10, 41°to 20° at T10-L4, and from a kyphotic curve of 92° to 65° at T3-T10 with no complications and his correction remains stable one-year post-operatively (Figure).

To our knowledge, this is the first reported case of current deformity correction strategies employing spinal osteotomies, thoracic pedicle screws, and vertebral de-rotation for the correction of the kyphoscoliosis associated with PS. This strategy is contrasted with the unsuccessful correction in previously reported cases, which may be attributable to their employment of second generation instrumentation using hooks, wires, or limited screw fixation. Conflicting recommendations have been given as to when surgical stabilization of spinal deformity in PS is warranted, but since the intelligence and life span of PS patients are normal, since spinal deformity may cause secondary restrictive pulmonary disease, and since severe kyphoscoliosis can cause neurological compromise, early surgical correction of kyphoscoliosis may be indicated.

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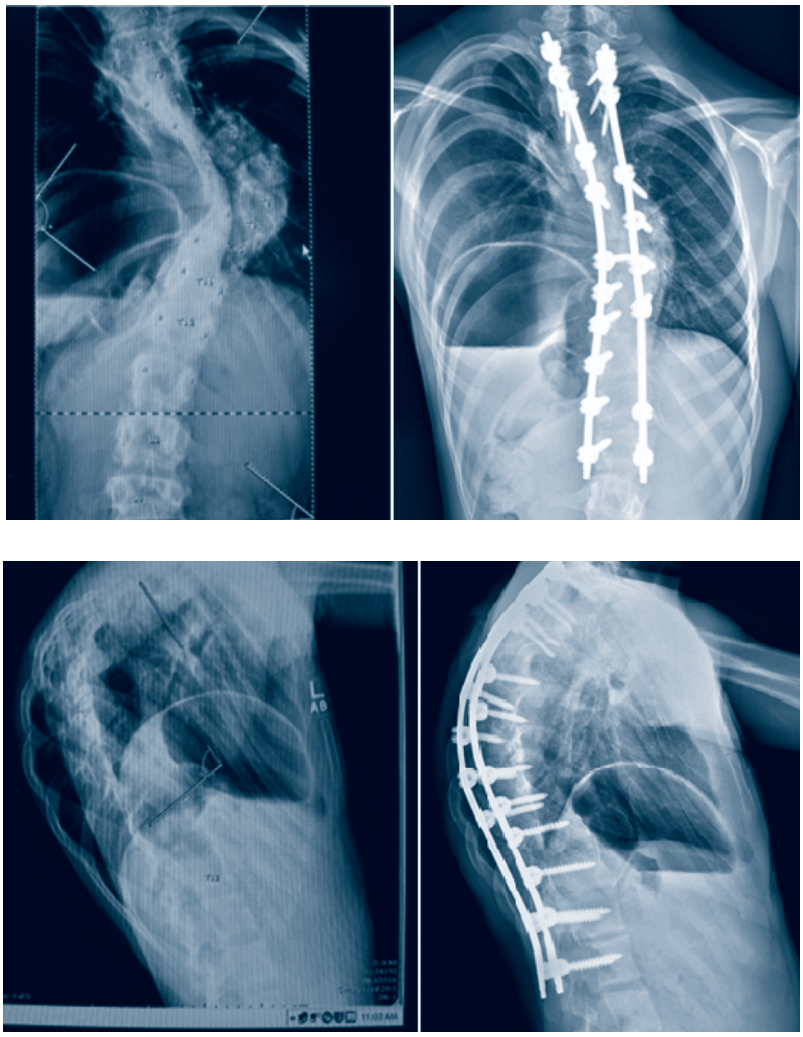

FIGURE: Comparison of Pre-Operative and One-Year Post-Operative Radiographs

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This case report is, by no means, a definitive statement on the safety or efficacy of surgically correcting kyphoscoliosis in PS. However, the fact that our patient's spinal deformity was effectively corrected without thrombotic complication and without curve recurrence one year post-operatively shows that safe and effective correction is possible. Future studies could aim to more thoroughly examine the outcome of current orthopaedic strategies in correction of spinal deformity in PS patients.

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Extreme Variations in Post-Adenotonsillectomy Admission Practices in 24 Pediatric Hospitals

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Adenotonsillectomy, the surgical removal of a patient's tonsils and adenoids, is one of the most commonly performed procedures in children in the United States. There are no current guidelines to determine whether a patient should be admitted for inpatient observation after undergoing adenotonsillectomy, leaving much room for physician and hospital variation.

Concern for postoperative complications may be an impetus for the decision to admit a patient after adenotonsillectomy. It is well known that children with obstructive sleep apnea (OSA) are at greater risk of postoperative airway collapse, respiratory depression, and hypoxemia than patients without the diagnosis.[1, 2, 3] This tendency can be exacerbated by the use of opioids for peri- and post-operative pain, particularly in the minority of children who metabolize codeine via an alternate pathway and may accumulate excess active metabolite in the bloodstream.[1, 4]

The objectives of this retrospective analysis were to 1) determine the average percent of adenotonsillectomy patients admitted based on their age and presence of OSA or other "flagged" comorbidities (complex chronic conditions such as cystic fibrosis, congenital abnormalities, etc). across 24 pediatric hospitals in the country, and 2) to identify practice variations if they exist amongst these group, using data from the Pediatric Health Information System (PHIS) collected over a one-year period.

Analysis of children undergoing adenotonsillectomy in twenty-four pediatric hospitals from July 2009 to June 2010 was conducted using PHIS. Patient characteristics were compared with hospital length of stay. Patients that were admitted overnight (defined as a length of stay > 1 day) were classified as inpatient cases. Children were divided into four groups based on age (0-2 years, 2–3 years, 3-5 years, and greater than 5 years) and presence/absence of comorbidities (+/- OSA, +/- "flags") and inpatient admission rates were compared and contrasted. (Table)

Across all hospitals, younger patients (0-2 years of age) are more regularly admitted as inpatient (86.7 percent inpatient admission rate) than all other age groups. In addition, patients with OSA are more likely to be admitted than their age-equivalent counterparts without obstructive sleep apnea, as are patients who are flagged with a clinical diagnosis of a complex chronic condition. Across all age groups, 46.9 percent of patients with OSA were admitted, while 21.6 percent of patients without OSA were admitted. Similarly, 68.7 percent of patients with PHIS "flags" were admitted, while only 28.8 percent of patients without these "flags" were admitted. (Table)

As the burden of health care costs rises, physicians must consider how they allocate hospital resources. It is unwarranted to admit every patient undergoing adenotonsillectomy, but it is crucial to avoid postoperative complications in high-risk patients. This data clearly shows that physicians are aware of the increased risk in certain patient population groups, reflected by their increased inpatient admission rates. These high risk groups include: age under two years, diagnosis of OSA, and diagnosis of a "flagged" comorbidity. However, although this data demonstrates clear trends for increased admission rates in certain groups, there is substantial practice variation. In patients that are not at high risk for surgery, such as a five-year-old patient without any comorbid diagnosis, the practice variation is even more significant.

It is our hope that this study, along with future ones, will help to establish

concrete guidelines for inpatient admission of pediatric adenotonsillectomy, for patients with and without comorbid conditions.

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	All age groups (n=29918)	0–2 Years (n=895)	2–3 Years (n=3277	3–5 Years (n=8370)	Older than 5 Yrs (n=17376)
ALL PATIENTS	31.1 (n=29918)	86.7 (n=895)	69.3 (n=3277)	33.7 (n=8370)	19.9 (n=17376)
– OSA	21.6 (n=22665))	85.7 (n=539)	68.6 (n=2273)	29.0 (n=6155)	15.5 (n=13698)
+ OSA	46.9 (n=7253)	88.2 (n=356)	70.9 (n=1004)	46.7 (n=2215)	36.5 (n=3678)
- Flags	28.8 (n=28143)	84.9 (n=717)	67.9 (n=3019)	31.5 (n=7875)	18.0 (n=16532)
+ Flags	68.7 (n=1775)	93.8 (n=178)	85.7 (n=258)	69.3 (n=495)	57.8 (n=844)
- OSA/ - Flags	37.6 (n=5282)	84.2 (n=378)	67.2 (n=1428)	27.5 (n=1607)	14.2 (n=1869)
- OSA/ + Flags	64.7 (n=649)	93.3 (n=84)	87.9 (n=131)	58.0 (n=181)	48.0 (n=253)
+ OSA/ - Flags	53.0 (n=2834)	86.2 (n=231)	69.5 (n=622)	42.9 (n=872)	33.0 (n=1109)
+ OSA/ + Flags	82.5 (n=570)	94.3 (n=83)	82.6 (n=90)	88.5 (n=162)	74.1 (n=235)

OSA = Obstructive Sleep Apnea, Flags = presence of at least one comorbidity flag

TABLE: Percentage of children in selected age and clinical groups admitted after adenotonsillectomy

Prospective Blinded Laboratory Assessment of Prophylactic Antibiotic Compliance in a Pediatric Outpatient Setting

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Advisors: Michael DiSandro, M.D. and Jenny Yiee, M.D., UCSF Pediatric Urology

The American Urology Association guidelines suggest that pediatric patients diagnosed with vesicouretal reflux (VUR) be placed on continuous antibiotic prophylaxis (CAP) in an attempt to prevent ascending urinary tract infections (UTI) that can lead to renal scarring.[1] While CAP is commonly prescribed in ambulatory care settings to impede the development of UTIs, it is important to note that patient compliance to recommended regimens remains to be a perpetual issue. Compliance rates for patients diagnosed with VUR and prescribed prophylaxis were shown to be as low as 40 percent.[2] Although compliance has been well studied in pediatric patients, prior studies for UTI prophylaxis have focused on using indirect methods such parental questionnaires and pharmacy claims data. The establishment of compliance by methods such as urine test analysis can allow for a more concrete perception of true compliance as subjective measurements tend to be inflated.[3]

To our knowledge we conducted the first prospective, single blinded study that objectively assessed compliance by measuring urine antibiotic levels in children being prophylactically treated for VUR. Children aged 0-18 years taking trimethoprim prophylaxis were recruited to take part in the study. The subjects were previously unaware of the study and approached to be enrolled during follow-up appointments. Urine samples were collected and sent to a commercial lab (NMS Labs) to determine the antibiotic levels. Of those approached, 97 percent consented to participate (n=54). Recruitment of eligible patients was prematurely ended because preliminary analysis of the data revealed very high compliance. The adherence rate was found to be 91 percent. Age, sex, self-report of compliance, duration of time on antibiotics, insurance status, history of breakthrough infection, surgery, history of pyelonephritis and hospitalization were not associated with compliance.

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Our compliance rate of 91 percent contradicts much of the reported values for the treatment of UTIs in the pediatric population and prophylaxis compliance, which is generally notoriously poor.[4,5] The referral of patients with VUR by a primary care provider to a pediatric urologist might facilitate compliance because the seriousness of the condition becomes more apparent, especially as it made clear that poor compliance will likely result in the need of surgery. Furthermore, a specialist can focus on a single issue for the duration of the entire visit to ensure that better parental clarity and understanding is achieved as compared to a primary care setting where multiple issues demand attention. Future studies should measure compliance in both other sub-specialty and general pediatric clinics. If proper compliance for prophylactic treatment is not established, perhaps pediatric urologist should be more involved with the care regardless of the future likelihood of surgery.

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Prevalence and Treatment of Methicillin-Resistant Staphylococcus Aureus (MRSA) in Hand Infections Presenting to an Urban Setting

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The prevalence of Methicillin-resistant Staphylococcus Aureus (MRSA) infection is increasing in the hospital setting.[1] The rise of antibiotic resistant pathogens continues in the modern American hospital despite evolving hygienic practices. As a major concern to all surgeons, proper prophylactic treatments can help reduce the rate of new MRSA infections and mortality. The National Surgical Infection Prevention Project (NSIPP) uses guidelines published in 1993, which state that cefazolin or cefuroxime are sufficient for surgery prophylaxis. The same report also indicates that vancomycin or clindamycin only be considered if the patient has a beta-lactam allergy. The NSIPP states that for best results, prophylaxis be started at least 60 minutes before operations and be continued for only 24 hours after completion.[2] Although cefazolin continues to remain the mainstay for prevention of wound infection, our study indicates that it is ineffective against MRSA. This study aims to demonstrate that clindamycin or vancomycin should become the preferred treatment in the prevention of MRSA infections.

Antibiotic	Cases susceptible (%)	Cases resistant (%)
Cefazolin	0 (0%)	41 (100%)
Clindamycin	38 (92.7%)	3 (7.3%)
Vancomycin	41 (100%)	0 (0%)
Tetracycline	35 (85.4%)	6 (14.6%)
Oxacillin	0 (0%)	41 (100%)
Gentamycin	41 (100%)	0 (0%)
Rifampin	41 (100%)	0 (0%)

TABLE: Antibiotic Resistance and Susceptibility to MRSA.

We retrospectively evaluated 82 patients who presented with hand infections to Washington Hospital Center from the years 2003–09; these patients were then operated upon. The specimens obtained during the initial evaluation and treatment were cultured and subjected to antibiotic susceptibility testing (Table). Only patients who had cultures positive for MRSA were selected for relevant data analysis. The following antibiotics were selected for testing susceptibility to MRSA: cefazolin, clindamycin, vancomycin, tetracycline, oxacillin, gentamycin, and rifampin. All information, including patient demographics, was obtained from patient charts.

Forty one of 82 (50 percent) patients had cultures testing positive for MRSA. Cefazolin and oxacillin had no effect in preventing or treating MRSA as all positive cultures (n=4) showed bacterial

resistance. Vancomycin, gentamycin, and rifampin were effective in all cases (n=41). Clindamycin was not resistant in 92.7 percent (n=38) of MRSA cultures. Tetracycline demonstrated efficacy in treating 85.4 percent (n=35) of MRSA cultures.

The choice of empiric antibiotic is becoming increasingly difficult since cefazolin has long been favored despite its ineffectiveness in preventing MRSA.3 The CDC has not yet published guidelines for MRSA prophylaxis during surgical procedures. Surgeons must rely on institutional guidelines or the National Surgical Infection Prevention Project's (NSIPP) recommendation, which cites a paper published in 1993. Given that these guidelines were published almost two decades ago, newer and more effective antibiotics should be considered for surgical wound prophylaxis.

The rate of community acquired MRSA hand infections in an urban setting is higher than previously suspected and the rate of MRSA positive cultures in our sample group is 50 percent (n=82). Resistance to cefazolin and oxacillin was found in all cultures whereas vancomycin, gentamycin, and rifampin demonstrated no resistance. Clindamycin and tetracycline had substantially more efficacy than cefazolin or oxacillin with MRSA susceptibility rates at 92.7 percent and 85.4 percent respectively. Although our patient sampling was relatively small, our subjects were good indicators of the general population presenting to an urban hospital. This data should be taken into account when managing seemingly routine hand infections given that the treatment options are different for MRSA infections. Specifically, based on our findings, cefazolin and oxacillin should be avoided as empiric antibiotics in favor of vancomycin, gentamycin, rifampin or clindamycin and tetracycline when preventing potential MRSA infections.

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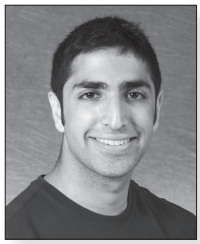
Corpus Callosum DTI Measurements in Neurofibromatosis Type I and Normal Controls



Amir Noor, MSII
Advisor: Nadja Kadom, MD, Children's National Medical Center

Neurofibromatosis type 1 (NF-1) is a common inherited disorder with a prevalence of approximately 1 in 2500-3000 births. The disease affects the brain in many ways, including structural abnormalities, such as macrocephaly and enlargement of the corpus callosum. The presence of cognitive deficits in NF-1 patients has lead to numerous investigations trying to find correlations between structural brain abnormalities and cognitive dysfunctions. Several studies have linked cognitive deficiencies in NF-1 patients to enlargement of the corpus callosum.[1,2]

Averaged Two Observers	NF-1	Controls	P-value
Skull (mm^2)	15326	14344	0.0001
CC-Skull Ratio	0.04900	0.03894	0.0001
Radial Diffusivity	0.00040	0.00037	0.023
Axial Diffusivity	0.00140	0.00148	0.0002
Apparent Diffusion Coefficient (ADC)	0.00078	0.00098	0.067
Fractional Anisotropy	0.71711	0.74969	0.0012

TABLE: *Averaged data of several measurements used to compare NF-1 patients to matched control patients without NF-1.*

The pathophysiological basis of corpus callosum enlargement (CCE) is not well understood. Four leading hypotheses to explain CCE have been proposed: 1) increased number of commissural fibers due to reduction in apoptosis; 2) excessive myelination resulting in larger axons; 3) increased extracellular fluid, and 4) myelinopathy associated with vacuolation, or a combination of all four of these factors.[3] Imaging parameters, such as diffusion tensor imaging (DTI) have been used in adults to further evaluate structural features of CCE in adult NF-1 patients.[3] DTI measures, such as mean diffusivity (MD), fractional anisotropy (FA), axial and radial diffusivity, allow for indirect assumptions regarding brain structure. MD reflects the size of the interstitial space that allows for overall molecular diffusion. FA relates to the directionality of diffusion that is mainly affected by the architecture of the environment around the interstitial space. Axial diffusivity (AD) represents the dominant direction

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of diffusion along the long axis of fibers. Radial diffusivity (RD) reflects the average diffusion of directions perpendicular o long axis. The goal of our study was to investigate the pathophysiological basis of corpus callosum enlargement in NF-1 patients through these DTI measurements.

Patients were consecutively selected from institutional data base with the inclusion criteria being: having an established diagnosis of NF-1, brain imaging with DTI sequence, abnormally high corpus callosum to skull ratio. Patients with complications of NF-1 that could affect size of the corpus callosum were not included in this study. Subjects were age and gender matched with normal controls that were randomly selected from the radiology database. Manual regions of interest (ROI) were placed over the corpus callosum for DTI measurements using DTI-Studio by two independent researchers, one blinded to diagnosis. Fifteen NF-1 patients and matched controls were analyzed. The corpus callosum to skull ratio was found to be significantly different between the experimental and control group (p=0.0001). For NF-1 patients we found: a trend to lower apparent diffusion coefficient (ADC, p=0.067), significantly higher radial diffusivity (p=0.023), significantly lower axial diffusivity (p=0.0002), and significantly lower fractional anisotropy (FA, p=0.0012).

The significantly lower axial diffusivity in NF-1 patients can indicate that there are more crossing fibers in the corpus callosum of in these patients than in the normal controls. Further studies using comparative DTI tractography may be helpful in further investigating this stipulation. The significant increase in radial diffusivity can be explained by a variety of factors that can account for a larger interstitial space, including thinner myelin sheaths, increased interstitial fluid, smaller axons, or a combination thereof. The trend of lower ADC may indicate low axonal diameter, as ADC has been shown to more strongly correlate with axonal diameter without the myelin sheath. In future studies we will correlate abnormal corpus callosum DTI markers with cognitive functions in NF-1 patients to see if relationships exist that can be used as predictors of cognitive deficits in young NF-1 patient.

The trend of lower ADC may indicate low axonal diameter, as ADC has been shown to more strongly correlate with axonal diameter without the myelin sheath.

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Idiopathic Transverse Myelitis and Arteriovenous Malformations: A Chart Review of 94 Patients for More Efficient Diagnoses

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Idiopathic transverse myelitis (ITM) is a clinical syndrome caused by a focal inflammation of the spinal cord causing demyelination and necrosis.[1] Spinal cord arteriovenous malformations (AVMs) are shunts fed by arteries normally supplying the neural tissue.[2] ITM and spinal cord AVMs present similarly in a clinical setting. Sensory changes in legs and some form of bowel or bladder dysfunction are common clinical characterizations. The similarity in presentation of these conditions poses a problem for quick and effective treatment due to their different etiologies. When an AVM is detected, treatment is aimed at halting the progression of the disease.[2] Diagnosis usually rests on a positive CT angiography test in spinal cord AVMs. In addition, there is a slight variance in sensory level, defined as the most caudal level at which sensation is intact. For ITM the most common sensory level in adults is the midthoracic region.[3] Most arteriovenous malformations result in a thoracolumbar sensory level.[4] For AVMs, prognosis depends on the extent of ischemia and necrotizing myelopathy present at the time of diagnosis, indicating the importance of the timely recognition of disease.[5] The delay in performing CT angiography to rule out AVMs and lack of knowledge of differentiating signs on presentation give cause for evaluation.

A retrospective review was performed of 94 cases of myelopathy referred for angiogram, comparing subjects whose

final diagnosis was ITM versus AVM. The aim was to assess any differences between ITM and AVM to provide more efficient diagnoses in the future. Gender demographics of the examined 94 patients with ITM and AVMs were relatively equal, with 55 percent males and 45 percent females. Substantially more males (76 percent) were affected by AVMs in our patient population compared to females (24 percent).

In terms of clinical characterization based on physical examination, ITM and AVM patients were found to have slightly impaired functioning in lower extremities in strength tests, while their upper extremities appeared unaffected. 80 percent of all patients were found to have sensory changes in their lower extremities, while just 7 percent were found to have sensory changes in their upper extremities. 75 percent of ITM and 59 percent of AVM patients experienced bladder or bowel dysfunction. As expected, the majority (60 percent) of ITM patients had an acute onset of disease, while 65 percent of AVM patients had a progressive onset. In terms of response to treatment, it was found that 55 percent of ITM patients showed improvement with steroids, while only 19 percent of AVM patients showed improvement. Also, 59 percent of AVM patients were found to have a vascular risk factor such as hypercholesterolemia, hypertension, or diabetes mellitus, in comparison to 45 percent of ITM patients.

These data demonstrate that while characterizing the clinical differences between ITM and AVMs still remains somewhat clinically ambiguous, there are clinical markers that can lead to a more timely diagnosis. While a positive spinal angiogram still remains the most accurate way of distinguishing an AVM, a lack of response to steroids also likely indicates that vascular etiology in the spine may be involved. A thorough patient history including onset of the disease, acute versus progressive, may prove to be more indicative of a particular diagnosis. Additional evaluation of the importance that risk factors such as hypercholesterolemia, hypertension, and diabetes mellitus play in AVMs may also lead to a more efficient diagnosis and therefore better prognosis for patients.

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Familial Aggregation of Panic Disturbances in Parkinson's Disease

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Panic disorder is one of the most common anxiety disorders in Parkinson's disease (PD) with a prevalence of 10 percent-30 percent.[1] In addition, there is a high prevalence of atypical 'panic-like' anxiety disturbances correlated with motor and antiparkinsonian medication fluctuations in PD.[2] One previous study reported distinct demographic and clinical features in individuals with PD and panic disorder as compared to PD subjects without anxiety, suggesting that panic disorder may be uniquely associated with PD and useful as a marker associated with disease etiology or prognosis.[3] To investigate the validity and significance of panic disorder as a phenotypic subtype in PD, this family study investigated the heritability of panic disorder and 'panic-like' symptoms in PD.

Participants were adults with idiopathic PD and their first-degree relatives. They were recruited from movement disorder practices in the greater Baltimore area. Panic disorder status was determined by a psychiatrist as part of a comprehensive psychiatric evaluation. Case probands with PD and panic disorder (PD-PANIC, n=20) and a comparison group of non-anxious PD probands (PD-NA, n=17), defined as having no history of any anxiety disorder, no current mood disorder or major psychotic disorder, were identified. The sample included 222 first-degree relatives (114 PD-PANIC relatives and 108 PD-NA relatives).

Evaluations to determine panic status in relatives were conducted by phone. When relatives were deceased, unreachable, or unwilling to participate; informant 'proxy' interviews were conducted by interviewing another family member or spouse.

Demographic and clinical variables in PD-PANIC and PD-NA probands were compared using t-tests and the chi-square statistic. Significance was set a priori at $p < 0.05$. The odds of panic disorder and 'panic-like' phenomena in first-degree relatives were estimated using logistic regression by the method of generalized estimating equations,

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which accounts for within-family correlation among relatives.[4]

Table 1 shows the demographic and clinical features of the PD-PANIC and PD-NA probands. As compared to the PD-NA probands, PD-PANIC probands were significantly younger (p=0.001), had a longer duration of PD (p=0.04), higher L-dopa doses (p=0.02), and greater ADL disability when in the 'off' state (p=0.03). However, they had comparable PD severity as measured by the Hoehn and Yahr stage and the UPDRS Motor sub-score.

As shown in Table 2, the prevalence and odds of panic disorder and 'panic-like' disorder is greater for relatives of PD-PANIC probands when compared to PD-NA probands [OR=16.13, p=0.01]. Regression analyses showed that the significant group differences in prevalence rate persisted after controlling for potentially confounding variables: younger age of PD-PANIC probands, presence of current depressive disorder in probands, higher prevalence of panic phenotype in female relatives, and higher prevalence of the panic phenotype in directly interviewed relatives.

The finding that panic disorder and 'panic-like' disturbances are familial in PD suggests that they may be genetically based and not simply accounted for by exposure to dopaminergic therapy or a reaction to motor impairment. The similarities between PD-PANIC probands and a previously described subgroup of 'younger-onset' PD patients suggests that panic-type anxiety may be an additional clinical marker for earlier onset PD and possibly an endophenotypic feature that co-segregates with relatives at increased risk for this PD variant.[5] Given the implications of these findings and the high prevalence of panic disorder in patients with PD, routine screening for panic phenomena in patients with PD should be considered.

Characteristic	PD-PANIC Probands (n=20)	PD-NA Probands (n=17)	P
Sex, No. (%)			
Male	11(55)	11(65)	0.55
Female	9(45)	6(35)	
Current age, years	61.2(7.6)	70.8(8.4)	0.001
Education, years	16 (3)	17(3)	0.32
MMSE Total	28.7(1)	28.8(1.3)	0.76
Age at PD Symptom Onset, years	49.1(9.3)	63.9(12.5)	0.0002
Age at PD Diagnosis, years	50.6(9.4)	64.7(12.4)	0.001
PD Symptom Duration, years	12.1(7.4)	6.9(6.9)	0.04
Cumulative LDOPA Use, mg	815.1(587.7), n=18	442.4(205.1)	0.02
Agonist Use, No. (%)	4(22), n=18	8(47)	0.12
Hoehn & Yahr Stage Score	2.3(0.9)	2(0.9)	0.12
Motor Fluctuations, No. (%)	11(73),n=15	3(18)	0.002
UPDRS Sub-scores			
I (Mentation, Mood)	3.9 (3), n=18	1.1(1.1)	0.001
II (ADL) Off	15.3(7.6), n=18	10.2(5.8)	0.03
II (ADL) On	13.1(8), n=16	9.8(5)	0.15
III (motor)	20.3(9.8), n=18	15.4(9.6)	0.16
IV (Therapy complications)	5.3(3.3), n=18	2.2(3.8)	0.02

Mean (SD): UPDRS – Unified Parkinson's Disease Scale; ADL – Activities of Daily Living

TABLE 1: Demographic and Clinical Features of PD-PANIC and PD-NA Probands

Characteristic of Relative	Odds Ratio (95% CI)	P
Group		
Case	16.13 (1.82–142.7)	0.01
Control	1.0	
Sex		
Female	4.08 (1.25–13.3)	0.02
Male	1.0	
Type of Interview		
Direct	3.14 (0.96–10.3)	0.06
Proxy	1.0	
Characteristic of Proband	**Odds Ratio (95% CI)**	**P**
Proband age		
PD-PANIC	0.984 (0.91–1.05)	0.55
PD-NA1.0		
Proband Depression Status		
Current Depression	1.08 (0..35–3..27)	0.90
No Depression	1.0	

TABLE 2: Adjusted Odds Ratios for Panic Disorder and "Panic-like" Disturbances

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Perceptions of Glycemic Control and Frequency of Type 2 Diabetes Fundus Photography

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The American Diabetes Association recommends annual fundus examination services for all type 2 diabetes patients.[1] However, not all patients, especially minority patients, receive testing on an annual basis.[2] Factors such as cost of service, time spent in the referral process, misinformation about the disease process, young age, and poor glycemic control prevent patients from receiving eye care.[3] Fundus photography has been proposed as a follow-up diagnostic tool to annual fundus examination, given their comparable efficacy.[4] Fundus photography was studied as opposed to dilated eye examination by a specialist, as the cost of service and referral wait-time is less of a burden for our underserved population. This study was designed to identify patient factors that contributed to a low rate of screening among our type 2 diabetes patients. These factors could be used to flag patients deemed high risk of not receiving these services.

351 patients were selected randomly from our photography database. The patient population most frequently self-identified as Hispanic (n=265) and female (n=218). The average age of patients was 52 ±10 years. Race and sex did not associate significantly with any variable. Patients in the study were asked to complete a bilingual questionnaire. Survey responses were grouped into four main categories for analysis: screen frequency, years with a diagnosis of type 2 diabetes, retinopathy, and perception of glycemic control. If a patient selected a poor or fair response for their glycemic control, they were grouped into the lower than moderate group. If the patient selected a good or excellent response they were grouped into the better than moderate group. Electronic medical records were then used to associate most recent hemoglobin A1C (HbA1c) and retinopathy diagnosis at the time of photography, to the responses.

These categories were then evaluated: The HBA1C level was associated with the patient responses to provide an objective measurement of glycemic control in this population. The responses consisted of lower than moderate (LTM; Most Recent HbA1C= 8.7 ± 2.0 percent); moderate responses (Most Recent HbA1c= 8.44 ± 1.9 percent); and better than moderate (BTM; Most Recent HbA1C=7.7 ± 1.9 percent). Data was analyzed using Fischer's exact test.

The frequency of receiving fundus photography was not significantly associated with the number of years diagnosed with type 2 diabetes, nor was it significantly associated with the patient's subjective level of control. The patient's perception of control was significantly associated with years diagnosed with type 2 diabetes — with patients who have had type 2 diabetes less than or equal to 10 years more likely to state BTM than LTM levels of control. Also, patient perception is not associated with a diagnosis of retinopathy. The presence of retinopathy is not associated with screen frequency, but is associated with years diagnosed with type 2 diabetes, as was expected.

Other studies have shown an association between measured levels of glycemic control and screen frequency in an established fundus photography program.[5] In this study, perception of glycemic control was not associated with rate of attendance. For example, those patients who state LTM levels of control were not less likely to attend eye screening services than a patient who states BTM levels of control. As a result, perceptions of glycemic control should not be used to classify patients as high risk of non-attendance in patient populations with similar demographics. Further research is needed to determine if other patient or provider variables, such as underlying mental illnesses, economic issues, or provider biases, influence the frequency of obtaining fundus photography.

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Associations	Fisher Exact P	Odds Ratio
Screen Frequency		
Years with T2DM	0.37	0.76
Subjective Level of Control	0.44	0.75
Subjective Level of Control		
Years with T2DM	0.014*	0.41
Retinopathy	0.15	1.62
Retinopathy		
Years with T2DM	<0.000001†	6.72
Screen Frequency	0.32	0.77

TABLE: Fisher's exact test testing and odds ratio of responses tested at 95% Confidence. Values with a P<0.05 are considered statistically significant.

Continued from p. 27

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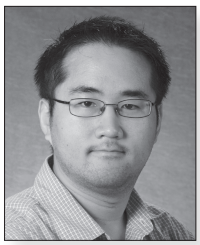
Validation of Cardiac Procedure Codes in Canadian Hospital Administrative Databases



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Cardiovascular diseases (CVD) are major chronic conditions with significant mortality, morbidity and resource utilization burden. In Canada, CVD has reduced the average life expectancy by 2.8 years.[1] While cardiac procedures are an effective method routinely used to treat CVD, they are often costly and should be closely monitored. Administrative databases, initially developed for the purpose of tracking patient volume and hospital activities, are potentially powerful tools for cardiac procedure surveillance due to their representativeness, availability, and capture of data over many years at relatively low cost.[2] However, they must first be validated against clinical registries in order to ensure accuracy.

The purpose of this study was to determine the validity of coding of cardiac procedures in the Canadian Institute for Health Information (CIHI) administrative databases, and compare them with the Cardiac Care Network (CCN) clinical registry of cardiac procedures. The CIHI cohort is a centralized population-based database that is collected by the hospital's administrative staff and contains only demographic information from all hospital discharges across Ontario. The CCN database is very cohort specific and is collected by trained nurse abstractors, including only patients who have undergone cardiac surgery. In addition to demographic information,

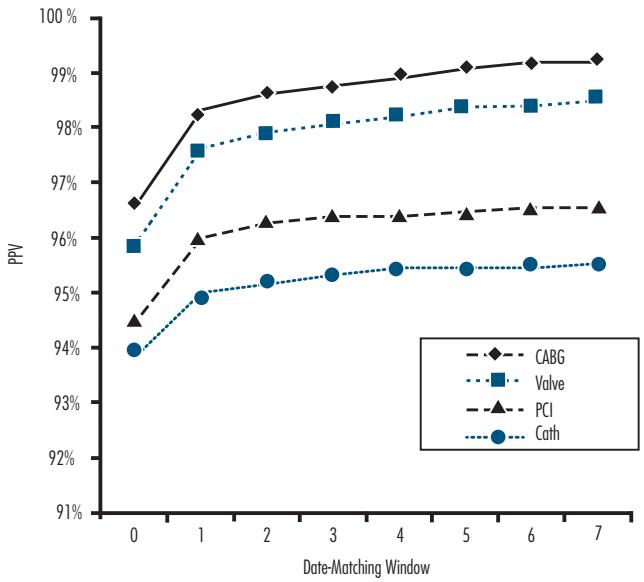

FIGURE: Increments in PPV between the administrative databases and the clinical registries were greatest when a one day window in terms of procedural dates were included. Further increases resulted in minimal increases in PPV

the patient's cardiac history, preexisting comorbidities and cardiac procedure data were collected. We hypothesized that widely used cardiac procedures, including percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) surgery, valve surgery, and cardiac catheterization would be accurately coded in administrative databases when compared with a clinical registry.

We examined the CIHI administrative database for all PCIs, CABG surgeries, cardiac valve surgeries, and cardiac catheterization procedures performed at 18 cardiac care centers in Ontario, Canada between the 2005 and 2006 fiscal years. Cardiac procedures were matched between the CIHI and the CCN databases whether they occurred on the same day in the same patient at the same hospital site. We determined the positive predictive value (PPV) of CIHI coded procedures as the numbers of matching procedures divided by total procedure counts in CCN. As the procedure dates can be a common source of mismatch, we repeated the analysis after expanding the allowable window for matching to ±1 day. To determine the extent of the time window's impact, we expanded the window for matching to ±7 days in a sensitivity analysis.

The PPV of the administratively coded CABG surgery from the CIHI databases was 97 percent. For valve surgery procedures, the accuracy of administrative databases was 96 percent. When compared against the CCN clinical registries, the coding accuracy of both PCI and cardiac catheterization were 94 percent. When the procedure date window was expanded from the same day to ±1 day, the PPV improved to 96 percent for PCI and exceeded 98 percent for CABG surgery, 97 percent for valve surgery, and 95 percent for cardiac catheterization.

In sensitivity analyses, we expanded the matching date window to ±7 days (Figure). The highest increase in PPV occurred when the date window was widened by ± 1 day, and there were only minimal increments in predictive value thereafter (Figure).

In summary, our evaluation suggests that administrative coding of CABG surgery, valve surgery, PCI, and cardiac catheterization procedures is valid, and therefore is a better alternative for outcomes research because they are readily available, cheaper to prepare and at least as accurate as a province-wide prospective registry.

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Design and Validation of the Aviation Laser Exposure Self-Assessment

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LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. This stimulated emission has the potential to cause materials to deform. Because the eye's optical system is capable of focusing radiation, it is particularly vulnerable.[1] Reports of aircrafts exposed to lasers are a growing concern due to the increasing availability and decreasing price of handheld lasers.[2] In the first six months of 2011, there were 671 cases reported to the Civil Aviation Authority (CAA) of the United Kingdom. Many countries have criminalized directing lasers at aircrafts and operational procedures to deal with exposure have been established.[3] While nearly all of the reported exposures have not been associated with long term physical damage, the increasing power of available lasers raises the possibility.

There are currently no guidelines for a rapid self-assessment to assist in determining if professional treatment should be sought after eye illumination from a laser. Eye damage, particularly retinal damage, can be painless, and may result in a delay in medical care.[3] Uncertainty can also send individuals to see a physician unnecessarily. The Aviation Laser Exposure Self-Assessment (ALESA) was created as a convenient means to assist in self-evaluation after a laser beam incident.

A review of laser incidents reported to the CAA was conducted. To center the ALESA on aspects of the incident that could be easily be identified, wavelength (color) dependent distances for perceived brightness levels were obtained and compared to the Nominal Ocular Hazard Distance (NOHD). An algorithm was created to indicate whether the experience had exceeded maximum permissible exposure and therefore warranted medical examination. A symptoms list and an Amsler grid were included to account for visual disturbances and general eye problems. Five experts in laser study and/or ophthalmology, in addition to a flight operations inspector, were consulted throughout the ALESA's creation.

A five point Likert-type scale was developed to gauge the attitudes of pilots and flight crew on the use of the ALESA.

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The study population consisted of 25 male and female pilots and flight crew members who have experienced aviation laser beam exposure. Because the participants were considered hard-to-find types of individuals, network sampling was used. After completing the ALSEA based on their most recent exposure, they indicated their level of agreement on six dimensions: time saving potential, likelihood of use, ease of completion, need, accuracy, and decrease in anxiety.

The proportion agreeing (responding favorably) was found to be significant in all aspects of the ALESA except for its ability to decrease anxiety. Content validity was established by the expert's judgment of the appropriateness of the ALESA and face validity was found by the participant's responses. The ALSEA was shown to be of use to the aviation community and the CAA is currently distributing the tool throughout the United Kingdom. None of the participants experienced permanent damage from their exposure and the probability of such damage at this time is extremely low. As the power of lasers purchased by the public increases in the future, it will be desirable to establish the sensitivity and specificity of the ALESA.

Stephanie Waggel with the London Metropolitan Police Service Air Support Unit.

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Losses to Follow-up Among Patients Registered for Care at a Tertiary HIV Referral Center in Chennai, India

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Highly active antiretroviral therapy (HAART) has dramatically improved survival and transformed HIV into a chronic disease requiring long term follow-up of HIV-infected patients. India has approximately 2.3 million HIV-infected persons and Antiretroviral Therapy (ART) is available in India via the public and private sectors. While services such as physician consultation and ART costs in the government sector are free, patients are generally required to pay for these and other services in the private sector. Unfortunately, it is difficult to ascertain the efficacy of ART treatment since there is limited data on loss-to-follow up (LTFU) rates and factors associated with LTFU in India.

Our case control analysis comprised a subset of patients registered for care at YRGCARE (a private non-governmental organization providing HIV clinical services in Chennai since 1993) between Jan. 1, 2004 and Dec. 31, 2009. Cases (LTFU) were defined as patients who did not have at least one visit between Jan. 1, 2010 and Dec. 31, 2010. "Project" patients included the subset of patients who were enrolled in clinical trials or projects and received some compensation for clinical care (e.g., free ART, free consultation, compensation for travel, etc.) and who were assigned retention staff. Logistic regression was used to identify factors associated with LTFU.

Of 7,995 patients included in the analysis, 68.2 percent were male with a median age of 34 years. The overall

loss to follow-up rate was 38.1 per 100 person-years p-y. Among those who were LTFU, there were 304 (3.8 percent) documented deaths. In univariate analysis, LTFU was less common in those with higher baseline CD4 count, higher CD4 count at last visit to the clinic, being enrolled in a project and having initiated ART. LTFU was more common among patients who were older and unmarried. In multivariate analyses, persons were significantly less likely to be LTFU if they were enrolled in a project (OR: 0.66; 95 percent CI: 0.58, 0.77), being initiated on ART (OR: 0.32, 95 percent CI: 0.26. 0.38) or married (OR: 0.71, 95 percent CI: 0.57, 0.88).

This clinical cohort exhibited a high-rate of LTFU as the majority of the losses occurred within the first month. Given the negative association of LTFU with being enrolled in a project, innovative strategies using incentives (e.g., conditional cash transfer, travel incentives) and peer-health workers in improving retention among patients needs to be evaluated for efficacy and cost-effectiveness, considering that cash incentives can improve HIV-positive patient follow-up.[1] Given the high rate of mortality among patients LTFU compared to documented deaths in the clinical cohort, the impact of these deaths on survival analyses need to be examined.

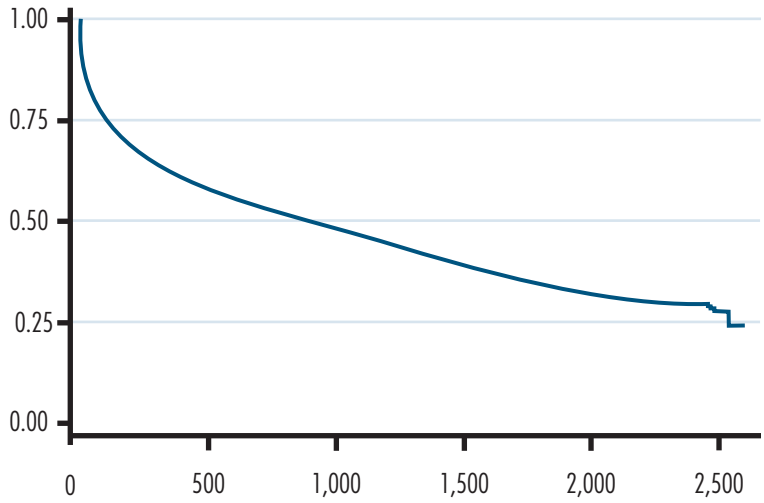

FIGURE: Kaplan-Meier curve of overall dropout from clinical care at YRGCARE

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The Rise of Ciprofloxacin-Resistant Urinary E. coli Among Older U.S. Outpatients, from 2000 to 2010

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Urinary tract infections (UTIs), including acute cystitis (infection of the lower urinary tract) and pyelonephritis (infection involving the kidney), are among the most frequently occurring infections in humans.[1] Among non-institutionalized elderly individuals, UTIs are the second most common type of infection, representing approximately 25 percent of all infections.[1]

We used data from The Surveillance Network (TSN) to describe in vitro E. coli antimicrobial resistance among isolates collected from older outpatients (ages 65 and older) in the United States from 2000 to 2010. Details of this surveillance system have been described previously.[3] Susceptibility testing is performed on site by each laboratory in accordance with FDA-approved testing methods and interpreted using Clinical and Laboratory Standards Institute (CLSI) recommended breakpoints. The objective of this study was to examine trends and prevalence of the antimicrobial resistance of urinary E. coli isolates to commonly prescribed antimicrobial agents among older outpatients (65+) in the United States from 2000 to 2010.

Susceptibility testing results from urinary isolates of E. coli (n = 2,555,999) were examined from U.S. outpatients from 2000 to 2010 (see Figure). The greatest increases in antimicrobial resistance from 2000 to 2010 were observed for ciprofloxacin 6.0 percent to 29.5 percent and TMP/SMX 16.3 percent to 26.8 percent, whereas nitrofurantoin 1.5 percent to 2.8 percent demonstrated a small increase (see Table). In 2010, ampicillin, amoxicillin-clavulanate, and tetracycline demonstrated resistance rates of 45.7 percent, 7.0 percent, and 24.9 percent, respectively.

E. coli antimicrobial resistance among older U.S. outpatients increased markedly for ciprofloxacin and TMP/

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DRUG	Total Isolates 2000–10	Percent Resistance 2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total Change 2000–10	P-value*
IMP/SMX	2,034,254	17.9	17.8	18.1	18.2	19.4	20.2	20.8	21.9	22.9	23	24.2	6.3	<0.0001
Ciprofloxacin	1,836,598	3	3.7	5	6.2	7.5	9.7	12.2	14.1	16	16.3	17.1	14.1	<0.0001
Ampicillin	2,002,221	38.2	38.1	38.2	38.2	38.2	39.4	40.5	41.5	42.4	42.4	43.4	5.2	<0.0001
Amox/Clav	759,749	5	4.1	5.3	4.5	3.7	3.8	5.6	8.2	9.9	6.6	5.3	0.3	0.0054
Nitrofurantoin	1,972,633	0.8	1	1.1	1.1	1	1.1	1.4	1.5	1.5	1.6	1.6	0.8	<0.0001
Tetracycline	580,328	22.6	22.1	22.1	21.1	21.9	22	23.5	23.7	24.4	24.5	24.9	2.3	<0.0001

NOTE: Isolates demonstrating intermediate susceptibility were not counted as resistant. Amox/Clav, Amoxicillin/Clavulanate; TMP-SMX, Trimethoprim/Sulfamethoxazole. Comparison of 2010 vs. 200 antimicrobial resistance rates

TABLE: Annual Rates of Antimocrobial Resistance in Urinary Eschericha coli Isolates to Select Antimibrobials in United States Outpatients, 2000–10.

Continued from p. 31

SMX from 2000 to 2010. Though rises in resistance have been reported previously for TMP/SMX, such substantial increases in E. coli resistance to ciprofloxacin have yet to be reported among older U.S. outpatients. These results highlight a disturbing trend of soaring resistance that will have a profound effect on how UTIs are treated in the outpatient setting. When resistant uropathogens such as E. coli are encountered, the likelihood of a clinical cure decreases and risk of recurrence increases.[3] Additionally, resistance significantly increases patient morbidity, costs of treatment, rates of hospitalization, and use of broader spectrum agents.[4] As the availability of efficacious antimicrobial therapies dwindles in the wake of rising antimicrobial resistance, the population that will be most severely affected will be older adults like the ones represented in this study.

The strengths of this investigation include the large number of isolates, the long time period for which data were collected, and the variety of agents examined. Although passive surveillance systems such as TSN are suitable for examining patterns in antimicrobial resistance , they are generally not used to guide therapeutic use of empiric agents. Local susceptibility data that are collected prospectively through active local surveillance are more appropriate when determining proper empirical therapy for the treatment of uncomplicated UTIs among individual patients.

In summary, our study shows that from 2000 to 2010 E. coli antimicrobial resistance to ciprofloxacin and TMP/SMX increased substantially among older outpatients in the United States, whereas lower resistance to nitrofurantoin remained low. Given the frequency with which UTIs are treated empirically, compounded with the speed that E. coli acquires resistance, judicious selection of empirical agents in the outpatient setting remains crucial.

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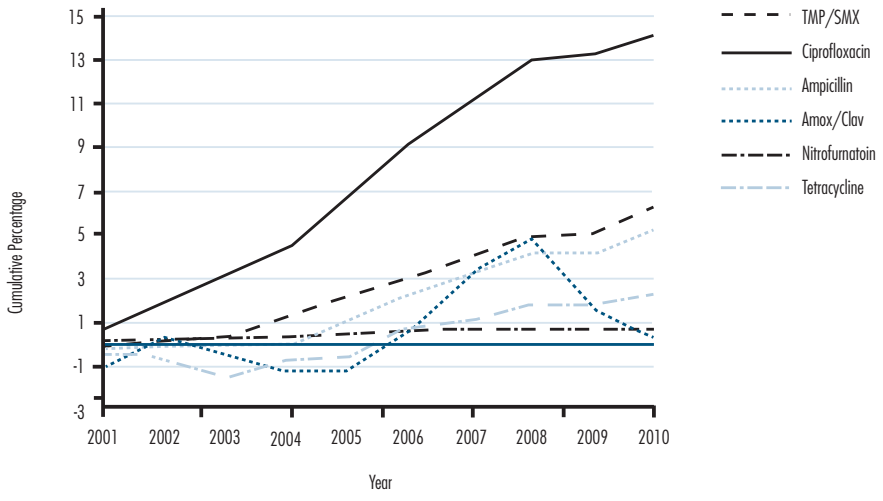


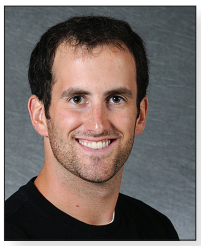
FIGURE: Cumulative change in antimicrobial resistance in urinary E. coli isolates, 2000–10.

Design and Conquer

The serendipity of collaboration, ingenuity, and innovation have often stemmed from the desire to create something new from a variety of perspectives and sources of knowledge.

This is exemplified in the medical field and incorporated in the theme of this year's edition of *Fusion*. Inspired by this integration of research and clinical care, the cover is a fusion of two different mediums by two second-year medical students; an anatomical sketch of a heart (Aaron P. Wessell) and a photograph of a statue from the National Museum of the American Indian (Benjamin J. Trevias).

The two mediums were chosen deliberately to showcase both contemporary and ancient elements to provide a dynamic composite cover.



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Submissions from the classes of 2013, 2014, and 2015, as well as the incoming class of 2016 for next year's edition of the journal will be accepted beginning in September 2012. For more information, please contact the Beaumont Society at *gwbeaumont@gmail.com*.

Contributions to the publishing costs of this journal are appreciated. If you would like to make a donation, please contact us at *gwbeaumont@gmail.com*. All proceeds will go toward the publishing costs for next year's journal.