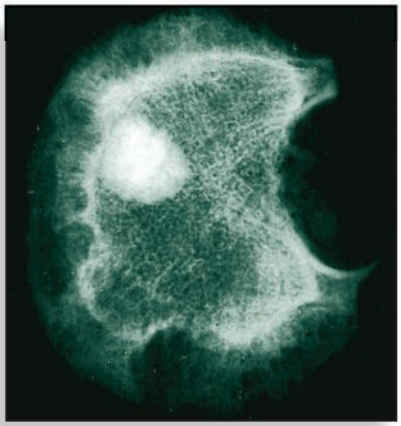
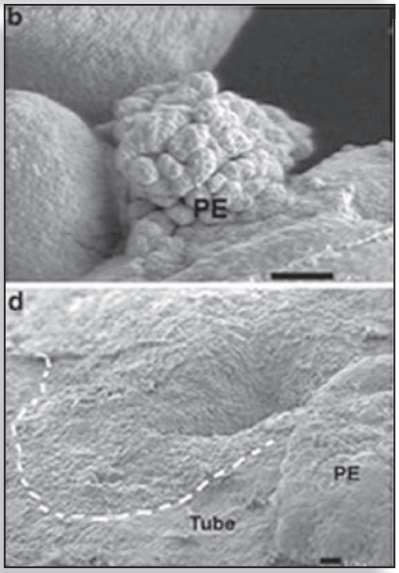
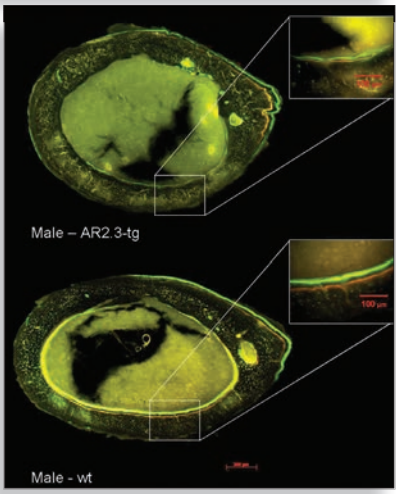
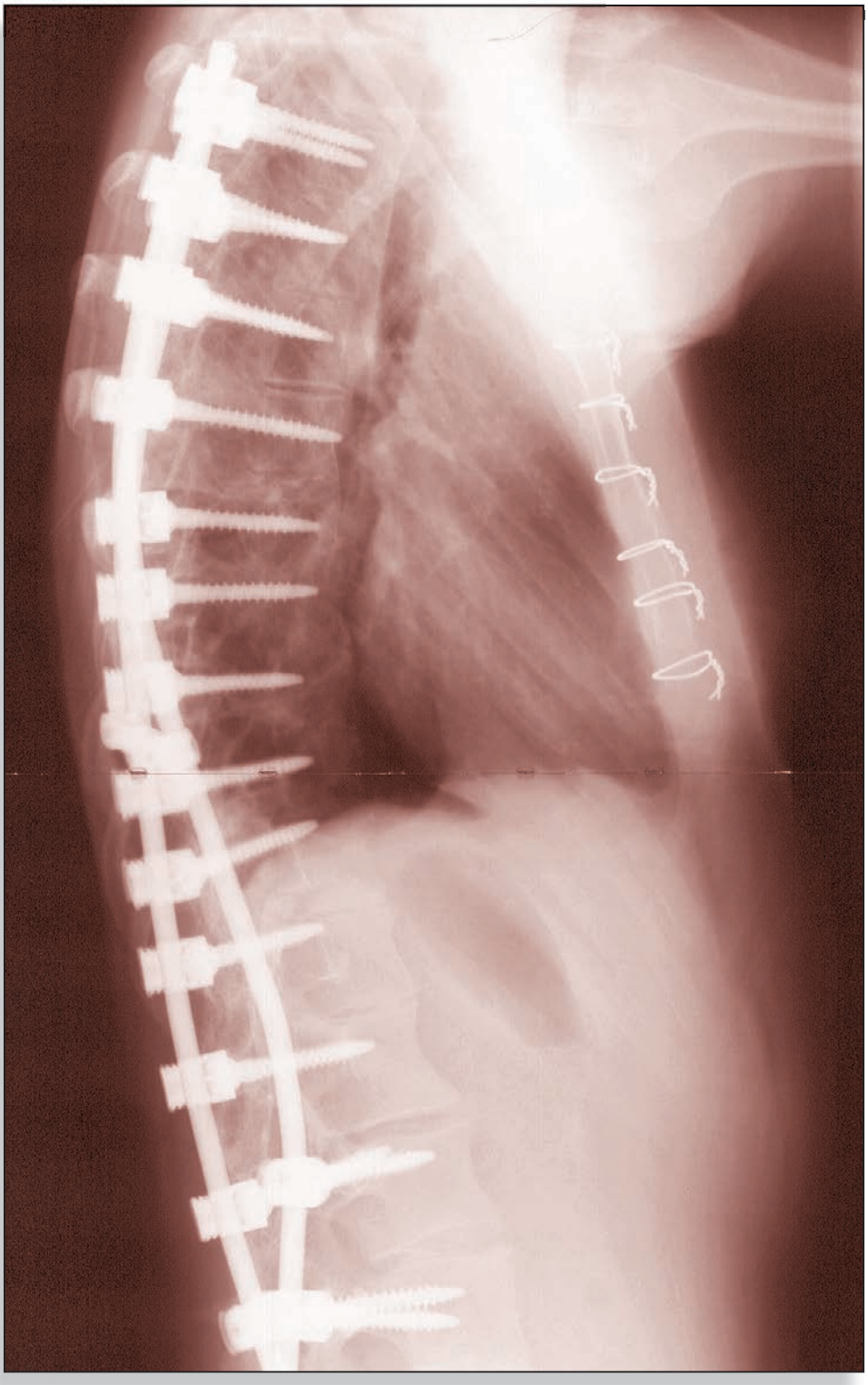
FUSION

THE WILLIAM H. BEAUMONT MEDICAL RESEARCH HONOR SOCIETY | V. II, SPRING 2008









A Student-run Scientific Journal Serving The George Washington University Medical Center

about the society. . .

BEAUMONT LEADERS

The 2007–08 officers of the William H. Beaumont Medical Research Honor Society include, front row from left, Amrita Karve, MSI representative; Najma Khorrami; Talya Bordin-Wosk, secretary. Middle row from left, Amit Bhakoo, treasurer; Steven V. Kardos, co-president; Ajay Wadgaonkar, co-president; Kasra Adham, vice president. Back row from left, Andrew H. Gordon, PhD; Sumi Bose.

The William H. Beaumont Medical Research Honor Society is an honorary research society of medical students that was established to 1935 honor Dr. William H. Beaumont, a pioneer in physiology research. The organization seeks to foster a continuing interest in biomedical research and to promote its value in the practice of medicine. As a part of this mission, the Society integrates current research topics with the curriculum; develops a research journal showcasing GW student research; makes available information on research opportunities and seminars throughout the area, including the William T. Gill Summer Fellowship for GW medical students; and highlights student and faculty research accomplishments at the annual GW Research Day.

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VOLUME II SPRING 2008

Fusion **is a publication of The George Washington University Medical Center's William H. Beaumont Medical Research Honor Society.**

This research journal is published by students in collaboration with the Office of the Dean; Office of Health Research, Compliance and Technology Transfer; and Medical Center Communications and Marketing.

Fusion

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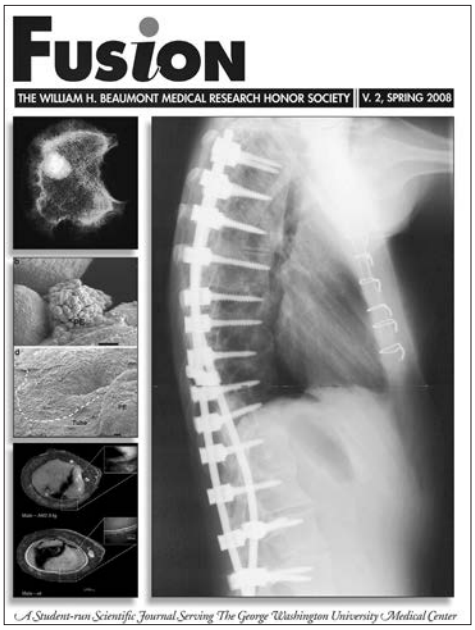


ON THE COVER: Clockwise from right, *Surgical Correction of Spinal Deformities after Heart Transplantation: A Case Series Report,* Brian Kaufman, MSII, p. 24; *What Role Do Androgens Play in Bone Growth?* Adrian A. Woo, MSI, p. 6; *A Three-Dimensional Model of Epicardial Development,* Tresa L. Nesbitt, PhD, MSI, p. 20; and, *Effect of Bone Density on Mechanical Properties after Percutaneous Vertebroplasty,* Nabila Hai, MSII, p. 28.

from the editors. . .

Medical students attending The George Washington University School of Medicine and Health Sciences routinely engage in biomedical research of a high quality. We are proud to present a diverse sampling of these research efforts with this publication, authored by our fellow medical students for presentation to The George Washington University Medical Center (GWUMC) community. The articles in the following pages span several disciplines, including basic science "bench" research, clinical "bedside" efforts, endeavors in global and public health, and medical education. From elaborate studies of leukemic cells, to new technologies in spinal surgery, to health travel experiences in rural India, GW medical students continue to impress in both the depth and scope of their work.

Putting together this journal was both rewarding and challenging. Members of the student editorial board, composed of officers of the William H. Beaumont Medical Research Honor Society, solicited submissions from the medical school Classes of 2010 and 2011. We received a strong response. The editors and editorial board, along with selected faculty, collectively and rigorously reviewed each submission. Responsibility for the content of the articles, however, remains with the respective author(s). In this second edition of *Fusion*, we are pleased to publish 31 submissions, reflecting an extraordinary range of our peers' research experiences.

The Dean's Office and Office of Student Opportunities continually offer superb guidance to medical students interested in undertaking research, and both deserve special recognition. In particular, both offices jointly fostered the creation of track programs in research, global health, health policy, and medical education, among others, to focus student interest and generate connections with experienced and talented faculty—at GWUMC and throughout the country. The diversity and quality of research in *Fusion* is a testament to their vision.

We thank James Scott, MD, FACEP, and W. Scott Schroth, MD, MPH, in the Office of the Dean; Anne Hirshfield, PhD, our faculty advisor in the Office of Research; Linda Dent and Debbie Goldstein in Medical Center of Communications and Marketing; as well as the numerous GWUMC faculty members who took time from their hectic schedules to review submissions. We also thank the editors of last year's debut edition of *Fusion*: Andrew Lerner, Vivek Patil and Rahul Arya. Their ingenuity, hard work and guidance helped lay the foundation for the journal you read today. Finally, we especially thank Thomas Kohout, in the Office of Communications and Marketing, for serving as our wonderful managing editor, and our editorial board for their diligence and hard work.

Medical research is an incredibly exciting endeavor, paving the way for important breakthroughs that expand our knowledge of the human body and enhance all dimensions of patient care. We hope the second annual edition of *Fusion* conveys this excitement to you.

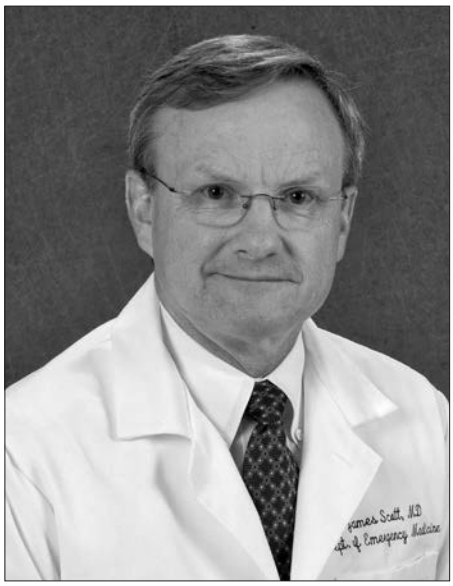
Warm regards,

Andrew H. Gordon, PhD, MSII

Steven V. Kardos, MSII

Ajay Wadgaonkar, MSII

from the dean's office. . .



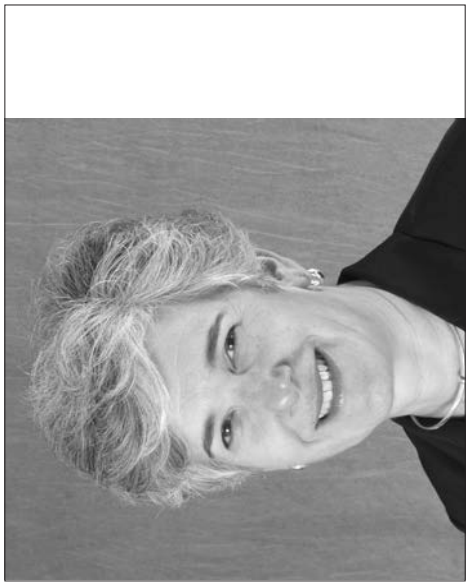
While it is indisputable that medicine is based on the foundations of science, how we define that science is anything but narrow in scope and quality. Laboratory research, clinical studies, community-based projects and outreach across the globe all qualify as scientific endeavors, if the results of these activities are scrutinized in an organized way, discussed openly and form the beginnings of ongoing inquiry. The first step in that process is to publish something based on the work and begin a dialogue about its importance, relevance and future directions. In this second edition of *Fusion*, students from The George Washington University School of Medicine and Health Sciences (SMHS) have gathered an impressive collection of contributions that span the experiences of scientific endeavors for SMHS students. I can say that I'm not only delighted to read about the activities of our students, but also extremely proud of their many and diverse accomplishments.

This journal was conceived by medical students in the William H. Beaumont Medical Research Honor Society and I commend them for their work. Specifically, I want to recognize Steven Kardos, Ajay Wadgaonkar and Andrew Gordon for their creativity and thoroughness in gathering, editing and organizing these projects. This journal would not exist without their hard work and vision. I also want to thank Associate Vice President Anne Hirshfield, PhD, for her guidance and mentorship of the Beaumont Society and her encouragement of the students working on this journal. We are also indebted to the Office of Student Opportunities which arranged many of these projects for the students, and the generous individual supporters in that office, specifically Dr. and Mrs. Gerald Lazarus, Dr. Christopher Barley, Dr. Keshav Narain, Dr. Charles Walkoff and Dr. Robert Rosenberg. In addition, none of this would have been possible without the active involvement of so many of our faculty in the research and other activities of our medical students. To all of you, a collective thank you.

I encourage you to take your time as you journey through these pages getting a glimpse of the variety of activities that are available to students at The George Washington University School of Medicine and Health Sciences. There is no doubt that this school has a unique geographic location which creates extraordinary opportunities for students to participate in ways that are much more difficult at other institutions. It is also true that, with those opportunities, comes a responsibility to discuss our discoveries in science, policy and health outreach in an open and critical way and to make our findings accessible to a larger audience. This journal, thanks to all those who contributed, is a great beginning. My congratulations to you all.

James L. Scott, MD
Dean, School of Medicine and Health Sciences,
Professor of Emergency Medicine

from the research office. . .

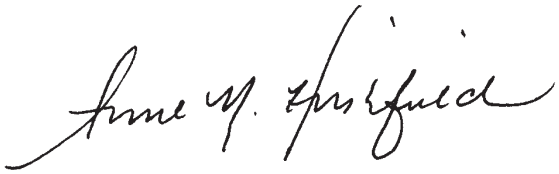


It is with great pleasure that I invite readers to explore the 2008 edition of *Fusion*, the second volume of a publication that was conceived and implemented by officers of The George Washington University's Beaumont Society. The William H. Beaumont Medical Research Honor Society was established at the GW School of Medicine in 1935 to promote student research. Although I have the honor of serving as this group's faculty advisor, the Society is entirely student run.

Fusion came into being because Beaumont Society officers wanted a vehicle for capturing and preserving the variety of experiences students had while carrying out research rotations and summer research projects, activities that many of our students undertake during their pre-clinical years. The officers wanted to communicate the excitement and value of these experiences to a wider audience. Last year's first edition was received with great enthusiasm and, as a result, members of the Society have decided that the publication should become an annual tradition.

This project is entirely student driven, and therefore a real testament to the students themselves who devoted a tremendous amount of time and effort to its production. Considering the demands of their medical education, this publication represents an outstanding achievement for those involved. In acknowledging all those who made this second volume of *Fusion* a reality, thanks go to the Office of Medical Center Communications and Marketing, who contributed their expertise in assuring the professional appearance of this publication. Acknowledgements and thanks are also due to the faculty research advisors who welcomed the student researchers into their programs and mentored their research activities and progress. Finally, we need to recognize contribution of the W.T. Gill Endowment, which provided summer stipends for most of the students whose work is described in this journal. Our gratitude for this invaluable resource cannot be overstated.

Many of the students whose work appears on these pages are among the best and most enthusiastic in their class. Some will undoubtedly go on to careers in research, joining the ranks of our many GW alumni who have made major contributions to collective knowledge about improving the health and well being of humankind. Others will not choose a research career, but will make their contributions in the clinical realm or in other forms of service. Whatever the future holds for these talented and dedicated students, their research experience will remain with them for the rest of their lives, informing their understanding of the nature and pursuit of knowledge.



Anne N. Hirshfield, PhD
Associate Vice President for Health Research

What Role Do Androgens Play in Bone Growth?

Adrian A. Woo, MSI
Principal Investigator:
Kristine M. Wiren, PhD
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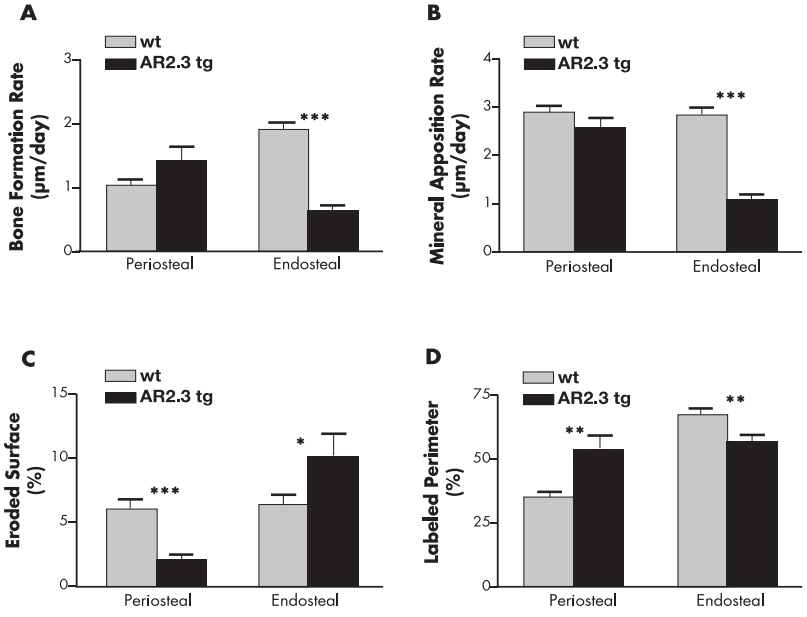
Osteoporosis causes deterioration of bone tissue, resulting in an increased fragility of bone and corresponding increased risk of pathologic bone fracture. Osteoporotic bone fracture, therefore, has become a serious public health dilemma and a leading cause of disability worldwide.[1] Studies suggest a link between osteoporosis and a hypogonadal state, potentially with reduced amounts of circulating androgens, in both men and women.[2,3] Therefore, androgens could play a role in the treatment of osteoporosis, yet the specific role of androgens in bone growth and maintenance remains unclear.

Hypogonadal elderly men appear to benefit from anabolic steroid treatment, developing increased lean body mass and gradual, yet progressive, increases in bone mineral density (BMD).[4] In a separate study, treating severely burned children with the androgen oxandrolone also increased BMD within three to six months.[5] However, studies of anabolic steroid abuse in developing teenagers suggest stunted growth as a result of premature closing of the epiphyseal growth plate in bone.[6,7] Thus more definitive studies are necessary to elucidate the role of androgens in bone growth and maintenance.

To examine specific androgen effects on bone, we genetically altered androgen receptors (ARs) otherwise normally present in F2 populations* of inbred mice. ARs in specific populations of osteoblasts were altered using different sections of the type I collagen promoter coupled to a rat AR gene sequence. A 3.6-kb portion of the type I collagen promoter directed AR expression in osteoblasts throughout the lineage, from bone marrow mesenchymal stem cells through to mature osteoblasts, while the 2.3-kb component targeted only the mature osteoblasts.[8] AR overexpression in male AR3.6-transgenic mice exhibited a complex phenotype, notably increased trabecular bone mass caused by reduced osteoclast activity. Also, cortical bone expansion was observed due to periosteal bone surface expansion, but without increased endosteal deposition. Femoral long bones of male AR3.6-transgenic mice demonstrated a more brittle phenotype, partially attributable to inhibition of bone growth on the endosteal surface.[9]

Overexpression of ARs in mature osteoblasts mimics the high level of endogenous AR expression documented at this stage of differentiation.[10] High-resolution micro-computed tomography showed no differences in total cross-sectional area. Cortical bone width and area were reduced with increased marrow cavity in male AR2.3-transgenic mice. These femurs also showed decreased biomechanical strength and quality in 4-point bending tests, as indicated by significant decreases in stiffness and maximum load, as well as more dramatic decreases in post-yield deflection (brittleness) and work-to-failure (data not shown). Dynamic histomorphometric analysis at eight weeks showed predominantly decreased endosteal labeling, with corresponding decreased periosteal and increased endosteal resorption (Figure 1C, D). Decreased bone formation and mineral apposition rates on the endosteal surface indicated that bone creation on this surface

FIGURE 1: RATES OF BONE FORMATION IN MALE AR2.3-TRANSGENIC MICE BY DYNAMIC HISTOMORPHOMETRY. After femurs were sectioned and polished to 40 μm, sections were subjected to fluorescent microscopy and labels were measured. A. Bone formation rate. B. Mineral apposition rate. C. Percentage of eroded surface. D. Percentage of labeled perimeter. Labels were measured at both periosteal and endosteal surfaces and show a significant lack of endosteal labeling in male AR2.3-transgenic mice. Data is shown as mean ± SEM. n = 8-20. *, $p < 0.05$ **, $p < 0.01$; ***, $p < 0.001$ (vs. gender-appropriate wild-type controls).



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BONE GROWTH *Continued from p. 6*

was significantly inhibited, while the periosteal surface was not significantly affected in this way (Figure 1A, B). These results indicate that effects of androgens differ at these surfaces, and are inhibitors of growth rather than anabolic in mature bone. This study, therefore, suggests androgen use in treatment of osteoporosis in fact could be deleterious rather than beneficial.

REFERENCES:

1. **CUMMINGS SR, MELTON LJ.** Epidemiology and outcomes of osteoporotic fractures. *Lancet,* 2002; 359:1761–67.
2. **DANIELL HW.** Osteoporosis after orchiectomy for prostate cancer. *J Urol,* 1997; 157:439–44.
3. **KENNY AM, RAISZ LG.** Mechanisms of bone remodeling: implications for clinical practice. *J Reprod Med,* 2002; 47:63–70.
4. **WANG C, CUNNINGHAM G, ET AL.** Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. *J Clin Endocrinol Metab,* 2004; 89:2085–98.
5. **MURPHY KD, THOMAS S, ET AL.** Effects of long-term oxandrolone administration in severely burned children. *Surgery,* 2004; 136:219–24.
6. **FAIGENBAUM AD, ZAICHKOWSKY LD, ET AL.** Anabolic steroid use by male and female middle school students. *Pediatrics,* 1998; 101:E6.
7. **JOHNSON MD.** Anabolic steroid use in adolescent athletes. *Pediatr Clin North Am,* 1990; 37:1111–23.
8. **KALAJZIC Z, LIU P, ET AL.** Directing the expression of a green fluorescent protein transgene in differentiated osteoblasts: comparison between rat type I collagen and rat osteocalcin promoters. *Bone,* 2002; 31:654–60.
9. **WIREN KM, ZHANG XW, ET AL.** Targeted overexpression of androgen receptor in osteoblasts: unexpected complex bone phenotype in growing animals. *Endocrinology,* 2004; 145:3507–22.
10. **WIREN KM, CHAPMAN EVANS A, ET AL.** Osteoblast differentiation influences androgen and estrogen receptor-alpha and -beta expression. *J Endocrinol,* 2002; 175:683–94.

*** F2 GENERATION MICE** were obtained by the mating of F1 generation mice showing the desired trait. The gene was inserted into the embryos of mice from a line of inbred mice from Jackson Labs.

Antiproliferative Properties of Thymosin Fraction 5 in HL-60 Human Promyelocytic Leukemia Cells

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For decades, the thymus gland was thought to be a functionless, vestigial organ in mammals. Through the pioneering work of Jacques Miller in the early 1960s, the thymus became appreciated for its vital roles in maturing T-cells and establishing the immune system. Shortly thereafter, Goldstein and White described the isolation of a family of hormone-like peptides from the thymus gland collectively called thymosins.[1] Since then, these small, biologically active peptides have been shown to possess important roles in diverse physiological processes ranging from immunomodulation to wound healing.

Interestingly, a number of these thymic peptides have anti-proliferative effects on various human and murine tumor cell lines. This finding, along with age-related correlations of thymic involution and an increased frequency of cancer, has led some to hypothesize the thymus's role in a possible immune surveillance mechanism of cancer suppression. Most recently, this idea was explored in HL-60 human promyelocytic leukemia cells.

HL-60 cells were initially derived from a patient with acute promyelocytic leukemia, a disease in which myeloid precursors undergo rapid proliferation without differentiating into mature cellular elements. This uncontrolled growth results in an accumulation of these non-functioning cells in bone marrow and blood, displacing their mature, active forms, ultimately leading to systemic complications. Previous work demonstrated the ability of thymosin fraction 5 (TF5), a partially purified protein extract from bovine thymus, to substantially suppress the proliferation of HL-60 cells in both concentration and time-dependent manners by what appears to be a distinct, non-lethal cytostatic mechanism.[3] Present efforts have been aimed at further purifying TF5 in order to isolate the effector peptide(s) responsible for this suppressive activity.

Purification by fast protein liquid chromatography (FPLC) followed by further purification by reverse phase high-performance liquid

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LEUKEMIA CELLS *Continued from p. 7*

chromatography (RP-HPLC) has yielded a subfraction of TF5 which retains the anti-proliferative activity of the parent extract. The near-term goals of this work are to sequence and chemically characterize this peptide as well as to see if its biological activity can be reproduced with a synthetic version of the molecule. Interestingly, the latest flow cytometry data obtained indicates that a significant number of the suppressed leukemic cells do indeed undergo apoptosis. Ultimately, elucidating the mechanism by which this peptide exerts its action will be helpful in determining whether it has real therapeutic potential.

REFERENCES:

1. **GOLDSTEIN AL, SLATER FD, WHITE A.** Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (thymosin). *Proc Natl Acad Sci,* U.S., 1966; 56:1010–17.
2. **GOLDSTEIN AL, BADAMCHIAN M.** Thymosins: chemistry and biological properties in health and disease. *Expert Opin Biol Ther,* 2004; 4:559–73.
3. **SPANGELO BL, POMPILIUS M, FARRIMOND DD, STEVENS N, NIEVA R, SHROFF S, BADAMCHIAN M, JOHNSON CR, JARVIS WD.** Presence of a peptide component of thymosin fraction-5 manifesting discrete cytostatic properties in HL-60 human promyelocytic leukemia cells. *Int Immunopharmacol*, 2005; 5:1317–29.
4. **SPANGELO BL, ROACH JD, HADI F, DAMAVANDY AA, PLIESKATT J, BADAMCHIAN M.** Thymosin fraction-5 possesses antiproliferative properties in HL-60 human promyelocytic leukemia cells: characterization of an active peptide. *Ann N Y Acad Sci*, 2007; 1112:305–16.

ER-Targeted Bcl-2 Mitigates Ethanol Toxicity More Significantly than Wildtype or Mitochondria-Targeted Bcl-2

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Apoptosis is the scientific term for the genetically programmed process of cell death.[1] The Bcl-2 family of proteins, which includes both pro- and anti-apoptotic members, is responsible for the regulation of apoptosis through control of caspase activity.[1] Bcl-2 protects cells from apoptosis by inhibiting Bax and Bak (both pro-apoptotic members) from releasing cytochrome c, an important co-factor for caspase activation, from mitochondria.[2] Previously, Bcl-2 was thought to be subcellularly localized only to mitochondria, however, recent work suggests that Bcl-2 is distributed in the rough endoplasmic reticulum (ER) as well.[3] Prior *in vivo* studies have shown that overexpression of wildtype Bcl-2 protects against ethanol toxicity, a known inducer of apoptosis.[4,5] Currently, it remains unclear whether ethanol toxicity protection is mediated through mitochondria or inside the ER.

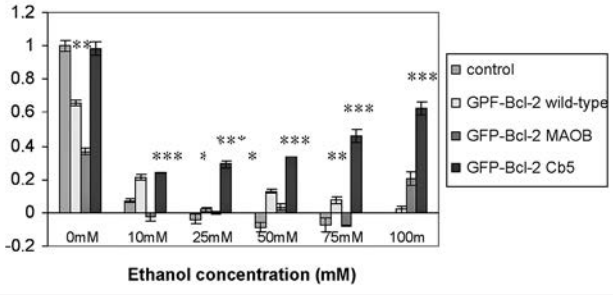

FIGURE 1: ER-targeted Bcl-2 rescues CHO695 from 24 hours of ethanol incubation more significantly than mitochondria or wildtype-targeted Bcl-2. Student's t-test was used to assess data significance. n=32; (*), (**) and (***) indicate significant results with a $p<0.05$, $p<0.01$ and $p<0.001$, respectively. Data are shown as means ± standard error.

Our team investigated whether the already known *in vivo* protection from ethanol is mediated through ER-Bcl-2 or mitochondria — Bcl-2. The team used three different fusion constructs encoding for green fluorescent protein (GFP): Bcl-2 fusion proteins were used to examine the Bcl-2 subcellular localization and determination of differential rescue from ethanol. A line of Chinese hamster ovary cells (CHO695) was transiently transfected with GFP: Bcl-2 wildtype plasmid, GFP: Bcl-2 MAOB (mitochondria target) and GFP: Bcl-2 Cb5 (ER target). Fluorescence microscopy was employed to verify the localization of the GFP: Bcl-2

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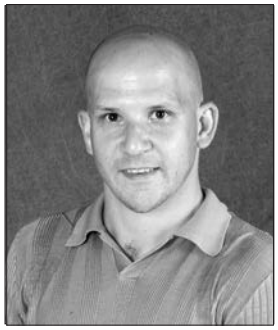
fusion proteins. Apoptosis was examined in response to ethanol in the presence of wildtype Bcl-2 or organelle-targeted Bcl-2 using the MTT apoptosis assay. We tested whether the subcellular localizations of Bcl-2 to the ER and the mitochondria would confer different levels of rescue against ethanol toxicity. GFP: Bcl-2 fusion proteins were appropriately localized, indicating successful organelle-targeting. Our *in vitro* model system confirmed the protective role of Bcl-2 wildtype against ethanol toxicity previously shown only *in vivo*.[4,5] Moreover, we found that the ER-targeted Bcl-2 significantly rescued CHO695 cells from ethanol toxicity, even at normally toxic concentrations, whereas wildtype and mitochondria-targeted Bcl-2 offered more limited protection (Figure 1). The same results were confirmed in another line of Chinese hamster ovary cells (CHO-Pro5) as well.

Therefore, our findings indicate that Bcl-2's known amelioration of ethanol toxicity is likely mediated through pathways in the ER. Such results could be utilized to elucidate a possibly new apoptotic pathway in which the ER is upstream of the mitochondria and in which Bcl-2 embedded in the ER membrane inhibits Bax translocation to the mitochondria. Research attempting to understand cellular changes induced by ethanol toxicity, and aiming to exploit a cell's own defense mechanism, could ultimately lead to treatments preventing alcohol toxicity.

REFERENCES:

1. **STRASSER A, O'CONNOR L, DIXIT VM.** Apoptosis signaling. *Annu Rev Biochem*, 2000; 69:217–45.
2. **ANTONSSON BS, MONTESSUIT S, LAUPER R, MARTINOU JC.** Bax oligomerization is required for channel-forming activity in liposomes and to trigger cytochrome c release from mitochondria. *Biochem J*, 2002; 345:271–78.
3. **ZONG WX, LI C, HATZIVASSILIOU G, LINDSTEN T, YU QC, YUAN J, THOMPSON CB.** Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis. *J Cell Biol,* 2003; 162:59–69.
4. **HEATON MB, MOORE DB, PAIVA M, GIBBS T, BERNARD O.** Bcl-2 overexpression protects the neonatal cerebellum from ethanol neurotoxicity. *Brain Res*, 1999; 817:13–18.
5. **WANG NS, UNKILA MT, REINEKS EZ, DISTELHORST CW.** Transient expression of wildtype or mitochondrially targeted Bcl-2 induces apoptosis, whereas transient expression of endoplasmic reticulum-targeted Bcl-2 is protective against Bax-induced cell death. *J Biol Chem,* 2001; 276:44117–28.

A New Focus in Breast Cancer Bone Metastasis: NF-κB Gene Suppression



Andrew H. Gordon, PhD, MSII
Advisor: J. Edward Puzas, PhD
Co-Collaborators: Regis J. O'Keefe, MD, PhD; Edward M. Schwarz, PhD; Randy N. Rosier, MD, PhD, Orthopaedic Research Laboratories and Department of Biomedical Engineering, University of Rochester Medical Center, Rochester, NY

Breast cancer is the most common malignancy seen in women. As many as one-third of women with early-stage breast cancer will die, with most developing complications arising from bone metastases, including severe pain, spinal cord compression and pathologic fracture.[1,2] In conjunction with the Center for Musculoskeletal Research and Department of Biomedical Engineering at the University of Rochester Medical Center, a novel way of suppressing this tumor burden in bone was discovered.

This required a tremendous appreciation for bone biology and how certain breast cancers prefer particular metastatic sites. In fact, some breast cancers uniquely display osteotropism, a significant ability to proliferate in bone.[1] These breast cancers with a preference for bone and the potential host metastatic site must each possess intrinsic biological properties favoring mutual attraction between one another.[3] As early as 1889, Paget put forth the "seed and soil" hypothesis that embodies this phenomenon, referring to the bone microenvironment as a fertile, rich "soil" in which breast cancer cells may grow as "seeds."[4]

A clinical hallmark of disrupted bone remodeling in aggressive breast cancers is osteoclast-mediated bone destruction.[1] Osteoclasts are bone-resorbing cells native to bone which are activated in response to cytokines produced by breast cancer cells.[5] Production of these cytokines is tightly controlled by the nuclear factor-κB (NF-κB) transcription factor.[6]

Targeting the NF-κB pathway holds significant promise in treating various cancers and

BREAST CANCER *Continued on p. 10*

diseases associated with abnormal bone resorption.[7,8] Since patients with bone cancers possess much lower relative survival rates than patients with soft tissue cancers,[9] verifying a target such as NF-κB, governing production of an array of

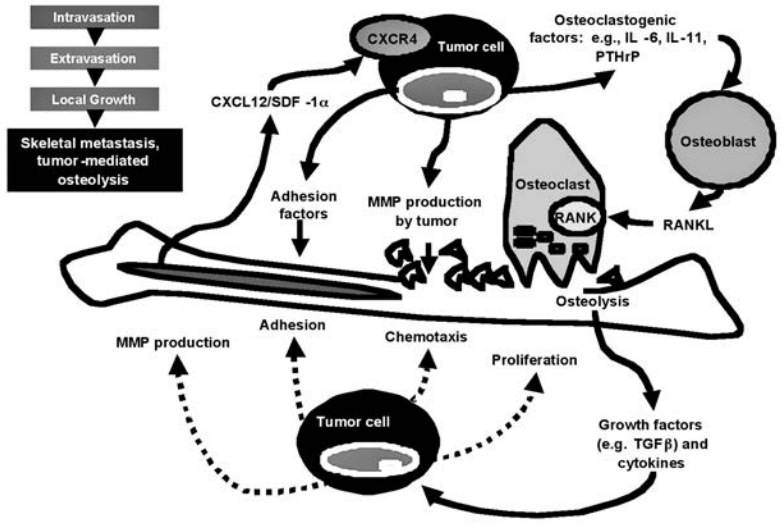


FIGURE 1: THE "VICIOUS CYCLE" OF CANCER-MEDIATED BONE DESTRUCTION. Chemical interactions between SDF-1α/CXCL12 and CXCR4 influence breast cancer cells to home in on bone, adhere to the bone linings and seed in the bone microenvironment. The tumor cells then secrete cytokines (i.e., PTHrP, TNFα, IL-1, IL-6, and IL-11), many of which are controlled by NF-κB signaling. This stimulates bone resorption by osteoclasts via osteoblast production of RANKL. Matrix metalloproteinase (MMP) production by tumor cells, also controlled by NF-κB signaling, also directly degrades bone. This bone destruction in turn releases from the bone matrix growth factors capable of stimulating tumor cells to destroy more bone, creating sustained "vicious" cycles of tumor-mediated bone resorption.

biological factors relevant to metastatic progression in bone, can be of great value.

Targeting the NF-κB pathway holds significant promise in treating various cancers and diseases associated with abnormal bone resorption.[7,8]

The bone-seeking MDA-MB-231 breast cancer cell line was genetically altered to suppress NF-κB-mediated gene expression. In its inactive state, NF-κB is bound to an IκB protein within the cytoplasm of cells. Phosphorylation of IκB frees NF-κB to move to the nucleus of cells to perform NF-κB-mediated gene transcription.[6] A single-point mutation of IκB (replacing one serine amino acid for an alanine) was stably infected in these breast cancer cells to prevent this phosphorylation and associated gene expression.[10] Blocking NF-κB signaling in MDA-MB-231 cells decreased *in vitro* cell growth, expression of the proinflammatory, bone-resorbing cytokine interleukin-6 (IL-6), and *in vitro* bone resorption by tumor/osteoclast co-cultures while reciprocally up-regulating production of the apoptotic enzyme caspase-3. Suppression of NF-κB transcription in these breast cancer cells also suppressed *in vivo* tumor-mediated bone destruction after intratibial injection of tumor cells in female immunosuppressed mice. Immunohistochemistry showed the resorptive lesions formed in bone by MDA-MB-231 cells express both the p65 subunit of NF-κB and IL-6 at the bone-tumor interface.[10] Taken together, these findings demonstrated an essential role for NF-κB signaling in breast cancer-mediated bone destruction. Strong rationale now exists to further investigate genetic and pharmacologic NF-κB inhibitors as standard or adjuvant therapies in these regards.

REFERENCES:

1. **GUISE TA, MUNDY GR.** Cancer and bone. *Endocrine Rev,* 1998; 19:18–54.
2. **DOMCHEK SM, YOUNGER J, FINKELSTEIN DM, SEIDEN MV.** Predictors of skeletal complications in patients with metastatic breast carcinoma. *Cancer,* 2000; 89:363–8.
3. **HART IR, FIDLER IJ.** Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. *Cancer Res,* 1980; 40:2281–7.
4. **PAGET S.** The distribution of secondary growths in cancer of the breast. *The Lancet,* 1889; 1:571-3.
5. **PEDERSON L, WINDING B, FOGED NT, SPELSBERG TC, OURSLER MJ.** Identification of breast cancer cell line-derived paracrine factors that stimulate osteoclast activity. *Cancer Res,* 1999; 59:5849–55.
6. **BARNES PJ, KARIN M.** Nuclear factor-κ B: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med,* 1997; 336:1066–71.
7. **YAMAMOTO Y, GAYNOR RB.** Therapeutic potential of inhibition of the NF-κ B pathway in the treatment of inflammation and cancer. *J Clin Invest,* 2001; 107:135–42.
8. **IOTSOVA V, CAAMANO J, LOY J, YANG Y, LEWIN A, BRAVO R.** Osteopetrosis in mice lacking NF-κ B1 and NF-κ B2. *Nat Med,* 1997; 3:1285–9.
9. **STORM HH.** Survival of adult patients with cancer of soft tissues or bone in Europe. *Eur J Cancer,* 1998; 34:2212–7.
10. **GORDON AH, O'KEEFE RJ, SCHWARZ EM, ROSIER RN, PUZAS JE.** Nuclear Factor-κ B–Dependent Mechanisms in Breast Cancer Cells Regulate Tumor Burden and Osteolysis in Bone. *Cancer Res,* 2005; 65:3209–3217.

Certain Piperazines Mimic Stimulant or Hallucinogenic Effects of Ecstasy

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Users of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") report the drug elicits a complex array of subjective effects, some stimulant-like and others hallucinogen-like.[1] Usage of MDMA has increased worldwide, particularly among young adults.[2] While regulatory control of MDMA exists in the United States, federal law enforcement agents continue to encounter and confiscate MDMA across the country.[3] In addition to widespread use of MDMA itself, certain "ecstasy" tablets also contain N-substituted piperazines which mimic the effects of MDMA.[4]

This study investigated how certain N-substituted piperazines, in comparison with MDMA enantiomers, may affect classical stimulant and hallucinogenic behaviors in mice trained to discriminate either S(+)-MDMA or R(-)-MDMA from saline. Previous studies suggest that the effects of S(+)-MDMA are more stimulant-like than those of R(-)-MDMA, while the actions of R(-)-MDMA are more hallucinogen-like than those of the S(+)- enantiomer.[5]

In a murine model, various N-substituted piperazines were administered and evaluated for head twitch response (a hallucinogen-like drug effect), increased locomotor activity (a stimulant-like drug effect) and MDMA-like discriminative stimulus effects in mice (analogous to subjective drug effects in humans). Various doses of l-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl) piperazine (TFMPP), 1-(3-methoxybenzyl) piperazine (m-MeO-BZP) or meta-chlorophenyl piperazine (*m*-CPP) were administered to mice to determine the effects on these behavioral endpoints.

The head twitch response is sensitive to serotonin 5-HT^2 agonist activity in the rodent and was therefore used to evaluate the hallucinogen-like effects of BZP, MeO-BZP, TFMPP, and *m*-CPP.[6,7] All N-substituted piperazines were tested for locomotor stimulant effects in a modified open field apparatus, and mice trained to discriminate S(+)-MDMA or R(-)-MDMA from saline were tested for stimulus generalization to all of the N-substituted piperazines.

The results of the current study indicated that BZP, TFMPP, and *m*-CPP dose dependently could fully substitute for S(+)-MDMA, but could not elicit R(-)-MDMA-like stimulus effects. In contrast, m-MeO-BZP dose dependently could partially substitute for both enantiomers. BZP profoundly stimulated locomotor activity, but did not elicit a significant head twitch response, while TFMPP elicited a dose dependent head twitch response but only suppressed locomotor behavior. Neither m-MeO-BZP nor m-CPP elicited head twitch behavior, as both compounds decreased locomotor activity dose dependently.

Of all the N-substituted piperazines studied, only BZP exhibited a clear stimulant-like pattern of behavioral effects, while only TFMPP exhibited hallucinogen-like effects in the head twitch assay. Based on the present findings, m-MeO-BZP and *m*-CPP cannot be described as stimulant-like or hallucinogen-like in the mouse. Still, various N-substituted piperazines demonstrate differing ecstasy-like drug effects in a murine model, and warrant further investigation and monitoring with regard to their addiction and abuse potential.

FIGURE 1: SUMMARY OF EXPERIMENTAL RESULTS.

Drug	Head Twitch	Locomotor	S(+)-MDMA Discrimination	R(-)-MDMA Discrimination
BZP	No	Increase	Full	None
MeO-BZP	No	Decrease	Partial	Partial
TFMPP	Yes	Decrease	Full	None
m-CPP	No	Decrease	Full	None

ECSTASY

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ECSTASY *Continued from p. 11*

REFERENCES:

1. **FANTEGROSSI WE, GODLEWSKI T, KARABENICK RL, STEPHENS JM, ULLRICH T, RICE KC, ET AL.** Pharmacological characterization of the effects of 3,4-methylenedioxymethamphetamine ("ecstasy") and its enantiomers on lethality, core temperature, and locomotor activity in singly housed and crowded mice. *Psychopharmacology,* 2003; 166(3):202–11.
2. **FANTEGROSSI WE, KIESSEL CL, DE LA GARZA II R, WOODS JH.** Serotonin synthesis inhibition reveals distinct mechanisms of action for MDMA and its enantiomers in the mouse. *Psychopharmacology,* 2005b; 181:529–36.
3. **LANDRY MJ.** MDMA: a review of epidemiologic data. *Journal of Psychoactive Drugs,* 2002; 34:163–169.
4. **YAROSH, H., KATZ, E., COOP, A., FANTEGROSSI, W.** MDMA-like behavioral effects of N-substituted piperazines in the mouse. *Pharmacol Biochem Behav*, 2007; 88:18–27.
5. **STEELE TD, NICHOLS DE, YIM GKW.** Stereochemical effects of 3,4- methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [3H]monoamines into synaptosomes from different regions of rat brain. *Biochem Pharmacol,* 1987; 36:2297–303.
6. **ORTMANN R, BISCOFF S, RADEKE E, BUECHE O, DELINI-STULA A.** Correlation between different measures of antiserotonin activity of drugs. Naunyn Schmiedeberg's, *Arch Pharmacol,* 1982; 321:265–270.
7. **PEROUTKA SJ, LEBOVITZ RM, SNYDER SH.** Two distinct central serotonin receptors with different physiological functions. *Science,* 1981; 212:827–829.

Anatomical Bases for Auditory Projections to Suprasylvian Visual Areas in the Cat Cerebral Cortex

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A fundamental organizational feature of the mammalian brain is the arrangement of sensory representations in the cerebral cortex. The cortical representation of vision is localized in the occipital lobe, auditory in the temporal lobe and somatosensory in the parietal lobe. Studies of a wide variety of mammals indicate a primary thalamic-recipient area for each sensory modality, designated as the primary sensory cortex. Most textbook depictions of the sensory representations show a large gap between locations of the primary sensory representations. However, these areas are not inactive, but rather they vigorously process sensory information. In fact, the sensory cortical organization of higher mammals is one in which primary processing areas are typically surrounded by secondary or higher-level processing areas.

In the cat brain, the lateral suprasylvian sulcus (LSS) contains representations of the visual modality thought to be exclusively populated by visual-specific neurons.[1,2] The visual LSS is laterally bordered by the dorsal zone of auditory cortex; recent electrophysiological experiments document the presence of auditory/visual bimodal cells in what otherwise appears to be the visual LSS.[3,4] While the sensory function of the LSS cells has been expanded with this finding, the transition/multisensory zones between auditory and visual regions have not been defined. It is not known whether

FIGURE 1. ORTHOGRADE PROJECTIONS FROM AI TO LSS. Injections of tracer result in labeled axons and terminals in the lateral bank of the LSS. Coronal sections are arranged from the left, anterior to posterior. Grey regions indicate location and extent of injection; each black dot represents a single bouton or axon terminal. Inset on left indicates the cortical level from which each coronal section originates. Scale bar = 1mm.

AUDITORY PROJECTIONS *Continued on p. 13*

AUDITORY PROJECTIONS

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there is a distinct border marking the edge between the two different representations, or if there is a gradual transition yielding a substantial area in which representations of both modalities overlap. In the latter case, inputs from different modalities that overlap within a given area can converge onto individual neurons, thereby inducing multisensory properties.[5]

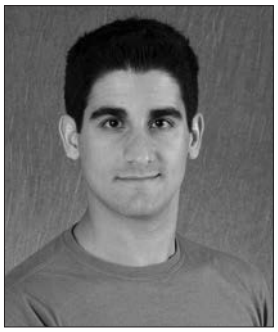
This problem was addressed in our study by labeling auditory projections to the LSS of the cat using neuroanatomical tract tracing methods. Adult cats (n=17) were anesthetized and, using sterile surgical techniques, biotinylated dextran amine (BDA) was injected into one of the following auditory cortical areas: anterior auditory field (AAF), primary auditory cortex (AI) or posterior auditory field (PAF). Standard cytochemical procedures were used to visualize the transported tracer and a computer-based digitizing microscope was used to plot the location of labeled axon terminals. In each case, labeled boutons were identified in the LSS region. Labeled terminal boutons were most concentrated at the outer lip of the LSS and the label became progressively reduced with depth along the lateral bank of LSS toward the fundus, as shown in Figure 1, for AI auditory projections to the LSS (each black dot=1 labeled bouton). This steady decrease in auditory projections down the bank of the LSS, as opposed to an abrupt demarcation, indicates a gradual transition from auditory to visual sensory modality.

Although the LSS has widely been regarded as a visual area of the cat cortex, these results show that multiple auditory cortical projections target the region in a gradated manner. Auditory projections are quite dense at the external lip of the sulcus and are gradually reduced within the depth of the bank of the LSS. Ultimately, the broad distribution of auditory input suggests that substantial portions of the visual LSS region may be a site of multisensory (visual and auditory) convergence. These overlapped projections may provide the anatomical basis for multisensory properties of neurons in higher mammals.

REFERENCES:

1. **MARSHALL WH, TALBOT SA, ADES HW.** Cortical responses of the anesthetized cat to gross photic and electrical afferent stimulation. *J Neurophysiol*, 1943; 6:1–15.
2. **PALMER LA, ROSENQUIST AC, TUSA, RJ.** The retinotopic organization of lateral suprasylvian visual areas in the cat. *J. Comp. Neurol,* 1978; 177:237–56.
3. **YAKA R, NOTKIN N, YINON U, WOLLBERG Z.** Visual, Auditory and Bimodal Activity in the Banks of the Lateral Suprasylvian Sulcus in the Cat. *Neurosci and Behavioral Phys,* 2002; 32:103–08.
4. **ALLMAN B, MEREDITH MA.** Multisensory Processing in 'Unimodal'Neurons: Cross modal Subthreshold Auditory Effects in Cat Extrastriate Visual Cortex. *J Neuorphysiol,* 2007; 98:545–49.
5. **MEREDITH MA.** Cortico-cortical Connectivity and the Architecture of Cross-modal Circuits. In: Spence C, Calvert G, Stein B (eds.) *Handbook of Multisensory Processes*, 2004; MIT Press:343–55.

A Role for Chemokine Gene Variants in Muscle Strength?



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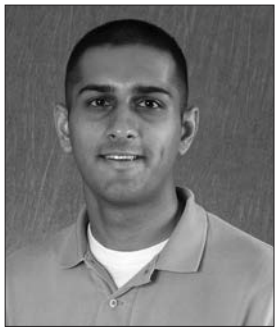

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The overarching goal of the Bone Health Program at Children's National Medical Center, in Washington, DC, is to contribute answers to the questions, "What makes bone strong?" and "can we make bones stronger?" More specifically, the program seeks to identify genetic variations that will help individuals better understand their risks for bone health and disease, while simultaneously providing them with recommendations for healthy behaviors that can reduce their risks of serious disease. To help achieve this goal, the Bone Health team partnered with Dr. Eric Hoffman, director of the Research Center for Genetic Medicine, to develop a program that explores answers to these questions in the clinic, operating room and laboratory.

Our Gill Fellowship summer projects were devoted to the study of chemokines. Chemokines are a large group of proteins that act as lures to attract white blood cells and are involved in various disease processes, including acute and chronic inflammation, infectious diseases and cancer. Although primarily associated with disease, the inflammatory response is also associated with muscle repair, regeneration and growth.[1] Muscle strength, as well as exercise, contributes to bone strength. The CC family of chemokines is the largest of its kind, and one of the most thoroughly characterized chemokines is CCL2.[1] CCL2 and one of its major receptors, CCR2, are encoded by genes CCL2 and CCR2, respectively. Given the widespread effects of CCL2 and CCR2, our goal was to investigate whether variants of the genes CCL2 and CCR2 influence skeletal muscle, bone and subcutaneous fat volume at baseline and after resistance training in healthy young adults.

Blood DNA samples and MRI images were obtained from the Functional Polymorphisms Associated with Muscle Size and Strength study of more than 1,200 college students enrolled in a 12-week resistance training program. Investigating the influence of genetic polymorphisms on bone geometry, fat volume, and skeletal muscle size and strength, variants in CCL2 and CCR2 were found to correlate with specific skeletal muscle and subcutaneous fat volume phenotypes. Although the study cohort had not participated in resistance training for one year, we found that individuals with the rare alleles consistently had the greatest baseline muscle strength and lowest subcutaneous fat volume. Strikingly, while the presence of the rare allele predicted baseline values of muscle strength and fat volume, it did not predict significantly higher values of muscle hypertrophy or gained strength after resistance training. This suggests that the ability of muscle to hypertrophy and gain strength are controlled by genes other than CCL2 and CCR2.[1,2]

… [I]ndividuals with the rare alleles consistently had the greatest baseline muscle strength and lowest subcutaneous fat volume.

In addition to providing a research experience, our fellowships also presented the opportunity to observe patient care in the orthopaedic outpatient clinic and operating room. More over, we traveled to the International Conference on Children's Bone Health in Montreal, Canada, to gain greater exposure to international research efforts in bone health.

REFERENCES:

1. **TIDBALL J.** Inflammatory processes in muscle injury and repair. *American Journal of Physiology — Regulatory Integrative and Comparative Physiology*, 2005; 288:R345–53.
2. **YU X, DLUZ S, GRAVES D, ZHANG L, ANTONIADES H, HOLLANDER W, PRUSTY S, VALENTE A, SCHWARTZ C, SONENSHEIN G.** Elevated expression of monocyte chemoattractant protein 1 by vascular smooth muscle cells in hypercholesterolemic primates. *Proceedings of the National Academy of Sciences of the United States of America,* 1992; 89:6953–57.

Cloning and Expression of Human Adenovirus Precursor Protein VI and Mature Protein VI

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There are 47 serotypes of the human adenovirus that are responsible for a group of respiratory diseases, including, but not limited to, 30 percent of all colds, pneumonia and bronchitis, in addition to gastroenteritis, viral pink eye and cystitis.[1] Epidemiological studies indicate an adenoviral agent as the cause of 5–10 percent of juvenile pneumonias and respiratory infections of children in North America.[2] Respiratory infection with adenoviruses is also regularly responsible for a number of infant deaths and considerable morbidity among some adult populations. Serological prevalence studies suggest that the average individual undergoes a minimum of two or three clinical episodes of adenoviral infection during childhood.[2] In addition, human adenovirus is one of the leading opportunistic infections that are a major cause of death in patients with Acquired Immune Deficiency Syndrome (AIDS) and deficient immune systems.[3]

The human adenovirus proteinase (AVP) is required for the development of infectivity.[4] AVP is synthesized by the adenovirus and packaged into nascent virus particles. When activated, AVP cleaves virion precursor proteins, thereby rendering the virus particle infectious. AVP requires two viral cofactors for maximal activity — adenovirus DNA and an 11-amino-acid peptide, known as pVIc, derived from the C-terminus of precursor protein VI (pVI). Protein VI also functions as a precursor "scaffolding" protein that plays a critical role in the assembly and maturation of the virus particle.[5]

The synthesis and investigation of the precursor and mature form of protein VI is essential because of its critical role in producing the pVIc cofactor required for proteinase activation and subsequent viral infection. To characterize the interactions of AVP and DNA that render the virus particle infectious, the genes for pVI and VI must be cloned, expressed in bacterial hosts and the resultant recombinant proteins purified. There is no evidence that pVI or VI has been successfully cloned or expressed prior to this experiment.

This experiment established a novel, stepwise protocol for cloning pVI and VI genes and expressing the proteins in bacterial hosts. The genes for pVI and VI were successfully amplified from genomic adenovirus DNA, digested with restriction enzymes, ligated into prepared pET expression plasmids and transformed into bacterial hosts. PCR of the purified plasmids verified successful cloning of the correct gene sequences. Proteins pVI and VI were then expressed in BL21-codon plus(DE3)-RIPL cells grown in ZYM-5052 auto-inducing medium. A 15 percent SDS-PAGE and an assay using AVP and Rhodamine fluorogenic substrate confirmed successful protein pVI and VI expression.

A supply of purified recombinant proteins pVI and VI was produced, purified and stored for future research. The end products of this investigation will be significant in developing drug targets for anti-viral agents and providing information for the study of other viruses with congruent activation mechanisms.

The end products of this investigation will be significant in developing drug targets for anti-viral agents … .

REFERENCES:

1. **BANIECKI M, MCGRATH W, MCWHIRTER M, ET AL.** Interaction of the human adenovirus proteinase with its 11-amino acid cofactor pVIc. *Biochemistry*, 2001; 40:12349–56.
2. **SCOTT-TAYLOR T, HAMMOND G.** Conserved sequences of the adenovirus genome for detection of all human adenovirus types by hybridization. *J. Clin. Microbiol*, 1992; 30:1703–10.
3. **MANGEL W, MCGRATH W, TOLEDO D, ET AL.** Viral DNA and a viral peptide can act as cofactors of adenovirus virion proteinase activity. *Nature*, 1993; 361:274–75.
4. **MCGRATH W, ABOLA A, TOLEDO D, ET AL.** Characterization of human adenovirus proteinase activity in disrupted virus particles. *Virology*, 1996; 217:131–38
5. **MATTHEWS D, RUSSELL W.** Adenovirus protein-protein interactions: molecular parameters governing the binding of protein VI to hexon and the activation of the adenovirus 23 K protease. *J. Gen Virol*, 1995; 76:1959–69.

The Role of Retinoic Acid in Neuronal Phenotype Determination

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An important element of the developing central nervous system is the acquisition of specific neuronal phenotypes. Retinoic acid plays a fundamental role in CNS development and is essential for normal growth of the embryo. The effect of retinoic acid on neurotransmitter phenotype determination can be analyzed by examining the expression patterns of specific molecular markers.

The expression of a gamma-aminobutyric acid (GABA)ergic or glutamatergic neuron can be examined in a model vertebrate organism such as Xenopus laevis, the African clawed frog. GABAergic neurons release GABA, the most abundant inhibitory neurotransmitter in the CNS, and glutamatergic neurons release glutamate, the most abundant excitatory neurotransmitter in the CNS. In order to visualize the expression patterns, whole-mount *in situ* hybridization was performed to examine the spatial expression of specific molecular markers during various stages of embryo development.[1,2] The xGAT1 and xVGlut1 RNA probes allowed visualization of the expression patterns of GABAergic and glutamatergic neurons, respectively.

It is believed that retinoic acid plays a fundamental role in the acquisition and patterning of GABAergic and glutamatergic phenotypes. Retinoic acid comes from the family of retinoids and has been correlated with causing birth defects in newborns, both when deficient and when present in excess.

Normal expression of xGAT1 is first observed at late neurula stages in the anterior portion of the developing spinal cord.[3] By tailbud stages, bilateral xGAT1 expression is apparent in all regions of the CNS, including distinct regions of the forebrain, midbrain, hindbrain and olfactory placodes.[3]

Normal expression of xVGlut1 is first detected at late neurula to early tailbud stage embryos with prominent expression in the developing trigeminal nerve and in the developing spinal cord.[4] By later tailbud stages, xVGlut1 mRNA is observed in the pineal gland, the olfactory placodes and more posteriorly in the trigeminal and facial cranial nerves.[4]

It is believed that retinoic acid plays a fundamental role in the acquisition and patterning of GABAergic and glutamatergic phenotypes. Retinoic acid comes from the family of retinoids and has been correlated with causing birth defects in newborns, both when deficient and when present in excess. These include abnormalities in facial bone structure, in the ears and eyes, and in brain mass. Retinoic acid acts as a signaling molecule regulating critical processes during early development, including axial patterning, cellular differentiation, tissue induction, proliferation and apoptosis.[5] The natural concentration of retinoic acid in the body is crucially important and is constantly regulated.

In Xenopus development, retinoic acid becomes important at the beginning of gastrulation (stage 10 of development two). This stage marks the point at which segregation of body layers begins to take place. It is known that excess concentrations of retinoic acid in the environment affect the formation of the neural tube. More specifically, the effect appears to be a truncated anterior of the CNS.

It is hypothesized that the expression patterns of the neurotransmitter markers will be altered by the presence of excess retinoic acid. To test this hypothesis, Xenopus embryos were exposed to various solutions of retinoic acid at different developmental stages.[2] *In situ* hybridizations were conducted, with xGAT1 and xVGlut1 RNA probes. Whole mount examination revealed obvious malformation in the embryo. The effect on expression patterns of the molecular markers needs to be further analyzed via histological sections. More specific findings regarding the role of retinoic acid and its affects on expression patterns of GABAergic or glutamatergic neurons will help in understanding the acquisition of a specific neuronal fate. Furthermore, it will help in understanding the development of congenital birth defects in humans.

REFERENCES:

1. SIVE, HL, GRAINGER, RM, HARLAND, RM. *Early Development of Xenopus Laevis: A laboratory Manual.* 2000. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

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2. **NIEUWKOOP PD, FABER J.** A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. *Normal Table of Xenopus Laevis* (Daudin), 1967. Amsterdam, North-Holland.

3. **LI M, SIPE CW, HOKE K, AUGUST LL, WRIGHT MA, SAHA MS.** The role of early lineage in GABAergic and glutamatergic cell fate determination in xenopus laevis. *Journal of Comparative Neurology*, 2006; 495:645–57.

4. **GLEASON KK, DONDETI VR, HSIA HL, COCHRAN ER, GUMULAK-SMITH J, SAHA MS.** The vesicular glutamate transporter 1 (xVGlut1) is expressed in discrete regions of the developing Xenopus laevis nervous system. *Gene Expr Patterns*, 2003; 3:503–07.

5. **DEGITZ SJ, HOLCOMBE GW, KOSIAN PA, TIETGE JE, DURHAN EJ, ANKLEY GT.** Comparing the effects of stage and duration of retinoic acid exposure on amphibian limb development: chronic exposure results in mortality, not limb malformations. *Toxicological Sciences,* 2003; 74:139–46.

A Potential Role for DHEA in Prostate Cancer

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Dehydroepiandrosterone (DHEA) is the most abundant endogenous steroid produced by the adrenals of men and women, although levels decline markedly with age. DHEA is increasingly consumed as a commercial nutritional supplement for its purported anti-aging effects, yet its usefulness, long-term safety and effects remain uncertain.[1]

DHEA can be metabolized by the prostate gland and can increase expression of secondary mediators for epithelial growth and differentiation. Furthermore, because it is a precursor to both estrogens and testosterone, controversy exists as to whether DHEA enhances or reduces the risk of prostate cancer.[2] Since supplements are not regulated by the Food and Drug Administration, The National Center for Complementary and Alternative Medicine (NCCAM) at the National Institutes of Health (NIH) plays a vital role in researching the effects of supplements such as DHEA.

Prostate cancer is the second most common type of cancer among men in the United States, and approximately one out of three men will become afflicted.[3] The prostate epithelium contains glandular cells that synthesize and secrete products of seminal plasma, including prostate-specific antigen (PSA), the major diagnostic marker for prostate cancer. These epithelial cells depend on androgenic stimulation for their viability and secretory ability and become malignant during prostate cancer. They are surrounded by a fibromuscular stroma that not only physically supports the glands, but also contributes to the endocrine and paracrine microenvironments of these cancer cells. Thus, the stromal cells help control the growth and differentiation of the epithelial cells, as well as play a critical role in mediating the responsiveness of these cells to steroid hormones.[4] My research aimed to determine what specific factors these stromal cells produced in response to DHEA and provide insight into how the stromal environment modulates conversion of healthy epithelial cells into malignant prostate cancer.

Prostate epithelial cancer cells were co-cultured with stromal cells and separated by a substance called Matrigel, which functioned like a permeable basement membrane, allowing the passage of soluble stromal mediators to the epithelial cells. After the cells were grown, treated with DHEA and harvested, the co-cultured stromal RNA was extracted. Subsequently, cDNA was synthesized and real-time reverse transcriptase PCR was performed to measure expression of 84 genes related to human growth factors.

Results from three separate experiments were comparable and showed the up-regulation of several specific genes, including FGF-1 (fibroblast growth factor), IGF-1 (insulin-like growth factor), FGF-7 (fibroblast growth factor) and HPRT-1 (hypoxanthine phosphoribosyltransferase). While these results are preliminary, they may provide some insight into what growth factors the stromal cells produce in response to DHEA and can be further explored as targets for stromal regulation of epithelial cell function. My research experience only scratched the surface investigating the role of DHEA in prostate cancer, but I gained useful research experience and am optimistic about NCCAM's future efforts on this important topic.

REFERENCES:

1. **ARNOLD, J.T. ET AL.** Androgen receptor or estrogen receptor-beta blockade alters DHEA-, DHT-, and E(2)-induced proliferation and PSA production in

DHEA *Continued on p. 18*

DHEA *Continued from p. 17*

human prostate cancer cells. *The Prostate*, 2007; 67:1152–62.

2. **ARNOLD JT, LE H, ET AL.** Comparative effects of DHEA vs. testosterone, dihydrotestosterone, and estradiol on proliferation and gene expression in human LNCaP prostate cancer cells. *American Journal of Physiology, Endocrinology, and Metabolism*, 2005; 288:E573–E584.

3. **THE NATIONAL CANCER INSTITUTE.** What you need to know about Prostate Cancer, 2005. Retrieved Oct. 20, 2007 from *www.cancer.gov/cancertopics/wyntk/prostate*.

4. **ARNOLD JT, ISAACS JT.** Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault. *Endocrine-Related Cancer*, 2002; 9:61–73.

Middle Ear Epithelial Cell Secretion of Cytokines with Cigarette Smoke Condensate Exposure

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Otitis media (OM), an inflammation of the middle ear, is a ubiquitous condition of early childhood accounting for 16 million physician office visits each year. OM is associated with hearing loss, delayed speech development and the potential for permanent middle ear damage. Environmental agents such as tobacco smoke have been shown to be a risk factor in the occurrence of OM in early childhood.[1] OM can be classified by its associated clinical symptoms, otoscopic findings, complications and duration. There are two classifications of OM, acute and chronic, which differ in duration and result from different etiologies. Acute OM often results from upper respiratory infections; whereas chronic OM is usually non-infectious and thought to be due to chronic inflammation and mucous hyper-secretion. Environmental agents such as cigarette smoke are highly correlated with the occurrence of chronic OM. Studies conducted by different federal agencies suggest exposure to tobacco smoke may be a significant risk factor in the development of chronic OM in early childhood. The direct mechanism by which cigarette smoke exposure results in OM remains under investigation.

The etiology of OM inflammation within the middle ear mucosa is primarily initiated, mediated and regulated by pro-inflammatory cytokines: TNF-α, IL-1β, IL-6 and IL-8.[2] The inflammatory cascade is initiated by micro-organisms and/or their products, leading to an increase in restrictive oxygen species (ROS) and causing activation of the NF-κB complex. NF-κB translocates to the nucleus and transcribes genes encoding for ICAM-1, TNF-α, IL-1β and several secondary pro-inflammatory cytokines.

Cigarette smoke induces pro-inflammatory cytokines in the lower respiratory system, and exposure of cigarette smoke to the rat lung induces redox-sensitive transcription factors NF-κB and AP-1, which are linked to the transcription of specific inflammatory cytokines and chemokines.[3] Cigarette smoke also induces the synthesis of glutathione and gamma-glutamylcysteine that is associated with AP-1 or an AP-1 like response element.[3] In addition to increasing transcription of inflammatory genes, cigarette smoke also decreases HDAC2 activity in the rat lung, in turn decreasing histone

SMOKE EXPOSURE *Continued on p. 19*

deacetylation, and thus increasing transcription of pro-inflammatory genes.

We hypothesized that cigarette smoke condensate (CSC) stimulation would activate NF-κB and ultimately increase pro-inflammatory cytokine secretion in mouse middle ear epithelium cells (mMEEC).

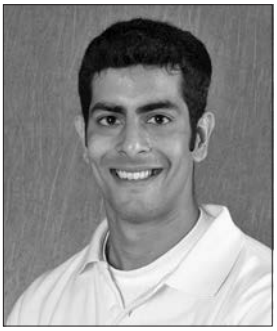
To investigate our hypothesis, we used a mouse inflammatory antibody array to measure 40 different pro-inflammatory cytokines from CSC stimulated mMEEC. The mMEEC was stimulated with 0 micrograms per microliter, 20 micrograms per microliter and 40 micrograms per microliter CSC for two, eight and 24 hours, respectively. The inflammatory antibody array was analyzed with chemiluminescence imaging. We observed a significant increase in pro-inflammatory cytokines IL-6, TNF-α, IFN-γ, IL-4 and IL-13. We plan to further analyze these cytokines of interest using Enzyme Linked Immunosorbent Assay (ELISA) kits to confirm our findings.

In conclusion, CSC activated pro-inflammatory cytokine secretion in mMEEC *in vitro*. These findings will bring us closer to understanding the biological mechanism responsible for the high correlation of OM with young children exposed to second-hand smoke.

REFERENCES:

1. **TUTKA P, WIELOSZ M, ZATONSKI W.** Exposure to environmental tobacco smoke and children's health. *International Journal of Occupational Medicine and Environmental Health*, 2002; 15:325–35.
2. **BARRETT TQ, KRISTIANSEN LH, OVESEN T.** NF-κB in cultivated middle ear epithelium. *International Journal of Pediatric Otorhinolaryngology,* 2002; 7:895–903.
3. **HELLERMANN GR, NAGY SB, KONG X, LOCKEY RF, MOHAPATRA SS.** Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. *Respiratory Research,* 2002. Retrieved May 29, 2007 from: *http://respiratory-research.com.*

Development of an *In Vitro* Model of Respiratory Tract Submucosal Glands



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A mucosal layer protects the respiratory tract epithelium against pathogens and environmental toxins. Mucin glycoproteins (mucins) are overproduced in chronic diseases of the respiratory tract, such as cystic fibrosis (CF) and chronic rhinosinusitis (CRS), and contribute significantly to disease morbidity and mortality.[1] Mucous hypersecretion predominantly results from goblet cell hyperplasia and/or submucosal gland (SMG) hyperplasia. *In vivo* and *in vitro* model systems are used to investigate mechanisms of mucin hypersecretion in the conducting respiratory tract epithelium. However, there is no established *in vitro* glandular model for the submucosa.

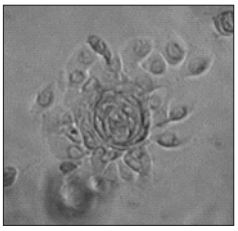

FIGURE 1: Microscopic view of gland-like structure formed by H-Tert immortalized tracheal bronchial epithelial cells when grown mixed with Matrigel on Day 9 after plating.

Presently, the standard *in vitro* models consist of differentiated primary human bronchial epithelial (HBE) or nasal epithelial cells grown on transwell plates coated with collagen at an air-liquid interface. Within two weeks, these cells differentiate into a conducting respiratory tract epithelium with cilia, basal and goblet cells.[2,3] The models secrete both MUC5AC and MUC5B mucins, which are *in vivo* markers for goblet cells and submucosal glands, respectively.[4] However, these cell models do not develop glands, although they are clearly pluripotent and express glandular proteins. We hypothesized that HBE cells would develop glands if grown in the appropriate context.

Submucosal glands are invaginations of the epithelium and are composed of ductal and secretory cells (both mucous and serous) that secrete mucins and host defense proteins into the mucosal layer. Cells from various tissues have already been shown to develop intestinal, mammary, salivary, pancreatic or oviduct glands when grown on Matrigel, a basement membrane complex isolated from murine tumors.[5,6] The main component of Matrigel is laminin, as well as collagen type IV, heparan sulfate and

SUBMUCOSAL GLANDS MODEL *Continued on p. 20*

SUBMUCOSAL GLANDS MODEL

Continued from p. 19

proteoglycans. At 37° C, Matrigel forms a three-dimensional gel that supports cell morphogenesis and differentiation.[7] Currently, there are no reports of respiratory tract gland (submucosal gland) development using Matrigel.

Two different approaches were used: respiratory tract-derived cells were either grown on top of, or mixed with, Matrigel. Cell media was changed as required and cells were periodically monitored by microscopy. Experiments were carried out using three different cell lines, including Calu-3 cells (cancer cell lines of SMG origin), H-Tert immortalized tracheal bronchial epithelial cells and normal human bronchial epithelial (HBE) cells.

Our preliminary results showed that respiratory tract-derived cells formed glandular-like structures but did not differentiate further after five days. A literature review indicates that epithelial cells from various tissues require seven to 15 days of growth on Matrigel to differentiate into glands, with subsequent apoptosis to form a glandular lumen.[7,8] Our data indicated that under these conditions, respiratory tract cells do not undergo apoptosis to form a lumen. Future experiments will focus on inducing apoptosis in order to facilitate differentiation of SMG and thus establishment of an *in vitro* model to investigate SMG development and hyperplasia in respiratory diseases such as CRS and CF.

REFERENCES:

1. **ROSE MC, VOYNOW JA.** Respiratory tract mucin genes and mucin glycoproteins in Health and disease. *Physiol Rev,* 2006; 86:245–78.
2. **WU R, ZHAO YH, CHANG MMJ.** Growth and differentiation of conducting airway epithelial cells in culture. *Eur Respir J,* 1997; 10:2398–403.
3. **BERNACKI SH, NELSON AL, ABDULLAH L, SHEEHAN JK, HARRIS A, DAVIS CW, RANDELL SH.** Mucin gene expression during differentiation of human airway epithelia *in vitro*. *Am J Respir Cell Mol Biol,* 1999; 20:595–604.
4. **REID C, GOULD S, HARRIS A.** Developmental expression of mucin genes in the human respiratory tract. *Am J Respir Cell Mol Biol,* 1997; 17:592–98.
5. **KLEINMAN HK, MCGARVEY ML, HASSELL JR, STAR VL, CANNON FB, LAURIE GW, MARTIN GR.** Basement membrane complexes with biological activity. *Biochemistry*, 1986; 25:312–18.
6. **TERRANOVA VP, AUMAILLEY M, SULTAN LH, MARTIN GR, KLEINMAN HK.** Regulation of cell attachment and cell number by fibronectin and laminin. *J Cell Physiol,* 1986; 127:473–79.
7. **KLEINMAN HK, MARTIN GR.** Matrigel: basement membrane matrix with biological activity. *Semin Cancer Biol,* 2005; 15:378–86.
8. **DEBNATH J, MUTHUSWAMY SK, BRUGGE JS.** Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods*, 2003; 30:256–68.

A Three-Dimensional Model of Epicardial Development

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Development of the epicardium is critical to proper heart formation. The epicardium provides all of the precursor cells that form the coronary system and supplies signals that stimulate cardiac myocyte proliferation. The epicardium forms from mesothelial cells (proepicardial [PE] cells) associated with the developing liver. Here, a model of epicardial development is presented. PE cells have been isolated from chicken embryos and cultured on a 3-D scaffold. This 3-D model system consists of a tubular scaffold engineered from type-I collagen and optimized to support the growth of embryonic cardiac tissues.[1,2,3]

Using scanning electron microscopy (SEM), we compared in vivo PEs with PEs cultured on the tube scaffold for three days. *In vivo* PE cells appeared as cobblestone cells covering the AV sulcus of HH stage-17 chicken hearts as well as the outflow tract of the heart. Cells on the tube also were observed to migrate as a sheet and possessed apical villi characteristic of *in vivo* PE cells.[4] Similarly, PE cells cultured on the tube scaffold displayed a cobblestone morphology and possess apical villi.[5] SEM data also revealed PE cells could migrate in a sheet-like fashion and inhabit pit-like areas of the tube scaffold.[5] Moreover, PE cells cultured on the collagen scaffold clearly maintained their morphological characteristics and closely resembled their *in vivo* counterparts. Cultured PE cells also expressed markers for both endothelial and smooth muscle cells, bearing a striking resemblance to *in vivo* cells undergoing vasculogenic processes.[5] Taken together, these data support the tube scaffold as a viable model for epicardial development.

REFERENCES:

1. EVANS HJ, SWEET JK, PRICE RL, YOST M, GOODWIN RL. Novel 3-D culture system for study of cardiac myocyte development. *Am J Physiol Heart Circ Physiol,* 2003; 285:H570–H578.
2. YOST MJ, BAICU CF, STONEROCK CE, GOODWIN RL, PRICE RL, DAVIS JM, EVANS H, WATSON PD, GORE CM, SWEET J, CREECH L, ZILE MR, TERRACIO L. A novel tubular scaffold for cardiovascular tissue engineering. *Tissue Eng,* 2004; 10:273–284.
3. GOODWIN RL, NESBITT T, PRICE RL, WELLS JC, YOST MJ, POTTS JD. Three-dimensional model system of valvulogenesis. *Dev Dyn,* 2005; 233:122–29.
4. VAN DEN EIJNDE SM, WENINK AC, VERMEIJ-KEERS C. Origin of subepicardial cells in rat embryos. *Anat Rec,* 1995; 242:96–102.
5. NESBITT TL, PATEL PA, YOST MJ, GOODWIN RL, POTTS JD. A 3-D Model of Coronary Vessel Development. *In vitro animal cellular: developmental biology,* 2007; 43:10–16.

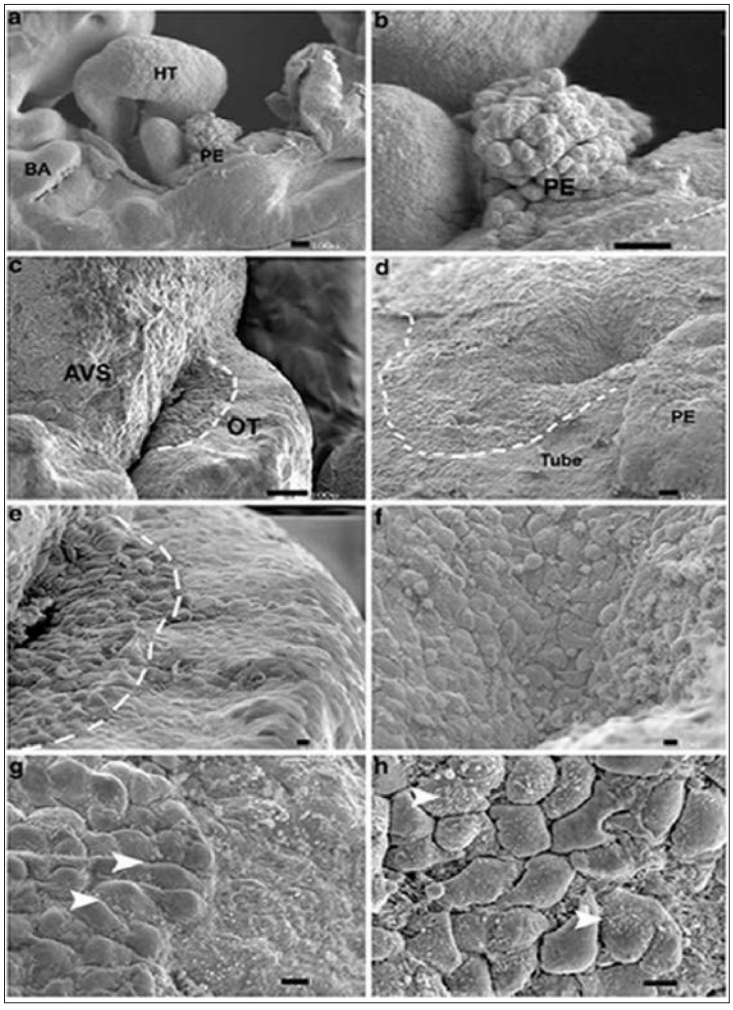

FIGURE 1: COMPARATIVE MORPHOGENESIS OF THE PROEPICARDIAL CELLS. A, B, C, E, G: Representative proepicardium (PE) morphogenesis in the stage-17 chicken embryo. A: Stage-17 chicken embryo showing the branchial arches (BA), heart tube (HT) and the proepicardium (PE). B: Higher magnification of (A) showing the PE extending from the liver toward the heart tube (HT). C: PE cells migrate as a sheet from the atrioventricular sulcus toward the outflow tract. The dashed line indicates the boundary of migrating cells. D, F, H: Representative images of PE cell morphogenesis on the tubular scaffold. D: Chicken PE cells cultured on the collagen tube exhibit a cobblestone appearance. The dashed line identifies the boundary of migrating PE cells on the collagen tube. E: Higher magnification of (C) shows that the *in vivo* cells migrate as a sheet over the HH stage-18 chicken heart. F: PE cells exhibit a cobblestone morphology and are found in pit-like areas of the tube scaffold. G, H: Arrows show the presence of apical villi on the surface of PE cells *in vivo* and on the collagen scaffold in G and H, respectively (scale bars equal 100 μm in A-C and 10 μm in D-H).

Improving MRI Detection of Creutzfeldt-Jakob Disease

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Creutzfeldt-Jakob disease (CJD) is a rare neurodegenerative prion disease characterized by the accumulation of abnormal prion protein leading to neuron loss, vacuolation and/or astrogliosis. CJD is characterized by rapid-onset dementia, rapid degeneration and death. In this study, Libyan-born Israelis at increased risk for the inherited form of CJD were examined at Sheba Medical Center (Tel Hashomer, Israel). The patients suffered from either familial or sporadic CJD. Our group previously reported similar radiological findings for familial cases when compared to the more common sporadic form.[1]

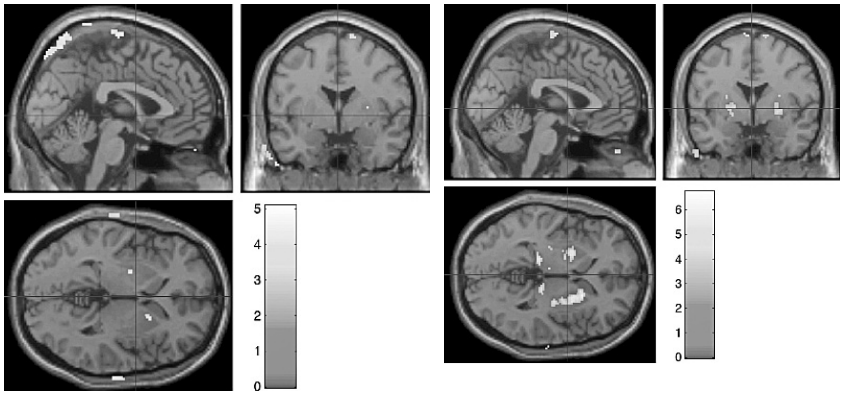

FIGURE 1: DIFFUSION COEFFICIENT (ADC) MAPS OF PATIENTS WITH CJD.
Comparing ADC maps of patients with controls showed more significant reductions in ADC within the thalamus and striatum using b=2000, suggesting greater sensitivity with high b-value (right).

This study analyzed two diffusion-weighted (DWI-MRI) sequences to evaluate the sensitivity of higher b-value DWI (b=2000 s/mm^2), as compared with the more common lower b-value sequence (b=1000 s/mm^2). DWI-MRI measures differences in proton movement indicative of tissue structure, and it has been demonstrated to be an early marker of CJD-related pathology with better sensitivity and specificity than other CJD criteria such as the cerebrospinal fluid 14-3-3 protein detection and EEG patterns.[2] DWI-MRI patterns in CJD patients typically include hyperintensities within the caudate nucleus, putamen, thalamus and cortical ribbon; these findings putatively correspond to vacuole formation, the hallmark histological finding of prion diseases.[3]

B-value is a function of diffusion gradient strength; a higher b-value is thought to be more sensitive to diffusion and has been used with success in detecting other diseases.[4] A high b-value DWI may provide earlier detection of CJD pathogenesis and improve understanding of the progression of imaging changes.

DWI scans of 10 patients and age- and gender-matched controls were analyzed using a voxel-based statistical software package. Comparisons were examined using Apparent Diffusion Coefficient (ADC) maps, a quantitative measure of proton diffusion capable of providing more robust statistical comparison. The comparison of control subjects to CJD patients showed greater sensitivity to basal ganglia and thalamic changes using high-value sequences, while the lower b-value detected more cortical changes. These findings suggest greater diffusion-weighted MRI sequences may be advantageous in detecting CJD-related changes.

REFERENCES:

1. FULBRIGHT RK, KINGSLEY PB, GUO X, HOFFMANN C, KAHANA E, CHAPMAN JC, PROHOVNIK I. The imaging appearance of Creutzfeldt-Jakob disease caused by the E200K mutation. *Magnetic Resonance Imaging*, 2006; 24:1121–29.
2. SHIGA Y, MIYAZAWA K, SATO S, FUKUSHIMA R, SHIBUYA S, SATO Y, KONNO H, DOH-URA K, MUGIKURA S, TAMURA H, HIGANO S, TAKAHASHI S, ITOYAMA Y. Diffusion-weighted MRI abnormalities as an early diagnostic marker for Creutzfeldt-Jakob disease. *Neurology*, 2004; 63:443–49.
3. KALLENBERG K, SCHULZ-SCHAEFFER WJ, JASTROW U, POSER S, MEISSNER B, TSCHAMPA HJ, ZERR I, KNAUTH M. Creutzfeldt-Jakob disease: comparative analysis of MR imaging sequences. *Am J Neuroradiol,* 2006; 27:1459–62.
4. ASSAF Y, MAYZEL-OREG O, GIGI A, BEN-BASHAT D, MORDOHOVITCH M, VERCHOVSKY R, REIDER-GROSWASSER II, HENDLER T, GRAIF M, COHEN Y, KORCZYN AD. High b value q-space-analyzed diffusion MRI in vascular dementia: a preliminary study. *J Neurol Sci,* 2002; 203-204:235–39.

Small Molecules that Induce p53 Signaling in Retinoblastoma Preclinical Animal Models

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Retinoblastoma is an intraocular malignancy that occurs in children when a germline mutation of RB1 is inherited and a somatic mutation inactivates the second RB1 allele.[1] Common modes of treatment to preserve vision include triple-drug chemotherapy, radiation, laser and cryotherapy. When chemotherapy is combined with focal treatments, up to 40 percent of patients do not respond and enucleation of the eye may be required to prevent metastatic retinoblastoma which is often fatal.[2] Current drugs have undesirable side effects, including an increased risk of developing acute myeloblastic leukemia or hearing loss from ototoxicity.[2,3] Since retinoblastoma is a rare disease (300 new cases in the United States each year), preclinical animal models that recapitulate the tumorgenicity are important for developing new chemotherapy treatments.[4]

The tumor suppressor RB1 gene is responsible for regulating cell proliferation, while the p53 gene regulates cell response to stresses such as DNA damage by inducing apoptosis.[5] In retinoblastoma, because the p53 gene is intact yet suppressed, it is a prime target for up-regulation to promote apoptosis and stop clonal expansion in tumors. The purpose of this research is to develop more effective, less toxic novel chemotherapeutic treatments using small molecules that up-regulate p53 to induce apoptosis in p53-suppressed retinoblastoma cells. By identifying compounds in a National Cancer Institute library that were previously shown to induce p53 in other types of cancer cells, we screened and selected small molecules that demonstrated cytotoxic effects in retinoblastoma cells.

One such p53 activator investigated was a small molecule that "Reactivates p53 and Induces Tumor cell Apoptosis" (RITA). In previous studies, RITA was shown to suppress the growth of osteosarcoma and fibro-sarcoma cells.[6] By analyzing the effects of RITA in RB1 tumor growth assays, RITA demonstrated potent cytotoxic effects. We calculated the 50 percent growth inhibitory concentration (GI50) from *in vitro* experiments, and correlated growth inhibition with Western Blot analyses. This data demonstrates suppression of cell proliferation while up-regulating p53 gene expression.

MS-275 IN OCULAR XENOGRAFT MODEL

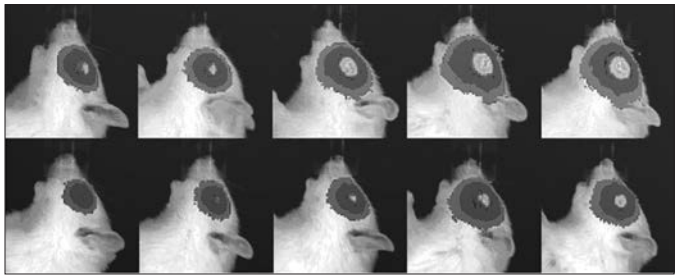

FIGURE 1: Y79-LUC CELLS WERE TRANSPLANTED INTO THE VITREOUS OF DAY-OLD RATS. On day-14 post-transplant the animals were treated by intraperitoneal injection of 20 mg/kg MS-275 or vehicle for 12 days (six treatments total) and injected with 150 mg/mL D-Luciferin for imaging with the IVIS Lumina imaging system.

To recapitulate the tumorgenicity properties of RB1 in animals, we used an ocular xenograft rat model. Human retinoblastoma cells were transplanted directly into the eyes of newborn rat pups to establish tumor growth. Using a microscope and the tip of an insulin syringe, the eyelids of anesthetized rat pups were surgically opened and the posterior chamber was infused with cultured cells. Tumor growth was established over 14 days and the rats were treated with drug and a control vehicle. MS-275 is a histone deacetylase inhibitor and known anti-tumor compound previously proven to suppress retinoblastoma cell growth *in vitro*. The rats were treated with either MS-275 or vehicle every other day for 12 days and were imaged using a Luciferin assay every other day. Our results indicate statistically significant inhibition of tumor growth with MS-275 treatment when

P53 SIGNALING *Continued on p. 24*

P53 SIGNALING *Continued from p. 23*

compared to control animals (Figure 1). At the end of the treatment period, MS-275 treated animals had 62 percent tumor growth inhibition when compared to vehicle-only control animals ($p = 0.038$).

Through the analysis of 140,000 compounds in the NCI library for small molecules that are good candidates for *in vitro* and ocular xenograft model experimentation, we plan to identify novel antitumor chemotherapies for the treatment of retinoblastoma.

REFERENCES:

1. **TSAI T, GOMBOS D, FULTON L, CONWAY R, O'BRIEN J, CRONIN J, MUTHIALU A.** Retinoblastoma and hypochondroplasia: a case report of two germline mutations arising simultaneously. *Ophthalmic Genetics*, 2005; 26;2.
2. **SMITS C, SWEN SJ, GOVERTS ST, MOLL AC, IMHOF SM, SCHOUTEN-VAN MEETEREN AYN.** Assessment of hearing in very young children receiving carboplatin for retinoblastoma. *European Journal of Cancer*, 2006; 42:492–500.
3. **LAURIE NA, GRAY JK, ZHANG J, LEGGAS M, RELLING M, EGORIN M, STEWART C, DYER MA.** Topotecan Combination Chemotherapy in Two New Rodent Models of Retinoblastoma. *Clin Cancer Res,* 2005; 11:7569–78
4. **DYER MA, RODRIGUEZ-GALINDO C, WILSON MW.** Use of preclinical models to improve treatment of retinoblastoma. *PLoS Med,* 2005; 2:e332.
5. **LAURIE NA, DONOVAN SL, SHIH CS, ZHANG J, MILLS N, FULLER C, TEUNISSE A, LAM S, RAMOS Y, MOHAN A, JOHNSON D, WILSON M, RODRIGUEZ-GALINDO C, QUARTO M, FRANCOZ S, MENDRYSA SM, GUY RK, MARINE JC, JOCHEMSEN AG, DYER MA.** Inactivation of the p53 pathway in retinoblastoma. *Nature*, 2006; 444:61–66.
6. **ISSAEVA N, BOZKO P, ENGE M, PROTOPOPOVA M, VERHOEF L, MASUCCI MARIA, PRAMANIK A, SELIVANOVA G.** Small molecule RITA binds to p53, blocks p53 — HDM-2 interaction and activates p53 function in tumors. *Nature*, 2004; 10:1321–28.

Surgical Correction of Spinal Deformities after Heart Transplantation: A Case Series Report

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Several studies have documented an association between congenital heart disease and scoliosis in children.[1-4] More recent research has shown that children undergoing any type of solid organ transplantation are placed at increased risk for developing a potentially limiting spinal curvature.[5] Among all solid organ transplantations, heart transplantation has been reported to be a major risk factor for the development of spinal deformity.[5] Armed with this knowledge, the pediatric orthopaedic research team at Morgan Stanley Children's Hospital of New York-Presbyterian Hospital reviewed the cases of patients undergoing scoliosis correction after orthotopic heart transplantation to establish any necessary changes in their perioperative care to ensure good surgical results.

… spinal deformity surgery can be successfully performed in children and adolescents who have undergone heart transplantation.

This study was a retrospective case series report of six heart transplant recipients with a diagnosis of scoliosis, requiring corrective spinal surgery between 1995 and 2007. The causative factor necessitating heart transplantation varied among the patient population. Two patients had congenital heart disease, two had congenital cardiomyopathy and one had Marfan syndrome. Hospital charts were reviewed to obtain clinical information regarding the heart transplantation, the spinal procedure and the patient's status (both cardiac and spinal) at the time of scoliosis correction. Diagnostic test results, including preoperative cardiac catheterizations, echocardiograms and preoperative Cobb angles, were obtained from the surgeon's notes. Intraoperative and follow-up information also was reviewed. Three different procedures were used to correct the scoliosis in the heart transplant patients. Four patients underwent a posterior spinal fusion and instrumentation, one patient underwent a posterior segmental instrumentation without fusion (growing rods),

SPINAL DEFORMITIES *Continued on p. 25*

and the last patient received an anterior release and posterior fusion with instrumentation.

The average age at time of heart transplantation was 7.8 ± 4.5 years. The children then underwent surgical correction for their scoliosis at a mean age of 13.3 ± 5.6 years. Average length of follow up after the scoliosis surgery was 21.5 ± 14.7 months. Two of the patients in the cohort died as a result of their cardiac condition. Three patients had complications following the scoliosis procedure. These complications included a fractured single rod construct in one patient, device migration after initial surgery in another patient and one instance of wound infection. None of the complications resulted in adverse sequelae. Appropriate curve correction and control was achieved for all six patients. The mean preoperative major curve was 70.2°. At the time of last follow up, the spinal curvature had corrected to a mean curve of 22.6° ($p<0.10$).

This case report demonstrates how spinal deformity surgery can be successfully performed in children and adolescents who have undergone heart transplantation. Especially with immunosuppressive therapy and numerous comorbid conditions, these patients represent an especially challenging cohort. Adequate surgical correction without serious adverse conditions, therefore, is best accomplished with meticulous preoperative planning and extensive multidisciplinary cooperation both intraoperatively and postoperatively. Despite the risks and challenges, correction of spinal deformity in pediatric heart transplant patients can significantly improve quality of life.

REFERENCES:

1. **BEALS RK, KENNEY KH, LEES MH.** Congenital heart disease and idiopathic scoliosis. *Clin Orthop Relat Res*, 1972; 89:112-6.
2. **FARLEY FA, PHILLIPS WA, HERZENBERG JE, ROSENTHAL A, HENSINGER RN.** Natural history of scoliosis in congenital heart disease. *J Pediatr Orthop*, 1991; 11:42–7.
3. **KAWAKAMI N, MIMATSU K, DEGUCHI M, KATO F, MAKI S.** Scoliosis and congenital heart disease. *Spine*, 1995; 20:1252–6.
4. **RECKLES LN, PETERSON HA, WEIDMAN WH, BIANCO AJ.** The association of scoliosis and congenital heart defects. *J Bone Joint Surg Am*, 1975; 57:449–55.
5. **HELENIUS I, ET AL.** Scoliosis after solid organ transplantation in children and adolescents. *Am J Transplant*, 2006; 6:324–30.

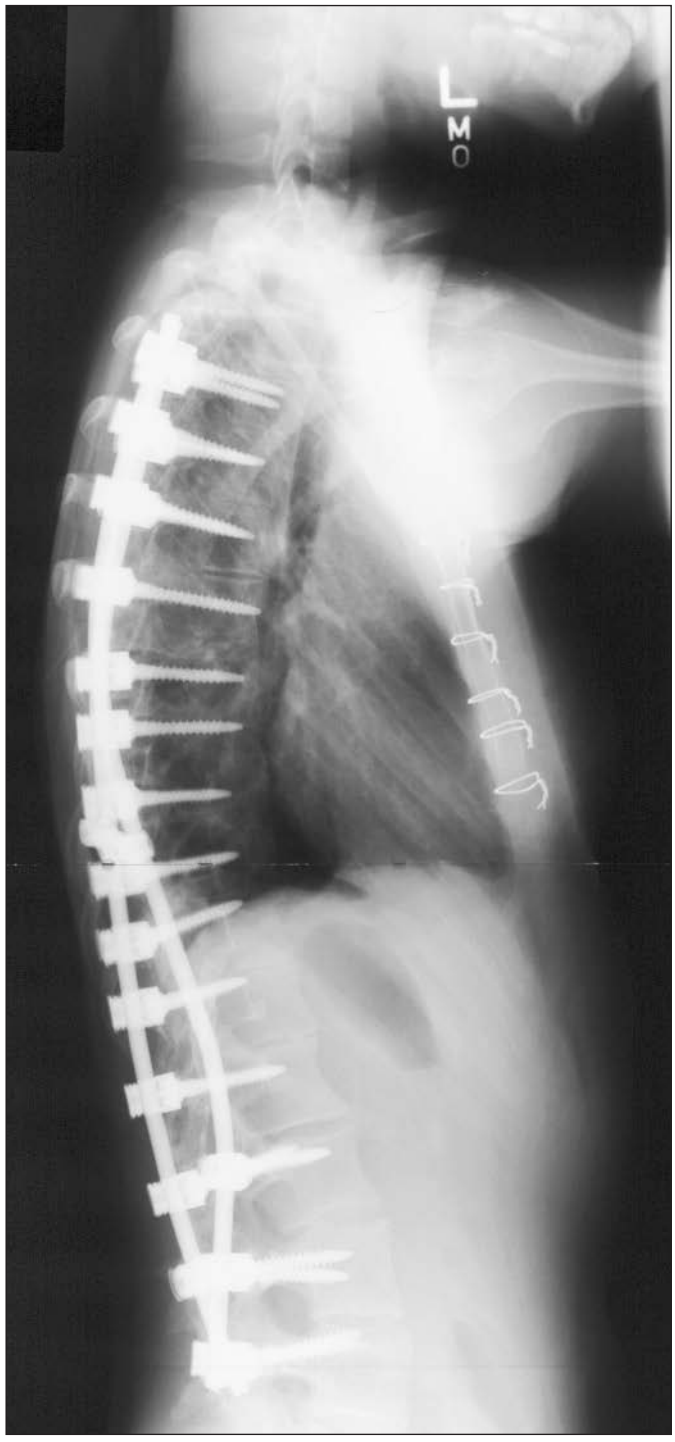

FIGURE 1: POSTOPERATIVE LATERAL X-RAY OF A STUDY PATIENT.
Note the posterior spinal instrumentation inserted during the scoliosis surgery as well as the anterior staples used to fuse the sternum following the heart transplantation.

Hip Arthroscopy and the Treatment of Femoroacetabular Impingement

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This project aimed to assess the long-term prognosis for patients who presented with femoroacetabular impingement (FAI) and underwent subsequent hip arthroscopy. FAI occurs when the femoral head does not have its full range of motion within the pelvic acetabulum. FAI is increasingly recognized as a cause of osteoarthritis in the hip through deterioration of the labrum and articular surface. Impingement itself is the premature and improper collision between the head and/or neck of the femur and the acetabulum. This causes a painful and decreased range of hip joint motion. FAI commonly presents as "cam-type" impingement, a result of excess bone forming around the head and/or neck of the femur; "pincer-type" impingement, an overgrowth of the acetabular rim; or a resulting FAI where the acetabulum is angled in such a way that abnormal impact occurs between the femur and the rim of the acetabulum.[1]

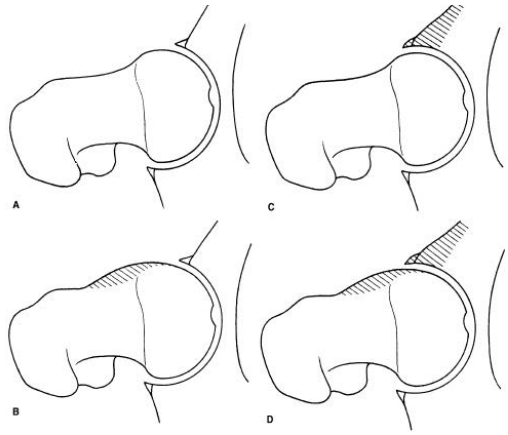


FIGURE 1: BALL-AND-SOCKET JOINT. A) Normal hip; B) Cam impingement; C) Pincer impingement; D) Combination of cam and pincer impingement.

When the excess bone present on the femoral head and/or neck abuts the rim of the acetabulum, the cartilage and labrum that line and cushion the acetabulum can be damaged. The extra bone can appear on X-rays as a small protrusion. When the protrusion repeatedly rubs against the cartilage and labrum, such as in athletes who perform repeated leg flexion and extension, the cartilage and labrum eventually may tear, causing pain. As more tissue tears, the femoral bone will impact with pelvic bone resulting in arthritis. Tears of the labrum can also fold into the joint space, causing a further painful restriction of hip motion.[2,3]

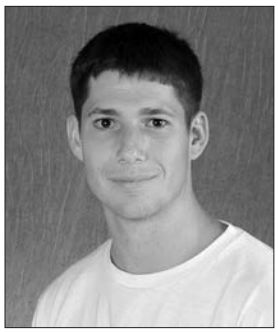
Arthroscopy of the hip has become an accepted surgical procedure with well-defined indications and expected outcomes, primarily due to recent advances in surgical instrumentation and techniques.[3] Hip arthroscopy is performed as an outpatient procedure using fluoroscopy for instrument placement. The procedure has been slower to evolve than arthroscopy of other joints because the hip is much deeper in the body. Furthermore, because the hip is a "ball-and-socket" joint, it is necessary to employ traction to expose the joint enough to fit the surgical instruments inside without causing further tissue damage. The basic principle of surgical treatment of FAI is to restore sphericity to the femoral head, thereby relieving the impingement. The surgery also aims to address the pathological changes in the labrum and articular cartilage.[1]

Studies have shown that the vast majority of hip arthroscopy patients return to sports and other physical activities at their peak level of performance prior to the onset of hip pain. The majority of patients clearly get better, but it is not yet clear to what extent the procedure stops the course of arthritis. Patients who have underlying skeletal deformities or degenerative conditions may not experience as much relief from the procedure as would a patient with simple impingement.

REFERENCES:

1. **CLOHISY JC, MCCLURE JT.** Treatment of anterior femoroacetabular impingement with combined hip arthroscopy and limited anterior decompression. *The Iowa Orthopaedic Journal*, 25:164–71.
2. **ESPINOSA N, BECK M, ROTHENFLUH DA, GANZ R, LEUNIG M.** Treatment of femoro-acetabular impingement: preliminary results of labral refixation. Surgical technique. *The Journal of Bone and Joint Surgery*, 2006; 88:925–35.
3. **SHUGARS RA, MORE RC.** Arthroscopic hip surgery. *The Association of Perioperative Registered Nurses Journal*, 2006; 82:975–98.

Novel "Non-Fusion" Technologies May Help Patients with Spinal Stenosis



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Technological advances, especially spanning the last two decades, have greatly advanced the field of spinal surgery. Many of these advances focused on creating alternatives to spinal fusion. Historically, spinal fusion has been widely used to treat degenerative pathologies in the spine. However, eliminating vertebral motion and placing greater stress on adjacent spinal segments limits the effectiveness of these fusions. As a result, new classes of non-fusion surgical devices have emerged, including interspinous spacers, facet replacement systems and posterior dynamic stabilization systems. These devices offer innovative, as well as minimally invasive, approaches to spinal surgery. The interspinous spacer, facet replacement system and posterior dynamic stabilization are each indicated to treat spinal stenosis.[1,2]

This retrospective study evaluated the percentage of patients who would be eligible to receive one of these non-fusion devices. Eligibility was defined as matching at least one indication but none of the contraindications for each device. There have been no published studies investigating the percentage of patients who may potentially benefit from any of these devices.

Patients were divided in two groups, the first including patients who had a lumbar fusion and the second consisting of patients who had any other lumbar surgery. Indications and contraindications in this study were adapted from IDE clinical trials for interspinous spacers, facet replacement and posterior dynamic stabilization. We evaluated the inclusion/exclusion criteria for multiple models in each category and selected the most stringent criteria. As a result, indications and contraindications were based on the inclusion/exclusion criteria for the IDE clinical trials of X Stop interspinous spacer, Anatomic Facet Replacement System and Stabilimax posterior dynamic stabilization.

Medical records of 100 patients who had lumbar fusions were cross-referenced with indications and contraindications for each device. Of these patients, 22 percent were eligible to receive an interspinous spacer, 30 percent were eligible to undergo facet replacement and 34 percent were eligible to receive posterior dynamic stabilization. Twenty-three percent of patients were eligible to receive all three devices, while 65 percent were not eligible to receive any device. Reviewing 100 patients who had a lumbar surgery other than fusion, 25 percent of patients were eligible to receive an interspinous spacer, 26 percent were eligible to undergo facet replacement and 27 percent were eligible to receive posterior dynamic stabilization. Twenty-five percent of patients were eligible to receive all three devices and 73 percent were not eligible to receive any of the devices.

Interspinous spacers, facet replacement and posterior dynamic stabilization, therefore, stand to impact 20–30 percent of lumbar surgeries.

As future research and technology expand the indications or eliminate the contraindications for these devices, the number of patients eligible to receive these devices may increase significantly.

As future research and technology expand the indications or eliminate the contraindications for these devices, the number of patients eligible to receive these devices may increase significantly. We predict that these devices will have a significant impact on spine surgery.

REFERENCES:

1. **HANLEY E.** The indications for lumbar spinal fusion with and without instrumentation. *Spine*, 1995; 20:143S–153S.
2. **ESSES S, HULER R.** Indications for Lumbar fusion in the Adult. *Clinical Orthopedics and Related Research*, 1992; 279:87–100.

Effect of Bone Density on Mechanical Properties after Percutaneous Vertebroplasty

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Vertebral compression fractures are the most common injuries resulting from osteoporosis, with an estimated incidence of 700,000 per year in the United States.[1] Among persons over 65, 33 percent of women have sustained a vertebral compression fracture, and this fracture rate is three times higher in women than in men. These fractures cause acute and chronic back pain, disability and increased mortality risk.[2] One treatment option is percutaneous vertebroplasty or injection of cement, typically polymethyl methacrylate (PMMA) acrylic bone cement, into the vertebral body. However, previous studies have shown that injecting an excessive amount of cement can increase the risk of both cement leakage and secondary adjacent fractures.[2] While the primary purpose of the surgery is to eliminate back pain, patients and practitioners expect other benefits, including mechanical reinforcement of the vertebra as well the prevention of adverse effects.

The primary objective of this study was to determine how bone mineral density (BMD) affects mechanical stiffness and strength of the vertebral body after vertebroplasty. A secondary objective was to determine how the relationships between mechanical properties and BMD vary with the amount of cement injected. The clinical goal of this research was to help physicians understand how BMD can identify a dose of cement that can restore mechanical properties to the greatest extent possible for a specific patient without increasing the risk of complications.

Bone mineral density of 13 vertebral columns from adult Caucasian female cadavers was measured with a dual-energy X-ray absorptiometry (DEXA) scanner. Vertebral bodies (n=126) were assigned to five groups based on cement treatment: intact, untreated, 4 percent fill, 12 percent fill and 24 percent fill. Non-intact specimens were compressed asymmetrically to simulate a wedge compression fracture before treatment with PMMA cement. Strength and stiffness were measured in axial compression.

The two most significant findings of the study suggest that: (1) the improvements in stiffness and strength resulting from vertebroplasty depend significantly on bone density, and (2) only the highest cement dose in the study (24 percent vertebral body fill, average of 7 mL) had an appreciable effect on either mechanical property. Regarding strength, only the 24 percent fill group improved strength over untreated levels, and greater improvements in strength were seen in patients with higher bone density. Unlike stiffness, however, the strength of the 24 percent fill group not only improved relative to the untreated group but was enhanced beyond the intact levels. The greatest improvements in stiffness would be expected in patients with higher bone density (who are the least likely to suffer a fracture or need vertebroplasty). Results suggest that highly osteoporotic patients may receive the least amount of improvement in

BONE DENSITY *Continued on p. 29*

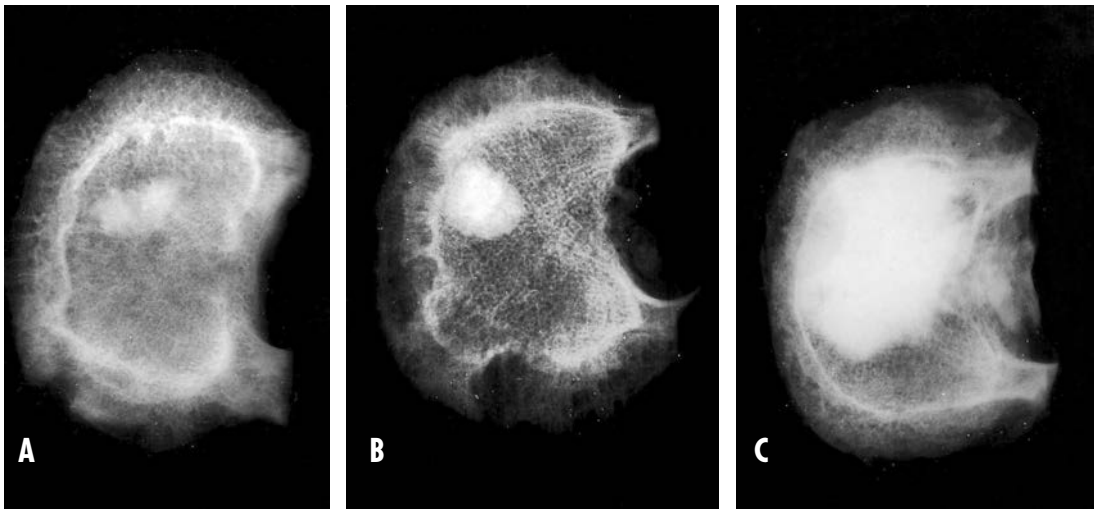

FIGURE 1: X-RAYS VERIFYING CONSISTENT VERTEBROPLASTY. All cement was injected using a transpedicular approach through the left pedicle with the needle tip in the anterior third of the vertebral body near the axial midplane. (A) L3 vertebra injected with 4 percent volume of cement. (B) T12 vertebra injected with 12 percent volume of cement. (C) L2 vertebra injected with 24 percent volume of cement.

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mechanical properties from vertebroplasty. For these patients, it may be advisable for clinicians to limit their expectations of vertebroplasty to pain relief but not mechanical benefit and to select a cement volume that will minimize risks of adverse events.

REFERENCES:

1. **RIGGS B, MELTON LR.** Involutional osteoporosis. *New England Journal of Medicine,* 1986; 314:1676–86.

2. **SILVERMAN S, MINSHALL M, SHEN W, ET AL.** The relationship of health-related quality of life to prevalent and incident vertebral fractures in postmenopausal women with osteoporosis. *Arthritis Rheum,* 2001; 44:2611–19.

*This study was published in the August 2007 issue of *Spine*: Graham J, Ahn C, Hai N, Buch B. Effect of bone density on vertebral strength and stiffness after percutaneous vertebroplasty," *Spine,* 2007; 32:E505–E511.

Identifying the Correlation between Pseudo-bulbar and Cognitive Impairment in Primary Lateral Sclerosis

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Primary Lateral Sclerosis (PLS) and Amyotrophic Lateral Sclerosis (ALS) both involve degeneration of neurons. In PLS, loss of corticospinal tract Upper Motor Neurons (UMN) results in stiffness and brisk reflexes. In ALS, there is loss of UMN and Lower Motor Neurons (LMN); as a result, one finds atrophy, fasciculation and diminished reflexes in addition to UMN findings.

Although Pringle *et al* have developed a classification scheme for PLS, there still remains a question as to whether PLS and ALS are indeed separate disease entities.[1] A previous study found that stiffness and spasticity are more commonly seen at the initial presentation of PLS and that wasting of the limb is rare, and concluded, "a patient presenting with spasticity who does not develop wasting within three years most likely has PLS."[2]

ALS patients often manifest cognitive changes of frontal lobe executive dysfunction to varying degrees, in some cases leading to fronto-temporal dementia (FTD). These impairments are most commonly seen in those ALS patients with bulbar impairment. Schreiber *et al* found, "there is a fronto-temporal pattern of cognitive dysfunction in ALS expressing itself early in the course of the disease and mainly with bulbar forms."[3] Furthermore, Lomen-Hoerth found in her study that half of patients showed executive function deficits.[4]

To further elucidate whether there is a distinction between ALS and PLS, our study aimed to determine if a correlation exists between bulbar dysfunction and cognitive impairment in the PLS population. Fifteen PLS patients and six age-matched, healthy volunteers participated in the study. The full battery of bulbar function measures were carried out on 14 of the patients and two of the volunteers. This work up included clinical examination measures for hyperreflexia in addition to two speech measures: the rate of speech and the Assessment of Intelligibility of Dysarthric Speech test. The measures of the rate of speech and clarity of enunciation declined in parallel; as speech became slower it also became more difficult to understand. Rate of speech also reflected the presence of hyperreflexia; brisk jaw jerks and gag reflex were associated with slow rates of speech as well. The rate of speech was thereafter used as the primary measure of bulbar function for comparison with the Mattis DRS-2 dementia score. The psychologist in our group obtained the dementia scores for 14 of the patients and six of the volunteers.

Preliminary analysis of the correlation between rate of speech and scores on the Mattis dementia scale does not suggest a correlation at this time. Regression analysis with a straight line fit was performed in an attempt to elucidate this relationship. We concluded that the relationship between cognitive function and bulbar dysfunction should be studied in a larger population of patients with PLS and compared to patients with ALS to better understand the common features of these disorders. Furthermore, understanding the underlying cortical pathology by imaging measures of atrophy as well as pathologic studies

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in PLS may further help explain the disease process itself.

REFERENCES:

1. **PRINGLE CE, HUDSON AJ, ET AL.** Primary lateral sclerosis. Clinical features, neuropathology and diagnostic criteria. *Brain*, 1992; 115:495–520.
2. **TARTAGLIA MC, ROWE A, ET AL.** Differentiation between primary lateral sclerosis and amyotrophic lateral sclerosis: examination of symptoms and signs at disease onset and during follow-up. *Archives of Neurology*, 2007; 64:232–36.
3. **SCHREIBER H, GAIGALAT T, ET AL.** Cognitive function in bulbar- and spinal-onset amyotrophic lateral sclerosis. A longitudinal study in 52 patients. *Journal of Neurology*, 2005; 252:772–81.
4. **LOMEN-HOERTH C.** Characterization of amyotrophic lateral sclerosis and frontotemporal dementia. *Dementia and Geriatric Cognitive Disorders,* 2004; 17:337–41.
5. **MURPHY J, HENRY R, ET AL.** "Establishing subtypes of the continuum of frontal lobe impairment in amyotrophic lateral sclerosis." *Archives of Neurology*, 2007; 64:330–34.
6. **ZHAI P, PAGAN F, ET AL.** Primary lateral sclerosis: A heterogeneous disorder composed of different subtypes? *Neurology*, 2003; 60:1258–65.
7. **KUNCL RW.** *Motor Neuron Disease*, 2002.

Clopidogrel in a Pediatric Population: Safety and Efficacy Results from a Single Center

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Although thrombotic diseases are less prevalent in children than in adults, complex pediatric patients, such as those with congenital heart disease, remain at risk for clot formation. Few guidelines exist for treatment of pediatric patients, leading pediatric practitioners to prescribe drugs, such as clopidogrel, off-label based upon extrapolation from adult experience and perceived clinical need.

Evidence-based information on clopidogrel, a thienopyridine derivative and potent antiplatelet, is limited to adults at risk for, or with a history of, ischemic stroke, acute coronary syndrome, peripheral arterial disease or are at risk of death from vascular disease.[1] Several large, randomized trials have evaluated the safety and efficacy of clopidogrel in adults using pre-determined dosing criteria, and generally found the drug to be safe and efficacious.[2,3,4]

Published material supporting the safety and efficacy of off-label use of clopidogrel in the pediatric population remains scarce. Several pediatric studies of clopidogrel performed outside of the United States are underpowered by small sample sizes. Two such studies performed at the Hospital for Sick Children in Toronto, Canada had cohorts of only 15–17 patients. Overall, both studies found clopidogrel to be well tolerated in children with minimal adverse events, the majority due to minor bleeding.[5,6]

We performed a study to provide descriptive information about off-label treatment practice with clopidogrel in a larger pediatric population. Patients (≤ 18 years) who were prescribed clopidogrel between March 2002 and August 2005 were retrospectively identified at Children's Hospital, Boston, MA. Data from the time of the first documented clopidogrel use to the most recent follow-up were collected and adverse events were classified according to seriousness and relationship to clopidogrel.

Among 90 patients, 53 percent were male, the median age was 6.7 years and the median weight was 23.6 kg at first clopidogrel use. Prescriptions were mostly for cardiac indications (96 percent) with the remainder for neurological indications (4 percent). Common cardiac indications included history of thrombosis involving a conduit or shunt, abnormal vasculature with potential for low flow and device placement. Median total dose was 1.3 mg/kg/day and duration of therapy varied greatly (median 45 days, < one day – four years). Minor bleeding occurred in three patients, all on concomitant acetylsalicylic acid (ASA). One patient experienced catastrophic bleeding after a vessel tear during catheterization. Another patient with poor ventricular function and a fenestrated Fontan (an operation performed on children with complex congenital heart defects) had baffle thrombosis and subsequent stroke while on clopidogrel and ASA.

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In conclusion, our study demonstrated that clopidogrel prescribed for children of all ages was well tolerated. Most prescriptions were for thrombosis prevention in patients with cardiac disease. Bruising and minor bleeding events occurred rarely. While various trials demonstrate the benefits of treatment with clopidogrel to prevent thrombotic events in adults, a need remains for further clinical trials examining the safety and efficacy of clopidogrel in children.[2,3,4] A qualitative prospective study would provide additional information about the burden of drug use on families, and could capture additional information about dosing, safety and efficacy that can improve the application of this drug in children.

REFERENCES:

1. *Physicians' Desk Reference.* 60 ed. Montvale, NJ: Thomson; 2006.
2. YUSUF S, ZHAO F, MEHTA SR, CHROLAVICIUS S, TOGNONI G, FOX KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*, 2001; 345:494–502.
3. CAPRIE STEERING COMMITTEE. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet*, 1996; 348:1329–39.
4. DIENER HC, BOGOUSSLAVSKY J, BRASS LM, CIMMINIELLO C, CSIBA L, KASTE M, ET AL. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. *Lancet*, 2004; 364:331–37.
5. FINKELSTEIN Y, NURMOHAMED L, AVNER M, BENSON LN, KOREN G. Clopidogrel use in children. *J Pediatr,* 2005; 147:657–61.
6. SOMAN T, RAFAY MF, HUNE S, ALLEN A, MACGREGOR D, DEVEBER G. The risks and afety of clopidogrel in pediatric arterial ischemic stroke. *Stroke*, 2006; 37:1120–22.

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Development of Class I HLA Typing Assay for HIV Clinical Trials in East Africa

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Highly polymorphic human leukocyte antigen (HLA) class I and II genes are important host genetic factors whose encoded molecules form intracellular complexes with pathogen-derived peptides and migrate to surfaces of infected cells and mediate adaptive immune responses. This process is fundamental to HIV infection and AIDS progression. While many relationships between HLA genotypes and HIV/AIDS are well established, scientists emphasize the need to study the influence of HLA alleles on the efficacy of HIV drugs.[1,2,3] This need is apparent in the development of a global HIV vaccine that targets the diverse populations of Sub-Saharan Africa, where an estimated 25 million people are infected with HIV.[4]

These HIV vaccine clinical trial participants are ideal candidates for studying HLA genotypes. Subsequent identification of these genotypes can in turn be used to interpret drug efficacy in participants.[1,5] "HLA typing" describes methods used to identify HLA alleles based on HLA gene sequences. Techniques usually involve conventional polymerase chain reactions (PCR) which incorporate sequence-specific primers (SSP), sequence specific oligonucleotide probes (SSOP/SSO) or sequence-based typing (SBT).[6] Real time PCR uses primer-probe combinations to distinguish HLA alleles based on fluorescence emissions and melting curve profiles, which are cheaper than SBT and do not require loading agarose gels which are prone to contamination. Many real-time PCR systems also use 384-well reaction plates, which produce large amounts of data in a short period of time. Such advantages have made it a popular method of HLA typing.[7]

I was part of a team developing an SSP-real time PCR assay for HLA class I typing to support ongoing HIV vaccine clinical trials in East Africa. During my time there, we developed an assay capable of distinguishing among 14 alleles of the HLA-A locus of HLA class I known to be predominant in 80–90 percent of East African populations. The completed system, which also will type the remaining HLA-B and

HLA TYPING

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HLA-C loci of HLA class I, will perform HLA class I typing for four individuals in less than two hours at a third the cost of SBT. Through such efficiency, our assay also can be performed in East African clinical trial field labs where other laboratory tests are performed at low costs.

Besides studying the efficacy of drugs in these settings, HLA genotyping data generated by our assay can be combined with HIV sequencing and epidemiological data from patients — using bioinformatics tools — to study the emerging selective pressures created by the AIDS epidemic.[8] Such premises were behind recently demonstrated "HLA footprints," which show how HIV viruses mutate and escape immunity in response to particular HLA types within a population.[9] This is just another suggestion for how large-scale HLA genotyping data can be used effectively.

Overall, through its ability to support the large-scale and cost-effective HLA genotyping needed for studying the efficacy of HIV vaccine candidates in East African clinical trials, this assay could be an important component in the design of a global HIV vaccine.

REFERENCES:

1. **CARRINGTON M, O'BRIEN SJ.** The influence of HLA genotype on AIDS. *Annu Rev Med*, 2003; 54:535–51.
2. **NOLAN D, GAUDIERI S, JOHN M, MALLAL S.** Impact of host genetics on HIV disease progression and treatment: new conflicts on an ancient battleground. *AIDS*, 2004; 18:1231–40.
3. **MCNICHOLL JM, DOWNER MV, UDHAYAKUMAR V, ALPER CA, SWERDLOW DL.** Host-pathogen interactions in emerging and re-emerging infectious disease: a genomic perspective of tuberculosis, Malaria, Human Immunodeficiency Virus Infection, Hepatitis B, and Cholera. *Annual Rev Public Health*, 2000; 21:15–46.
4. **EDITORS HOFFMANN C, ROCKSTROH JK, KAMPS BS, EDS.** *HIV Medicine,* 2006. 14th edition.
5. **VARDAS E, BUTTÒ S, GLASHOFF R, MALNATI MS, POLI G, CLERICI M.** Preparing for phase II/III HIV vaccine trials in Africa. *Microbes Infect*, 2005; 7:1436–44.
6. **ADAMS SD, KRAUSA P, MCGINNIS M, SIMONIS TB, STEIN J, MARINCOLA FM.** Practicality of High Throughput HLA Sequence-Based Typing. *ASHI Quarterly*, 2001;25.
7. **FANER R, CASAMITJANA N, COLL J, CARO P, PUJOL-BORRELL R, PALOU E, JUAN M.** Real-Time PCR Using Fluorescent Resonance Emission Transfer Probes for HLA-B Typing. *Hum Immunol,* 2006; 67:374–85.
8. **CAO K, MOORMANN, AM, LYKE KE, ET AL.** Differentiation between African populations is evidenced by the diversity of alleles and haplotypes of HLA class I loci. *Tissue Antigens*, 2004; 63:293–325.
9. **KLENERMAN P, MCMICHAEL A.** "Finding Footprints among the Trees." *Science*, 2007; 315:1505–07.

Gastric Stimulation for Vomiting, Nausea and Related Symptoms Associated with Gastroparesis Using Enterra™ System

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Gastroparesis is a stomach disorder characterized by delayed gastric emptying. Patients with gastroparesis suffer from a number of gastrointestinal (GI) symptoms, including bloating, nausea, vomiting, early satiety, postprandial fullness, epigastric pain and heartburn. Gastroparesis has become an increasingly common medical condition among the United States population, affecting more than 1.5 million people. No cure for this disorder currently exists.

Non-operative therapies for patients with gastroparesis include diet modification, drug therapy (including Prokinetics and Anti-emetics) and nutritional feeding via gastric or jejunal feeding tubes. However, these approaches can negatively affect the patient's quality of life. Surgical procedures include gastrectomy, pyloroplasty and gastrojejunostomy. However, these procedures have provided limited success and are prone to complications.

The George Washington University Medical Center is one of 10 clinical sites in the U.S. participating in a novel treatment study for patients with gastroparesis. Currently, 50 patients are enrolled in the study, with plans to enroll more patients in the future. This clinical trial is a double-blinded, randomized study to evaluate the safety and effectiveness of gastric stimulation on the frequency of nausea and vomiting in patients with gastroparesis. The procedure involves a surgical implant of an Enterra™ electrical stimulator, provided by Medtronic, Inc., onto the body of the stomach. Interim results of the clinical trial, organized by Medtronic, show that gastric stimulation reduces symptoms of nausea and vomiting in patients for whom drug therapy was unsuccessful. The goal of this study is to induce a sustained reduction in the often debilitating symptoms of this disease.

Patients were asked to fill out a monthly diary of their symptoms prior to surgery to assess their baseline frequency of nausea and vomiting, early satiety, bloating, postprandial fullness, epigastric pain, epigastric burning, chest pain and chest burning. Several study-related tests are performed to determine if the patient qualifies for the study. An implant is placed inside the abdomen onto the body of the stomach and the electrical stimulation begins at the time of surgery. After six weeks, patients are randomized to either the "On" or "Off" position for three months, then set to the opposite group for three additional months. Patients are required to attend scheduled follow-up appointments at three, six, nine and 12 months post-implantation and annually thereafter until the study is closed. At each follow-up appointment, subjects are assessed for symptom severity, frequency, complications and adverse events, health status, quality of life and normal daily activities. The following symptoms are assessed using a Likert scale of zero (absent) to four (extremely severe): vomiting, nausea, early satiety, bloating, postprandial fullness, epigastric pain, epigastric burning, chest pain, chest burning and other symptoms.

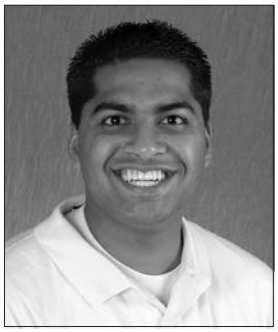
The results from baseline to 12 months noted a decrease in the frequency of vomiting, nausea, early satiety, bloating, postprandial fullness, epigastric pain, epigastric burning, chest pain, chest burning and other symptoms. Secondly, there was a significant decrease in vomiting at each follow up appointment: three, six, nine and 12 months. Lastly, there was a significant decrease in the total severity scores and frequency scores at each three month interval up to 12 months.

This study demonstrated that Enterra™ gastric stimulation is an effective and safe treatment option for the reduction of the severity and frequency of symptoms associated with gastroparesis. This treatment has improved the quality of life for many people and has allowed them to carry out a normal lifestyle and feel in control of their symptoms. Results of this study will be used to support a pre-market approval application (PMA) to the U.S. Food and Drug Administration.

REFERENCES:

1. MEDTRONIC. *Gastroparesis Fact Sheet.* 2007; Retrieved July 14, 2007 from *http://wwwp.medtronic.com/Newsroom/LinkedItemDetails.do?itemId=1101836121811&itemType=fact_sheet&lang=en_US*.
2. FOX J, FOXX-ORENSTEIN A. *Gastroparesis*. 2006; Retrieved July 14, 2007 from *http://www.gi.org/patients/gihealth/gastroparesis.asp*
3. MEDTRONIC. *Enterra™ Therapy: Gastric Electrical Stimulation (GES)*. 2007; Retrieved July 14, 2007 from *http://www.medtronic.com/neuro/enterra/*.

Breast-Specific Gamma Imaging as an Adjunct Modality for the Diagnosis of Invasive Breast Cancer



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Mammography has remained the modality of choice for breast cancer screening with a sensitivity of 78-85 percent.[1-4] However, this percentage declines significantly for women with dense breasts and sub-centimeter cancers. Limitations such as these have resulted in the development of adjunct imaging modalities to improve breast cancer detection.

One such method involves nuclear medicine techniques, in which physiological properties of tumors are utilized to effectively make a diagnosis. Most common among these is the use of 99mTc-Sestamibi with a general purpose gamma camera. Although this modality has improved breast cancer detection by a large margin, it has been found to be inadequate in the discovery of non-palpable lesions and those less than one centimeter in size due to intrinsic resolution limitations.[5–8]

FIGURE 1: BREAST SPECIFIC GAMMA IMAGING. Patient with a multifocal infiltrating ductal carcinoma of the left breast seen as separate areas of increased radiotracer uptake in the craniocaudal view.

To resolve these difficulties, a small field-of-view camera was developed that allows high-resolution detection of breast lesions.[9] This technique, Breast Specific Gamma Imaging (BSGI), is a substantial improvement over the general purpose gamma camera model. The design of BSGI allows one to reliably image sub-centimeter lesions due to its improved resolution and shape which is optimized for breast imaging. Furthermore, images acquired from the device are in projections correlated to the medial-lateral and craniocaudal views of traditional mammography, allowing images to be interpreted in a straightforward manner. Until now, there have been limited studies defining the sensitivity of this emerging modality. This study is the largest to date, determining the sensitivity of BSGI for the diagnosis of invasive breast cancer.

Our project was a retrospective review of 139 consecutive women with biopsy-proven invasive carcinoma from November 2004 to June 2007. The images acquired from the BSGI were subjectively categorized based on the degree of focal radiotracer uptake. Lateralization and region of the focal area of increased radiotracer uptake were noted and compared to the laterality and location of the biopsy proven invasive cancer. The clinical report in the patient's record was used for BSGI interpretation and cases were not reinterpreted for the study.

Out of the 149 invasive cancers identified, 146 were recognized with BSGI, totaling a sensitivity of 98 percent for the BSGI detection of invasive breast cancers. There were three cancers unidentified by BSGI, each being of grade 1 histology and sub-centimeter lesion size. One explanation for the inability of BSGI to find these three pathologically proven invasive cancers may be due to the posterior and otherwise difficult to image area of the breast. In the case of one patient, the small size of the breast along with a posterior location may be the cause for the non-visualization of the lesion. Despite these limitations, BSGI was still able to identify 40 percent (2/5) sub-centimeter, grade 1 tumors. Moreover, our study showed four out of 149 infiltrating ductal carcinoma cases that were pathologically categorized as multifocal. BSGI was able to correctly identify the multifocality in all instances.

Our results demonstrate that BSGI is a highly sensitive method of diagnosing invasive breast cancer. This study, using the largest sample size of invasive cancers studied with BSGI to date, along with data in the literature, also suggests that BSGI is a reliable adjunct imaging modality.

REFERENCES:

1. ROSENBERG ED, HUNT WC, WILLIAMSON MR, ET AL. Effects of age, breast density, ethnicity and estrogen replacement therapy on screening mammographic sensitivity and cancer stage at diagnosis: review of 183,134 screening mammograms in Albuquerque, NM. *Radiology,* 1998. 209:511–18.
2. KOLB TM, LICHY J, NEWHOUSE JH. Comparison of the performance of screening mammography, physical examination and breast US and evaluation of factors

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that influence them: an analysis of 27, 825 patient evaluations. *Radiology*, 2002; 225:165–75.

3. **KHALKHALI I, MENA I, JOUANNE E, ET AL.** Prone scintimammography in patients with suspicion of carcinoma of the breast. *J Am Coll Surg*, 1994; 178:491–97.
4. **KHALKHALI I, CUTRONE JA, MENA IG, ET AL.** Scintimammography: the complementary role of Tc-99m sestamibi prone breast imaging for the diagnosis of breast carcinoma. *Radiology*, 1995; 196:421–26.
5. **MEKHMANDAROV S, SANDBANK J, COHEN M, ET AL.** Technetium 99-m MIBI scintimammography in palpable and nonpalpable breast lesions. *J Nucl Med,* 1998; 39:86–91.
6. **ARSLAN N, OZTURK E, ILGAN S, ET AL.** Tc-99m MIBI scintimammography in the evaluation of breast lesions and axillary involvement: a comparison with mammography and histopathological diagnosis. *Nuc Med Commun*, 1999; 20:317–25.
7. **FENLON HM, PHELAN NC, O'SULLIVAN P, ET AL.** Benign versus malignant breast disease: comparison of contrast-enhanced MR imaging and Tc-99m tetrofosmin scintimammography. *Radiology*, 1997; 205:214–20.
8. **PALMEDO H, BENDER H, GRUNWALD F, ET AL.** Comparison of fluorine-18 fluorodeoxyglucose positron emission tomography and technetium-99m methoxyisobutylisonitrile scintimammography in the detection of breast tumours. *Eur J Nucl Med*, 1997; 24:1138–45.
9. **MAJEWSKI S, KIEPER D, KEPPEL C.** Optimization of dedicated scintimammography procedure using small detector prototypes and compressible breast phantoms (abstr). Presented at the IEEE Medical Imaging Conference, Lyon, France. October 2000.

Religious Experience in Epilepsy

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The ancient association between epilepsy and having a concurrent religious/spiritual experience has persisted through the ages and remains to the present day. People with epilepsy have been reported to have heightened religious experiences and spiritual orientations from a clinical perspective. In particular, people with temporal lobe epilepsy (TLE) have been described as intensely religious, preoccupied with the study and practice of spirituality/religion and frequently report intense spiritual/religious experiences. Waxman and Geschwind (1975) proposed an Interictal TLE Personality citing "hyperreligosity," an excessive preoccupation with religion/spirituality, as a distinct behavioral trait among its probable characteristics.[1]

The TLE Interictal Personality was further investigated by Bear and Fedio (1977) using a structured personality inventory and hyper-religiousness was among the set of 18 personality traits consistently observed in TLE patients.[2] The proposition of an interictal TLE Personality has been controversial and our attempt to verify this syndrome, in addition to other studies, have been met with mixed results.

Our study evaluated the religiousness/spirituality of TLE patients as compared with patients with primary generalized epilepsy and other types of focal epilepsy to determine whether TLE patients are more religious/spiritual than other epileptics. We hypothesized that TLE predisposes individuals to a heightened sense of religiousness/spirituality, also associated with neuroplastic changes, the net effect of which is to enhance sensory activation of the limbic system. A systematic survey of religious experience in epilepsy, with a focus on TLE, was conducted with a more comprehensive questionnaire on spirituality/religion than previously administered.

Data for this study was collected from adults admitted to the New York University Comprehensive Epilepsy Center inpatient video-EEG monitoring unit between April 16, 2001 and Sept. 16, 2002. The study population consisted of 151 patients with various types of epilepsy. The Brief Multi-Dimensional Measure of Religion/Spirituality for use in Health Research (BMMRS) was used to assess individual religiousness/spirituality.[3] The BMMRS is a 39-item questionnaire developed by the Fetzer Institute in association with the National Institute on Aging (1999) that examines 11 different domains of religiousness/spirituality which have been identified as related to physical and mental health.

We found TLE patients are no more religious/spiritual than other epilepsy patients

RELIGION AND EPILEPSY *Continued on p. 36*

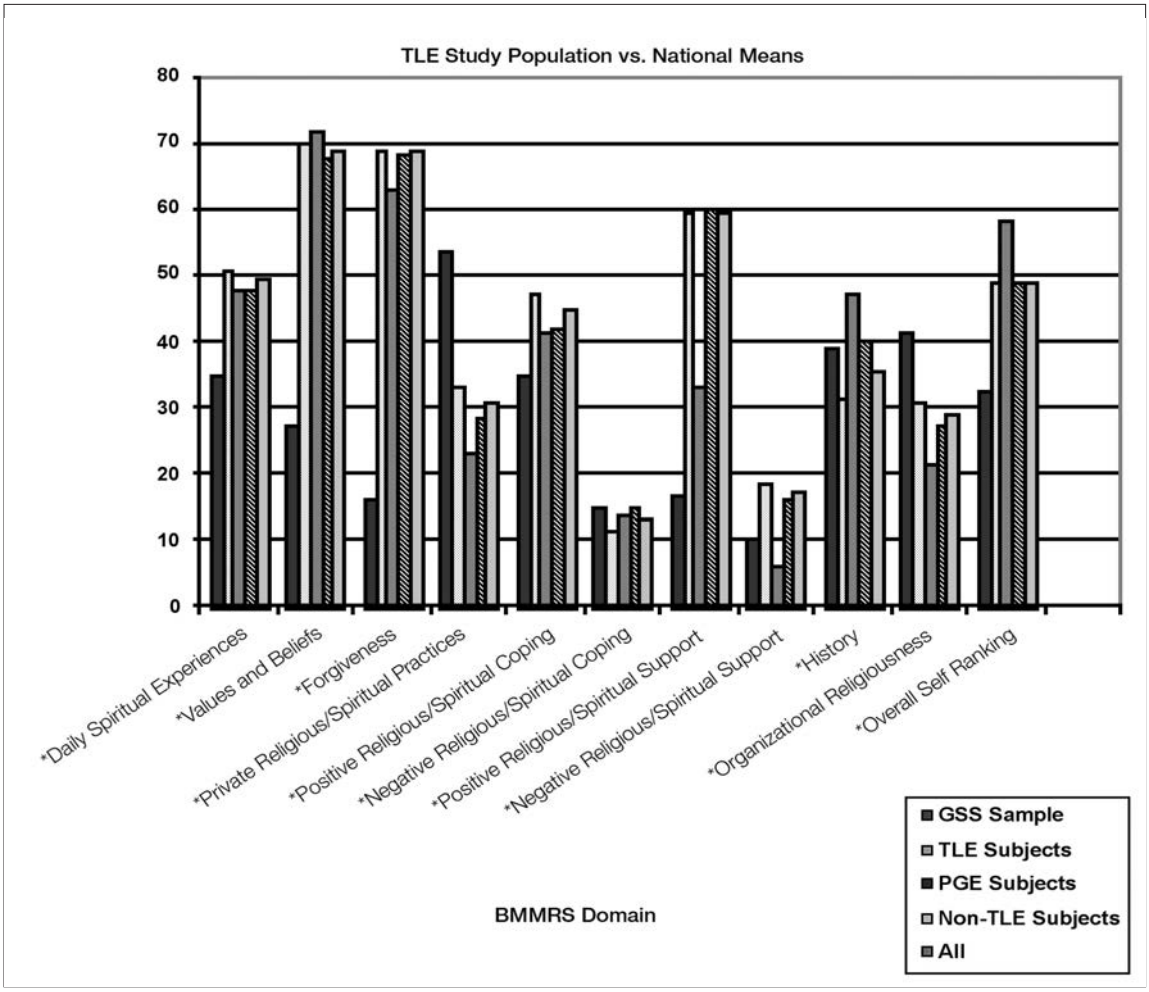

FIGURE 1: BMMRS DOMAIN SCORES REPRESENTED BY OUR STUDY POPULATION (TLE SUBJECTS) VS. THE GENERAL POPULATION (GSS SAMPLE). Scores are scaled from 0-100 with higher scores indicating experiences with a greater degree of religiousness/spirituality.

*Significant Differences (p value < .05) in the BMMRS mean scores of TLE subjects vs. the general population (GSS Sample) were found in nine out of the 11 BMMRS domains:

Daily Spiritual Experiences ($p < .0001$); Religious/ Spiritual Values and Beliefs ($p < .0001$); Forgiveness ($p < .0001$); Private Religious/ Spiritual Practices ($p < .0001$); Positive Religious/Spiritual Coping ($p = .0010$); Positive Religious/Spiritual Support ($p < .0001$); Organizational Religiousness ($p = .0017$); Negative Religious/Spiritual Support ($p = .0430$); Overall Self-Ranking ($p < .0001$).

There were no significant differences in Religious/Spiritual History and Negative Religious/ Spiritual Coping domains.

RELIGION AND EPILEPSY *Continued from p. 35*

and report fewer religious conversion experiences. Upon comparison of the study population with the BMMRS normative population distribution data from the 1998 General Social Survey, we found epilepsy patients as a whole are more religious/spiritual than the general population.[4]

Our findings refute the purported "hyperreligiosity" associated with TLE. No direct causal relationship can be established between seizure discharges in the temporal lobe and deepened religiousness/spirituality. The religious/spiritual preoccupation and intense religious conversion experiences proposed as part of the constellation of TLE personality traits is not supported by our data. Heightened religiousness/spirituality may be a trait common among all epileptics, regardless of epilepsy type and seizure origin.

REFERENCES:

1) **GESCHWIND N, WAXMAN SG.** The interictal behavior syndrome of temporal lobe epilepsy. *Archives of General Psychiatry*, 1975; 32: 1580–86.

2) **BEAR DM, FEDIO P.** Quantitative analysis of interictal behavior in temporal lobe epilepsy. *Archives of Neurology*, 1977; 8:454–67.

3) **FETZER INSTITUTE/NATIONAL INSTITUTE ON AGING WORKING GROUP.** Multidimensional Measurement of Religiousness/ Spirituality for Use in Health Research: a report of the Fetzer Institute/National Institute on Aging Work Group, 1999; Kalamazoo, MI: John E. Fetzer Institute.

4) **DAVIS JA, ET AL.** *General Social Survey.* The National Data Program for Social Sciences, 1998; National Opinion Research Center, University of Chicago.

A Geometrical Forefoot Model: Relationship to Foot Function

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In collaboration with the Motion Analysis Laboratory of Leon Root, MD, biomechanical foot function was studied using a geometrical forefoot model designed to assess and treat pedal pathology. Malleolar valgus index (MVI) and center of pressure excursion index (CPEI) were developed to measure static and dynamic foot function.[1] Many sophisticated and complex foot models have been created, however, none have been used for clinical decision making.[2,3] A simplified mathematical approach was developed by Demp to model metatarsal length patterns from dorsoplantar X-rays by unique conic curves and their eccentricities.[4,5] Our study explores the association between these model parameters and the biomechanical foot function in healthy asymptomatic individuals.

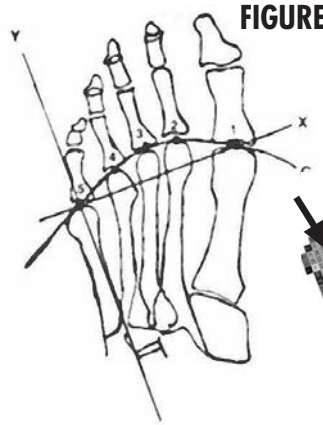

FIGURE 1

The Demp forefoot model (Figure 1) constructs a conic curve through the metatarsophalangeal joint (MTPJ) centers. Five MTPJ x,y coordinates are used to solve the equation for a unique conic curve:

$$AX2 + BX + CXY + DY + EY2 = 0$$

where A, B, C, D and E are the resulting five coefficients. In this study, medial and lateral aspects of each MTPJ were calculated with a 3-D digital pointer. The midpoint of each medial and lateral MTPJ joint space was considered to be the MTPJ center. A forefoot coordinate system was generated such that the x-axis projected from the fifth MTPJ to the first MTPJ, the y-axis was perpendicular to that vector, and the origin was at the fifth MTPJ. Using Maple software, conic curves were created and the possible curve types were: ellipse, parabola and hyperbola. Eccentricity, which is a constant that describes the shape of a conic curve, was calculated as follows:

$$E = (P \times F1)/(P \times D1) = (P \times F2)/(P \times D2)$$

where P is the pressure, F is the focus and D is the distance from the center to a vertex. We hypothesized that feet with normal rectus alignment and function had a hyperbolic conic curve describing their MTPJ relationships with an eccentricity greater than one.[4,5]

Barefoot plantar pressures were measured during one's comfortable walking speed with the Novel emed X system. The plantar loading parameters (peak pressure, peak force, pressure-time integral) were measured in each anatomical region of the foot (hallux, first–fifth MTPJs, medial arch, lateral arch, medial heel and lateral heel) using a masking algorithm. In Figure 2, the maximum pressure throughout the stance phase of a healthy asymptomatic person (*right*) and a person with diabetes and HV (*left*) is illustrated. The areas of high pressure are indicated by the arrows. Note that the subject with diabetes and HV has higher plantar pressures and a reduced CPEI indicative of overpronation as compared with the healthy individual. Using our simplified geometric forefoot model, conservative or surgical treatments may be modified to optimally convert a pathological foot to a healthy asymptomatic foot with improved biomechanical function.

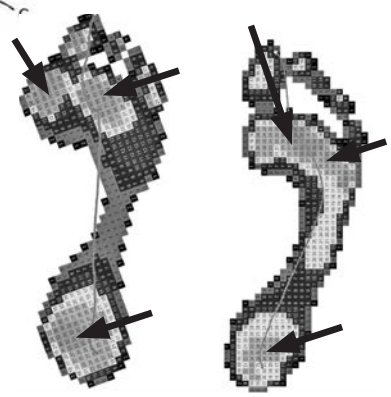

FIGURE 2

REFERENCES :

1. **SONG J, ET AL** Foot type biomechanics: comparison of planus and rectus foot types. *JAPMA*, 1996; 86:16–23.
2. **CAMACHO DL, ET AL** A three-dimensional, anatomically detailed foot model: A foundation for a finite element simulation and means of quantifying foot-bone position. *J Rehab Res Dev,* 2002; 39:401–10.
3. **GILCHRIST LA, WINTER DA** A two-part, viscoelastic foot model for use in gait simulations. *J. Biomech,* 1996; 29:795–8.
4. **DEMP PH** A mathematical model for the study of metatarsal length patterns. *JAPMA*, 1964; 54:107–110.
5. **DEMP PH** Geometric Models that Classify Stuctural Variations of the Foot. *JAPMA*, 1998; 88:437–41.

FIGURE 1: CONIC CURVE MODEL.
FIGURE 2: PEAK PLANTAR PRESSURES: healthy (right), HV (left).

public health issues. . .

Barriers to Therapeutic Cancer Vaccine Development

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Therapeutic cancer vaccine development is an exciting and rapidly evolving discipline of biotechnology. However, it is undoubtedly burdened by a variety of obstacles that need to be addressed before success can be achieved.

Therapeutic vaccines energize the body's immune system to recognize and attack established tumors that are otherwise ignored by normal physiologic immune surveillance.[1] Prophylactic vaccines, on the other hand, are administered to healthy individuals and are designed to prevent infection from cancer-causing viruses.[2]

As of August 2007, two prophylactic cancer vaccines had been approved by the Food and Drug Administration (FDA). In 2000, ENGERIX-B was approved to prevent primary liver cancer as a result of chronic hepatitis B infection. In 2006, Gardasil was approved to prevent infection from some of the most common strains of human papillomavirus that cause cervical cancer.[2] Currently, the scope for cancer prophylaxis is limited because few cancers are caused by viruses.[3]

Therapeutic cancer vaccines, however, have the potential to combat nearly any type of established tumor. Yet, so far none have reached the market by earning FDA approval.[4,5] This lack of success can be attributed to both scientific and regulatory barriers.

As a collection of altered self cells, tumors contain many of the same identification markers as normal cells of that tissue. That, in part, makes it difficult to convince the immune system to attack and eradicate tumors.[6] While a variety of tumor-associated antigens have been identified and many immunotherapeutic strategies have been tested, objectively defined clinical responses are rare. The reasons for this include the inability of current approaches to generate efficient T-cell responses, the presence of regulatory cells that inhibit T-cell responses and tumor escape mechanisms.[7]

While scientific innovation remains the principal factor for determining success, a paradigm shift in three regulatory factors could greatly facilitate the development process.

First, the current regulatory system is geared to support single-agent drug approvals, yet combinations may be necessary. There is an increasing trend in therapeutic vaccine development toward the use of multiple antigens and adjuvants. Attacking cancers from several angles may yield synergistic and clinically significant results.[1]

Second, traditional efficacy endpoints, which are based on quantitative tumor assessments, may not always adequately assess clinical benefit (i.e., survival and quality of life). Tumor response and patient response are not necessarily mutually inclusive.[8] Thus, when a candidate vaccine meets specified safety standards, various efficacy criteria should be considered to account for this potential discrepancy.

While a variety of tumor-associated antigens have been identified and many immunotherapeutic strategies have been tested, objectively defined clinical responses are rare.

And third, current designs of clinical trials can be made more efficient and effective. The adaptive clinical trial approach, which employs Bayesian statistical methods, offers a scientifically rigorous alternative to the classic design.[9] This approach is suitable in studies as information accrues during a trial, potentially allowing for smaller and more informative trials and enabling patients to receive better treatments. Furthermore, this approach better handles combination therapies and multiple endpoints.[10]

Implementing these changes may help bring quicker approvals of safe and efficacious

VACCINE BARRIERS *Continued on p. 39*

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therapeutic cancer vaccines. That, in turn, would encourage further innovation, leading to improved treatments that can increase the duration and improve the quality of life.

REFERENCES:

1. **PARDOLL D, ALLISON J.** Cancer immunotherapy: breaking the barriers to harvest the crop. *Nat Med*, 2004; 10:887–92.
2. **SOBOL RE.** The rationale for prophylactic cancer vaccines and need for a paradigm shift. *Cancer Gene Ther,* 2006; 13:725–31.
3. **PBR STAFF.** Can therapeutic vaccines fulfill their promise? *Pharmaceutical Business Review*. Jan. 24, 2006. *http://www.pharmaceutical-business-review.com/article_feature.asp?guid=6A311756-BFD0-4F94-9D6B-7F3F514275A9*.
4. **EASTMAN P.** FDA Commissioner pledges help in bringing new immunotherapies to cancer patients. *Oncology Times,* 2007; 29:29–30.
5. **FOX JL.** Uncertainty surrounds cancer vaccine review at FDA. *Nat Biotechnol*, 2007; 25:827–28.
6. **PARDOLL D.** Does the immune system see tumors as foreign or self? *Annu Rev Immunol,* 2003; 21:807–39.
7. **WARD RC, KAUFMAN HL.** Targeting costimulatory pathways for tumor immunotherapy. *Int Rev Immunol*, 2007; 26:161–96.
8. **SCHLOM J, ARLEN PM, GULLEY JL.** Cancer vaccines: moving beyond current paradigms. *Clin Cancer Res*, 2007; 13:3776–82.
9. **GOTTLIEB S.** 2006 Conference on adaptive trial design, Washington, DC. U.S. Food and Drug Administration. July 10, 2006. Retrieved from *http://www.fda.gov/oc/speeches/2006/trialdesign0710.html*.
10. **BERRY DA.** Bayesian clinical trials. *Nat Rev Drug Discov*, 2006; 5:27–36.

Increasing Awareness and Access to Emergency Contraception

Each year, approximately 750,000 to 850,000 adolescent women become pregnant in the United States, and of these pregnancies, 74 to 95 percent are unintended or unwanted.[1] Teenage mothers are less likely to receive timely prenatal care and are more likely to smoke during pregnancy. Babies born to teenage mothers are at increased risk of low birth weight, pre-term birth, serious chronic illnesses or developmental delays.[2] Due in part to these significant outcomes, understanding teen pregnancy and assisting teens who wish to delay childbearing is an issue of increasing public health and medical importance.

Recent estimates suggest that approximately 46 percent of high school students and 80 percent of college students between 18 and 24 have had sex.[3] The National Longitudinal Study of Adolescent Health found that sexual activity during adolescence is associated with inconsistent or no use of contraception.[4] Marginalized youth, including youth of color, youth in foster care, and low-income youth are more likely to be confronted with financial, cultural and institutional barriers to obtaining health care which may contribute to their increased risk of negative sexual outcomes.[5] Developing health programs to address teen pregnancy, especially among high-risk populations, is a major challenge requiring multi-level approaches.

In 2006, the Harlem Health Promotion Center (HHPC) began developing an Emergency Contraception (EC) awareness campaign to address the dire need for enhanced contraception options amongst its high-risk Black and Latino patient populations. To date, there is a deficiency in culturally specific and tailored intervention materials for this population, and research indicates high rates of teen pregnancy coupled with a lack of awareness about EC.[6] This project titled "EC as 1, 2, 3" utilizes community-based participatory research and social marketing techniques.

This model relied on a youth development theory, and allowed youth to actively participate. For the summer of 2006, a group of 10 Black and Latino "youth experts" between the ages of 15 and 20 were selected to participate. These "experts" assisted with major tasks, including incorporating the findings of formative research with experiential knowledge. The participants also worked with a Harlem-based filmmaker and design students from Parsons New School of Design. Additionally, as the Youth Advisory Board Coordinator, I developed and coordinated a comprehensive youth development curriculum which included leadership and professional

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development, critical social analysis and media training for the affiliated youth.

In May 2007, the project materials, which included an educational DVD, brochure, posters and Web site were launched for use with the Center's Mobile Health Team outreach to high-risk youth and for use in training and educational sessions with medical, nursing, and public health students and clinical providers. The overall project succeeded in producing outstanding educational materials for youths. As one youth stated, "they were ready to hear our voice and make it an echo for change."

REFERENCES:

1. ABMA J, CHANDRA A, MOSHER WD, PETERSON L, PICCININO L. Fertility, family planning, and women's health: new data from the 1995 national survey of family growth. Hyattsville, MD: National Center for Health Statistics; 1997.
2. VENTURA SJ, MATHEWS TJ, HAMILTON BE, VENTURA SJ, MATHEWS TJ, HAMILTON BE. Births to teenagers in the United States, 1940–2000. *Natl Vital Stat Rep*, 2001; 49:1–23.
3. GRUNBAUM JA, KANN L, KINCHEN SA, ET AL. Youth risk behavior surveillance: United States, 2001. *J School Health*, 2006; 72:313–28.
4. MANLOVE J, RYAN S, FRANZETTA K. Contraceptive use and consistency in U.S. teenagers' most recent sexual relationships. *Perspect Sex Reprod Health*, 2004; 36:265–75.
5. AUGUSTINE J, ALFORD S, DEAS N. Youth of color: at disproportionate risk of negative sexual health outcomes. *Advocates for Youth*, 2004.
6. COHALL AT, GUPTARAK M, BADI A. EC as 1, 2, 3 project proposal. Harlem, NYC: Harlem Health Promotion Center, 2005; 32.

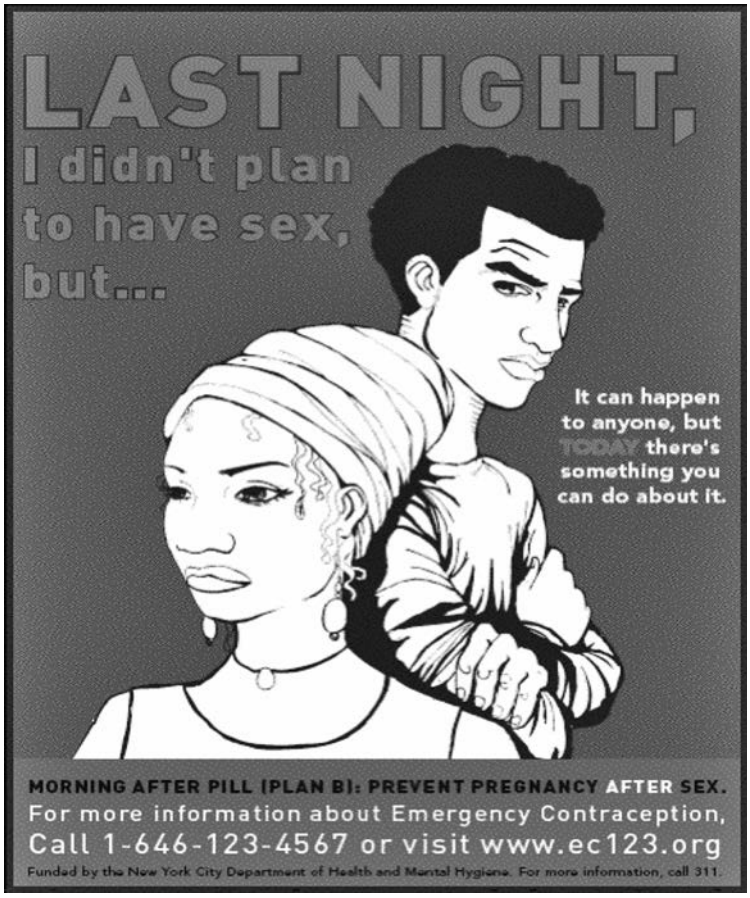

FIGURE 1: ADVERTISEMENT FOR "EC AS 1,2,3" CAMPAIGN FOR EMERGENCY CONTRACEPTION.

travel abroad. . .

Medical Relief Efforts in Jammu, India

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Since India attained independence in 1947, Jammu and Kashmir have been in a state of political and social turmoil due to tensions between Hindus and Muslims. In 1990, tensions between the groups peaked and many families had no choice but to leave their homes and start fresh. Seventeen years later, Kashmiris are still trying to get their lives back together and move on from the terror they experienced.

FIGURE 1: MOTHER BATHING HER DAUGHTER AT A WATER PUMP IN A MIGRANT CAMP IN JAMMU.

In the summer of 2007, I traveled with support from the American Association of Physicians of Indian Origin to Jammu, India. Under the guidance of Dr. K.L. Chowdhury and his staff, I worked inside migrant camps and at the Shirya Bhatt Charitable Hospital in Jammu, India.

Diabetes has increased in prevalence within the Kashmiri migrant community. About 25 percent of the adult population aged 18 and over suffers from diabetes, and nearly 33 percent from hypertension. The clinic suggested gathering information on the management costs of diabetes in terms of medications and monitoring, as well as the type of diet and the current blood glucose levels. I prepared a survey and went to the "quarters" of about 80 families and found that approximately 25 percent of their monthly income went toward diabetes medications and blood sugar testing for one person.

More valuable was learning about the lifestyle changes each family made upon migration. Each family was given a 10' by 15' room in which to live in. A group of about 10 families was expected to use one communal bathroom, one water pump and to live without privacy from their neighbors. In that one room, children would sleep, play and study while their parents cooked and washed clothes.

Health care was also a luxury. I remember coming across a man lying on the dirt floor in front of his quarter with a measured hemoglobin of 3 and fever of 104 degrees. His children, ages 10 and 8, stood above his head, his wife and mother pressing his feet and legs. His elder brother stared at him with fear in his eyes. His pale skin and weak, unmoving body made me think he was going to pass away before me. The nearest hospital was 20 miles away, and there was no ambulance to take him, nor were the funds available to pay for his care. A previous doctor had recommended a blood transfusion, but there was no Type A blood type match in his family.

As a medical student, I was unable to help. I could not offer any physical comfort. Instead, he gave something to me. He reaffirmed my reasons for aspiring to become a physician: to provide relief and support. I want to work in this type of community: where resources are sparse, and the need is great. Dr. Chowdhury and his staff amazed me with their tireless efforts to aid this community and demonstrated how effort and passion can create positive change.

A Place of Learning: The Mondaña Clinic, Ecuador

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During the three-hour canoe ride to Mondaña, the sun pierces through the dense foliage of the Ecuadorian rain forest as the canopy rises and falls along the river's shore. My goals while in this beautiful setting were to involve myself in the community healthcare system and patient population through education, conversation and treatment. In the quiet of the headwind, I wondered what challenges lay ahead.

The Mondaña Clinic was started in 1995 by The Yachana Foundation to address the needs of a large, medically underserved population. The Clinic, located along the Upper Napo River in the Ecuadorian rain forest, now serves local Mondaña and 25 surrounding communities, with a patient population of more than 10,000. These communities are spread over a wide geographic area, many accessible only by canoe or long hikes, making access to health care difficult. Nonetheless, the clinical staff makes regular visits. I traveled on such a visit and was privileged to share meals with families where I learned about the local history, culture and health. The Mondaña Clinic is the cornerstone of health care in the area, and visits are a critical part of the Clinic's responsibilities. They represent the beginnings of a growing effort to provide for marginalized populations within remote regions of Ecuador.

FIGURE 1: APPROXIMATE LOCATION OF THE MONDAÑA CLINIC IN ECUADOR.

In the Clinic, the lack of modern diagnostic technology forces doctors to rely on signs and symptoms to differentiate diagnoses and treat patients. I was challenged to learn these practical skills promptly. During my time in the rain forest, I saw a variety of illnesses, the most common of which were parasitic infections, the common cold or gripe, and malnutrition. I learned a great deal about the ways in which these illnesses are being addressed and treated. For example, because many of these communities have no running or potable water, the Yachana Foundation developed a cheap water filtration system to improve community sanitation and thereby reduced parasitic and bacterial infections. To address insufficient health education, I spent time teaching and interacting with the students from the local Yachana High School. I had the opportunity to discuss health and hygiene and to design and implement a first responder and sex-education course.

The Yachana Foundation was started to address the health care needs of the underserved communities along the Upper Napo River and has now broadened its focus to include education, eco-tourism, rain forest conservation, micro enterprise and micro lending. The Foundation is so deeply involved in the lives of the surrounding communities that it has become an integral part of health, education and preservation of the Upper Napo River. This was a unique opportunity for me to learn about and make an impact on the health care and education of a large, underserved community. I observed, firsthand, what one organization is doing to preserve one of the world's richest biological resources and improve the lives of its inhabitants. It came as no surprise to me when I discovered that, in the indigenous Quichua language, the word Yachana means, "a place of learning."

REFERENCES:

1. Foundation for Integrated Education and Development: Community Healthcare in the Amazon Rain Forest. Retrieved from *http://www.funedesin.org/html/health-care.htm*. Accessed Oct. 8, 2007.

other abstracts. . .

Development of a Longitudinal Genetics Curriculum

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Genetics is an ancient science. Yet, recently, with the description of the DNA molecular structure and the completion of the sequencing of the human genome, much more information relevant to the practice of medicine is being revealed. The evolution of the field from a basic descriptive science of Mendelian genetics, drosophila and other organisms, to cancer biology, molecular immunology, cardiovascular genomics and pharmacogenomics has made the management of curricula change in U.S. medical schools seem slow and fragmented. The ensuing gap between available information and knowledge of medical students, residents and practitioners, poses a challenge for teaching and the dissemination of this vital information.

Pre-curriculum medical student surveys indicated deficient knowledge and comfort with genetics. Ninty-seven percent of the students were comfortable speaking with patients about family history, but 31 percent did not feel comfortable discussing a diagnosis of a genetic disease.

Although most medical schools have developed genetics courses for first- and second-year medical students, the integration of basic science and clinical medicine has lagged. In addition, exposure to genetics in the early years of medical school and having to recall and apply it in the clinical years is a difficult task for most students. This educational shortcoming calls for the implementation of a genetics curriculum for medical students in their clinical years. Addressing this disparity in genetics education during the medical school years can improve the knowledge base of future clinicians in these regards.

In recognition of this educational deficit, researchers at The George Washington University School of Medicine and Health Sciences (SMHS) designed a genetics curriculum for third-year medical students. A needs assessment, and pre- and post-test about medical genetics was developed. As a pilot study, the survey and pre-test were administered to third-year students during their OB/GYN clerkship. The curriculum in genetics reinforced genetic concepts learned in the first two years of medical school and introduced their clinical application. The curriculum, administered through Blackboard®, was case-based using an organ-system approach, and introduced students to relevant online genetics resources. Students were presented clinical vignettes on genetic diseases and answered clinically relevant questions about these disorders. The curriculum was designed with principles of adult learning theory to emphasize skills in self-directed learning. After completing the curriculum, students took a post-test and their scores were compared to their pre-test scores. Students then completed an exit survey. In the pilot study, 17 students completed the needs assessment, and pre- and post-test. Twelve of 17 students showed improved scores, with a mean increase of 11.5 percent. Five of 17 failed to demonstrate improvement, with a mean decline of 4.5 percent.

Pre-curriculum medical student surveys indicated deficient knowledge and comfort with genetics. Ninety-seven percent of the students were comfortable speaking with patients about family history, but 31 percent did not feel comfortable speaking about a diagnosis of a genetic disease. In the exit survey, students expressed the need for a more robust genetics education curriculum beginning in the second year. They indicated overall satisfaction with our experimental curriculum, particularly the online resources, and recommended its future use.

The addition of this curriculum into the current GW SMHS curriculum would make medical genetics education more longitudinal, reinforce genetics concepts in a clinical setting, and assist students in developing self-directed learning skills to be better equipped to find genetics information throughout their careers. The curriculum will be administered to 180 GW medical students between July 2007 and June 2008.

index. . .

BASIC SCIENCE ABSTRACTS

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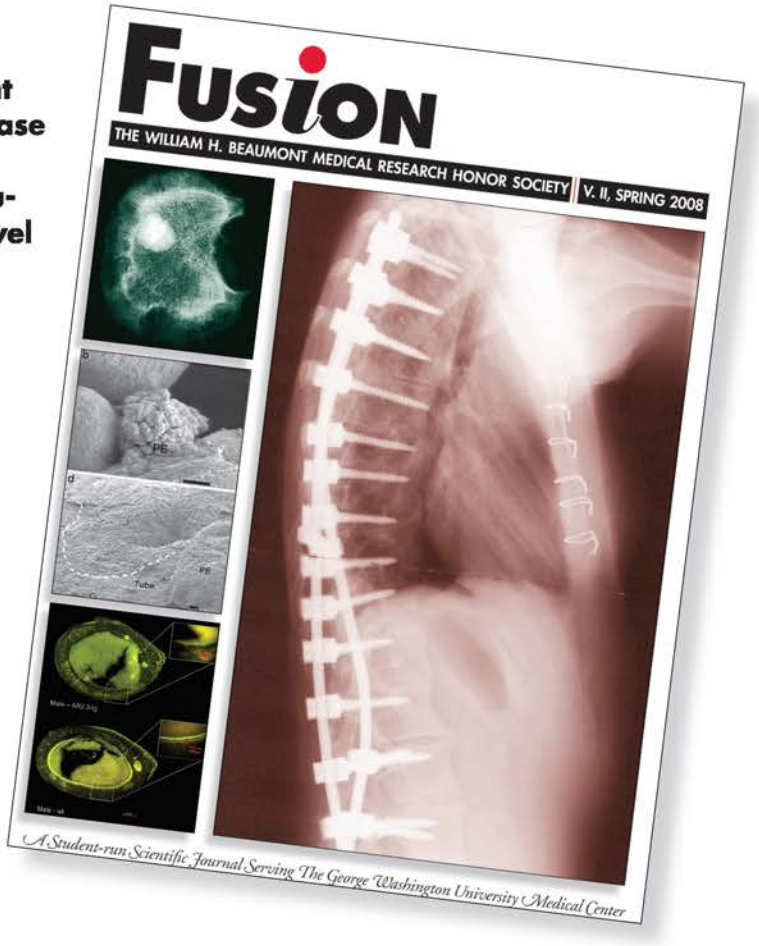
planning ahead. . .

Fusion **is the annual student-run scientific journal of the GW William H. The Beaumont Medical Research Society created to showcase student achievements in basic science and clinical research, public health, medical education and international health-related travel experiences.**

Submissions for next year's issue of the journal will be accepted beginning September 2008 from the Class of 2011 as well as the incoming Class of 2012. More information about the submission process will be provided during the summer of 2008. If students have any questions or comments, 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.



FUSION

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