Annual Medical Student Research Forum 2012

Wednesday, August 8th
10:00 am to 2:00 pm

Room 111 and the Atrium
Lee Medical Building

Sanford School of Medicine
The University of South Dakota
414 East Clark Street
Vermillion, SD

Program and Abstracts

UNIVERSITY OF
SOUTH DAKOTA
SANFORD SCHOOL OF MEDICINE

Program Schedule

10:15 - 12:05 Oral Presentations (Lee Med Room 111)

Medical Summer Research Program participants

10:15-10:25 Marnie Schuneman: Analysis of hemoglobin A1c levels and microvascular complications in type 1 diabetic patients using the national type 1 diabetes (T1D) clinic exchange registry

10:25-10:35 Ryan Geraets: Examining the mechanisms by which Notch signaling promotes principal cell differentiation and determining whether Notch signaling is involved in maintaining expression of mature principal cell genes.

10:35-10:45 Aaron Clem: Regulation of RNA II expression in *Enterococcus faecalis*

10:45-10:55 Luke Hofkamp: Acetylated Alpha-Tubulin and Gli2 Identify Subsets of High Grade Prostate Intraepithelial Neoplasia (HGPIN)

10:55-11:05 Mark Mingo: A multi-valent vaccine approach that elicits broad immunity for influenza A

11:05-11:15 Eammon Grosek: An Ounce of Screening; the Feasibility of Self-Samplers to Screen for HPV in Native American Populations

11:15-11:25 Allison Abitz: Ubiquitin and expression and trafficking of choline transporter

11:25-11:35 Jared Drenkow: Targeting CRMP2-associated signaling complex as a possible therapeutic target in the treatment of variant late-infantile NCL

11:35-11:45 Cory Sykora: Effects of Hypothyroidism on Expression of Dopamine D2 Receptor Isoforms in Hamster Striatum, Nucleus Tractus Solitarius and Hippocampus

11:45-11:55 Joshua M. Doorn: - A mouse model of Kufor Rakeb syndrome, a form of monogenic parkinsonism

11:55-12:05 Alexandra Higgins: Differential Modulation of Nociceptive versus Non-Nociceptive Synapses by Endocannabinoids

12:05-1:00 Lunch & Poster Session (Lee Med Atrium)
Posters presented by:
Rachel Thies, Sanford Children's Research Center
Scholarship Pathways Program

1:00-2:00 Keynote Address (Lee Med Room 111)
Keith A. Hansen, MD, Department of Obstetrics & Gynecology Chair, Sanford School of Medicine:
"Oncofertilty:2012"

Medical Student Research Program Abstracts
Oral Presentation, Lee Med Building Room 111

Analysis of hemoglobin A1c levels and microvascular complications in type 1 diabetic patients using the national type 1 diabetes (T1D) clinic exchange registry

Marnie Schuneman MSII, Brad Uhing PhD, Julie Kittelsrud CNP

Avera McKennan Research Institute, Avera McKennan, Sioux Falls, SD

Contact: margaret.j.schuneman@usd.edu, brad.uhing@avera.org

Background: There is a well-established association between patients with type 1 diabetes and various microvascular complications. Many risk factors have been linked to these complications including disease duration, age, lifestyle, and long-term control of blood sugar levels (hemoglobin A1c).

Methods: In this study we analyzed the national T1D clinic exchange registry to assess the effects of hemoglobin A1c levels and the risk of diabetic neuropathy, retinopathy, and nephropathy. Patients were enrolled in the national T1D registry by completing a diabetic questionnaire and consenting the release of their medical and diabetic records. Patient information was assigned an anonymous identification number and entered into the online database. Within established ranges of Hb A1c levels, patients were separated by type of microvascular complication based upon various lab values, physical exam findings and diabetic history.

Results: Results will be reported once obtained.

Examining the mechanisms by which Notch signaling promotes principal cell differentiation and determining whether Notch signaling is involved in maintaining expression of mature principal cell genes.

Ryan Geraets, MSI and Kamesh Surendran, PhD

Sanford Children's Health Research Center, Sanford Research/USD, Sioux Falls

Contact: Ryan.D.Geraets@coyotes.usd.edu;Kameswaran.Surendran@sanfordhealth.org

Background: The immature collecting duct cells of the kidney differentiate into principal and intercalated cell types during embryogenesis. Inheritance of mutant forms of aquaporin2 (Aqp2) and arginine-vasopressin receptor 2 (Avpr2), which are specifically expressed in mature principal cells, results in Nephrogenic Diabetes Insipidus (NDI). A recent study determined that inactivation of mind bomb-1 (mib-1), a facilitator of both Notch and Wnt signaling pathways, in mice results in reduced numbers of principal cells and a NDI like phenotype.

Methods: HoxB7-Cre; RBP-$J^{f/\Delta}$ mice in which the RBP-J, a mediator of the Notch signaling pathway, is inactivated in the immature collecting duct cells were analyzed to study the requirement of Notch signaling in principal cell differentiation. Kidneys from HoxB7-Cre; RBP-$J^{f/\Delta}$ and wild type littermates were harvested at various time points during embryogenesis. Kidney sections were analyzed for markers of principal cells (Aqp2), intercalated cells (Foxi1), and cell proliferation (PSER10) by immunohistochemistry. Kidney RNA was also extracted from these mice to examine expression of Aqp2 and Avpr2 genes by quantitative real time reverse transcriptase polymerase chain reaction (qRTPCR).

Secondly, to examine the ability of Notch signaling to regulate the expression of Aqp2 and Avpr2, M1 and $MPKCCDC_{14}$ principal cell lines were transfected with either notch intracellular domains 1 and 2 (NICD1, NICD2) or the dominant negative master mind (dnMamL). NICD1 and NICD2 activate the notch signaling pathway whereas dnMamL inhibits the signaling pathway. RNA was isolated from transfected cells and the expression of Aqp2 and Avpr2 was analyzed by qRTPCR.

Results: Immunohistochemical staining of kidney sections and qRT-PCR of kidney RNA from HoxB7-Cre; RBP-$J^{f/\Delta}$ and control littermate mice revealed an increase in intercalated cells and a decrease in principal cells. We detected very few proliferating intercalated cells in either the wild type or Notch signaling deficient kidneys at different stages of embryogenesis. Surprisingly, the transfection of $MPKCCDC_{14}$ with NICD1,2 resulted in a decrease of AVPR2.

Conclusions: Inactivation of RBP-J results in decreased expression of Aqp2 due to reduced number of principal cells. The mechanism by which Notch signaling ensures the correct ratio of principal to intercalated cell differentiation is not by suppressing the proliferation of intercalated cells. Whereas Notch signaling promotes principal cell differentiation during development, activation of Notch signaling in a mature principal cell line suppresses the expression of Avpr2 and may be involved limiting water reabsorption by the mature principal cells.

Regulation of RNA II expression in *Enterococcus faecalis*

Aaron Clem, MSI and Keith Weaver, PhD.
Division of Basic Biomedical Sciences, Sanford School of Medicine at USD, Vermillion

Contact: aaron.clem@usd.edu, Keith.Weaver@usd.edu

Background: Toxin-antitoxin (TA) systems are used by bacterial plasmids as a method of ensuring their retention, programming for death any daughter cells that do not inherit a copy of the plasmid (Weaver, 2009). Surprisingly, such TA systems have also been identified on bacterial chromosomes, in which their role is not yet clear. Several chromosomal homologues of the *Enterococcus faecalis* plasmid pAD1 *par* TA system have been found associated with genes involved in carbohydrate metabolism, suggesting a physiological role in metabolic regulation, possibly modulating the activity of membrane-localized transporters (Weaver, 2009).

Methods: This study investigates the production and decay of RNA I (the toxin) and RNA II (the anti-toxin) of the chromosomal *par* homolog EF0409 in the *E. faecalis* strain OG1RF and a mutant deleted for the EF0409 locus with a copy of the RNA II gene maintained episomally. Media containing glucose or mannitol was given a 2% inoculation of the target strain. Time course experiments (9 hour span) were performed and analyzed through spectrophotometry and northern blotting. Densitometry was performed to quantify relative RNA I and RNA II amounts.

Results: In the OG1RF strain grown in glucose-containing medium, RNA II concentration was initially very low, rose exponentially, peaked at or shortly after 4 hours, and decreased rapidly thereafter. A similar trend was observed with the mutant strain expressing RNA II episomally; however, after peak concentration was reached, the amount of RNA II decreased more slowly than was observed in OG1RF. When OG1RF was grown in mannitol-containing medium, a slower growth rate was observed by optical density measurement, and peak RNA II concentration appears to be reached at a later point in time, although further experiments with an extended time span must be conducted to determine exactly when.

Conclusion: Expression of EF0409 increases during log phase and drops rapidly as cells enter stationary phase. No major differences in the decay of RNA II were observed between OG1RF and a mutant expressing RNA II episomally, which indicates that RNA II regulation of cultures grown in glucose media is similar whether the *par* homologue is present in the genome or on a plasmid. These results indicate that the DNA encompassing the RNA II gene is sufficient for the observed regulation, and context, including surrounding genes and expression of RNA I, are not important for the observed pattern of RNA II expression. Data regarding the effect of growing OG1RF cultures in mannitol media suggests a later time of peak RNA II concentration, perhaps coincident with later entry into stationary phase, although further research into this is necessary to accurately characterize its production and decay over time.

Acetylated Alpha-Tubulin and Gli2 Identify Subsets of High Grade Prostate Intraepithelial Neoplasia (HGPIN)

Luke Hofkamp PhD, MSII[1] (lhofkamp@usd.edu), Catherine Stoos MD[2],
Joel Ziebarth MD[2] (Joel.Ziebarth@usd.edu), and Barry Timms PhD (btimms@usd.edu)[1]

[1] Division of Basic Biomedical Sciences, Sanford School of Medicine, The University of South Dakota, Vermillion, SD 57069
[2] Department of Pathology, Sanford Health Systems, Sioux Falls, SD 57105

Background: High grade prostate intraepithelial neoplasia (HGPIN) is thought to be the precursor lesion to prostate cancer. The use of extended core biopsy series has decreased the correlation of finding HGPIN on biopsy and diagnosis of cancer on re-biopsy. Currently, there is no clinical mechanism to ascertain which HGPIN lesions will progress to cancer. In this study we observed differences in protein expression that characterized subsets of HGPIN lesions previously unrecognized by histopathology.

Methods: Serial sectioned archived radical prostatectomy and prostate biopsy specimens were analyzed by immunohistochemistry for expression of acetylated alpha-tubulin (AcT), Ki67, Gli2 and vascular endothelial growth factor (VEGF). These sections were analyzed for differential protein expression in HGPIN lesions. Expression levels were analyzed using the Aperio Imagescope Positive Pixel Count Algorithm.

Results: We have identified two distinct subsets of HGPIN through differential expression of AcT, Ki-67, Gli2 and VEGF. These subsets were classified as HGPIN negative (HGPIN-) and HGPIN positive (HGPIN+). The HGPIN- subset expression of AcT, Ki-67, Gli2 and VEGF correlates closer to values seen in benign prostate tissue; while HGPIN+ subsets overexpress AcT, Ki-67, Gli2 and VEGF at levels more similar to those observed in regions of cancer. AcT and Gli2 were further evaluated in biopsy material and a significant correlation of expression was seen between prostatectomy and biopsy.

Conclusions: Immunohistochemical analysis of cytoskeletal, developmental and proliferation proteins revealed a consistent differential expression pattern between two distinct subsets of HGPIN. Since 5% of all prostate biopsies contain only HGPIN and no cancer, the potential to differentiate between subsets of HGPIN and potential for progression to cancer is of considerable clinical value.

A multi-valent vaccine approach that elicits broad immunity for influenza A

Mark Mingo MSI, Victor Huber, Ph D
Division of Basic Biomedical Sciences, Sanford School of Medicine at USD, Vermillion
Contact: Mark.Mingo@usd.edu, Victor.Huber@usd.edu

Background: Influenza A viruses are classified into subtypes based on the surface proteins hemagglutinin (HA) and neuraminidase (NA). The major antigenic protein for influenza virus is HA, which constantly changes to escape host immunity, leading to annual vaccine updates. Alternatively, radical changes in HA expression can occur through genetic reassortment events in intermediate mammalian species, typically the pig. It is likely that a novel influenza virus that acquires the ability to spread to humans and with a high transmission rate and pathogenicity will create a pandemic. At this time, the majority of pre-pandemic vaccine plans focus on making vaccines rapidly after a virus begins to spread within the human population, which will lead to a 6-9 month lag time during the early stages of a pandemic, while the vaccine is being made. Using this approach, it is very difficult to limit the early effects of a pandemic in humans. We hypothesize that vaccination using HAs that stimulate immunity toward both swine and human HAs would prevent pandemics by limiting transmission between these two natural hosts of influenza A virus.

Methods: Since the HA generates most of the immune response, we used DNA shuffling to create chimeric HAs using 5 parental viruses that were selected to elicit broad immunity. Using DNA vaccines to generate immunity toward these HA-DNA constructs, we selected constructs that demonstrate broad reactivity toward parental HAs. These HA genes were cloned into plasmids, allowing them to be used to generate virus reassortants using a technique called reverse genetics. Reverse genetics is a laboratory process that allows for creation of influenza viruses using the individual 8 genes that make up the influenza A virus. Mice were used as the initial mammalian model to test the chimeric HAs, and further research is planned in pigs. Hemagglutination inhibition assays, microneutralization assays, and ELISA were used to measure the antibodies generated.

Results: Using reverse genetics and DNA shuffling we were able to create and rescue viruses expressing chimeric HA. Screening of chimeras using a DNA vaccine approach was implemented to determine the overall immune responses before generating viruses for future vaccinations. The selected HAs were expressed on a mouse-adapted influenza background for testing in a mouse model, and also a live attenuated background for testing in a swine model.

Conclusion: Chimeric influenza vaccines can act as a pandemic preventative plan, based on the results seen previously for generating an immune response to a chimeric DNA vaccine. Future studies will be performed in the swine model to determine the efficacy of the novel chimeric viruses that were created.

An Ounce of Screening; the Feasibility of Self-Samplers to Screen for HPV in Native American Populations

Eammon Grosek, MSI and Delf-Schmidt Grimminger, MD **Contact emails:**
eammon.grosek@gmail.com, delf.schmidt-grimminger@usd.edu

Background: In the US, cervical cancer is the fourth most common malignancy in women following breast, colorectum, and endometrium. There will be an estimated 12,170 new cases of women diagnosed with cervical carcinoma in the United States in 2012, and an estimated number of 4220 women will die from their cervical cancer. Despite falling incidence, cervical cancer remains the tenth leading cause of cancer death in the US. Risk factors for cervical cancer include Human Papillomavirus infection (HPV), multiple sexual partners, smoking, poor nutrition, immunosuppression (diabetes, HIV) and high parity. Cervical cancer affects disproportionately Native American women in South Dakota. In the Aberdeen area the age adjusted mortality rate for cervical cancer is several times the national average. Of the 33,000 women of screening age on the Pine Ridge reservation only 10,000 actually receive regular pap smears. The introduction of a HPV self-sampling tool that can capture cervical could be a step towards providing better access to screening for Native American women here is South Dakota.

Methods: This project consisted of analyzing patient cervical samples acquired from a self-sampler for HPV DNA. This process will start by isolating DNA using the Qiagen Minelute DNA kit which purified the sample, yielding pure DNA. Once the DNA is isolated it will be amplified by using a PCR reaction, where the sample is also being biotinylated. This PCR DNA probe will then be denatured and will be probed with the Roche HPV Genotyping Linear Array. If HPV is present the HPV DNA probe will bind to the corresponding HPV type on the linear array. The DNA is hybridized to Streptavidin Horseradish Peroxidase and then visualized through a reaction, catalyzed by the proxidase, producing a blue band precipitate on the array where the corresponding HPV genotype is present on the linear array strip.

Results: We concluded that every patient obtained cervical self-sample was able to capture some cervical human DNA because every cervical patient sample tested positive with the linear array for the presence of the β-globin genes. Comparing the effectiveness of the Self-Sampler with the Physician Sample; 116 HPV infections were found in patients from the physician screen. Of these, 45 (39%) were also found in both of the self-samples and 30 (26%) were found in only one self-samples. Therefore we can conclude that the self-samplers were able to capture 75 (65%) of the infections found in the physician sample and 41 (35%) of the infections were not found.

Conclusions: The ability of the self-sampler to capture cellular material in the cervix can be affirmed with confidence due to the capture of the β-globin gene in virtually every sample. The results of the self-sampler's ability to detect HPV genotypes consistently are encouraging, but data analysis is only in a preliminary stage, leaving many questions to explore. Does the self-sampler sometimes miss the infected area of the cervix? What deviations in results are due to old infections that are lapsing or new infections that are emerging? Can extra-cellular HPV that is present but not actively infecting tissue skewing results? How does the traditional cytology of patients relate to their HPV infection profile? Such further considerations will provide a more complete picture of the Self-Sampler's feasibility as a screening tool for native communities.

Ubiquitin and expression and trafficking of choline transporter

Allison Abitz, MSI and Yifan Li, MD, PhD

Division of Basic Biomedical Sciences, University of South Dakota, Vermillion, SD

Contacts: Allison.Abitz@usd.edu and Yifan.Li@usd.edu

Background: Autonomic nervous system, consisting of sympathetic nervous system (SNS) and parasympathetic nervous system (PSNS), plays a critical role in regulation of cardiovascular functions. Our research is particularly interested in the PSNS dysfunction in various cardiovascular diseases. One of our research aims is to investigate whether and how altered choline transporter (CHT) affects PSNS function. Recent studies reveal that CHT protein undergoes dynamic trafficking between the cytosol and the plasma membrane. Ubiquitination is an important mechanism to regulate protein trafficking. However, whether ubiquitination is involved in CHT trafficking is unknown. As a preliminary study, this summer project was to examine the effects of manipulation of ubiquitination on CHT protein expression.

Methods: Model: This study used a cholinergic cell line, SN56, as an in vitro model. All experiments were conducted in cells cultured in DMEM containing 10% FBS. Pharmacological manipulation: Cells were treated with following reagents for 24 hours: PYR41, an ubiquitin activating enzyme (E1) inhibitor (0.1 to 0.5 µM), MG132, a proteasome inhibitor (0.1 to 0.4 µM), and LDN57444, a selective UCHL1 (a deubiquitinating enzyme) inhibitor (0.1 to 0.5 µM). Gene manipulation: The plasmid expressing ubiquitin (pRK5-Ub, 1 ug/ml) and siRNA targeting ubiquitin gene ubb (2 nM) were used to overexpress or knock down ubiquitin, respectively. The plasmid or siRNA were pre-mixed with Lipofectamine and then added into cells. Following the overnight transfection, cells were cultured in fresh media for additional 24 hours. Western blot: At the end of treatments, cells were washed and lysed, protein concentration was assayed and adjusted, and samples were mixed with loading buffer. Proteins in samples were separated by 10% SDS-PAGE, transferred onto nitrocellulose membranes, and blotted using primary antibodies against CHT, ubiquitin, actin or GAPDH. Bands were visualized by appropriate secondary conjugated with fluorescence and Li-Cor scanner.

Results: Treatment with PYR41 for 6 hours showed inconsistent results. Treatment with PYR41 for 24 hours showed reduced polyubiquitination and CHT protein levels. Overexpression of ubiquitin increased polyubiquitinated proteins but decreased CHT expression compared with the transfections with two control plasmids. In contrast, gene knockdown of ubb, one of the major ubiquitin genes, reduced polyubiqutinated proteins but increases CHT protein level, suggesting that CHT may be subjected to ubiquitin-proteasome mediated degradation. Treatments with proteasome inhibitor, MG132, increased polyubiquitinated proteins and CHT protein. Interestingly, the light band above the main CHT band was reduced. Fractionation of cytosol and plasma membrane proteins indicated that the treatments with MG132 reduced membrane fraction of CHT but seemingly increased cytosolic CHT. Treatment with LDN57444 reduced CHT protein.

Conclusion: Overall results indicate that manipulation of ubiquitination alters CHT expression and localization. These data provide rationales and foundation work to further investigate the role of ubiquitin and ubiquitination in CHT expression, trafficking, and function.

Targeting CRMP2-associated signaling complex as a possible therapeutic target in the treatment of variant late-infantile NCL

Jared Drenkow, Tarah Nelson, Seung Yon Koh, Jill Weimer, PhD

Sanford Children's Research Center, Sanford Research/USD, Sioux Falls

Contact: Jared.Drenkow@usd.edu, jill.weimer@sanfordhealth.org

Background: Neuronal Ceroid Lipofuscinoses (NCL) are a class of lysosomal storage disorders characterized by progressive loss of vision, mental and motor deterioration, seizures, and premature death. NCLs are caused by mutations in at least 14 different CLN genes, where different mutations affect the onset and severity of the disease. Mutations in the CLN6 gene lead to a variant late infantile form of the disease which manifest around the age of 2 with children not surviving past their third decade. CLN6 is crucial in the developing brain, as well as in maintenance of the nervous system. CLN6 has been shown to directly associate with collapsin mediator response protein-2 (CRMP2) and kinesin light chain 4 (KLC4), and in this study we explore stabilization of this complex by LKE (lanthionine ketimine-(R)-5-ethyl ester) as a possible therapy to prevent or delay onset of vLINCL.

Methods: To study the effects of LKE on vLINCL, the $Cln6^{nclf}$ mouse (analogous to human phenotype) were given LKE via a special food blend. Mice underwent neurobehavioral assessment using a rotarod and survival was monitored over 12 months. Pathologically, mice will be monitored for changes in cortical size, loss in GABAergic interneuron and glial activation, all hallmarks of NCL pathogenesis) following LKE treatment. Additionally, to expore the role of the CRMP2-CLN6 complex in neurodevelopment, the number of migrating neuronal cells in embryonic mice was analyzed. Lastly, to better understand the formation of this signaling complex. The interaction domain between CRMP2 and CLN6 were mapped using dot blots of CRMP2 peptide fragments.

Results: Peptide mapping have allowed us to narrow down the domains of interaction CRMP2 with CLN6 and KLC4. Furthermore, Enzymes activity assays have demonstrated alterations in the activity of PPT1 and TPP1, known NCL-related enzymes. We have also found that there are nominal changes in cortical thickness during embryonic development. This supports postnatal degeneration as the underlying cause of brain volume loss rather than developmental abnormalities. Stabilization of the CCK complex with LKE appears to have little or no affect on motor coordination deficits in the viLNCL mice but we are unsure of whether it can influence cell survival or delay cortical and thalamic degeneration. Therefore, ongoing study will help determine if LKE is a viable treatment option for NCLs.

Conclusions: Overall, this investigation helps in understanding the interaction of CRMP2, CLN6, and KLC4 and how these interaction partners lead to neurodevelopment in the developing brain.

Effects of Hypothyroidism on Expression of Dopamine D2 Receptor Isoforms in Hamster Striatum, Nucleus Tractus Solitarius and Hippocampus

Cory Sykora, Evelyn Schlenker, PhD, and Kathleen Eyster, PhD

Division of Basic Biomedical Sciences, Sanford School of Medicine at USD, Vermillion

Contact: Cory.Sykora@usd.edu, Evelyn.Schlenker@usd.edu

Background: In past experiments, hypothyroid hamsters exposed to hypoxia showed excitation of breathing with dopamine D2 receptor agonist bromocriptine treatment, while bromocriptine depressed ventilation in euthyroid hamsters. However, expression of D2 receptors in the nucleus tractus solitarius (NTS) and the striatum (areas associated with regulation and control of breathing) were similar between the two groups. A potential mechanism to explain these physiological differences may be in the expression of dopamine D2 receptor isoforms. The dopamine D2 receptor exists as two alternatively spiced isoforms, short (D2S) and long (D2L), with each having different neuronal distributions, D2S being predominantly presynaptic and inhibiting dopamine release and D2L being postsynaptic, responding to the dopamine released from the presynaptic neuron. We hypothesized that the underlying differences observed in ventilation between the hypothyroid and euthyroid hamsters following bromocriptine treatment and hypoxia may be a result of alterations in the expression of the two D2 isoforms in brain regions associated with ventilation in the two groups of hamsters.

Methods: Female golden Syrian hamsters were rendered hypothyroid (n=3) with the addition of 0.04% propylthiouracil (PTU) to tap water for 5 months while another set of female hamsters received just tap water to drink, composing the euthyroid group (n=3). Following ventilatory studies, animals were sacrificed, with brains removed, sliced, and microdissected with 20- gauge needle in the striatum, hippocampus, and the NTS. Microdissected samples were lysed with Tri-Reagent and total RNA was extracted and quantified. Reverse transcription of RNA was used to make complementary DNA (cDNA) which was purified, and quantified. PCR reactions were then performed on the cDNA samples using primers specific for the genes coding for the dopamine D2S and D2L isoforms. Samples were then run on a Southern blot, quantified for D2S and D2L expression, and normalized to the expression of the housekeeping gene, GAP-DH.

Results: Both D2S and D2L were present in all brain regions and there was no difference in GAP-DH levels (loading control). In the striatum D2L and D2S levels were similar to each other and not affected by PTU treatment. In the hippocampus of the hypothyroid hamster expression of dopamine isoform D2S was significantly larger than that of the D2L isoform (P=0.05). A similar trend was seen in the euthyroid group. In the NTS, D2S levels were greater than D2L levels in the hypothyroid group, but not in the euthyroid group.

Conclusion: We successfully detected and distinguished D2L and D2S isoforms in all three brain regions of female hamsters. Although trends and differences appear in the data a limitation of this study was the low number of samples used in the two groups.

A mouse model of Kufor Rakeb syndrome, a form of monogenic parkinsonism

Joshua M. Doorn, Tyler Jepperson, and Michael C. Kruer
Sanford Center, 2301 East 60th Street, *Sioux Falls*, SD, 57104

Contact: josh.doorn@coyotes.usd.edu, michael.kruer@sanfordhealth.org

Background: The identification of single gene causes of Parkinson Disease (PD) has led to significant advances in the understanding of disease pathogenesis. For example, the PD gene products Parkin, PINK1, and DJ-1 have been shown to interact in a common biological pathway, integrating ubiquitin ligase activity and mitochondrial quality control, biological processes long implicated in PD. A recently-identified monogenic, early-onset form of PD is caused by mutations in *ATP13A2*, initially called Kufor Rakeb Syndrome (KRS). KRS features parkinsonism, dementia, and neurobehavioral signs. *ATP13A2* encodes a P5-type ATPase which may transport inorganic ions. Similar to what has been accomplished for other forms of monogenic PD, the study of *ATP13A2*-associated PD has the potential to shed light on currently unappreciated biologic pathways that are nevertheless of critical importance to PD neurobiology. To advance our understanding of *ATP13A2*-associated PD, we characterized key phenotypic features of an ATP13A2 knockout (KO) mouse.

Methods: Motor deficits were gauged by a variety of measures, while several behavioral neuroscience paradigms were used to characterize other phenotypic features. Rotarod and inverted pole testing were used as general indicators of neuromotor dysfunction. The dish test was used as a surrogate marker of anxiety and abulia. The hanging tail test was employed as a marker of depression and has also been shown to precipitate abnormal dystonic movements in diseased mice. Akinesia was tested by holding a mouse by the tail with only forepaws remaining on a level surface and observing the number of steps taken in a time-limited manner. Bradykinesia was tested by placing the forepaws of a mouse on a bar above the benchtop and determining how long it takes the mouse to remove both forepaws. Finally, muscular rigidity was measured by placing the mouse's forepaws on a horizontal pole 20 cm above the bench top and measuring the time to fall.

Results: Analysis of knockout and wild type mice at two months of age shows significant differences in rotarod, dish, and muscular rigidity performance. However, our preliminary data was unable to confirm this effect at three months of age.

Conclusions: Significant differences were found between 2 month-old knockout and wild type mice in rotarod, dish, and muscular rigidity performance. Although these preliminary findings will need to be longitudinally confirmed in larger cohorts, our data suggests that *ATP13A2* knockout mice may display a progressive neurodegenerative phenotype.

Differential Modulation of Nociceptive versus Non-Nociceptive Synapses by Endocannabinoids

Alexandra Higgins, MSI and Brian Burrell, PhD
Neuroscience Group, Division of Basic Biomedical Sciences, Sanford School of Medicine at USD, Vermillion, SD

Contacts: Alexandra.Higgins@usd.edu, Brian.Burrell@usd.edu

Background: Chronic pain is a major health concern and the most common symptom for which patients seek medical care in the United States. Many of the pharmacological therapies used for pain management have undesirable properties (e.g. addiction and respiratory depression), thus it is important to develop new pain management strategies. Endocannabinoids, which are lipid modulatory neurotransmitters, have been proposed as a potential therapy for chronic pain, but recent evidence has suggested that endocannabinoids have sensitizing effects that may actually enhance the perception of pain. Sensitization processes that contribute to chronic pain can involve changes in both nociceptive (pain-sensing) and non-nociceptive afferents. Therefore, it is possible that endocannabinoids have different effects on pain versus non-pain pathways.

The leech central nervous system (CNS) is well characterized in terms of identity of individual neurons, their functions, and their synaptic connectivity. The identity of nociceptive and non-nociceptive sensory neurons and the synaptic targets of these afferent cells are known in considerable details in the leech CNS. Thus, the leech is a useful model system to study the endocannabinoid-based modulation of pain. Specifically, I examined the effects of endocannabinoids on synaptic transmission by nociceptive vs. non-nociceptive sensory neurons.

Methods: Ganglia were dissected and placed in a recording chamber in which synaptic transmission between identified neurons was measured. The effects of the endocannabinoid 2-arachidonoyl glycerol (2AG) were tested on nociceptive and non-nociceptive synapses. GABA-A receptor antagonist, bicuculline, was applied with 2AG in order to determine whether the drugs induced potentiation via the same mechanism. TRPV-1 antagonist SB366791 was applied with 2AG to determine whether the 2AG-induced potentiation utilized a TRPV-like receptor as 2AG-induced depression does.

Results: 2AG induced depression at nociceptive synapses, but actually potentiated non-nociceptive synapses. Bicuculline, which also causes potentiation at non-nociceptive synapses, occluded the potentiating effects of 2AG. Additionally, application of a TRPV-1 antagonist blocked the potentiating effects of 2AG.

Conclusions: Endocannabinoid induced long-term potentiation in non-nociceptive synapses and could explain the sensitizing effects of endocannabinoids that have been reported by others. This potentiation may be the result of a decrease in inhibitory input (disinhibition) given that GABA-A receptor antagonists occluded 2AG-induced potentiation. Since endocannabinoids enhance signaling at non-nociceptive synapses, cannabinoid-based therapies may not be useful in the treatment of allodynia and mechanical hyperalgesia because these likely involve increased activation of non-nociceptive Aβ afferents in response to a somatosensory stimulus. However, cannabinoid-based treatment may be effective if the pain involves only spontaneous nociceptive C-fiber activity that does not occur in response to a stimulus.

Invited Research Presentation

Poster Session, 12:00-1:00 pm, Lee Med Atrium

Rachel Thies
The Safe Passage Study: A Prospective Study on the Role of Prenatal Alcohol Exposure in SIDS and Stillbirth

Scholarship Pathways Program

Poster Session, 12:00-1:00 pm, Lee Med Atrium

Adam Binneboese
Localization of orofacial motor representation in the corona radiata, internal capsule, and cerebral peduncle in the non-human primate

Joseph Carda
Association between BMI and lower limb ligament injury

Laura Danielson
Preventing endoleaks in endovascular aneurysm repair

Nichole Gilbert
Lifestyle change challenge

Keary Johnson
Improvement of diabetes management with use of point of care hemoglobin A_1C testing

Josie Kerk
South Dakota Diabetes Coalition state plan development

Deanna Lassegard
Exploring a national healthcare system: United Kingdom- NHS

Teresa Maas
Comparison of life science education in rural and urban South Dakota

Courtney Nelson
The impact of eEmergency on quality outcomes for patients with chest pain

Jeremy P. Pepin
USMLE Step 1 Prep: A comprehensive review of the cardiovascular system

Daniel Terveen
The impact of insurance status on hospital charges in pediatric patients in MDC 5

Jared Velgersdyk
Utility of increased chlamydia screening in high risk women

Brian T. Westerhuis
Generation and analysis of a novel mouse model of medulloblastoma

Vanessa Wookey
Evaluating symptom distress in cancer patients

Invited Research Presentation Abstract

The Safe Passage Study: A Prospective Study on the Role of Prenatal Alcohol Exposure in SIDS and Stillbirth

Rachel Thies, MSI, Amy Elliott, Ph.D.
Sanford Children's Research Center, Sanford Research/USD, Sioux Falls
Contact: Rachel.Thies@usd.edu, amy.elliott@sanfordhealth.org

Background: Compelling epidemiologic, physiologic, and pathologic data suggest that maternal drinking during pregnancy, sudden infant death syndrome (SIDS), and stillbirth may be inter-related in important ways. The Prenatal Alcohol in SIDS and Stillbirth (PASS) Network proposes a community-linked prospective study to investigate the role of prenatal alcohol exposure in the risk for SIDS and stillbirth, as well as other adverse pregnancy outcomes, including fetal alcohol syndrome (FAS). This study will involve women from two areas plagued by high rates of perinatal mortality and prenatal alcohol exposure: American Indians in Northern Plains and Coloured (mixed race ethnicity) in South Africa. Importantly, we use the term "American Indian" in accordance with local tribal pride and approval (Don Warne, MD, MPH, Executive Director, Aberdeen Area Tribal Chairman's Health Board). We use the term "Coloured" because it is the name this population of mixed ancestry in the Western Cape uses itself, and it reflects its unique and vibrant culture and history, as well as race; it is not considered perjorative in any way in South Africa today. Approximately12,000 women will be asked to participate in the proposed Phase II of this study. Phase I was a feasibility study designed to demonstrate the feasibility of collecting multiple types of data longitudinally from the same woman throughout pregnancy and through her infant's first year of life. This multidisciplinary study is designed to answer complex and important questions about the inter-relationships between alcohol, SIDS, stillbirth, and fetal alcohol spectrum disorders (FASD), and the influence of genetic and environmental interactions in the pathogenesis of this spectrum of adverse pregnancy outcomes potentially related to maternal drinking.

Methods: Data about the mother and infant will be collected during the prenatal period and through the infant's first year of life. Information on exposures during the prenatal period, fetal physiological development, infant neurobehavioral measures, maternal and infant genetic factors, infant hearing and EEG, and brain and placental tissue pathology will be collected.

Results: The study is still in Phase II and enrolling women to participate in the study at all clinical sites. These sites are divided into the Northern Plains and South Africa. The Northern Plain sites include Sanford Health Sioux Falls, SD, Rapid City Regional Hospital Rapid City, SD, Indian Health Services Pine Ridge, SD, and the Altru Health System Spirit Lake Reservation, ND. The physiology data is analyzed at the Physiology Assessment Center at Columbia University New York, NY. There are also additional sites where tasks such as pathological analysis and data coordination and analysis are performed.

Conclusions: Data from the PASS study has not yet been released. Once the data is analyzed, the goal of the Safe Passage study is to provide information and evidence to decrease infant mortality. This could possibly include public health awareness and health promotion utilizing information gained in the study.

Scholarship Pathways Program Abstracts

The Scholarship Pathways Program is an elective opportunity developed to enrich the medical school experience by promoting rigorous independent scholarship and scholarly excellence as well as produce leaders in medical education, research and service. The program spans all four years and develops critical thinking and independent learning skills. At the end of their first summer in the program, students create a poster summarizing their project and progress to date. The included abstracts represent each student's work in progress.

Localization of orofacial motor representation in the corona radiata, internal capsule, and cerebral peduncle in the non-human primate

Adam Binneboese, MS II; Robert J. Morecraft, PhD
University of South Dakota Sanford School of Medicine

Contact: adam.binneboese@usd.edu, robert.morecraft@usd.edu

Background
Nearly 800,000 Americans suffer from stroke each year, and this disorder is the leading cause of long term disability. The most common form of stroke is middle cerebral artery (MCA) occlusion which often results in compromised circulation and damage to the subcortical white matter forming the corona radiata (CR) and internal capsule (IC). Although the general anatomical regions subject to damage in this area have been elucidated, much less is known about the subtle organization of descending orofacial motor pathways through this territory. Common symptoms following damage to the CR and IC often include facial paresis, dysarthria, and dysphagia. However, these sets of symptoms occur following injury to different parts of the CR and IC and their severity and time course of recovery are varied indicating a more widespread distribution of orofacial pathway organization than currently recognized. Thus, the frequency of occurrence of orofacial dysfunction following MCA stroke may correlate well with the existence of a potentially dispersed distribution of multiple orofacial pathways. Additionally, examining orofacial representation in the CR and IC may assist in interpreting adverse motor consequences in the aftermath of neurosurgical procedures such as deep brain stimulation and subcortical leucotomies aimed to treat retractable psychiatric and movement disorders.

Methods
Using 9 rhesus monkeys we studied the trajectories of 5 corticobulbar pathways through the CR, IC and cerebral peduncle (CP). The pathways studied originated from the head region of the primary motor cortex (M1), ventral lateral premotor cortex (LPMCv), supplementary motor cortex (M2), rostral cingulate motor cortex (M3) and caudal cingulate motor cortex (M4). Each monkey was anesthetized and these head regions were injected with anterograde tracers. The tissue was fixed and processed using immunohistochemical methods. The location of each descending orofacial motor pathway was charted using an Olympus BX51 microscope interfaced with Neurolucida data collection software.

Results
In the CR, pathways originating from M2, M3 and M4 arched over the caudate and pathways originating from M1 and LPMCv arched over the putamen. In the IC, pathways were found to be widespread, partially overlapping and topographically organized. M3, M2, LPMCv, M4, and M1 occupied anterior to posterior positions respectively. As each fiber system progressed inferiorly in the IC, they shifted posteriorly to lie within the posterior limb of the IC and overlap increased. In the CP, pathways occupied the medial half from superior to inferior levels, and overlap increased inferiorly.

Conclusions
Our finding of dispersed orofacial pathways in the CR and superior IC correlate well with the frequent occurrence of orofacial dysfunction following MCA infarction. On the other hand, this widespread organization may correlate with favorable levels of recovery. In contrast, more severe orofacial motor deficits are likely to arise from lesions that occupy more inferior levels of the IC and throughout the CP due to the progressive and extensive overlap of fibers traversing this brain region. The compact and commixed nature of motor fiber organization at inferior CP levels and the midbrain-pontine isthmus suggest a vulnerable region of passage for comprehensive disruption of corticobulbar projection fibers.

Association between BMI and lower limb ligament injury

Joseph Carda, MS II; Judith Peterson, MD
University of South Dakota Sanford School of Medicine

Contact: Joseph.M.Carda@coyotes.usd.edu, sodakrehab@sodakrehab.com

Background
Every year millions of Americans undergo orthopedic surgery to repair torn knee and ankle ligaments. Many of these injuries are suffered by athletes, who endure excessive and strenuous physical activity. Though such athletic activities are a risk factor, it is also important to understand the underlying causes of ligament damage in the average patient. One potential contributing factor may be obesity. The joints in overweight patients are subjected to a larger work load over a longer period of time. Thus, Body Mass Index (BMI) could be an independent predictor of ligament damage in the joints on the lower limb. This study will explore the association between BMI and ligament tears by comparing the prevalence of injuries that require surgery.

Methods
Patients who have been diagnosed with damage to ligaments in the knee or ankle will complete a simple survey once an orthopedic surgeon has determined that their injury will require surgery. Data collection will include height, weight, type of injury, and additional information about exercise habits. From this data we will be able to calculate the patients BMI and compare the frequency of injury between healthy, overweight, and obese patients. We will also compare the rate of injury in active vs. non-active patients.

Next Steps
Activities in the next two years will include patient recruitment, data collection, and statistical analysis.

Preventing endoleaks in endovascular aneurysm repair

Laura Danielson, MS II [1]; Patrick Kelly, MD, FACS[2]
[1]University of South Dakota Sanford School of Medicine
[2]Sanford Vascular Associates, Sanford Health, Sioux Falls, SD

Contact: Laura.Danielson@usd.edu; Patrick.Kelly@sanfordhealth.org

Background

Abdominal aortic aneurysms (AAAs) are an expansion of a segment of the aorta to over 3 cm in diameter. These occur in 3-9% of men 50 years and older and can also occur in women. The primary danger with AAAs is aneurysm rupture leading to hemorrhage, severe abdominal pain, cardiovascular collapse, and death. Unfortunately, AAAs are generally asymptomatic until shortly before rupture, making them difficult to diagnose unless found on ultrasound, CT scan or MRI. For AAAs that are determined to be at risk for rupture, intervention is based on diameter and patient symptoms. Two main surgical options exist for those requiring intervention: open surgery or endovascular aneurysm repair (EVAR). Open surgery through a laparotomy can be a very successful treatment for aneurysms but is contraindicated in more frail patients.

EVAR consists of the insertion of a stent-graft system to cover the aneurysm, creating a normal-sized channel for blood flow and blocking blood from entering the distended areas. This decreases the risk of rupture by reducing the wall strain (due to blood flow) that adds pressure to the vulnerable aneurysmal walls. A decrease in aneurysm diameter is generally seen after EVAR. Endoleaks can occur when there is blood flow between the wall of a stent-graft and the arterial wall. There are four different types of endoleaks, distinguished by the reason for the leakage of blood. This project will address all types, with a focus on type 2 endoleaks which involve continued filling of the aneurysm sac by lumbar arterial branches or the inferior mesenteric artery.

Methods

This project explores a novel method for removing the possibility of Type 2 endoleaks by blocking the openings of arteries into the aneurysmal sac during a standard EVAR. Currently, if an endoleak is detected by routine scanning, additional intervention may become necessary. If this project is successful, it will 1) decrease morbidity and mortality due to AAAs, 2) decrease frequency and cost of scans, and 3) limit need for additional intervention.

Next Steps

The project is currently in the midst of exploring potential blocking materials, methods of insertion, and design of a simulated aneurysm to test the concept. By the end of the project, it is hoped to have a patented method of preventing endoleaks that can proceed to human clinical trials.

Lifestyle change challenge

Nichole Gilbert, MSII; Susan Anderson, MD
University of South Dakota Sanford School of Medicine

Contact: nichole.gilbert@coyotes.usd.edu; susan.anderson@usd.edu

Background
Almost 30% of children and 60% of adults in the United States are either overweight or obese. Unhealthy lifestyle puts patients at higher risk for heart disease, stroke, high blood pressure, diabetes. Simple changes to lifestyle such as healthier diet, decreased stress, and increased activity, offer significant benefits and decrease risk of chronic disease. Other benefits include increased joint stability to prevent osteoporosis, improved mood, decreased anxiety, enhanced self-esteem and improved memory. Most chronic conditions are largely preventable with small behavior adjustments.

Methods
This study aims to create a flexible lifestyle change program that helps participants in their efforts to improve their health. The eight-week program allows participants to cater their lifestyle change to fit their life. Participants add one positive, healthy lifestyle change each week to add eight new healthy habits to their everyday life. Participants can work together to make small changes to improve their overall health and reduce their cardiovascular disease risk.

Results
While still in the middle of the first pilot study of the eight-week program, the ease at which participants were able to add healthy changes to their life has yet to be measured. Preliminary results show that most participants expressed that improving their health is important to them. In addition, 78% stated time or lack of motivation as a major prevention to practicing healthy lifestyle habits, and 56% stated that they are very highly motivated to improve their lifestyle.

Conclusions
Decreasing risk of chronic diseases can be very simple and is directly linked to improving your health and wellbeing. While most people are aware that improving their lifestyle is important, many have a lack of motivation or things in their life that prevent them from doing so. This program will help participants consider and implement small lifestyle changes that have the potential to make a big difference in their health.

Improvement of diabetes management with use of point of care hemoglobin A_1C testing

Keary Johnson MS II; Janet Lindemann MD
University of South Dakota Sanford School of Medicine

Contact: keary.johnson@coyotes.usd.edu; janet.lindemann@usd.edu

Background
Nearly 26 million Americans have diabetes and another 79 million have prediabetes. One of the standards of care for these patients is laboratory hemoglobin A1c (HbA1c) testing every three months. The HbA1c level reflects the average blood glucose for the previous three months. Patients may not receive the results of this test until the next clinic visit, delaying the time when they can discuss the results with the physician. The use of point of care HbA1c (POCHbA1c) testing at a clinic visit would yield immediate results for the patient and the physician to use for diabetes management. The aim of this study is to determine if POCHbA1c testing helps to lower HbA1c levels and improves long-term diabetes management.

Methods
Thirty patients with a baseline HbA1c within the last year will be recruited into the study. The thirty patients will randomly be divided into two groups, A and B. Group A will receive standard HbA1c testing every three months for two years. Group B will receive POCHbA1c testing every three months for two years.

Results
The HbA1c levels of the two groups will be compared to determine if there is an improvement with POCHbA1c testing.

Conclusion
Based on the study hypothesis, we expect a significant drop in HbA1c levels using POCHbA1c .

South Dakota Diabetes Coalition state plan development

Josie Kerk; Dawn Hahn, RN-BC
University of South Dakota School of Medicine and South Dakota Diabetes Coalition

Contact: Josephine.Kerk@coyotes.usd.edu; dhahn@sdqio.sdps.org

Background
The South Dakota Diabetes Coalition (SDDC) aims to improve outcomes of those patients with diabetes in the state of South Dakota. The Coalition is currently working on a new state plan for 2014-2016. A data collection tool has been created and is in the process of implementation. By examining survey feedback, this project will guide the SDDC in developing their objectives in advocacy, education, and public awareness.

Methods
Survey subjects will include three separate survey groups: endocrinologists, patients with diabetes, and SDDC members. Surveys were distributed in person, at clinics, and online, respectively. Responses will be summarized and compared with previous data to suggest needs and objectives to the SDDC.

Next Steps
Data collection is in progress. Once data collection is complete, the results will be analyzed and formatted for presentation to the SDDC. Final conclusions will guide the SDDC in development of the 2014-2016 state plan.

Exploring a national healthcare system: United Kingdom- NHS

Deanna Lassegard, MS II; Barry Timms, PhD
University of South Dakota Sanford School of Medicine

Contact: Deanna.lassegard@usd.edu, barry.timms@usd.edu

Background
The United States spends more on health care than any other developed country. Despite the increase in spending, the United States has a relatively lower life expectancy, higher infant mortality, and greater number of people living with chronic diseases. Such concerns are part of the ongoing debate in Congress regarding health care spending and reform. This project compares our current health care system a representative national health care system in the United Kingdom.

Project description
During one month in the United Kingdom, I collected data via direct observation of patient care, interview of doctors and patients, and witness firsthand how the National Healthcare System (NHS) operates. Using patient survey databases from the United States and the United Kingdom, the systems were compared in regard to patient satisfaction with their respective health care system. Additional comparisons were made using WHO data, morbidity and mortality statistics, and national funding and costs of healthcare.

Outcome
Final analysis for all parameters is pending. Some preliminary results are outlined as follows.

For the calendar years 2009-2010, nation-wide patient self-reported hospital satisfaction scores for the US and UK were nearly identical: 69% of US patients would recommend their hospital compared to 65% of UK patients.

In the World Health Organization (WHO) 2000 rating of healthiest nations, the USA ranks 37th, while the UK ranks 18th, with UK spending 9.3% of the country's GDP on health care compared to 16.2% in the US. Additionally, in the WHO life expectancy report for males and females, the USA ranks 24th while the UK ranks 14th.

Next Steps
The health care system in the United States is expensive. When looking to reform our health care system, national health care systems such as the UK-NHS may have features that can guide health care reform process. This project will continue to compare the systems and report on pros and cons of each as well as outlining possible cost-saving measures that might be implemented here.

Comparison of life science education in rural and urban South Dakota

Teresa Maas[1], Paul Thompson[2], Samuel Shaw[3], and Peter F. Vitiello[1,2]
University of South Dakota Sanford School of Medicine[1], Sanford Research/USD[2], South Dakota Department of Education[3]

Contact: teresa.maas@coyotes.usd.edu, peter.vitiello@sanfordhealth.org

Background

During the next decade, the U.S. demand for scientists and engineers is expected to increase at four times the rate of other occupations. Between 2008 and 2018, South Dakota is projected to need an additional 8,000 workers in health care alone. In an effort to support this growth, The Sanford Program for the Midwest Initiative in Science Exploration (PROMISE) is collaborating with many other programs across the state to enhance learning and promotion of the life sciences. While previous national studies show rural areas are faced with a number of unique challenges that may limit progress, there has been little work to identify how locale differences affect high school education in South Dakota. By making these comparisons, South Dakota educational programs may be able to develop unique strategies to improve rural life science education.

Methods

The National Center for Education Statistics (NCES) recently created the urban-centric locale code system to classify school communities based on both population size and distance from an urban area. Using these locale categories, South Dakota school ACT test scores between 2007 and 2011 were analyzed. Analysis of variance (ANOVA) tests were done to evaluate significance differences between school locales.

In order to identify factors that might contribute to science education disparities between locales, a survey for South Dakota life sciences teachers (biology, anatomy & physiology, environmental, agricultural) was developed. The survey questions educators about their student population, educational background, professional training, classroom resources, and financial support. After creating an e-mail database from school websites, the survey was distributed to 282 South Dakota teachers via SurveyMonkey®.

Results

When looking at the science subject scores of the ACT, as well as the English, reading, math, and composite scores, South Dakota city students scored significantly higher than students in town and rural areas. From 2007-2011, the correlation between student performance and school locale was a continuous trend. Because the educator survey is currently in distribution, data analyses are planned for Spring 2013.

Next Steps

Based on trends in ACT scores, similar analyses will be completed using Dakota STEP subject scores. High school seniors will also be surveyed about science course selection, available learning opportunities, and future plans for education and employment. Upon receipt of completed educator and student surveys, analyses will be conducted to investigate significant differences in life science education between rural and urban high schools. Results from all analyses will be shared with other programs to enhance educational endeavors.

The impact of eEmergency on quality outcomes for patients with chest pain

Courtney Nelson, MS II; Donald Kosiak, MD; Sarah Kappel, RN
University of South Dakota Sanford School of Medicine, Avera Health

Contact: courtney.nelson@usd.edu, don.kosiak@avera.org, sarah.kappel@avera.org

Background

Timeliness of care in emergent cardiac conditions is crucial for reducing morbidity and mortality. Recent literature has concluded that patients with emergent cardiac conditions presenting to Critical Access Hospitals (CAHs) may not be receiving adequate and timely treatment, mainly due to inadequate access to specialty resources and delayed activation of transfers. Avera's eEmergency began in 2009 as a pilot program to offer around the clock care and support to rural Critical Access Hospitals. Board certified emergency medicine physicians and experienced emergency nurses are available to document, initiate transfers, and consult with rural physicians. Collaboration between rural clinicians and trained emergency medicine clinicians allows for improved adherence to evidence based practices, and thus more complete care for patients presenting to rural CAHs. Today, Avera's eEmergency services serves 57 hospitals over 6 states, offering services to over one million rural residents.

Methods

Avera's "Chest Pain Project" allows physicians and staff in rural hospitals to be in immediate contact with a board certified emergency medicine physician. Direct communication between rural and specialty clinicians has allowed for a higher quality of care by improved compliance with evidence-based medicine. Avera's "Chest Pain Project" currently involves 20 rural sites and specifically focuses on the improvement of four aspects of chest pain: Median time to EKG, administration of aspirin, median door-to-needle time for fibrinolysis candidates, and median door-to-transfer time for PCI candidates. Preliminary data recordings for the four aspects of chest pain management began in September of 2011, and is currently collected and analyzed monthly.

Results

As of May 2011, 188 patients presented with chest pain to CAHs participating in the "Chest Pain Project". The median time to EKG for participating CAHs is now less than 10 minutes. All sites have met 100% compliance with aspirin therapy. Three patients have been considered eligible for and received fibrinolysis, with an average door-to-needle time of 41 minutes. Of the 188 patients presenting with chest pain, 26% required transfer to tertiary facilities for PCI.

Conclusions

Preliminary data compiled from all sites participating in Avera's "Chest Pain Project" shows improvement in the four aspects of chest pain management. Median time to EKG and compliance with aspirin therapy appear to be the most impressive improvements, and are most likely a result of the availability of EKG machines and aspirin in rural CAHs. While compliance with fibrinolytic therapy has been impressive, the average door-to-needle time is greater than the recommended 30 minutes may be due to limited experience of clinicians and availability of supplies. Improvements in median time to transfer have also been observed, however the greatest challenge to improving transfer times is the geographical distance between rural CAHs and tertiary facilities. While the geographical distance may not be improved, the elapsed time between presentations to the initiation of transfer may improve with the assistance of eEmergency services. Data from sites not currently participating in the study will allow for comparisons beyond the monthly trends shown with preliminary data, and will help to identify strengths and weakness of emergency systems in CAHs.

USMLE Step 1 Prep: A comprehensive review of the cardiovascular system

Jeremy P. Pepin, MS II; Barb Goodman, PhD

University of South Dakota Sanford School of Medicine

Contact: Jeremy.Pepin@coyotes.usd.edu, Barb.Goodman@usd.edu

Background

Traditionally, medical school curriculum has been taught as subject-based blocks. Recently, many medical schools have shifted to an organ/systems-based approach. As a result of this new approach, students may prefer to study for their USMLE Step 1 in an organ/systems-based approach. However, organ/systems-based study material for the USMLE Step 1 is limited. The purpose of this project is to create a review program for the cardiovascular system for future students to use in preparing for the USMLE Step 1.

Methods

Outlines were made for different subjects specific to the cardiovascular system, including anatomy, embryology, histology, physiology, microbiology, pharmacology and pathology. Each subject was then broken down into modules and created as PowerPoint® presentations, which varied by complexity and/or region of the cardiovascular system. Within each module, review questions were included to help students determine their comprehension of the covered material before proceeding to the next module. With the aid of freelance medical graphic designers and medical professors, figures were designed to help improve the understanding of the material being presented. These modules will be uploaded to a website to allow for ease of access by students. The design of the program allows reviewers to select topics asynchronously.

Next Steps

Currently, the text portion for the anatomy modules has been completed. Next, images will be added. Outlines for histology and physiology have been initiated and should be ready to put into a PowerPoint® format within the next month. Still to be completed are the subjects of microbiology, pharmacology and pathology. Outlines for these three subjects will be created over the next year. The website for the program is under development.

The impact of insurance status on hospital charges in pediatric patients in MDC 5

Daniel Terveen, MSII; Benson Hsu, MD

University of South Dakota Sanford School of Medicine

Contact: Daniel.Terveen@usd.edu, Benson.Hsu@sanfordhealth.org

Background
Insurance disparities such as lack of insurance are a major focus of healthcare reform. Uninsured patients can lead to significant cost shifting by hospitals in order to recoup lost revenue. We hypothesize that pediatric patients with private insurance will have higher charges at discharge than those with public or no insurance.

Methods
We conducted a retrospective study using hospital discharge data from the Healthcare Cost and Utilization Project (HCUP) Kids' Inpatient Database (KID) year 2009. Pediatric patients age 0-20 in Major Disease Category 5 (Cardiovascular) were stratified by primary payer status. There were 20,164 patients with primary insurance identified as Medicaid, 22,387 identified as Private, and 2,286 uninsured. Hospital region, size, ownership, and teaching status were also examined accounting for age, gender, and race. Outcome variables were compared by univariate analyses using $\chi 2$ for all categorical variables and weighted analysis of variance (ANOVA) for the variables: total charges, length of stay, and number of procedures.

Results
Pediatric patients in MDC 5 with private insurance had lower total charges than Medicaid patients and higher total charges than uninsured patients. Total charges were $84,487 for all privately insured patients, $103,411 for Medicaid-covered patients, and $44,171 for uninsured patients (P<.001). Length of stay, total charges, and number of procedures for uninsured patients were 37%, 47%, and 49% lower, respectively, than for the privately insured, and the same categories for privately insured patients were 30%, 18%, and 9% lower than for those insured by Medicaid (P<.001).

Conclusions
Medicaid patients had higher total charges, longer length of stay and a greater number of procedures performed than private or uninsured pediatric patients. These differences in cost and utilization may impact decisions in coverage shifts as the US moves towards universal insurance.

Utility of increased chlamydia screening in high risk women

Jared Velgersdyk, MSII; James Barker, MD
University of South Dakota Sanford School of Medicine

Contact: jared.velgersdyk@coyotes.usd.edu, Jim.Barker@usd.edu

Background
Chlamydia trachomatis is a gram negative bacterium whose pathological symptoms often go unnoticed for extended periods of time, especially in women. As of January 2000, all 50 states and the District of Columbia have regulations which require reporting of chlamydia cases. During 2010 alone, more than 1.3 million cases of chlamydia were reported to the CDC. However, the prevalence is estimated at 2.8 million infections. Untreated, such infections in women can lead to urethritis, cervicitis, pelvic inflammatory disease, ectopic pregnancies, and even infertility. “Screening females aged <25 years is ranked by the National Commission on Prevention Priorities as one of the 10 most beneficial and cost-effective prevention services, but it also is among the most underutilized.” This study’s aim is to increase the testing of chlamydia in high risk women (ages 18-25) and compare the number of positive results, to previous data.

Methods
This study will collect samples from all participating female patients under age 25 years who present to the Avera Downtown Health Clinic. Samples will be sent to South Dakota Department of Health for analysis. SDDOH utilizes the Gen-Probe Aptima Combo 2, which has a sensitivity and specificity of 96.1% and 98% respectively. This assay utilizes a target amplification nucleic acid probe to detect rRNA from C. trachomatis. Through this study, the subjects who test positive will receive free treatment for their infection.

Results
The number of positive results during the pre-collection phase will be compared to the number of positive results during the data collection phase – six month periods each. The data will be analyzed to determine statistical significance.

Next Steps
This study hopes to reduce the prevalence of *Chlamydia trachomatis* through increased screening of high risk women. This will be accomplished by increased screening of high-risk women and providing treatment to those who test positive.

Generation and analysis of a novel mouse model of medulloblastoma

Brian T. Westerhuis, MS II; Haotian Zhao, PhD
University of South Dakota School of Medicine

Contact: Brian.Westerhuis@coyotes.usd.edu; Haotian.Zhao@sanfordhealth.org

Background
Medulloblastoma is one of the most common childhood cancers**.** Dysregulation of Sonic hedgehog/Patched (Shh/Ptch) signaling represents an important mechanism underlying medulloblastoma formation. Shh signaling drives the rapid postnatal proliferation of cerebellar granule neuron progenitors (GNPs), but its overactivation induces medulloblastoma development from GNPs in human and mice. We showed previously that transcription factor *Atonal Homolog 1* (*Atoh1/Math1*) is a crucial molecular target in Shh-dependent medulloblastoma. Atoh1 plays a crucial role in the maintenance of GNPs and collaborates with Shh signaling to induce medulloblastoma formation from GNPs.
Innovative strategies to more safely and specifically treat medulloblastoma are needed. Knowledge of tumor cell growth and migration may help guide our search for therapies targeting specific oncogenic mechanisms. However, detailed understanding of the motility features and cytoskeletal elements of invading/migrating medulloblastoma cells is lacking. This study will characterize the invasion and migration of medulloblastoma cells *in vivo*. We utilized Cre-loxP technology to generate a mouse medulloblastoma model in which tumor initiation from individual GNPs can be regulated by tamoxifen-inducible Cre activity.

Methods
Transgenic strains were generated in which Atoh1 expression is temporally and spatially regulated by Cre-mediated DNA recombination. Two different promoters were used: the human cytomegalovirus (CMV) major immediate-early promoter/enhancer, or a synthetic CAG promoter which consists of the CMV promoter fused to a minimal chicken beta-actin gene promoter. In these mice, Atoh1 expression will be activated after removal of the loxP-flanked STOP cassette between the promoter and Atoh1 cDNA followed by an IRES-LacZ cassette. After crossing with the *Math1-CreER* strain, the resulting neonatal mice were treated with tamoxifen and LacZ expression was examined by whole-mount β-Gal staining of brains.

We also inserted cDNA for HA-tagged Atoh1 (Atoh1-HA) into a *ROSA26* targeting vector in which it is preceded by the CAG promoter and a *loxP*-flanked Neo-STOP cassette. The targeting vector was then introduced into embryonic stem (ES) cells by electroporation. Correctly targeted ES cell clones were selected by Southern blot hybridization.

Results
Eight transgenic lines were generated with CMV promoter, while six lines were generated with a synthetic CAG. LacZ expression was undetectable in transgenic strains made with CMV promoter. In contrast, mice made with CAG promoter exhibit robust Cre-dependent LacZ expression in the cerebellum.

Atoh1-HA cDNA were successfully targeted into the ubiquitously expressed *Rosa26* locus in ES cells. Two clones were used to generate multiple chimeras.

Conclusions
Conditional Atoh1 transgenic strains were generated and will be used to establish a mouse medulloblastoma model to study tumor cell migration and invasion.

Evaluating symptom distress in cancer patients

Vanessa Wookey, MSII; Heidi McKean, MD
University of South Dakota Sanford School of Medicine
Avera Medical Group Oncology and Hematology

Contact: vanessa.wookey@coyotes.usd.edu, heidi.mckean@avera.org

Background
Distress can be caused by a variety of physical, social, or psychological/emotional factors and manifests itself in various ways in cancer patients. It negatively affects emotions, coping abilities, and cancer treatments. Distress can also reduce adherence to treatments, quality of life, and patient survival rates. Although the prevalence of distress in cancer patients is reported at 35%, it is frequently undiagnosed.

Recognizing and treating distress is an important element in the treatment of cancer patients. Routine screening for distress is now recommended in oncology clinics, allowing patients to receive the necessary interventions or referrals. Nonetheless, distress is unrecognized in more than half of cancer patients. This study aims to help physicians and other healthcare team members better recognize distress in cancer patients by identifying some common symptoms that cause distress.

Methods
Cancer patients undergoing chemotherapy complete a Distress Management survey on the first visit to their oncologist after their first chemotherapy session. Patients will rank their overall distress using a 0-10 scale, 10 representing the most distress, and identify specific symptoms causing the distress, including practical, family, emotional, spiritual, and physical problems. The data from the Distress Management survey from patients 18 years of age or older will be used for analysis. This study will first identify the most common symptoms of distress in the entire patient population. Basic descriptive statistics, such as mean, standard deviation, and confidence intervals, will be calculated for the demographic data, overall distress and the distress variables. The data will be analyzed to discover if any statistically significant differences exist between men and women, different age groups, marital status, level of overall distress, type of cancer diagnosis, or stage of cancer.

Results
Results are pending.

Next Steps
The next step in this project is to begin data collection. Ultimately, results will help establish a baseline and improve understanding of prevalence and severity of distress in oncology patients in the region.