North Dakota Tribal College Research Symposium 2022 Thursday, April 21, 2022 Cankdeska Cikana Community College

Maternal Risk of Pre-eclampsia: Influence of Fetal rs1205 Genotype

Crystal Azure¹, Hailey Davis¹, Craig Poitra¹, Tyler Parisien¹, Lyle G. Best¹ ¹Turtle Mountain Community College

Background and Purpose:

We have previously identified rs1205 and two other C-reactive protein (CRP) gene variants, the maternal genotypes of which, increase the risk of pre-eclampsia (PE). These findings have been replicated in two non-American Indian populations. Most analyses of genetic PE risk assume that maternal genotype confers risk, whereas the fetal genotype may be determinative and the maternal genotype simply correlated with fetal genotype.

Method:

We enrolled only offspring of mothers known to be heterozygous for the rs1205 variant of CRP and experiencing either PE affected or normal pregnancies. Thus eliminating the maternal genetic influence of this variant. Offspring were then genotyped to determine if fetal rs1205 genotype was associated with PE.

Results:

Offspring of 23 of 30 normal pregnancies and 9 of 18 PE pregnancies carried the rs1205 T allele in a dominant genotype (Fisher's exact chi square p=0.058). Multivariate logistic regression analysis adjusted for maternal age, nulliparity and BMI gives an odds ratio of 0.288, p=0.085, 95% CI 0.070-1.188.

Conclusion:

Among 48 women, all heterozygous for the rs1205 allele, both chi-square and multivariate adjusted logistic analysis strongly suggest a trend toward reduced risk of PE among pregnancies with fetal T allele dominant genotypes. This is in accord with previous findings of reduced risk associated with maternal T allele genotype.

Great Horned Owl (Bubo virginianus) Diets in South Bismarck

Abby Decoteau* and Alicia Andes

Environmental Science Department, United Tribes Technical College, 3315 University Dr. Bismarck, ND 58504

Great Horned Owls are not an uncommon sight in North Dakota. Chances are you have seen them perched in trees gazing for prey or the quick but silent flash of an owl at night swooping down to capture its prey. Much is known about owl diets but details into specific areas is not. Owls are good at adapting to their environment and can learn how to hunt in any habitat. In this study, I observed, collected, and dissected owl pellets in the months of May-August to determine how their diets are impacted by location and reproduction stage in the Bismarck area. We hypothesized that there would be a significant difference between a nesting owl pair diet at one site compared to a fledging diet at another site. To achieve this, I located owls at United Tribes Technical College and surrounding parks and tree nurseries. I collected freshly regurgitated pellets and dissected them to identify prey remains. All data was recorded and analyzed using Excel. I failed to reject my hypothesis, as my data suggest that owls use different prey types at different locations and during different life stages. Owl's should be considered as a natural source of pest management and may be useful in controlling United Tribe Technical Colleges Richardson's ground squirrel population.

Investigation of antimicrobial properties of Sage, Artemisia ludoviciana, extract and smoke Ferlin Knight* and Mandy Guinn

Environmental Science Department, United Tribes Technical College, 3315 University Dr. Bismarck, ND 58504

White sage, Artemisia ludoviciana, is commonly found in the native grasslands of the Great Plains region. Historical plains tribes utilized the plants from these landscapes as foods, medicines, shelter and for ceremonies. For many tribal nations, sage leaves were steeped as tea, crushed and used to heal headaches or nosebleeds, and applied as an ointment for wounds. Many Indigenous people also utilized sage as a sacred plant for ceremonies, using it to cleanse and purify the person or the environment. Sage is still commonly used for smudging homes to clean and purify the air of germs, sickness, and bad spiritual energy. This project blended traditional knowledge with western science to study the anti-microbial properties of sage. Using a zone of inhibition assay, we measured the anti-microbial properties of both sage smoke and sage extract on Escherichia coli and Staphylococcus aureus. Our null hypothesis was that there is no significant difference in the smoke vs extract of the antibacterial properties in the silver sage plant. The results showed zones of inhibitions on all the Staphylococcus plates treated with the extract and smoked sage treatments. None of the E-coil plates exhibited a zone of inhibition. Thus, my results suggest that sagebrush inhibits and prevents the growth of Staphylococcus aureus. This research is significant because sage is a cheaper alternative to costly medications, and it is found commonly found in our region. Additionally, this research validates the traditional ecological knowledge (TEK) and medicinal uses that tribal communities have innately known for centuries.

Comparison of Aqueous and Soil Contaminants in New Town, ND, Selected Locations Francis Short Bull*, Kerry Hartman, Audrey LaVallie Department of Environmental Science, Nueta Hidatsa Sahnish College, 301 College Dr., New Town, ND. 58763

A number of water and soil samples were collected in the New Town, ND, municipal area and from rural areas adjacent to the New Town area; water samples were taken from tap water as well as outdoor bodies of water. A current concern on the Fort Berthold Reservation is the possibility of fracking waste and salt water disposal infiltrating local water supplies due to the large-scale oil industry in the area. Trihalomethanes were recently reported as being at higher than desirable levels in the Four Bears residential areas, although this may be due to water exposure to extensive decay of organic waste material.

Two soil samples per area were taken and extracted by organic solvent for further analysis for semivolatiles. Aqueous samples were also extracted for semivolatiles, and all samples were evaluated for chlorine, nitrates, phosphates and other contaminants. Semivolatile analysis was carried out by GC-MS, with several semivolatiles identified. Soil samples were further evaluated for metal and nonmetal content in relation to not only deleterious levels, but also in terms of nutrient supply for plant growth. At that point correlation to sample location was done to determine underlying causes for differences in nutrient levels.

A Bioinformatics Approach to Elucidate the Role of the Metal Binding Protein Metallothionein-3 through Protein-Protein Interactions

Gazal Kalyan, Seema Somji, Donald A. Sens, and Scott H. Garrett* Department of Pathology, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND 58202-9037

Metallothioneins are small (~7kD) cysteine-rich family of proteins with a capacity of formation of metal clusters within its two domains. They are engaged in numerous metal-ion related functions such as detoxification of metals, Zn2+ and Cu2+ homeostasis, retention, and transportation of metal ions. The role of Metallothionein-3 (MT-3) is mainly studied in the brain; however, overall, it is comparatively less explored than its other isoforms. Sequence and structural analysis of MT-3 was performed to evaluate how its function differs from the other members of the metallothionein family. The protein-protein interaction network of MT-3 with the topmost heavily associated proteins was evaluated using STRING. Furthermore, the amount of gene expression of all metallothioneins in various tissues was compared. Kidney is a focal target of toxicological effects through poisonous heavy metal exposure (Pb, Hg, As, and Cd) by industrial waste, air or water pollution, and medicinal drugs. Therefore, continuing the previous studies in our lab, the mortal culture of human proximal tubule (HPT) cells was used as our experimental model. A list of binding partners of MT-3 was obtained through pull-down experiments. The binding partners enolase-1, tropomyosin, β -actin, and aldolase-A were studied in detail through structure-based molecular docking. Theoretical binding energy (ΔG) and Kd were estimated to assess the strength of binding. In conclusion, it was observed that several binding partners were cytoskeleton proteins indicative of the role of MT-3 as a key moderator of cell motility, cell shape, cell division, and cell structural organization.

Long-term arsenite exposure induces the expression of stem cell related genes in normal urothelial cells

Josaphina Juarez^{*}, Aaron Mehus, Scott H Garrett, Don A Sens, Seema Somji Department of Pathology, School of Medicine and Health Sciences, 1301 N. Columbia Road Stop 9037, University of North Dakota, Grand Forks, ND 58202-9037

Environmental exposure to arsenite (As3+) has a strong association with the development of human urothelial cancer (UC) and this cancer is the 5th most common cancer in men and the 12th most common cancer in women. Muscle invasive urothelial cancer are grouped into basal or luminal molecular subtypes based on their gene expression profile. The basal subtype is more aggressive and is characterized by the high expression of keratins (KRT) 1, 5, 6, 14, and 16 and

the EGFR, whereas the luminal subtype is less aggressive and express the genes PPARγ and FOXA1. Our previous studies show that As3+-transformed urothelial cells express basal genes and when injected into immune compromised mice form tumors that have areas of undifferentiated/basal cells as well as areas that show squamous differentiation, a marker of poor prognosis. It is postulated that these undifferentiated cells are the stem cells that are driving the growth and spread of the tumor. The goal of this study was to determine if long-term As3+-exposure could induce the expression of genes related to self-renewal/stemness in the normal bladder epithelial cells. For this purpose, the immortalized UROtsa cells were passaged in 1 mM As3+. Every five passages samples were collected and the expression of stem cell related genes was determined and the ability to form colonies in soft agar was assessed. Our data shows that long-term As3+-exposure induces the expression of stem cell genes SOX2, NANOG, POU5F1 (OCT-4) and CD44 and the expression of the genes is not correlated to the ability of the cells to form colonies in soft agar. In conclusion, our study shows that long-term As3+ exposure induces the expression of genes related to self-renewal/stemness prior to the transformation of the urothelial cells by arsenite.

Proteomic alterations within MCF10A human breast epithelial cells from chronic As3+- or Cd2+-transformation

Aaron A. Mehus*, Nicholas Bergum, Peter Knutson, Scott H. Garrett, Donald A. Sens, and Seema Somji

Department of Pathology, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, North Dakota

Epidemiological studies have established an association of breast cancer (BC) development with exposures to arsenic (As) and cadmium (Cd). Triple-negative/basal-like BCs (TNBC) are aggressive and do not express ER (estrogen receptor) or PR (progesterone receptor), and lack overexpression of receptor tyrosine-protein kinase erbB-2 (HER2). The MCF10A cells are a basal-like immortalized human breast epithelial cell line that is ER/PR-negative and HER2-low. We have previously transformed these cells by long-term environmentally relevant exposures to As3+ and Cd2+ (1µM). These transformed cells readily form colonies in soft agar, a hallmark of carcinogenesis. We sought to determine proteomic alterations within MCF10A cells that arise from chronic As3+- or Cd2+-transformation. Tandem mass tag (TMT) quantitative proteomics was performed on parent MCF10A, As-MCF10A, and Cd-MCF10A cells followed by pathway analysis. Real-time qPCR and Western blotting was used to validate genes and proteins, respectively. A total of 6,349 proteins were quantified. Cell division and adhesion were the main pathways disrupted when comparing As-MCF10A to parent while cell division and cell cycle were top pathways altered when comparing Cd-MCF10A to parent. When comparing As- to Cdtransformed cells, key pathways dysregulated were migration, proliferation, and adhesion. Data highlighting the molecular changes that occur between a normal breast epithelial cell and one that has been transformed with As3+ or Cd2+ is limited. These results present a unique opportunity to exploit pathways that may aid the development of therapeutics or discover prognostic biomarkers for TNBC that has developed from As3+ or Cd2+ exposures.

Factors contributing to Tribal College Students' Academic Success: Student Voices

Pat Conway, Jennifer Boeckel, Chris Dahlen, Karl Haefner, Danielle Myers-Wilson, Sarah Olimb, Michael Parker, Karen Saari, Brent Voels, and Cynthia Lindquist Cankdeska Cikana Community College, Essentia Institute of Rural Health

Tribal colleges play a key role in increasing "higher education opportunities to American Indians through programs that are locally and culturally based, holistic, and supportive" (AIHEC, 2021). Completion of math courses are a common barrier to academic persistence in tribal colleges and other higher education institutions. In 2013, the College adopted an innovative method of teaching mathematics using the HAWKES Learning Program to address lack of success in math courses. After five years, this qualitative study was conducted; 40 students were interviewed. They articulated factors that supported them in math and led to academic persistence and factors that were barriers. About one-third of the students had attended college intermittently, some for more than 20 years. Most said that flexible class schedules, the ability to complete math courses successfully. They more frequently commented on factors other than the math-related issues that influenced their success in math and persistence in their college career: individual, family, and community factors influenced their academic success, especially non-academic barriers such as health and work. A robust model of factors interacting to influence tribal college student academic persistence resulted.

Maternal Risk of Pre-existing or Gestational Diabetes and Genetic Variant rs523288 Craig Poitra¹, Tyler Parisien¹, Bethany Davis², Lyle G. Best^{1,3} ¹Turtle Mountain Community College, Belcourt, ND; ²Translational Genomics Research Institute (TGen), Phoenix, AZ; ³University of North Dakota, Grand Forks, ND

Background: Pre-existing type II diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM) are associated with adverse pregnancy outcomes, including fetal macrosomia, stillbirth, and neonatal metabolic disturbances.1 Powe et al2 have demonstrated an association between GDM and selected genetic variants in cohorts of predominantly European ancestry. Our aim was to explore whether there was an association between T2DM/GDM and the genetic variant rs523288.

Methods: This study genotyped 6/23 cases of T2DM/GDM respectively and 110 controls from a case/control study of pre-eclampsia in an American Indian community. Genotyping of rs523288, was conducted by real-time PCR using a TaqMan assay. Analysis was conducted on additive and dominant genetic models using chi-square and both univariate and multivariate logistic regression statistical methods.

Results: The genotypic distribution satisfied Hardy-Weinberg equilibrium and the dominant A allele model was significant for association by chi square testing (p=0.042). Univariate and multivariate analysis of additive and A allele dominant models failed to reach statistical significance. Multiparity, body mass index (BMI) and pre-eclampsia were all independently, positively and significantly associated with T2DM/GDM.

Conclusion: There is evidence of association between T2DM/GDM and rs523288 in an A allele dominant model. Multiparity, BMI and concurrent pre-eclampsia are strongly and independently associated with T2DM/GDM, as reported in the literature.

Schlafen 12 induces luminal differentiation of Arsenite (As3+) transformed UROtsa cells Sarmad Al-Marsoummi^{*}, Seema Somji, Scott H. Garrett, Donald A. Sens. Department of Pathology, School of Medicine and Health Sciences, 1301 N. Columbia Road Stop, University of North Dakota, Grand Forks, ND 58202-9037

Human urothelial cancer (UC) is the fifth most common cancer in the United States. UC has been linked to chronic environmental exposure to arsenite (As3+). Urothelial cancers are classified into non-muscle invasive (NMIUC) and muscle-invasive urothelial cancer (MIUC). Genetically, MIUCs are grouped into more aggressive basal or less aggressive luminal subtypes. Schlafen 12 (SLFN12) is a protein expressed in humans but not rodents. SLFN12 induces differentiation in enterocytes, breast cancer, and prostate cancer and reduces lung adenocarcinoma's aggressiveness. The role of SLFN12 in urothelial cancer has not been investigated. This study aims to examine the role of SLFN12 in urothelial cancer. We used adenoviral vectors to overexpress SLFN12 in arsenite-transformed UROtsa cells (UROtsa-As+3), that previously established in our lab, as a model of urothelial cancer. We analyzed cell viability by crystal violet assay and cell proliferation by flow cytometry. RT-qPCR was used to analyze mRNA levels of different luminal and basal urothelial differentiation markers. Overexpressing SLFN12 in UROtsa-As+3 cells reduced cell proliferation and increased the expression of luminal differentiation markers (GATA3, FOXA1, PPARG) and luminal keratins (KRT7, KRT8, KRT18). Moreover, SLFN12 reduced the expression of basal keratins (KRT1, KRT5, KRT13, KRT14, KRT16, KRT6a, KRT6c). Our data show that SLFN12 is a molecular signal that can reduce the aggressive behavior of urothelial cancer. Therapeutics that increase SLFN12 expression will reduce the aggressiveness of urothelial cancer and improve the survival of patients with urothelial cancer.

Big Horn Sheep Transplantation on Fort Berthold Reservation: Summation and Interpretation of Herd Data Concerning Demographics, Adaptation to Habitat, Migration and Survival Rates

Shaundeen Smith*, Kerry Hartman, Audrey LaVallie

Department of Environmental Science, Nueta Hidatsa Sahnish College, 301 College Dr., New Town, ND. 58763

The Lewis and Clark expedition first reported Big Horn Sheep near the Yellowstone and Missouri river in present day North Dakota and the last known native Big Horn Sheep was killed near the Little Missouri River in 1905, according to ND State Fish and Game Management. It is estimated that unregulated hunting and disease significantly killed off large numbers of sheep. In 2020, thirty Big Horn Sheep were transplanted to the Fort Berthold Reservation from Montana, and placed into high quality habitat areas near Mandaree and Twin Buttes, ND, although the ideal range is limited in size.

GPS collars were placed on 2-3 males and females in each herd, providing information on herd movement and habits. The current study here was undertaken in conjunction with the MHA Tribal Game and Fish Department and allowed collection and interpretation of data on numbers of the sheep, travel patterns, population growth, demographics of the herds, adaptation to habitat, survival rates, diseases encountered (and transmission rates) as well as dietary requirements. Additional information on tourism, home construction and oil drilling/development in the sheep habitat will also be collected and evaluated in terms of effects on the transplanted sheep herds.

Tracking of Pollutants (Deposition in Soil) Released in Close Proximity to a Flare Site

Tannah Houle*, Kerry Hartman, Audrey LaVallie Department of Environmental Science, Nueta Hidatsa Sahnish College, 301 College Dr., New Town, ND. 58763

The oil industry in and around New Town, ND, has created a number of changes in air, water and soil quality, although specific patterns in the release of contaminants is not well known. Methane gas flaring, in particular, can release substantial amounts of methane, VOCs, and CO2, as well as benzene, NOx, SO2, metals and black carbon upon combustion. The original gases released from the well can also include other alkanes, H2S and additional CO2. Deposition of varied forms of these contaminants may result from interaction with wind and rain. In this study, monitoring of soil components very close to the point of release of flared materials was undertaken, with topical soil samples taken at 50, 100, 150 and 200 ft from a flare source. These samples were compared to non-flare affected soils (similar series) for nitrate, phosphate, carbon and metal content, and were extracted

The Soil Food Web: Foundation of Regenerative Agriculture

Mike Parker Cankdeska Cikana Community College

The two major, and divergent, paradigms of commercial food production for more than the past 50 years have been what are known as conventional agriculture and organic agriculture. The former is a chemical-intensive operation modeled after the "green revolution" which emphasizes high-yielding monocultural cropping systems whereas the latter method harkens back to the more natural way of farming that existed before the advent of synthetic fertilizer and chemical pesticides.

While conventional farming initially did what it promised in higher yields of commodity crops to feed an exploding human population, it has also had negative consequences such as ground water contamination from leaching fertilizer, an increasing use of insecticides, herbicides, and fungicides along with a boom or bust economy for the farmers involved to name but a few. Organic farming, which is a certification program for those growers who want to totally eliminate all synthetic chemical applications and rely only on naturally occurring, or organic inputs, has been growing in popularity but has been plagued with poorer yields and weed control problems.

The last few years have brought about an alternative production method commonly known as regenerative agriculture. This type is a similar model compared to organic farming as far as the elimination of man-made inputs but realizes that the traditional organic methods were still failing to imitate nature whose ecosystems, which include prairies and forests, create annual biomasses that far exceed man-made agriculture and do so without invasive "weeds" that seem to compete with the desired crops for biomass production.

The undergirding of regenerative ag is the actual regeneration of the soil to a state where it existed before it came under the influences of human agriculture. Within a healthy soil ecosystem exists a food web of micro and macro organisms that recycle nutrients and make them available to the next crop of plants. We will investigate in more detail how this happens.

Compost Bioreactor

An Alternative Compost Making Process

Mike Parker, Starla Littlewind, Derik Bull Cankdeska Cikana Community College

Traditional Compost

Most aerobic compost is made from a wide variety of organic substrate materials. These materials are chosen and proportioned in the compost 'pile' accordingly to approximately create an overall carbon to nitrogen ratio of 30:1 along with an average moisture content of 60%. It is under these conditions that that the microorganisms (bacteria) present can initiate rapid growth by consuming the available substrate while giving off products of respiration such as CO2 and heat. These thermophilic organisms thrive in this environment until about 165F when the oxygen becomes depleted and registers under 6 ppm indicating that the pile is going anaerobic. To prevent this from happening the pile is mechanically turned to introduce oxygen and any needed moisture and amendments. Another outcome of the turning is to produce a homogenous pile. This cycle is repeated until the reheating has ceased due to the pile substrate being broken down sufficiently to where accelerated bacterial growth is not encouraged.

Johnson Su Method

A few years ago, the Johnson-Su Method, named after its creators, was introduced and has been shown to have much potential. It is an aerobic static pile which eliminates the need for turning, and in doing so, it sequesters more carbon by not respiring as much CO2 and it allows fungal colonization of the pile since fungi is not being destroyed in the turning process. An inoculum extract can be made from the finished product for seed treatment, foliar application, or as a soil drench.

This poster delves into the making of a Johnson-Su bioreactor and the initial loading of it.