PROGRESS REPORT SUMMARY	GRANT NUMBER	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR	FROM	THROUGH
RANKIN, GARY O PHD	03/01/2011	02/29/2012

APPLICANT ORGANIZATION

MARSHALL UNIVERSITY

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)

WEST VIRGINIA IDEA NETWORK OF BIOMEDICAL RESEARCH EXCELLENCE (WV-INBRE)

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☐ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☐ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☐ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☐ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☐ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary Targeted/Planned Enrollment Format Page.

PERSONNEL ROSTER

Name, Degree	Department	Non-Host Institution: State, Country
Jr. Investigators		
Baria, Kim, RN	Health Research And Education	Charleston Area Medical Center: WV, USA
Barr, Taura, PHD, RN	Nursing	West Virginia University: WV, USA
Belay, Tesfaye, PHD	Biological Sciences	Bluefield State College: WV, USA
Chen, Yi C, PHD	Biology	Alderson-Broaddus College: WV, USA
Cushman, Kenneth, PHD	Biology	West Liberty University: WV, USA
Hankins, Gerald, PHD	Biology	West Virginia State University: WV, USA
Harris, Rob, PHD	Biology	West Virginia State University: WV, USA
Huggins, Luke, PHD	Biology	West Virginia Wesleyan College: WV, USA
Kaushal, Gagan, PHD	Pharmaceutical & Admin Science	University Of Charleston: WV, USA
Li, Bingyun, PHD	Orthopedics	West Virginia University: WV, USA
Linger, Rebecca, PHD	Pharmaceutical & Admin Science	University Of Charleston: WV, USA
Luo, Haitao, PHD	Biology	Alderson-Broaddus College: WV, USA
Morris, Gary, PHD	Science And Mathematics	Glenville State College: WV, USA
Park, Maiyon, PHD	Biochemistry And Microbiology	
Sal, Melanie, BS, PHD	Biology	West Virginia Wesleyan College: WV, USA
Salisbury, Travis, PHD	Pharm, Physiol & Toxicology	
Shurina, Robert, PHD	Biology	Wheeling Jesuit University: WV, USA
Stoilov, Peter, PHD	Biochemistry	West Virginia University: WV, USA
Troyer, Timothy, PHD	Chemistry	West Virginia Wesleyan College: WV, USA
Zhang, Hunter, MD, PHD	Physiology And Pharmacology	West Virginia University: WV, USA
Investigators		
Aguilar, Jarrett, PHD	Natural Science & Mathematics	West Liberty University: WV, USA
Blough, Eric, PHD	Biological Sciences	
Boskovic, Goran, PHD	Biochemistry & Microbiology	
Brock, Robert, PHD	Physiology And Pharmacology	West Virginia University: WV, USA
Callery, Patrick, BS, PHD	Basic Pharmaceutical Sciences	West Virginia University: WV, USA
Collier, Simon, PHD	Biological Sciences	
Crick, Darrell, PHD	Chemistry	Concord University: WV, USA

Name, Degree	Department	Non-Host Institution: State, Country
Cuff, Christopher F, PHD	Microbiol, Immunol Cell Biol	West Virginia University: WV, USA
Davis, Mary E, PHD	Physiology & Pharmacology	West Virginia University: WV, USA
Delidow, Beverly, PHD	Biochemistry & Microbiology	
Dementieva, Yulia, PHD	Mathematics	Emmanuel College: MA, USA
Denvir, James, PHD	Biochemistry And Microbiology	
Egleton, Richard, BS, PHD	Pharmacology, Physiology & Tox	
Fan, Jun, BS, PHD	Biochemistry & Microbiology	
Fisher, Kimberly, PHD	Physical Sciences	Bethany College: WV, USA
Gannett, Peter, PHD	Basic Pharmaceut Sci	West Virginia University: WV, USA
Georgel, Philippe, PHD	Biological Sciences	
Gibson, Laura F, PHD	Pediatrics	West Virginia University: WV, USA
Griffith, Robert, PHD	Basic Pharmaceutical Sciences	West Virginia University: WV, USA
Grover, Lawrence, PHD	Pharmacology, Physiology & Tox	
Hardman, Wanda Elaine, PHD	Biochemistry And Microbiology	
Harper, Kathy, PHD	Biology Clinical	West Virginia State University: WV, USA
Henderson, Angela, RN	Trials Exercise	Charleston Area Medical Center: WV, USA
Hollander, John M, PHD	Physiology	West Virginia University: WV, USA
Huber, Jason, BS, PHD	Basic Pharmaceutical Sciences	West Virginia University: WV, USA
Ivanov, Alexey, PHD	Biochemistry	West Virginia University: WV, USA
Joyce, Maureen, BS, MPH	Pharmacology, Physiology & Tox	
Kim, Jung Han, PHD	Pharmacology, Physiology & Tox	
Kim, Seung-Yun, PHD	Comp Sci, Math & Engineering	Shepherd University: WV, USA
Kreisberg, Robert, PHD	Biology	West Liberty University: WV, USA
Lukomski, Slawomir, PHD	Microbiology, Immuno &Cell Bio	West Virginia University: WV, USA
Maher, John, PHD	Vp For Research	
Mangiarua, Elsa, PHD	Pharmacology, Physiology & Tox	
Neal, William, MD	Pediatric Cardiology	West Virginia University: WV, USA
Niles, Richard, PHD	Biochemistry And Microbiology	
O'Donnell, James, PHD	Psychiatry And Behavioral Medi	West Virginia University: WV, USA
Olfert, Mark, BS, PHD	Exercise Physiology	West Virginia University: WV, USA

Name, Degree	Department	Non-Host Institution: State, Country
Omar, Hatim, FAAP, MD	Pediatrics	University Of Kentucky: KY, USA
Patton, Brian	Information Technology	
Primerano, Donald A, PHD	Biochemistry & Microbiology	
Rankin, Gary O, PHD	Pharmacology, Physiology & Tox	
Reardon, Dean		University Of Charleston: WV, USA
Rojanasakul, Yon, PHD	Baic Pharmaceutical Sciences	West Virginia University: WV, USA
Sanders, Vickie, MA	Wv-Inbre Office (Wvu)	West Virginia University: WV, USA
Santanam, Nalini, PHD	Pharmacology, Physiology & Tox	
Schafer, Rosana, PHD	Microbiology, Immunology And C	West Virginia University: WV, USA
Schaller, Michael, BS, PHD	Biochemistry	West Virginia University: WV, USA
Serrat, Maria, PHD	Anatomy And Pathology	
Sheil, James M, PHD	Micro, Immun & Cell Bio	West Virginia University: WV, USA
Shiemke, Andrew, BS, PHD	Biochemistry	West Virginia University: WV, USA
Stover, Shawn, PHD	Biology	Davis & Elkins College: WV, USA
Valentovic, Monica, PHD	Pharmacology, Physiology & Tox	
Watson, Valerie, MS	Micro, Immun & Cell Bio	West Virginia University: WV, USA
Webb, Kristen, BS	Wv-Inbre Office (Mu)	
Webster, Amber, BA	Wv-Inbre Office (Mu)	
Weed, Scott, PHD	Neurobiology And Anatomy	West Virginia University: WV, USA
Wonderlin, William, PHD	Biochemistry And Molecular Pha	West Virginia University: WV, USA
Yearsley, Lisa, BS	Pediatrics	University Of Kentucky: KY, USA
Yu, Hongwei, PHD	Biochemistry & Microbiology	
Zeng, Wei-Ping, PHD	Biochemistry And Microbiology	

SUBPROJECT DESCRIPTIONS

Administrative Core

WV-INBRE PROGRAM DIRECTOR;
WVU & MARSHALL U: ADMINISTRATIVE CORE (0001)

TYPE: Administrative Core
%IDeA $: 19.000% **IDeA $:** 690,277

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Rankin, Gary O PHD	Pharmacology, Physiology & Tox	
Davis, Mary E PHD	Physiology & Pharmacology	West Virginia University, Wv Usa
Griffith, Robert PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa
Mangiarua, Elsa PHD	Pharmacology, Physiology & Tox	
Primerano, Donald A PHD	Biochemistry & Microbiology	
Sanders, Vickie MA	Wv-Inbre Office (Wvu)	West Virginia University, Wv Usa
Santanam, Nalini PHD	Pharmacology, Physiology & Tox	
Sheil, James M PHD	Micro, Immun & Cell Bio	West Virginia University, Wv Usa
Shiemke, Andrew BS, PHD	Biochemistry	West Virginia University, Wv Usa
Watson, Valerie MS	Micro, Immun & Cell Bio	West Virginia University, Wv Usa
Webb, Kristen BS	Wv-Inbre Office (Mu)	

Total # human subjects expected for entire study: 0
Total # human subjects enrolled to date: 0

SUBPROJECT DESCRIPTION

The Administrative Core (AC) is composed of the Principal Investigator, Program Coordinator, Core Directors (Genomics, Bioinformatics, ACoRN), Summer Program Coordinators, HSTA Coordinator and Evaluation Coordinator. WV-INBRE office staff at Marshall University and West Virginia University provides administrative support to all activities of the AC. The AC works closely with the PI and is responsible for directing the day-to-day operations of WV-INBRE. The AC also works closely with the Steering Committee and External Advisory Committee to continually work to improve the overall research and student/faculty development and training activities of WV-INBRE. A major responsibility for the AC has been to establish, facilitate and evaluate a network of biomedical researchers at predominately undergraduate institutions (PUIs). The AC also works with Core Directors to establish workshops and training sessions and to facilitate the operation of the Summer Research Program. AC members also normally prepare two newsletters a year and work with the IT Coordinator to help maintain the WV-INBRE website (www.WV-INBRE.org). Specific Core activities are described in the appropriate subproject descriptions and progress reports.

SUBPROJECT PROGRESS

The current funding year is the third year of Phase II of WV-INBRE. The lead institution was Marshall University with West Virginia University serving as a partner lead institution. The AC, led by PI Dr. Gary Rankin, Marshall University, has been involved in many activities during the current grant year (Y11). One of those activities was conducting a competition among partner institution (PUI) faculty for new major PUI research awards. The overall scores and critiques were reviewed by the AC and submitted to External Advisory Committee (EAC) members for their review and recommendation. Based on EAC recommendations, three applications were selected for funding. These projects were selected to be funded starting in May, 2012 and will be conducted by Dr. Yi Chen, Alderson-Broaddus College, Dr. Joseph Horzempa, West Liberty University, and Dr. Qing

Wang, Shepherd University. The AC is also working to replace EAC member, Dr. Kenneth Ramos who resigned. The AC and EAC are discussing a replacement for Dr. Ramos. In addition, the AC also coordinated the very successful Summer Research Program during the summer of 2011, which culminated with the Summer Research Symposium held at Marshall University on July 28, 2011.

Dr. Sheil published a WV-INBRE newsletter for the fall of 2011. Information Technology Coordinator Brian Patton has been working with Elsa Mangiarua to continue the redesign and updating of the WV-INBRE website: http://www.wv-inbre.net/. Many areas of the website have already been updated, and work is in progress to update the remaining portions of the website and change the overall design.

Faculty at the partner institutions competed for Faculty Research Development Awards (FRDAs) to begin in Y11. Four applications totaling $90,000 were selected for funding with awards being provided to Alderson-Broaddus College (1), Shepherd University (1), and the University of Charleston (2). Based on the Y10 competition for major PUI research awards, Dr. Tesfaye Belay, Bluefield State College was selected to receive a major PUI research award for Y11 for his project entitled “Effect of stress on pathogenesis of Chlamydia trachomatis and immune responses in a mouse model”. Dr. Belay is a previous FRDA recipient. In addition, carryover funds were obtained to purchase equipment to upgrade cores at Marshall University and West Virginia University and to provide new equipment at eight PUIs.

Steering Committee and External Advisory Committee Meetings: A Steering Committee meeting was held on July 27, 201 at Marshall University and attended by three External Advisory Committee (EAC) members (Tew [Chair of the EAC], Cutler and Yang). Dr. Fornsaglio, who could not attend, reviewed all meeting materials and contributed to the report. Their EAC report is attached. A second meeting is being planned for early April, 2012.

Additional Meetings and Presentations: (1) Drs. Rankin, Sheil and Primerano attended the IDeA Networks of Biomedical Research Excellence (INBRE) PIs and PCs meeting at NIH on October 4, 2011. (2) Dr. Rankin also met with other Southeast Region PIs on September 21, 2011 in New Orleans, LA to discuss best practices in each INBRE in our region. (3) Dr. Rankin attended the Southeast Regional IDeA Meeting in New Orleans, LA on September 22-24, 2011. Dr. Rankin also co-chaired an oral session entitled Cancer I. (4) Drs. Rankin, Sheil and Primerano helped the West Virginia University COBRE PI, Dr. Laura Gibson, to plan and host the 2011 West Virginia IDeA Meeting. The meeting was held on October 21-22, 2011 at Waterfront Place Hotel in Morgantown, WV. Dr. Rankin presented an update on WV-INBRE, while Dr. Primerano discussed genomics and next generation sequencing. Dr. Santanam gave an oral presentation entitled “Epicardial fat biomarkers in patients with coronary artery disease: WV-Appalachian Heart Study”. (5) Dr. Rankin also prepared a presentation entitled “Update on NIH EPSCOR-Like Programs” for the West Virginia Science and Research Council meeting on January 26, 2012.

The AC is continuing to develop the links between WV-INBRE and the West Virginia University Health Sciences and Technology Academy (HSTA) program headed by PI, Dr. Ann Chester. The WV-INBRE/HSTA Coordinator, Valerie Watson (WVU) has established interactive connections with the HSTA regional clubs throughout the state and representatives of the WV-INBRE partner institutions. Ms. Watson coordinated talks by INBRE undergraduate research interns and high school science educator research interns at some HSTA club meetings. Informational brochures were sent to HSTA Field Site Coordinators in 18 West Virginia counties. In addition, a WV-INBRE-HSTA Task Force has been formed to explore ways to enhance the WV-INBRE and HSTA interactions. WV-INBRE also utilized one of its four supplements to enhance WV-INBRE HSTA activities. Funds from this supplement were used to place eight undergraduates in

WV-INBRE funded research labs at the five partner institutions during the academic year of 2010-2011 and two students have been placed in two laboratories in 2011-2012 to date. Seven WV high school science educators were placed in biomedical research labs at Marshall University, Shepherd University, University of Charleston, West Virginia State University to conduct summer research projects.

Dr. Santanam, Evaluation Coordinator, conducted an internal WV-INBRE evaluation meeting on November 10, 2011. A decision was made to update the way the WV-INBRE database was maintained and Dr. James Denvir was appointed to make the necessary changes and updates. An external review of WV-INBRE is scheduled for late March to evaluate interactions between the AC and the PUIs.

In Y09, WV-INBRE PI Gary Rankin was awarded four ARRA supplements (3P20RR016477-09S1-S4). These supplements supported summer research experiences for PUI students and middle and high school science educators (S1), advanced translation research (S2 and S4) and enhanced the research opportunities for HSTA graduates at the PUIs and HSTA high school science teachers (S3). During Y10, S1 was completed, while S2 (see SPID 0036) and S4 (see SPID 0037) are active research projects, and S3 has actively recruited HSTA graduates to work on WV-INBRE funded research projects during the academic year and recruited HSTA high school science teachers to work on biomedical research projects during the summer. S3 ended during Y11, while S2 and S4 were awarded no cost extensions for one year.

Recently, WV-INBRE was given permission to establish a Center for Natural Products Research as part of the PUI research plan. The Center will coordinate natural products research in the area of finding and developing chemotherapeutic agents. An organizational meeting is planned for early April.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

WV-INBRE PROGRAM EVALUATION (0029)

TYPE: Administrative Core

%IDeA $: 1.000% **IDeA $:** 20,000

INVESTIGATOR, DEGREE	**DEPARTMENT**	**NON-HOST INSTITUTION: STATE, COUNTRY**
Santanam, Nalini PHD	Pharmacology, Physiology & Tox	

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

The overarching objectives of Phase II WV-INBRE are:

1. Develop and enhance the multi-disciplinary statewide network research base by providing research support to faculty, postdoctoral fellows and graduate students at the participating institutions. The network will maintain a scientific focus that will strengthen and build the lead and partner institutions.
2. Provide research opportunities for undergraduate students and to serve as a pipeline for undergraduate students to continue health research careers.
3. Work with partner institutions to enhance research activities for undergraduate students.
4. Enhance science and technology knowledge of the state's workforce.
5. Strengthen and develop stronger relationships between WV-INBRE and other NCRR biomedical research programs (e.g. COBRE and SEPA/HSTA) to enhance biomedical research opportunities for West Virginia's students and participating undergraduate faculty.

The role of the Evaluation Core is to conduct annual program evaluation and provide summative data that demonstrates the accomplishments of WV-INBRE and also provide formative feedback on areas of WV-INBRE program that needs improvement in the subsequent years. WV-INBRE evaluation core accomplishes their goals by performing (i) an internal evaluation utilizing internally-generated survey tools to evaluate the progress made within the program and (ii) an external evaluation performed by soliciting the assistance of professional organizations such as AAAS and evaluating individual objectives of the WV-INBRE program.

Internal Evaluation:

The Internal evaluation monitors the accomplishments of WV-INBRE funded investigators both in relation to their research progress and their improvements in research infrastructure. The Internal evaluation is administered by sending survey forms to all the INBRE funded investigators from primarily undergraduate institutions (PUI) within the state of West Virginia (WV) at the beginning of the Fall semester (Aug-Sep). The survey forms are designed to gather information assessing the progress made in relation to the PUI investigator's research accomplishments as well as assessing the improvements in research infrastructure at their respective institutes, over the previous funding year. The survey also collects data on institutional commitment and infrastructure advancement over the past year. Some of the data being collected from the surveys include: (i) the number of publications, abstracts and presentations by WV-INBRE funded investigators and students (ii) the number of grants submitted for external funding (iii) improvements in research facilities and infrastructure in network institutions (iv) institutional commitment to PUI funded investigators and (v) monitor the increase in recruitment of new faculty and students in the STEM areas in these institutions (increase in pipeline). We also track the progress and accomplishments made by the undergraduate students who participated in the annual WV-INBRE summer program after their graduation.

In addition to the annual surveys, the progress of WV-INBRE funded projects by the PUI investigators are periodically assessed by two ways (i) the principal investigators present their work-in-progress to the Administrative core (AC) and the External Advisory Committee (EAC) during the spring steering committee

meeting and (ii) the members of the AC assess the progress made by the funded INBRE investigators by contacting both the individual investigators and their mentors (during Fall) and presnt their findings at the annual Evaluation meeting held each fall. At the end of the evaluation meeting, assessment reports are sent to the PUI investigators and their respective mentors.

Additionally, members of the AC periodically visit some of the network institutes, to assess progress in infrastructure made in these funded institutions. During the Fall Evaluation retreats the AC also evaluates the progress made by each of the WV-INBRE funded cores, i.e. bio-informatics core, genomics core and imaging core.

External Evaluation:
In phase 2 of the funding cycle, in addition to the internal evaluations, the Evaluation Core also conducted the external evaluation. The first external evaluation meeting was conducted in March of 2010.

AAAS Evaluation meeting: In March of 2010, AAAS helped evaluate the WV-INBRE Bio-informatics and genomics cores. The AAAS provided a 26 page evaluation report on their findings and recommendations. Several of the recommendations have been included as part of the services provided by the two cores in the subsequent years.

SUBPROJECT PROGRESS

Reports from the Internal Program Evaluation Meeting held November 2011:

Surveys were sent out to nine network institutions that are part of the WV-INBRE network. From each institute, one lead investigator and one institutional official were identified to complete the surveys. Completed surveys were received from seven institutes. The data obtained from the surveys indicated that, this past year there were twelve papers published, one published abstract and twenty five presentations at national and regional meetings by network investigators. A total of $9,333,299 of extramural funding was obtained this past year by the various network institutes. Funding sources included NSF and NASA-EPSCOR. Several of the network institutions have either provided matching funds for research or provided improved infrastructure for WV-INBRE investigators. There has been an increase in research partnerships between the two lead institutions and network institutions but not within the WV-INBRE network investigators, which needs to be addressed. Such partnership will strengthen the research base in West Virginia. The summer internship program continues to draw the best students from the network. This past year funds were also available for 3 HSTA high-school teachers to have summer research experience. This year 75 students had applied for this program which is an increase from previous years and 36 interns were selected. At the summer research symposium that was held on July 28th 2011 at Marshall University campus, 56 posters and 6 oral presentations were made by both INBRE investigators, HSTA high-school teacher and INBRE supported students.

Online Resources in progress: With the assistance from the information technology expert, Mr. Brian Patton and the Biostatistician Dr. James Denvir, we have started establishing a student database that will list all the students who have been associated with WV-INBRE since its inception. This database will help in tracking the progress made by WV-INBRE associated students over the years.

We are also in plans of setting up online evaluation surveys accessible to all WV-INBRE funded investigators. We anticipate that user-friendly online surveys will help improve responses from the investigators and help gather complete data.

External Evaluation March-April 2012: WV-INBRE is planning on conducting the second external evaluation meeting the spring of 2012. The evaluation core is currently in the process of planning for this meeting. With the recommendation from the AC, the topic to be evaluated this year is “The

Interactions between the administrative core and PUI investigators". The evaluation core has already identified, Dr. David Essig, Professor, Department of Biology, Geneva College, Pennsylvania, to be the chair of the external evaluation team. A three member review team will help with the evaluation to be held in March-April 2012.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

Career Development

INBRE: WVU & MARSHALL U: TRAVEL AWARDS (0011)

TYPE: Career Development

%IDeA $: 0.000% **IDeA $:** 10,000

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Griffith, Robert PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa
Belay, Tesfaye PHD	Biological Sciences	Bluefield State College, Wv Usa
Huggins, Luke PHD	Biology	West Virginia Wesleyan College, Wv Usa
Mangiarua, Elsa PHD	Pharmacology, Physiology & Tox	

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

The WV-INBRE offers travel awards to partner faculty and students. A maximum of $1,500 per travel award is normally allowed. Funds were to be used for travel expenses, lodging, meals, registration, and other justifiable expenses. So that funds from the current grant year can be used to support the request, the travel was to be completed before April 15, 2011. There are no fixed deadlines; applications are considered as they are received. The highest priority are given to faculty members and students who would be presenting biomedical research they have conducted during the WV-INBRE summer research program or at their home institutions. Students can be supported to attend meetings with their summer or institutional mentor. No funds can be spent to support the summer mentors' travel. Travel to meetings by faculty and students who would not be presenting research, but anticipate that attendance will enhance their educational or profession development or is beneficial to the WV-INBRE would be considered if requested. Meetings centered on biomedical research receive priority. Other meetings, if adequately justified, would be considered for awards.

SUBPROJECT PROGRESS

This year we supported travel for PUI faculty and students to present results of WV-INBRE-sponsored research at national meetings from the following institutions: Dr. Kaushal and two students, University of Charleston, $2,955; one student, Bluefield State College, $1,113.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

INBRE: WVU & MU SUMMER RESEARCH FELLOWSHIP PROGRAM (0013)

TYPE: Career Development

%IDeA $: 1.000% **IDeA $:** 38,325

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Mangiarua, Elsa PHD	Pharmacology, Physiology & Tox	
Griffith, Robert PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa
Niles, Richard PHD	Biochemistry And Microbiology	
Salisbury, Travis PHD	Pharm, Physiol & Toxicology	
Shiemke, Andrew BS, PHD	Biochemistry	West Virginia University, Wv Usa
Wonderlin, William PHD	Biochemistry And Molecular Pha	West Virginia University, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

The objective of the summer fellowship program is to provide partner institution faculty a meaningful research experience that will stimulate their interest to develop mentored research programs in biomedical science that will lead to receipt of extramural funding. Additionally, the experience gained will better enable them to mentor undergraduate students doing research at their home institutions.

SUBPROJECT PROGRESS

Two faculty members from the partner institutions, Dr. Kimberly Fisher from Bethany College and Dr. Gary Morris from Glenville State University, participated as 2011 summer fellows. They presented their research at the 2011 Summer Research Symposium. For 2012, there will be two faculty members participating as summer fellows. Dr. Jennifer Franko from Bethany College will work at West Virginia University with Dr. Rosana Schafer. Dr. Gary Morris from Glenville State College will return to Marshall University to work in the lab of Dr. Travis Salisbury. Both fellows will present their research at the 2012 Summer Research Symposium which will be held on July 26th at West Virginia University.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

WV-INBRE HEALTH SCIENCES AND TECHNOLOGY ACADEMY (HSTA) (0034)

TYPE: Career Development

%IDeA $: 3.000% **IDeA $:** 104,878

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Watson, Valerie MS	Micro, Immun & Cell Bio	West Virginia University, Wv Usa
Aguilar, Jarrett PHD	Natural Science & Mathematics	West Liberty University, Wv Usa
Belay, Tesfaye PHD	Biological Sciences	Bluefield State College, Wv Usa
Chen, Yi C PHD	Biology	Alderson-Broaddus College, Wv Usa
Crick, Darrell PHD	Chemistry	Concord University, Wv Usa
Hankins, Gerald PHD	Biology	West Virginia State University, Wv Usa
Harris, Rob PHD	Biology	West Virginia State University, Wv Usa
Huggins, Luke PHD	Biology	West Virginia Wesleyan College, Wv Usa
Ivanov, Alexey PHD	Biochemistry	West Virginia University, Wv Usa
Kim, Seung-Yun PHD	Comp Sci, Math & Engineering	Shepherd University, Wv Usa
Kreisberg, Robert PHD	Biology	West Liberty University, Wv Usa
Reardon, Dean		University Of Charleston, Wv Usa
Santanam, Nalini PHD	Pharmacology, Physiology & Tox	
Sheil, James M PHD	Micro, Immun & Cell Bio	West Virginia University, Wv Usa
Shiemke, Andrew BS, PHD	Biochemistry	West Virginia University, Wv Usa
Troyer, Timothy PHD	Chemistry	West Virginia Wesleyan College, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

WV-INBRE will work directly with the HSTA program to engage high school graduates who demonstrate interest in biomedical research. Students who participate in the HSTA program in their high schools maintain competitive GPAs and already demonstrate interest in math and science. The HSTA program is a well-established and renowned program initiated by funding received from the NCRR's Science Education Program Award (SEPA) program. WV-INBRE will encourage and assist HSTA students to continue to expand upon their research experiences by entering WV-INBRE funded biomedical research programs at one of our PUIs throughout the state of West Virginia. Thus, the NCRR-funded WV-INBRE and HSTA programs propose to interface with each other to form a regional model program whereby high school graduates who have participated in biomedical research-focused training through the HSTA initiative will be provided opportunities to further develop their interest in biomedical research at an undergraduate institution in the WV-INBRE network.

The interface between WV-INBRE and HSTA will initially focus on two phases of the program: (1) assist in recruiting HSTA graduates into WV-INBRE PUIs, and (2) engage HSTA graduates in the WV-INBRE-funded biomedical research programs at these institutions. A WV-INBRE/HSTA Coordinator will direct this effort and will work directly with the WV-INBRE Administrative Core. In developing phase 1, the coordinator will establish interactive connections with the HSTA regional clubs throughout the state and representatives of the WV-INBRE partner institutions. Therefore, the direct involvement of WV-INBRE investigators and their

student interns with HSTA graduates will be supported throughout the formative high school level training years of the HSTA students.

WV-INBRE-funded faculty project investigators at the PUIs have shown interest in developing programs to attract qualified high school graduates to the biomedical research programs at their institutions and will work to provide contact and mentoring resources for the HSTA students and graduates. Thus, we hope to establish a viable means to continue the training and development of these students throughout their 4 years of undergraduate education. In developing phase 2, the coordinator will work closely with the HSTA program, the PUIs, and the WV-INBRE database manager to strengthen the tracking of these students as they progress through their careers and better document how many of these students pursue a biomedical research related career and the nature of their career choices.

SUBPROJECT PROGRESS

In phase 1, the WV-INBRE/HSTA Coordinator hoped to establish interactive connections with the HSTA regional clubs throughout the state and representatives of the WV-INBRE partner institutions and has been successful. The coordinator has maintained contact with all 15 HSTA Field Site Coordinators (FSCs). They provided information concerning the 78 HSTA clubs in the 29 high schools in 26 counties in West Virginia. A list of HSTA teachers and their contact information, the various HSTA club meeting dates, and locations were provided. The FSCs distributed brochures at their HSTA club meetings. Coordinator spoke at the Mon/Marion HSTA orientation and participated in a group panel discussion about INBRE. INBRE-funded researcher, Dr. Yi Charlie Chen of Alderson-Broaddus College, and a summer 2010 /2011 INBRE intern, Rebekah Sine spoke about their INBRE research and experiences at a Tucker County High School HSTA club meeting. INBRE-funded researcher, Dr. Gagan Kaushal, of University of Charleston spoke at a South Charleston High School HSTA club meeting about his research. At these meetings a presentation and informational brochures were presented to students concerning the opportunities of the WV-INBRE program.

In phase 2, the WV-INBRE/HSTA Coordinator has used information provided by the HSTA database, HSTA FSCs, and representatives at the PUIs to locate and contact HSTA graduates with biomedical science majors at the PUIS. The WV-INBRE grant received a NCRR ARRA Supplement directed toward the HSTA initiative. Funding was provided for research positions and supplies for 12 HSTA graduates at the PUIs. During the 2010-2011 academic year, 8 undergraduates were placed in the WV-INBRE funded research labs with the following mentors: Christina Sargent and Sasha Richmond in Tesfaye Belay's lab at Bluefield State College; Jeremy Lloyd in Darrell Crick's lab at Concord University; Anthony Johnson in Robert Harris' lab at West Virginia State University; Amber Wilson in Jarrett Aguilar's lab and Kyle McGill in Robert Kreisberg's lab at West Liberty University; and Jacob Wagoner in Timothy Troyer's lab and Morgan Miller in Luke Huggins' lab at West Virginia Wesleyan College. HSTA coordinator (Valerie Watson) and other INBRE representatives (Andrew Shiemke, Gary Rankin, Elsa Mangiarua, Nalini Santanam) spoke at a number of HSTA symposia during April-May 2011. Talks were given at the following symposia: Braxton/Webster counties, Marion/Monongalia counties, Tucker/Barbour counties, Taylor County, Preston County, Greenbrier/Fayette counties, Eastern Panhandle Region, and Kanawha County. INBRE representatives also acted as judges for the science projects. Five high school science teachers were funded for 9-week summer research internships that began on June 13, 2011. Wendy Lee from Musselman High School and Denise Gipson from Jefferson High School were mentored by Dr. Seung-yun Kim at Shepherd University. Timothy Clifton from Herbert Hoover High School and Tiffani Smith from Huntington High School were mentored by Dr. Robert Harris at West Virginia State University. Rene Norman from Sissonville High School was mentored by Dr. Dean Reardon at University of Charleston. Two additional teachers were funded for internships this summer through other funding: Johnathan Baldwin from Scott High School was mentored by Dr. Gerald Hankins at West Virginia State University and Brian McNeel from Cabell Midland High School was mentored by Dr. Richard Egleton at Marshall University.

Students and high school science educators presented their research at the 11th annual INBRE symposium held Thursday July 28, 2011 at Marshall University. In addition to a poster, Denise Gipson gave an oral presentation summarizing the research performed in Dr. Seung-yun Kim's lab. During the month of July, the following INBRE representatives participated in HSTA Summer Institutes at West Virginia University, Marshall University, and West Virginia State University: Ashley Gerard, Dr. Gerald Hankins and Dr. Robert Harris of West Virginia State University, Dr. Elsa Mangiarua of Marshall University, and Valerie Watson, and 2011 INBRE summer interns: John Baldwin, Hannah Cavender, Timothy Clifton, Carissa Dunn, Ryan Johnson, Brian McNeel, Tiffani Smith and John Phillip Thomas.

During the 2011-2012 academic year, 2 undergraduates were placed in the WV-INBRE funded research labs with the following mentors: Sasha Richmond in Tesfaye Belay's lab at Bluefield State College and Amber Wilson in Jarrett Aguilar's at West Liberty University.
INBRE/HSTA task force group was created in March 2011 and meets on a monthly basis during the academic school year to better coordinate the initiatives of INBRE and HSTA programs. INBRE members include: Dr. James Sheil, Valerie Watson, Vickie Sanders, Dr. Elsa Mangiarua, Dr. Andrew Shiemke, Jim Denvir and HSTA members include: Dr. Ann Chester, Kas Kasten, Cathy Morton-McSwain, Sara Hanks, Merge McMillion, and Summer Kuhn .

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PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?
N 2. Will vertebrate animals be used next year?
N 3. Will recombinant DNA experiment(s) be conducted next year?
N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:
N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

Education & Training

INBRE: WVU & MU SUMMER RESEARCH INTERNSHIP PROGRAM (0009)

TYPE: Education & Training

%IDeA $: 7.000% **IDeA $:** 254,039

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Mangiarua, Elsa PHD	Pharmacology, Physiology & Tox	
Blough, Eric PHD	Biological Sciences	
Brock, Robert PHD	Physiology And Pharmacology	West Virginia University, Wv Usa
Callery, Patrick BS, PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa
Collier, Simon PHD	Biological Sciences	
Egleton, Richard BS, PHD	Pharmacology, Physiology & Tox	
Georgel, Philippe PHD	Biological Sciences	
Gibson, Laura F PHD	Pediatrics	West Virginia University, Wv Usa
Griffith, Robert PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa
Grover, Lawrence PHD	Pharmacology, Physiology & Tox	
Hardman, Wanda Elaine PHD	Biochemistry And Microbiology	
Hollander, John M PHD	Exercise Physiology	West Virginia University, Wv Usa
Huber, Jason BS, PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa
Kim, Jung Han PHD	Pharmacology, Physiology & Tox	
Li, Bingyun PHD	Orthopedics	West Virginia University, Wv Usa
Lukomski, Slawomir PHD	Microbiology, Immuno &Cell Bio	West Virginia University, Wv Usa
O'Donnell, James PHD	Psychiatry And Behavioral Medi	West Virginia University, Wv Usa
Olfert, Mark BS, PHD	Exercise Physiology	West Virginia University, Wv Usa
Rankin, Gary O PHD	Pharmacology, Physiology & Tox	
Santanam, Nalini PHD	Pharmacology, Physiology & Tox	
Schafer, Rosana PHD	Microbiology, Immunology And C	West Virginia University, Wv Usa
Schaller, Michael BS, PHD	Biochemistry	West Virginia University, Wv Usa
Serrat, Maria PHD	Anatomy And Pathology	
Shiemke, Andrew BS, PHD	Biochemistry	West Virginia University, Wv Usa
Stoilov, Peter PHD	Biochemistry	West Virginia University, Wv Usa
Valentovic, Monica PHD	Pharmacology, Physiology & Tox	

Yu, Hongwei PHD	Biochemistry & Microbiology	
Zhang, Hunter MD, PHD	Physiology And Pharmacology	West Virginia University, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

The goal of the summer research internship program is to provide a meaningful research experience for PUI students that will stimulate their interest in pursuing careers in biomedical sciences. Faculty from Marshall University and West Virginia University will provide research projects and mentoring for students during the 9-week program. During the course of the program, supplemental activities including lectures and seminars on various topics related to biomedical research and careers in the biomedical sciences are presented to the students. They are encouraged to consider obtaining post-graduate training in the biomedical sciences following graduation. In the last week of the program, a summer research symposium will be held where students will present the results of the their project before their peers and WV-INBRE members and faculty members. Students will be encouraged to present their research at other meetings in West Virginia and throughout the country.

SUBPROJECT PROGRESS

Sixty-eight students from the partner institutions (PUIs) applied for the Summer Research Program in Y11. Twenty-two students were accepted with eleven conducting research at Marshall University and eleven at West Virginia University under the mentorship of faculty at the lead institutions. The program was concluded on July 28, 2011 with a Summer Research Symposium held at Marshall University. Dr. Terrence J. Monks, Professor and Chair of Pharmacology and Toxicology, College of Pharmacy, University of Arizona Health Sciences Center was the keynote speaker. Students and faculty from the Summer Program, PIs holding major PUI research awards, those supported by Faculty Research Development Awards and students and high school teachers supported by the WV-INBRE--HSTA initiative made oral and poster presentations; a total of 56 poster and 6 oral presentations were made by participants. In the fall of 2011, the 2012 Summer Research Program was promoted at all WV-INBRE partner institutions through presentations or through announcements mailed to the institutions. For the 2012 program, a Mentors Directory listing prospective research mentors and available projects for the summer research program, as well as application forms for the summer student internship and faculty fellowship programs, were developed and placed on the WV-INBRE website. Electronic submission of applications was available. Currently, the supplemental activities of the summer program for Y11 are being developed. Three summer interns made presentations at the 9th Annual Undergraduate Research Day at the Capitol in Charleston, WV on January 26, 2012. Summer interns presented their summer research at the Orthopaedic Research Society (ORS) Annual Meeting in San Francisco, CA (Feb 2012), the American College of Sports Medicine (ACSM) Annual Meeting in Denver, CO (June 2011), the American Association for Cancer Research 102nd Annual Meeting in Orlando, FL (April 2011), and the American Association for Cancer Research Metabolism and Cancer Special Conference in Baltimore, MD (October 2011).

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

Infrastructure

WV-INBRE: MU & WVU BIOINFORMATICS CORE (0002)

TYPE: Infrastructure

%IDeA $: 4.000% **IDeA $:** 150,905

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Davis, Mary E PHD	Physiology & Pharmacology	West Virginia University, Wv Usa
Boskovic, Goran PHD	Biochemistry & Microbiology	
Denvir, James PHD	Biochemistry And Microbiology	

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

The Bioinformatics Core provides investigators at the lead and partner institutions access to bioinformatics analysis tools and expert assistance in utilizing these tools for data analysis and discovery. Access to Vector NTI is provided for gene sequence analysis. Analyses of gene expression data, including Ingenuity Pathways Analysis (IPA), are provided in conjunction with the Genomics Core and for expression results generated off-site. In addition, IPA access or analyses are provided to assist investigators in generation of hypotheses.

WV-INBRE sponsors a Bioinformatics Workshop every summer for investigators at the partner and lead institutions. These workshops focus on methods and techniques relevant to the research projects supported by WV-INBRE and are presented by content experts, such as NIH-NCBI personnel and training staff for software supported by WV-INBRE. Bioinformatics Core personnel have developed courses on use of bioinformatics tools at the lead institutions and will assist partner institutions in developing courses or exercises to enhance existing courses.

SUBPROJECT PROGRESS

The Bioinformatics Core (BC) assists investigators in the analysis of sequence-related data, either in conjunction with the Genomics Cores or directly with investigators (some have microarray data from external vendors and others have PCR array data). BC members provide assistance with experimental design, statistical analysis and use of Ingenuity Pathways Analysis (IPA) tools in the interpretation of results. The BC supports access to IPA and sequencing/expression analytical tools (Partek, SAM and others). These have resulted in 6 publications in peer reviewed journals, with an additional paper in press, and 16 presentations at national or regional meetings. BC resources have supported 18 grants, including 7 funded NIH grants, 2 pending NIH grants, a grant pending for DARPA, 2 grants funded by local sources, and 5 grants that were not funded (NIH, NSF, IARPA and a private foundation).

Computational facility at WVU. In partnership with the CoBRE-funded WVU Center for Neuroscience Genomics Core and the WV Clinical Translational Science Institute, the WV-INBRE has established and equiped a computational facility to support analyses of large or complex datasets using bioinformatic or statistical approaches. The facility also functions as a classroom lab, and is in use for teaching of bioinformatics to graduate students.

New personnel

With the departure of Dr. James Denvir from WVU, to lead the bioinformatics/biostatistics efforts at Marshall University, WVU is in the process of hiring a new bioinformaticist/biostatistician. This position is being funded by CoBREs at WVU (Neuroscience and Cancer), WV-INBRE, and the emerging School of Public Health. The pool of candidates includes many individuals with

experience analyzing high dimensional data from expression or NexGen datasets, and using various algorithms for developing new methods for extracting meaningful relationships from these datasets. On campus interviews began in January.

Participation in Program of Excellence: Dr. James Denvir, Bioinformatics Core Co-Director, is participating as a potential mentor for the Southeast INBRE Region Program of Distinction Bioinformatics Internships for Undergraduate Student. If the project is selected by undergraduate student(s), Dr. Denvir, collaborating with a faculty researcher, will mentor the student intern in bioinformatics analysis of biological data.

We are currently using Partek for NextGeneration Sequence analysis. Dr. Denvir is additionally using CASAVA to analyze data generated by the Illumina HiSeq 1000 at Marshall University, and MACS to analyze Chip-seq data. We are currently initiating a month-long trial of CLC Genomics Server and Workbench with a view to purchase and install before the beginning of the next budget year. We anticipate this software will enable us to centralize access to and analysis of Next Generation Sequencing data.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

WV-INBRE: WVU & MARSHALL U: EQUIPMENT GRANTS (0010)

TYPE: Infrastructure

%IDeA $: 8.000% **IDeA $:** 280,806

INVESTIGATOR, DEGREE	**DEPARTMENT**	**NON-HOST INSTITUTION: STATE, COUNTRY**
Griffith, Robert PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

As part of the Faculty Research Development Program, grants are made available to partner institutions and researchers to purchase equipment that will enhance the biomedical research infrastructure at the partner institution. The maximum that could be requested was $30,000.

SUBPROJECT PROGRESS

In order to enhance the research capabilities of the PUI schools we allocated funds to purchase needed laboratory equipment that could be directly related to ongoing biomedical research projects. The grants were limited items of equipment with a maximum of $30,000. We made the following awards: Dr. Aguilar, West Liberty University, $6,139; Dr. Kreisberg, West Liberty University, $30,000; Dr. Fultz, West Virginia State University, $30,000; Dr. Chen, Alderson-Broaddus College, $26,200; Dr. Stover, Davis & Elkins College, $18,501; Dr. Wing, Shepherd University, $25,925; and Dr. Sal, West Virginia Wesleyan College, $30,000.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

GENOMICS CORE (0033)

TYPE: Infrastructure

%IDeA $: 4.000% **IDeA $:** 156,114

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Primerano, Donald A PHD	Biochemistry & Microbiology	
Boskovic, Goran PHD	Biochemistry & Microbiology	
Cuff, Christopher F PHD	Microbiol, Immunol Cell Biol	West Virginia University, Wv Usa
Dementieva, Yulia PHD	Mathematics	Emmanuel College, Ma Usa
Denvir, James PHD	Biochemistry And Microbiology	
Fan, Jun BS, PHD	Biochemistry & Microbiology	
Georgel, Philippe PHD	Biological Sciences	
Ivanov, Alexey PHD	Biochemistry	West Virginia University, Wv Usa
Kim, Jung Han PHD	Pharmacology, Physiology & Tox	
Salisbury, Travis PHD	Pharm, Physiol & Toxicology	
Zeng, Wei-Ping PHD	Biochemistry And Microbiology	

Total # human subjects expected for entire study:	10
Total # human subjects enrolled to date:	10

SUBPROJECT DESCRIPTION

The main objective of the Genomics Core Facility is to enable the genomic research goals of WV-INBRE research projects. We provided the following services in Y11: (1) next generation sequencing, (2) microarray-based gene expression profiling and pathway analysis, (3) automated DNA sequencing and access to DNA/RNA sequence analysis software, (4) access to real-time PCR instrumentation, NanoDrop spectrophotometers and Agilent BioAnalyzer for DNA/RNA analysis and (5) access to the Luminex 100 for multiplex protein detection. The Core will provide centralized genomic, bioinformatic and biostatistical services that are not available to individual labs and provide training in methods needed for completion of the research projects. These services will position project investigators to gather critical data efficiently and analyze it with the most up to date bioinformatic and statistical tools. All Genomics Core Facility services are available to investigators at Marshall University (MU), West Virginia University (WVU) and WV-INBRE Partner Institutions

SUBPROJECT PROGRESS

Genomics Core Staffing and Instrumentation

Dr. Donald Primerano served as the director of the Genomics Core Facility. He oversaw all Genomics Core services and insured that all projects were completed in a fair and timely fashion. Drs. Primerano and James Denvir oversaw the development of next generation sequencing (NGS) and delivery of all core facility services. Dr. Primerano advises Drs. Boskovic and Fan in the development of NGS and microarray protocols, ensures that projects are prioritized and completed in a timely fashion, provide training to COBRE investigators in NGS applications, and advertise Core services via seminars and the MU website. Drs. Primerano, Denvir, Boskovic and Fan meet with clients at the outset for planning of experimental design and for the review of the dataset at the

completion of the work.

Dr. Denvir was hired as an Assistant Professor in the Department of Biochemistry and Microbiology at Marshall University in July 2011. He performs analysis of NGS datasets and assist in downstream pathway analyses. He is well acquainted with Casava, the software suite provided by Illumina for immediate data processing from the HiSeq 1000, and has attended training sessions in the use of Tuxedo Suite software and CLC Genomics Workbench. The latter is currently being trialed by the Genomics Core, and the intention is to install and support use of this software in the near future. Dr. Denvir also has expertise in MACS for analysis of Chip-Seq data and with IGV for the visualization of analyzed data. He will continue to evaluate and select new analytical tools needed for NGS applications as these become available, develop new forms of analysis and consult with investigators after the analysis is complete. He will work with MU Computing Services to ensure that our servers and storage devices are operating efficiently and have up to date operating systems.

Dr. Boskovic develops protocols for NGS applications, prepare samples for NGS, operate the HiSeq1000 sequencer, perform routine maintenance of HiSeq sequencer and c-Bot cluster station, and manage raw data output. He examines and records quality metrics from all runs on the HisSeq1000. Dr. Boskovic continues to serve as microarray facility manager and perform sample QCs, labeling, hybridization onto microarray slides, and microarray scanning. Dr Boskovic will additionally provide pathway analysis on expression data sets. He insures that the HiSeq1000 and Microarray scanner receive preventative maintenance

Dr. Fan directs the operation of conventional DNA sequencing/genotyping, constructs NGS libraries and manages ACoRN genomic DNA banks. DNA sequencing, genotyping, and genomic DNA banking are carried out by Dr. Fan and Jennifer Yu. Drs. Boskovic and Fan troubleshoot any problems with DNA/RNA extraction, NGS library construction, and microarray methods. Dr. Fan oversaw DNA sequencing and performed genotyping and LDLR gene sequencing related to the Familial Combined Hyperlipidemia project (SPID #0026).

Genomics Core Equipment: The Core is currently equipped with an Illumina HiSeq1000, a Linux server with 48 Terabytes for storage of sequencing data, 48 CPUs and 128GB memory, a Mac OS X Server with 12 CPUs, 6TB storage and 48GB memory, Agilent DNA Microarray Scanner, Agilent Microarray Hybridization Station, two ABI Model 7000 Sequence Detection Systems (for RT PCR), one StepOnePlus Real Time PCR system, three Agilent 2100 Bioanalyzers (two of which handle high sensitivity DNA Chips), 1 Perkin Elmer, 2 Qubit and 2 NanoDrop spectrophotometers, Applied Biosystems 3130 Genetic Analyzer, Luminex 100 system, 5 MJ/BioRad PTC200 Thermal Cyclers, 1 Savant Speed Vac Concentrators and 1 Fast Prep FP120 Homogenizer. All instruments except the FP120 are kept on service agreements. The Agilent Scanner has been upgraded to 2 micron resolution to allow for scanning of Agilent high density arrays (1.2 million features per array). The ABI3130 Genetic Analyzer (four capillary) can sequence 96 templates per day or ~500 STR genotypes per day is sufficient for the needs of the WV-INBRE program and the university.

Collaborations and Publications: The Genomics Core has a strong record of supporting the genomic and statistical needs of statewide research programs and individual investigators. This includes ongoing collaborations with the WV-INBRE and pending grant applications for the Center for Nutrition and Cancer and the WV Cancer Genomics Network.

Table 1. Genomics Core Collaborations with funded/pending applications

Title: WV-INBRE Phase II
P.I.: Gary Rankin PhD
Agency: NIH/NCRR
Roles: D. Primerano, Director of Genomics Core and Appalachian Cardiovascular Research Network
Status: Funded

Title: West Virginia Cancer Genomics Network
P.I. Richard Niles, PhD (MU)
Agency: WV Division of Science and Research (Higher Education Policy Commission)
Role: Donald Primerano, co-investigator and Director of NGS Facility
Status: Pending

Title: COBRE Center For Nutrition and Cancer
P.I. Elaine Hardman, PhD (MU)
Agency: NCRR/NIGMS NIH
Role: Donald Primerano, co-investigator and Director of NGS Facility
Status: Pending

Genomics Core staff members have authored three publications and were acknowledged in two publications in the past year.

Publications
1. Dementieva, Y., J. Denvir, L. Wei, D. A. Primerano, T. L. Green, P. Wehner, M. R. Flood, D. Calica, B. Freeman, M. Huff, S. Dodson, C. Hill, A. Francis, K. McIntyre, R. Kreisberg, S. Warren, H. Blackwood, M. Davis, H-M Lee. Identification of genes contributing to cardiovascular disease in overweight and obese individuals from West Virginia. WV Medical Journal 108: 23-31 (2012)
2. Varney, M. E., J. Buchanan, Y. Dementieva, W. E. Hardman, V. E. Sollars. A high omega-3 fatty acid diet has different effects on early and late stage myeloid progenitors. Lipids 46(1), 47-57 (2011)
3. Cieply, B., Riley, P., Pifer, P., Widmeyer, J., Addison, J., Ivanov, A., Denvir, J., Frisch, S. Suppression of the epithelial-mesenchymal transition by grainy head-like-2. Cancer Research, 2012. In Press.

Acknowledgments:
1. Bhullar, J., and V. E. Sollars. YB-1 expression and function in early hematopoiesis. Immunogenetics 63, 337-350 (2011)
2. Akinsete, J. A., G. Ion, T. R. Witte, W. E. Hardman. Consumption of high ω-3 fatty acid diet suppressed prostate tumorigenesis in C3(1) Tag mice. Carcinogenesis. 33:140-8 (2012)

Y11 usage: The Genomics Core provided the following services to WV-INBRE, COBRE, MU, and WVU during the reporting period (last column in the following table).

	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	2011-Pres	TOTAL
Agilent Microarray (arrays/year)	54	100	41	80	188	320	112	40	935
ABI 3130 (capillary sequencer) templates/year				480	1117	866	1294	1407	723	1614	966	8467
ABI Model 7000s and StepOnePlus (RT-PCR) runs/year						117	92	64	145	240	276	283	88	1305
Agilent Model 2100 Bioanalyzers (runs/year)	95	133	55	87	100	45	98	24	637
Illumina HiSeq1000 (#runs/year)	NA	NA	NA	NA	NA	NA	NA	6	6

External Advising and Training:
Dr. Ivana Yang, an established microarray investigator at the University of Colorado in Denvir and a member of the WV-INBRE External Advisory Committee, served as our external advisor on experimental design and interpretation of data. She also advised in the selection of the Illumina

NGS system and participated in the review of NGS pilot grant applications. In order to stay current with microarray technology and methods of data analysis, core staff members are required to attend workshops or national meetings in their specific areas. Drs. Boskovic, Dr. Fan and Primerano attended the Association of Biomolecular Resource Facilities (ABRF) in February 2011. Drs. Denvir, Boskovic, Fan, Primerano and J.H. Kim will present a poster entitled “Identification of Potential Susceptibility Variants for Obesity and Type 2 Diabetes in the TALLYHO Mouse” at the ABRF in March 2012.

Next Generation Sequencing Pilot Grants

In Y10 and Y11, the WV-INBRE program funded six biomedical research pilot grants that required the use of next generation sequencing (NGS) technologies as part of the experimental design. These technologies include whole genome sequencing, whole exome sequencing, RNA-Seq, Chip-Seq, Methyl-Seq, microbiome studies and related high throughput methods. The primary purpose of these awards was to allow investigators to gather preliminary data for investigator-initiated grant applications in the biomedical sciences. This solicitation for applications was open to applications from investigators at West Virginia University, Marshall University and WV undergraduate institutions that are part of the WV-INBRE network. Funds ($12,000 per awardee) were used for the acquisition of NGS supplies or services. NGS data from two of the six projects (#1 and #4) has been analyzed and will be presented at conferences in March 2012. Data from projects #2 and #5 is being analyzed at present. The following sections describe the progress of each of the six pilot projects. A table showing protections against risks for each project is given in the Appendix materials.

NGS Project #1

Title

Oral Health Disparities Among Elders With and Without Cognitive Impairment: Microbiome Analysis
Subproject investigator. Chris Cuff PhD

Introduction

An ongoing, multi-Institution, interdisciplinary research project at West Virginia University and Duke University is investigating the link between oral health and mental function in older citizens of West Virginia in an NIH-sponsored study. As part of this study, we are performing microbiome analysis of oral sub-gingival plaque samples by high throughput DNA sequencing. The goal of this work is to determine risk factors linking oral health and cognitive impairment.

Hypothesis/Specific Aim

It is our hypothesis that microbial community composition influences the severity of oral disease, which plays a role in cognitive impairment, including development of Alzheimer’s disease. The Specific Aim of this study is to perform molecular identification of bacterial phylotypes in subgingival plaque samples from participants in clinical research aimed at addressing the role of oral health in cognitive impairment.

Methods

Twenty-two subgingival plaque samples from clinically healthy and diseased periodontal sites were obtained from study participants as part of their oral evaluations following informed consent. From each participant, multiple plaque samples (2-6) from several sites were obtained and pooled into ‘healthy’ and ‘diseased’ sites based on clinical evaluation and pocket probing depths of 5mm. DNA was isolated from these samples using commercially available kits, and PCR amplification of a portion of the gene encoding the 16S rRNA subunit was accomplished using the method of Bartram et al. (Appl. Environ. Microbiol. 2011 June; 77(11): 3846–3852). PCR products were sequenced in 2 flow cells by the Marshall University Genomics Core Facility. Resulting sequences are being analyzed using Quantitative Insights into Microbial Ecology (QIIME) computer program

running in the Amazon Web Services cloud computing system.

Results and Discussion
Approximately 135 million paired-end reads were obtained from the successful sequencing run. Post sequencing data processing has involved de-multiplexing the sequences and assigning the sequences to the correct sample identifier. Next, the paired end reads were processed to join the 2 ends at overlapping regions of high quality sequences (Q score 30 or greater), and extraneous sequences used for multiplexing and sequencing were removed, yielding analyzable sequences of ~160 bases. About 30% of the starting data set successfully passed through quality filtering and are currently being processed by QIIME. Preliminary analysis of a subset of high-quality processed sequences indicates that the sequencing and data analysis pipeline is a success. Each study sample is providing a 'microbial signature' that is consistent with oral bacteria, and differences in microbial populations within individual samples make it possible to predict whether the sample was obtained from 'healthy' or 'diseased' sites, i.e. high numbers of sequences corresponding to disease-associated 'red complex' bacteria such as Prevotella sp. and Porphyromonas sp., as well as Treponemes can be detected some but not all samples. Ongoing work will center on completing the analysis and then linking these findings to other parameters of oral health and cognitive function to determine whether a distinct microbial signature is predictive of decreased cognitive function.

NGS Project #2
Title: Epigenetic Modulations of Breast Cancer by Omega-3 Fatty Acids Mediated By Changes in Histone Post-Translational Modifications.

Subproject investigators: Philippe Georgel PhD and Elaine Hardman PhD

Introduction: The goal of the proposed research was to identify breast cancer-related genes epigenetically regulated through changes in histone post-translational modifications (PTM) involved in imprinting events mediated by omega-3 fatty acid diet. Our preliminary work has identified two histone PTM, H3K18 acetylation (H3K18ac) and H3K4 di-methylation (H3K4me2) as globally modulated in breast tissue of the offspring of mice whose maternal diet was rich in omega-3 fatty acids. Our original transgenic mouse model linked that increased H3K18ac and H3K4me2 was associated with a reduced susceptibility to breast cancer. Our preliminary gene expression profiling experiments revealed that a fairly large number of genes were differentially expressed in mice as a function of diet (omega-3 vs. an omega-6 FA-rich diet). To investigate the epigenetic role of H3K18ac and H3K4me2 on preventing breast cancer (BCa) in response to different FA diets, we planned to perform a chromatin immuno-precipitation experiments, using anti H3K18ac and anti H3K4me2 antibodies followed by genome-wide sequencing of MeCP2-associated fragments to identify all targeted genes whose expression level is dependent on changes in the histone PTMs above-listed.

Specific aims: To investigate the genetic location susceptible to changes in H3K18ac and H3K4me2 in BCa cells isolated from mammary glands of F1 generation mice fed with a corn oil (CO) diet whose mother consumed using either a canola oil (CA) or CO-based diet [CO (maternal diet)/CO (offspring diet] vs. CA/CO diets during gestation and lactation of the F1 mice, we proposed the two following Specific Aims:
Specific Aim 1: Generation of a genome-wide map of H3K18ac and H3K4me2-interacting sequences by chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq). Specific Aim 2: Match genetic loci affected by H3K18ac and H3K4me2 to the genes which expression has been shown to be modulated by maternal diet changes from CO-CO to CO-CA.

Methods: 1. Generation of genome-wide K3K18ac and H3K4me2 maps: To confirm that specific

histone PTMs in offspring can be affect by the maternal diet, mammary tissue from 130 days old CO-CO or CA-CO groups F1 mice was isolated. The biological location of the two PTMs is to be investigated by Chromatin Immunoprecipitation-based Sequencing (ChIP-Seq) leading to the generation of epigenomic maps corresponding to the different diets. ChIP DNA was prepared as described by Rada-Iglesias and colleagues (2005) using a MeCP2 ChIP compatible antibody (Abcam ab2828). The DNA fragment library was to be prepared from separate biological samples using the ABI SOLID System 2.0 Fragment Library Preparation protocol, and then sequenced by the Marshall University Genomics Core Facility using the Illumina HiSeq1000 system. Once completed, this should allow us to identify genes that are differentially regulated by changes in H3K18ac and H3K4me2 signal following maternal consumption of the omega-3 rich diet compared to maternal consumption of the omega-6 rich diet.

Results and Discussion: The ChIP has been performed and libraries have been prepared and sequenced in the Illumina HiSeq1000 system. We are now in the process of analyzing the results from the deep sequencing runs.

NGS Project #3
Title: Identification of genomic binding sites of transcriptional repressors Snail and ZEB in epithelial cells undergoing epithelial-mesenchymal transition (EMT) by ChIP-Seq.

Subproject investigator: Alexey Ivanov, PhD

Introduction: Epithelial-mesenchymal transition (EMT), the generation of motile mesenchymal-like cells from epithelia, has been increasingly recognized as a major contributor to the metastatic process in breast as well as in many other human epithelial cancers. EMT has been associated with cancer cell acquisition of stem cell properties, increased invasion and resistance to chemotherapeutic drugs. Key inducers of EMT are developmentally regulated transcription factors Snail and ZEB. Snail and ZEB are over-expressed in breast adenocarcinomas, and their increased expression is an independent prognostic factor for metastasis, poor survival and disease relapse in patients with breast carcinoma. At the molecular level, Snail and ZEB function to repress gene transcription by binding to E-box DNA element. The role of Snail and ZEB in triggering EMT is well established. However, the molecular mechanism of their action in this process is not fully understood. It is unknown, for example how Snail or ZEB, being repressors, activate hundreds of genes. We have previously characterized gene expression changes in human mammary epithelial cells undergoing Snail/ZEB-induced EMT using microarrays and identified more than three thousand Snail/ZEB-regulated genes.

Hypothesis: Most genes down-regulated by Snail/ZEB are likely directly bound and repressed by Snail/ZEB; while genes up-regulated by Snail/ZEB are likely indirect Snail/ZEB targets activated through secondary transcriptional and post-transcriptional events.

Specific Aim: We propose to identify Snail and ZEB genomic binding sites using ChIP-Seq in the same cell culture model where we have analyzed Snail/ZEB-induced transcriptome changes and correlate Snail/ZEB promoter occupancy with the identified target genes.

Methods: We utilized the same system of tet-inducible expression of Snail1, Snail2 or ZEB1 in HMLE cells, which we have previously used for gene expression profiling. Two ChIP protocols, from Dr. Peggy Farnham's lab and from Dr. Richard Myer's lab were compared in assay performance. Both labs are members of the ENCODE consortium. Next generation sequencing will be performed at the Genomics Facility of University of Southern California.

Results and Discussion: It is of crucial importance to have good, ChIP-validated antibody to

perform ChIP-Seq experiments. First, we evaluated the efficiency of several commercially available antibodies to Snail1 (4 antibodies), Snail2 (2 antibodies) and ZEB1 (5 antibodies) to ChIP positive control E-Cadherin promoter, the best characterized direct target of Snail and ZEB. One antibody for Snail1 and one for ZEB1 have previously been published to work in ChIP. The published Snail1 antibody performed poorly and the published ZEB1 antibody performed satisfactory in our antibody ChIP validation. In fact, we found that some other antibodies from our screen perform good (one for Snail1 and one for Snail2) or better (one for ZEB1) compared to the published ones, and will be used in further experiments. Second, we compared the two ChIP protocols and optimized Farnham's lab protocol for our cell line. Currently, we are conducting our large scale ChIP with ZEB1 antibody, and resulting immune precipitated DNA will be sent to the USC Genomics Core for NGS.

NGS Project #4
Title: Identification of Potential Susceptibility Variants for Obesity and Non-Insulin Dependent Diabetes in the TALLYHO Mouse
Introduction: The TALLYHO/Jng (TH) mouse is an inbred polygenic model for Type 2 diabetes characterized by obesity, insulin resistance, hyperlipidemia, and hyperglycemia. Genetic outcross experiments with lean non-diabetic strain of C57BL/6 (B6) mice revealed major susceptibility loci for obesity on chromosome 6 (Tabw2) and for diabetes on chromosome 4 (Tanidd4). For both loci, the TH alleles are associated with disease susceptibilities. Currently, the Tabw2 locus has been fine mapped to 8-Mb interval and the Tanidd4 locus to 15.8-Mb interval.
Methods: In order to identify potential susceptibility variants for Tabw2 and Tanidd4 loci, we performed whole genome sequencing of the TH mouse genome in two 2 x 100 paired end read strategies using an Illumina HiSeq1000 next generation sequencer. Libraries were prepared and sequenced using standard Illumina protocols.
Results and Discussion: We generated ~60 Gb of TH sequence with an average coverage of ~25X. Seventy five percent of the readout aligned to the mouse reference B6 genome (Build 37). In our initial test, we compared the TH genome to the B6 genome using Illumina CASAVA software and identified 4,749,554 SNPs. Among the SNPS, 38,147 mapped to the Tabw2 interval and 83,122 to the Tanidd4 interval. In order to refine these sets, we compared the TH, which contains Swiss lineage, variant set to those variants in three Swiss-derived non-obese, non-diabetic strains (A/J, AKR/J and Balb/cJ). We identified 17,360 SNPs and 28,297 SNPS within the Tabw2 and Tanidd4 intervals, respectively, that were not found in any of the three non-diabetic strains.
In conclusion, we applied a whole-genome sequencing strategy in combination with mapping information to identify a set of candidate genes for the obese and diabetic phenotypes in TH mice. Further defined and systemic filtration of the variants (e.g. based on variant effect of function) will facilitate identification of the causal genes and variants.

NGS Project #5

Title: Characterizing the AHR cistrome in human MCF-7 breast cancer cells

Subproject investigator: Travis Salisbury, PhD

a) Project summary & Hypothesis:
The Aryl Hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates transcriptional responses to exogenous and endogenous chemicals. Ligand-activated AHR induces toxicity, transcription of phase I and phase II drug metabolizing enzymes and changes in cellular growth. The AHR is likely to control cellular responses by regulating the expression of genes. Obesity is a risk factor for several types of cancer. Prior reports and our data indicate that adipocytes secrete signaling molecules that induce breast cancer cells to grow rapidly. We have discovered that blockade of AHR activity in breast cancer cells inhibits cancer cell responsiveness

to adipokines. Given that the AHR is a ligand-activated transcription factor, we hypothesize that AHR regulation of gene expression determines the maximal level of cancer cell growth that can occur in response to adipokines. The objectives of this proposal are to define the genome-wide set of cis elements bound by the AHR (the AHR cistrome) in the human MCF-7 breast cancer cell line to further investigate how the AHR controls cancer growth by regulating gene expression.

b) Methods & Results: Chromatin immunoprecipitation coupled with next generation sequencing (ChIP-Seq) was used to define the AHR cistrome in MCF-7 cells. AHR-ChIP-Seq was performed with chromatin isolated from MCF-7 cells treated with DMSO (vehicle) or the AHR agonist 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). With total chromatin input controls, Model-based Analysis of ChIP-Seq (MACS) was performed to generate peaks of sequence tag enrichment to identify specific AHR DNA binding site. Using MACS and peak finding, we identified the binding of the AHR to a region upstream from the CYP1B1 gene known to contain 5 AHR binding GCGTC motifs (the specific peak region identified by MACS was ~650 to 1100 bp upstream from the CYP1B1 transcription start site). In TCDD treated MCF-7 cells, this peak had a false discovery rate (FDR) of less than 1.5 % with an AHR binding motif within 13 bp of the peak summit (area of highest sequence build up, and predicted to the precise AHR binding site). The CYP1B1 promoter spanning peak in DMSO treated MCF-7 cells had a FDR of less than 4.5% and an AHR binding sequence motif within 57 bp of the peak summit. Using MACS we also identified the AHR binding to the CYP1A1 gene promoter in regions containing AHR binding motifs with FDRs ranging from less 3% to less than 6 %. Thus, with MACS we have identified AHR binding to known AHR binding motifs that have been shown to have transcriptional activity. Conventional AHR-ChIP confirmed recruitment of AHR to CYP1A1 and CYP1B1 gene promoters, providing further validation of our AHR-ChIP-Seq experiments. With total input as a control, MACS analysis of AHR-ChIP-Seq identified ~134,000 peaks in TCDD-treated MCF-7 cells and ~97,700 peaks in vehicle-treated MCF-7 cells. Currently, we are validating additional AHR gene targets and will use bioinformatics to further characterize the biology of AHR binding as it pertains to cancer growth.

NGS Project #6
Title: Global Analysis of DNase I Hypersensitivity Sites in Treg Cells
Subproject investigator: Wei-ping Zeng, PhD

Introduction: Regulatory T (Treg) cells function as active suppressors of immune responses of conventional T cells and other immune cells. They play important regulatory roles in immune responses to infections and cancers. Therefore, understanding the pathogenesis and designing new therapies of infectious diseases and cancers will benefit from studies of regulation of genes important for Treg cell function and frequencies. In the past, many molecules expressed by Treg cells have been identified to mediate their immune suppressive function. In addition, other genes expressed in Treg cells, including those important for homeostatic maintenance of the Treg cells, have been analyzed with gene expression arrays. However, little is known about the regulation of these genes in Treg cells. DNAse I hypersensitive (DHS) sites often contain important cis elements, such as locus control regions, enhancers and sometimes silencers.

Methods: To study the regulation of the important genes in Treg cells, we wish to obtain a map of the global distribution of the DHS sites in Treg cells. Towards this goal, we are adapting a method for DNase-Seq analysis for high throughput on the Illumina next generation sequencer.

Result and Discussion: Since the Treg cells are a minor population of CD4 T cells, before applying the method to this rare population of cells we determined the best experimental conditions using total CD4 T cells. We have tried different concentrations of DNAse I, temperatures and times of digestion of the nuclei. In addition, we made several modifications to improve the yields of DNA at various steps. After pulling down the DNA fragments derived from ends generated by DNase I

digestion, we performed PCR to determine whether such isolated DNA fragments are indeed enriched for the DNase I hypersensitive sites by examining a previously known DHS site at the CD4 gene. Preliminary results showed that the sequence of CD4 DHS site is preferentially amplified compared with nearby sequences with no DHS site. This result indicated successful enrichment of the DHS sequences. In the future, we will attempt to apply the same conditions to study of the Treg cells.

PROTECTION AGAINST RESEARCH RISKS

Y 1. Will human subjects be involved next year?
If yes, provide complete the above Targeted/Planned Enrollment Table and the Inclusion Enrollment Report. Provide the date of IRB approval and enclose with transmittal.
05/06/2011 (Cuff Project)

Y 2. Will vertebrate animals be used next year?
If yes, provide the date of Institutional Animal Care and Use Committee (IACUC) approval and enclose with transmittal.
05/06/2010
If no approval date, please explain.
Is this IACUC approval date different from the date reported last year?

Y 3. Will recombinant DNA experiment(s) be conducted next year?
If yes, provide the date of Office of Recombinant DNA Activities (ORDA), NIH approval:
EXEMPT

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

CENTER FOR NATURAL PRODUCTS RESEARCH (0040)

TYPE: Infrastructure

%IDeA $: 4.000% **IDeA $:** 148,500

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Rankin, Gary O PHD	Pharmacology, Physiology & Tox	
Chen, Yi C PHD	Biology	Alderson-Broaddus College, Wv Usa
Crick, Darrell PHD	Chemistry	Concord University, Wv Usa
Hankins, Gerald PHD	Biology	West Virginia State University, Wv Usa
Harris, Rob PHD	Biology	West Virginia State University, Wv Usa
Huggins, Luke PHD	Biology	West Virginia Wesleyan College, Wv Usa
Troyer, Timothy PHD	Chemistry	West Virginia Wesleyan College, Wv Usa
Valentovic, Monica PHD	Pharmacology, Physiology & Tox	

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

For several years, investigators within the WV-INBRE network has expressed an interest in natural products research and a number of Faculty Research Development Awards were made to start drug discovery projects related to natural sources. The External Advisory Committee recomended that WV-INBRE look toward organizing researchers around the state to promote synergistic interactions for the discovery of new chemotherapeutic agents, a common theme among early projects and part of the research emphasis of WV-INBRE, from natural products. To that end, WV-INBRE has sought and received approval from NIH to move forward to develop the Center for Natural Products Research. Dr. Rankin will serve as the initial Director for the Center as it is organized, and EAC member and COBRE PI, Dr. Stephen Cutler, Chair, Department of Medicinal Chemistry, University of Mississippi, will serve as consultant to the developing center which is currently being organized.

SUBPROJECT PROGRESS

Researchers from Alderson-Broaddus College, Concord University, West Virginia State University, West Virginia Wesleyan College, Marshall University and West Virginia University have expressed an interest in being part of the Center. Equipment has been purchased for researchers at Alderson-Broaddus College, Concord University and West Virginia State University to facilitate natural products research projects at these PUIs. A full organizational meeting of interested investigators will be held in late March or early April and projects selected for funding in Y12.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

Pilot Subproject

WV-INBRE: WVU & MARSHALL U: FACULTY RESEARCH DEVELOPMENT AWARD (0007)

TYPE: Pilot Subproject

%IDeA $: 3.000% **IDeA $:** 122,300

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Griffith, Robert PHD	Basic Pharmaceutical Sciences	West Virginia University, Wv Usa
Belay, Tesfaye PHD	Biological Sciences	Bluefield State College, Wv Usa
Cushman, Kenneth PHD	Biology	West Liberty University, Wv Usa
Huggins, Luke PHD	Biology	West Virginia Wesleyan College, Wv Usa
Kaushal, Gagan PHD	Pharmaceutical & Admin Science	University Of Charleston, Wv Usa
Kim, Seung-Yun PHD	Comp Sci, Math & Engineering	Shepherd University, Wv Usa
Linger, Rebecca PHD	Pharmaceutical & Admin Science	University Of Charleston, Wv Usa
Luo, Haitao PHD	Biology	Alderson-Broaddus College, Wv Usa
Sal, Melanie BS, PHD	Biology	West Virginia Wesleyan College, Wv Usa
Sheil, James M PHD	Micro, Immun & Cell Bio	West Virginia University, Wv Usa
Yu, Hongwei PHD	Biochemistry & Microbiology	

Total # human subjects expected for entire study: 0

Total # human subjects enrolled to date: 0

SUBPROJECT DESCRIPTION

Faculty Research Development Awards (FRDAs):

To enhance the research base at WV-INBRE Primarily Undergraduate Institutions (PUIs), we provide funds to support biomedical research projects to be carried out at the PUIs. These grants benefit the WV-INBRE as a whole as well as the PUIs, and each focused on a biomedical research project. Project proposals could be for initiation of a new research endeavor or to continue an ongoing project. Individual investigators at each PUI could apply for a maximum of award of $40,000. Funded projects were required to involve undergraduates in the research. Continuation of funding for previously funded projects required solid evidence of research progress.

SUBPROJECT PROGRESS

Faculty Research Development Awards granted this year were to: Dr. Kaushal, University of Charleston, $13,200; Dr. Linger, University of Charleston, $10,000; Dr. Kim, Shepherd University, $28,800; and Dr. Luo, Alderson-Broaddus College, $40,000.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

Y 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

Chlamydia for Belay's Project

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

Research Subproject

KINETIC AND MOLECULAR DYNAMICS CORRELATIONS IN CYTOCHROME P450 (0023)

TYPE: Research Subproject

%IDeA $: 5.000% **IDeA $:** 172,385

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Aguilar, Jarrett PHD	Natural Science & Mathematics	West Liberty University, Wv Usa
Gannett, Peter PHD	Basic Pharmaceut Sci	West Virginia University, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

The cytochrome P450 enzymes mediate the metabolism of various xenobiotic and endogenous compounds and can bioactivate pro-carcinogens such as benzo-[a]-pyrene. Many drug-drug interactions are caused by the effect of one of the drugs on the activity of the P450 isoforms involved in the metabolism of the second drug. Some isoforms demonstrate atypical kinetics for the metabolism of certain substrates. We and others have suggested that simultaneous binding of two substrates in the active site (a two-site model) is responsible for most atypical kinetic profiles. Dapsone and structurally related substrates, have been shown to activate CYP2C9 metabolism of flurbiprofen, naproxen, and piroxicam. The kinetic data suggest both substrate and activator are present in the active site. CYP2C9 polymorphisms result in reduced enzyme catalytic activity and greater activation by effector molecules as compared to wild-type protein, with the mechanism(s) for these changes in activity not fully elucidated. The molecular dynamics for several mutations involving key sites for possible catalytic activity will be studied by molecular dynamics and the resulting kinetics data will be compared to determine if these key sites are responsible for the changes in activity between various mutants of CYP 2C9. The mutations will be prepared via site directed mutagenesis, and the enzyme expressed and purified for kinetic analysis. Kinetic analysis will be conducted using an HPLC with a fluorescence detector. The data obtained will be compared to that obtained from the molecular dynamics data for same mutations. The distance between the active H of the substrate and the heme iron appears to be important to substrate metabolism. The kinetics for these mutations will be compared to these distance changes and correlations between the two studied. The mutants are R108I and N204I as they are responsible for substrate binding within the active site. Other mutants E300I, S209A, T304A, and N474I will be studied as they may determine binding orientation of the effector. This will provide information that would allow one to control the kinetics by proper choice of substrate and effector and provide a greater understanding of the active site mechanism of cytochrome P450 2C9.

SUBPROJECT PROGRESS

We first had an issue with our site-directed mutagenesis and our plasmid became smaller than that of our wild type protein. We attempted many measures to circumvent this problem and determined to sequence the plasmid. It was noted after many trials that we were missing a Lac I repressor. This was decided would not effect our project as our soul purpose for the plasmids was to produce protein. The missing LacI only meant the expression of CYP2C9 was always on without control. This is no longer an issue and site directed mutagenesis has been used successfully to generate all of the mutants that are presented in this proposal.

The expression of mutants has been an even greater time consumer. The expression of mutated protein is a 3 day process in which the protein content must be constantly monitored. It is an issue that many 3 day trials are unsuccessful at producing the mutated proteins. The most viable reason that we can deduce is that many mutations of proteins can be toxic to the cells. In many cases this means we may not be able to express these mutations. We have recently taken steps to use chaperones to assist in protein folding as to minimize the toxicity of the mutated proteins. We have been able to express several of the mutations and working on getting the chaperones into those

that do not express. The current list of proteins that have been expressed are N474I, T304A and S209A. We are working on E300V currently and have expressed R307L which has an impact on this project.

The purification of the expressed protein is being carried out according to protocol received from the lab of Dr. Timothy S. Tracy. We have successfully purified the wild type form of the protein and 2 of the expressed mutated forms. Purification is a week long process that takes approximately 8 hours of steady work to reach a stopping point. The time factor and use of undergraduates at this point make it very difficult to manage schedules to be sure we can cover 8 hours in a day. The addition of a half time technician would help in this area.

With the success of expression and now purification we are in the process of beginning our kinetic analysis. The kinetic analysis is a faster process and it is foreseeable to carry out 2 experiments a day. Leaving us with the task of increasing rate of purification so that kinetic analysis can continue at an acceptable rate.

The molecular modeling part of the experiment has been completed in terms of looking at correlations of distances and hydrogen bonding. We are still trying to determine a viable way to address stacking. MM-PBSA has been run for the majority of our mutations in silico. We are in a hold to see if this data will correlate with the kinetics.

We have been successful and an additional centrifuge, obtained from an equipment grant, will allow us to speed up our processes and finish the kinetics an d put out publications. We are submitting an area grant to the NIH before the February 25th deadline. We were close but after discussions with my mentor decided we needed to change a few things to make it more competitive. My writing skills need improved and I am looking for a good grant writing workshop. The publications will begin to come forth upon completion of kinetics. I have had the unfortunate experience that although the modeling data is promising on its own it needs to be completed with actual kinetics data. This has been mentioned to me at several of the meetings at which we presented posters.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

EFFECT OF STRESS ON PATHOGENESIS AND IMMUNITY DURING CHLAMYDIA GENITAL INFECTION (0039)

TYPE: Research Subproject

%IDeA $: 5.000% **IDeA $:** 174,247

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Belay, Tesfaye PHD	Biological Sciences	Bluefield State College, Wv Usa
Yu, Hongwei PHD	Biochemistry & Microbiology	

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

Genital infection by Chlamydia trachomatis is the most common bacterial sexually transmitted disease (STD) worldwide. The fact that not all infected women develop chlamydia-induced sequelae would suggest that certain socio-biological factors such as stress status may contribute to how an infection evolves into complications. However, the effect of stress on the susceptibility, intensity and complication of chlamydial infection are unknown. The central objective of this proposal is to utilize a reliable mouse model of coldstress and genital chlamydia infection that reproduces the pathological and immunological processes in humans to investigate the effect and mechanism(s) of stress-induced hormones on: (a) the susceptibility to and intensity of genital chlamydial infection; (b) the development of pathologies such as tubal inflammation and infertility; and (c) the host innate and acquired immune responses to Chlamydia. Our hypothesis is that cold-stress increases the severity of chlamydia genital infection and development of complications by modulating the immune response against Chlamydia. Based on our preliminary results, we predict that coldstress application leads to decreased resistance to chlamydia genital infection via induction of elevated stress hormones that are immunosuppressive. Aim #1 will continue analyzing the effect of cold water stress on the cytokine, chemokine and receptor dynamics in mice infected intravaginally with Chlamydia and assess the influence of cold-stress on the trafficking and distribution of immune cells in different regions of the genital tract during genital chlamydial infection. Aim #2 will continue determining the effect of cold water stress on ascending chlamydial infection and the histopathological changes of the genital tract of infected animals. Aim #3 will continue directly assessing the effect of cold-stress on Chlamydia-induced infertility by determining the effect on pregnancy and number of pups. Aim #4 will investigate and define the possible mechanism (s) how Chlamydia clearance is impaired due to the impact of releasing NE in our stress mouse model. The findings from this project may aid in developing an approach to reduce complications of chlamydal genital infection and ultimately infertility in the host.

SUBPROJECT PROGRESS

Aim #1: To assess the influence of stress on distribution pattern of immune cells in different regions of the genital tract during Chlamydia trachomatis infection

The purpose of this aim was to determine the localization of variety of immune cells with surface markers during Chlamydia genital infection within the regions of the genital tract of mouse stress model using flow cytometry analysis. Cells were harvested by collagenase Type 1 treatment of genital tract of infected mice. Fluorescein-labeled monoclonal antibodies directed against murine CD3, CD4, CD8, CD54 (ICAM-l), CD71, CD 102 (ICAM-2), MadCAM-l, and NK were used to identify the cell surface markers in the genital tract of mice. In general, the total number of immune cells in stressed mice was reduced; however, no statistically significant difference between stressed and non-stressed was obtained. Increased infiltration of leukocytes into the genital tract of stressed or non-stressed infected mice was obtained. Further flow cytometry experiments are underway to localize neutrophils, lymphocytes and dendritic cells or adhesion molecules in the regions of the genital tract of stressed mice during chlamydia infection. These data will aid to examine whether there is a direct or indirect correlation between immune cells and chlamydia load in the genital tract

during stress conditions of the model.

Aim #2: To assess the effect of cold-stress on Chlamydia-induced infertility in the mouse model

This aim was undertaken to examine the effect of stress on induction of infertility in a mouse model. Cold-stress was induced by immersing mice in cold water for 5 minutes daily for 24 days. Stressed and non-stressed groups of mice which received Depo-Provera on day 17 were infected intravaginally with 107 IFU of C. trachomatis in a volume of 30 µl of phosphate-buffered saline (PBS) while under anesthesia. Infected mice were monitored along with cervico-vaginal swabbing at 3-day intervals during primary and secondary course of infections. After recovery from Chlamydia genital infection, all mice were mated with proven breeder male mice and monitored for 19-21 days. Our results showed that exposure of mice to 24-day stress resulted in a greater intensity of Chlamydia genital infection. The percentage of pregnant mice and the number of pups was calculated. We noted that 10 out of 19 (52%) stressed mice were infertile compared to 2 of 19 (10%) non-stress mice. Cold-water induced stress also resulted in a decreased mean number of embryos (4 to 6) in stressed mice compared to mean number of embryos (7 to 9) in non-stressed mice. These results suggest that stress may increase complications of immunopathogenesis resulting from C. trachomatis genital infection and infertility. Further experimentation is underway to determine the effect and mechanism of stress in modulation of fertility in mice. (Abstract submitted to the General Meeting of American Society for Microbiology, San Francisco, CA, June 2012).
Aim #3: To determine the effect of cold-induced stress on the histopathological changes of the genital tract during Chlamydia infection in the mouse model

Comparative analysis of the immune responses and histopathologic analysis in primary infection versus secondary Chlamydia genital infection in stressed and non-stressed mice was initiated at the Pathology Laboratory Service in the Medical School of Marshall University. Preliminary data of the cervical regions of mice representing stressed and non-stressed mice showed no significant difference of hispathology. However, gross examination revealed that stressed and infected mice had fluid-filled and heavily extended uterus unlike non-stressed infected mice. Evaluation of each anatomical site of uterine horn and oviduct for acute inflammation, chronic (lymphocytes) and plasma cells and fibrosis for chronic inflammation was initiated before the relocation of the Pathology Laboratory Service in the Medical School of Marshall University.

Aim #4: To elucidate the mechanism(s) by which stress increases susceptibility to Chlamydia trachomatis genital infection in the model

Our study showed that increased susceptibility to Chlamydia infection is accompanied by multiple immunosuppressive effects may be due to the inhibition of recruitment of T cells into genital tract, suppression of proinflammatory cytokines, chemokines by stress hormones such as noradrenaline (norepinephrine). The primary focus of this aim is to investigate how the stress hormone, norepinephrine (noradrenaline) impacts immune-mediated protection against Chlamydia trachomatis infection. We will test whether □ADR antagonists; propranolol (non-selective), or atenolol (selective) blockade affects Chlamydia clearance during genital infection. We speculate that administration of antagonists will reduce the Chlamydia burden in genital tract of stressed mice to levels similar to non-stressed infected mice. An alternative approach, using a noradrenaline receptor knockout mouse, is under consideration.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

Y 2. Will vertebrate animals be used next year?

If yes, provide the date of Institutional Animal Care and Use Committee (IACUC) approval and enclose with transmittal.

02/09/2011

If no approval date, please explain.

Is this IACUC approval date different from the date reported last year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

Y 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

Chlamydia

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

PI3K, AKT AND ERR-ALPHA PATHWAYS IN FLAVONOID-INHIBITING TUMORIGENESIS (0032)

TYPE: Research Subproject

%IDeA $: 4.000% **IDeA $:** 125,000

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Chen, Yi C PHD	Biology	Alderson-Broaddus College, Wv Usa
Luo, Haitao PHD	Biology	Alderson-Broaddus College, Wv Usa
Rojanasakul, Yon PHD	Baic Pharmaceutical Sciences	West Virginia University, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

In this study, we tend to expand our understanding on the effect of flavonoid kaempferol and how kaempferol regulates VEGF expression and angiogenesis in ovarian cancer cells. We timed VEGF secretion, and studied in-vitro angiogenesis by kaempferol treatment. Gene expression was examined by qRT-PCR, ELISA, Western Blotting, or luciferase assay, and pathways were examined by manipulating genetic components with plasmid or siRNA transfection. It was found that kaempferol time-dependently inhibited VEGF secretion, and suppressed in-vitro angiogenesis. Kaempferol down-regulated ERK phosphorelation as well as NFkappaB and cMyc expression, but promoted p21 expression. Examination of relationship between these genes suggested a novel ERK-NFkappaB-cMyc-p21-VEGF pathway, which accounts for kaempferol's angioprevention effects in ovarian cancer cells. These data supplements our comprehension of the mechanisms behind kaempferol's biological influence in ovarian cancer cells, and better characterized kaempferol toward chemoprevention.

SUBPROJECT PROGRESS

We have successfully completed these specific aims proposed in our previous proposal.
Aim 1. Identify the roles and molecular mechanisms of flavonoids in inhibiting EGFR, PI3K, AKT, and ERRα in ovarian cancer cells. We found that the flavonoid kaempferol inhibited PI3K/AKT, and ERRα regulated VEGF expression in ovarian cancer cells. We published our results in peer-reviewed scientific journals.
Aim 2. Determine flavonoid-inhibiting signaling pathways that affect ovarian tumor growth. We found that both HIF dependent (PI3K/AKT) pathway and HIF independent (ERRα and cMyc) pathways were involved in the ovarian tumor growth inhibition. We published our results in peer-reviewed scientific journals.
Aim 3. Determine whether kaempferol inhibit ovarian tumor angiogenesis, and identify which signaling molecules mediate kaempferol-inhibiting tumor angiogenesis. We found that kaempferol inhibit ovarian tumor angiogenesis through inhibiting HIF-1α, VEGF, cMyc and ERRα signaling molecules. We published our results in peer-reviewed scientific journals.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?
N 2. Will vertebrate animals be used next year?
N 3. Will recombinant DNA experiment(s) be conducted next year?
N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:
N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

SEX STEROID HORMONES AND EPIGENETICS IN MENINGIOMAS (0038)

TYPE: Research Subproject

%IDeA $: 5.000% **IDeA $:** 174,407

INVESTIGATOR, DEGREE	**DEPARTMENT**	**NON-HOST INSTITUTION: STATE, COUNTRY**
Hankins, Gerald PHD	Biology	West Virginia State University, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

Meningiomas comprine approximately 30% of primary central nervous system tumors in the United States, however their pathobiology is poorly understood. Over 90% of meningiomas are benign while 5% are atypical and 3-5% are malignant. Complete surgical resection is the treatment of choice for benign meningiomas. Surgical resection is often difficult since approximately one-half of benign intracranial meningiomas arise in the skull base. For skull base meningiomas the surgical complication rate can be as high as 30 to 40% even in expert hands. The female to male incidence ratio in adults is 2:1 for intracranial tumors and 10:1 for spinal tumors, while no such sex difference exists for meningiomas in children. Therefore, the female sex steroid hormones progesterone and □-estradiol are suspected factors in meningioma tumorigenesis. However, no mechanisms have been demonstrated for female sex hormones in

meningioma formation or progression. We recently reported evidence that a steroid responsive gene, deleted in liver cancer-1 (DLC1), may function as a tumor suppressor in meningiomas. Our microarray data indicate that a number of steroid responsive genes are differentially expressed between meningiomas and normal meninges. We also found that steroid hormones and their antagonists can alter the growth of meningioma cells and that histone deacetylase inhibitors induce a decrease of meningioma cell growth in culture. Our long-term goal is to develop strategies to prevent or slow meningioma tumor growth that can serve as alternatives or adjuncts to surgery. The central hypotheses of this study are 1) that meningioma tumorigenesis is driven in part by actions of female steroid hormones and 2) that the tumorrigenesis may be mediated in part by progesterone and estrogen receptor containing chromatin-modifying complexes. We propose to test our hypothesis by pursuing the following three specific aims:

1) To treat meningioma cells with progesterone or 17β-estradiol and assess the expression of several genes that are differentially regulated between meningiomas and normal meninges.

2) To evaluate the effects of inhibitors of DNA methylation or histone de-acetylation on the growth of meningioma cells in vitro and on expression of genes that are differentially expressed between meningiomas and normal meninges.

3) To determine whether the promoters of the differentially regulated genes in specific aim 1 and 2 are bound by progesterone receptor, estrogen receptor, ETS2, or the histone acetyltransferase p300.

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3) To determine whether the promoters of the differentially regulated genes in specific aim 1 and 2 are bound by progesterone receptor, estrogen receptor, ETS2, or the histone acetyltransferase p300.

SUBPROJECT PROGRESS

During the past year we have focused on specific aims 1 and 2.

One graduate student and an undergraduate student have focused on fibroblast growth factors

and their receptors and have demonstrated that both fibroblast growth factors and their receptors are expressed by meningioma cells. Further, growth factor message levels are modulated by steroid hormones. Treatment of meningioma cells with antagonists of FGF receptors decreases the proliferation of the cells and the phosphorylation of ERK1 and 2. Together, these results provide more evidence that steroid hormones may affect the expression of fibroblast growth factors (FGF2 and FGF9) in meningiomas and also that an FGF autocrine loop plays a role in meningioma cell proliferation, partially by signaling through ERK1/2.

Of other genes identified to examine in specific aims 1 and2, we have found that the expression of the cyclin dependent kinase p27 kip1 (CDKN1B) is modulated in meningioma cells by both the progesterone antagonist Mifepristone and the histone deacetylase inhibitor butyric acid. The transcriptional repressor KLF10 (Krueppel-like factor 10 or TIEG-1) was down regulated by a factor of 32 by the estrogen receptor antagonist, ZK164015 in more aggressive lines of meningioma, while Mifepristone had no effect. Since PR is typically higher in lower grades of meningioma while ER increases in higher grades, we are examining this in cells from lower grade tumors. KLF10 expression also was modulated by butyric acid.

Although SLC20A2 (GLVR-2, Pit2) was not highlighted in the grant, we found that its expression was significantly down regulated by Mifepristone. Expression was also modulated by HDAC inhibitor treatment. Little is known about this gene outside of its function in the kidney, although it has been focus of gene therapy work as the Gibbon ape leukemia virus receptor 2, and we speculate that it may be involved in the calcification of the tumors.

The work of one graduate student who graduated during the past year was published during the summer: Manohar S, Harlow M, Nguyen H, Li J, Hankins GR, Park M, Chromatin modifying protein 1A (Chmp1A) of the endosomal sorting complex required for transport (ESCRT)-III family activates ataxia-telangiectasia mutated (ATM) for PanC-1 cell growth inhibition. Cell Cycle 10 (15): 2529-2539, 2011. Although Dr. Park has left Marshall University and Dr. Travis Salisbury has replaced her as the mentor, we are still collaborating on studies on chromatin modifying proteins. Dr. Salisbury's research on the aryl hydrocarbon receptor complements the grant research given the parallels and crosstalk between AHR and steroid receptors.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

MECHANOTRANSDUCTION, INTRACELLULAR SIGNALING AND VASCULAR BIOLOGY (0022)

TYPE: Research Subproject

%IDeA $: 5.000% **IDeA $:** 175,256

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Harris, Rob PHD	Biology	West Virginia State University, Wv Usa
Blough, Eric PHD	Biological Sciences	

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

A fundamental problem in biology is to understand how cells are able to sense and respond to environment cues. The integration of chemical signals such as growth factors and cytokines with mechanical stimuli is not well understood. The place where cascades involved in solid-state (mechanical) signaling and soluble (chemical) signaling converge and the manner in which they interact is no doubt complex. This research project is designed to investigate signaling events associated with both chemical and mechanical stimuli. Cells of the vascular system are continuously exposed to the effects of mechanical forces such as stretching and fluid shear stress. These forces, which are created by the pulsatile nature of blood flow when the heart contracts and relaxes, have a marked influence on cell structure and function. The adaptations of these cells, including enhanced growth and migration, seem to be important in the pathological conditions that accompany cardiovascular diseases such as atherosclerosis, hypertension, and restenosis. Cardiovascular disease remains a major cause of morbidity and mortality in the U.S. and the economic and human costs associated with these pathologies are enormous. This has resulted in an intense research interest in the mechanisms which regulate contraction, migration, and growth of vascular smooth muscle cells (VSMC). While it is now clear that mechanical forces imposed on cells of the vessel wall are important factors in the initiation and progression of pathological changes, the molecular mechanisms involved in these adaptations are not fully understood. In addition, it is now clear that the basic mechanism of smooth muscle contraction can only be explained in light of actin remodeling. However, the exact nature of cytoskeletal reorganization and the mechanisms regulating these changes are not well known. The overall goal of this project is to elucidate the acute responses in cytoskeletal reorganization that occur during mechanical stress of VSMC and to determine the intracellular signaling mechanisms that are involved. Utilizing molecular approaches combined with fluorescence microscopy, and relying on the precise changes in cell orientation and actin cytoskeletal reorganization as endpoints for quantitative assessment of responsiveness to mechanical strain, we will evaluate the role of various cytoskeletal structures on the response of VSMC to stretch. Further, we will make a systematic determination of the effects of various types of mechanical stress on activation of cell signaling molecules. In addition, we will evaluate the effects of resveratrol, a purported cardioprotective molecule, for its potential effects on stretch-induced cell signaling and receptor mediated cellular hypertrophy. The use of pharmacologic and molecular techniques to stabilize, destabilize or down-regulate specific cytoskeletal components is expected to provide clear answers concerning the role of specific components in mechanotransduction and the cell orientation response. The inhibition or down-regulation of specific signaling proteins is expected to provide information concerning pathways regulating mechanosensing and transduction. The knowledge gained may be useful in the development of therapeutic agents regulating mechanotransduction mechanisms contributing to cardiovascular pathologies.

SUBPROJECT PROGRESS

Among the changes in gene expression that we have found to be associated with mechanical stress is the the giant protein titin. The role of titin in organizing and stabilizing contractile filaments in sarcomeres of striated muscle is well understood. Titin forms filaments in cardiac and skeletal muscle that provide elasticity in relaxed cells but limit cell lengthening and as such contribute to overall stiffness. Since its discovery nearly a decade ago, the function of smooth muscle (SM) titin

remains unclear, but as in striated muscle, it is reasonable to postulate that SM titin may contribute to stiffness. Unlike striated muscle, SM cells respond to stretch in a complex manner that involves an extensive change in morphology and reorganization of their cytoskeleton. SM cells may thus respond to changes in mechanical load with alterations in titin expression. In this study, titin expression was determined (qRT-PCR) in A7r5 cells which were exposed to cyclic (dynamic) or static (step) stretch. There was an early decrease in expression of titin in response to 1hr (19%) and 2hrs (77%) of 15% unidirectional cyclic stretch (UCS). It is during this time that the actin cytoskeleton is relatively unloaded as it is breaking down and reforming. At 6hrs of UCS, when the cytoskeleton had remodeled to a lower stretch orientation, the decline in titin mRNA expression was less than the 2hr value (38% vs 77%). In contrast to UCS, cells responded to 1hr of 15% static stretch with an increase in titin expression (35%). In cells treated with cytochalasin D to block polymerization of actin stress fibers, there was a marked increase in titin expression in unstretched cells (335%) which increased further after 6hrs of UCS (530%). Together, these data suggest that SM cells may alter expression of titin in order to modulate internal stiffness. We believe that SM titin may play a sensory role for the cell.

Hypertrophy of VSMC leads to increased vascular stiffness and an increased risk of cardiovascular disease. Although not well understood, recent data suggests that resveratrol (RV) may have beneficial effects for the prevention of cardiovascular disease. RV is a polyphenolic compound found in high concentrations in grape skin and red wine and is known to have anti-oxidant and anti-carcinogenic properties. Here we investigate the potential of RV to inhibit the growth of VSMC following stimulation with the PGF2α analog fluprostenol (Fp). It has been suggested that PGF2α is a mediator of vascular cell growth through a process that may involve the production of intracellular reactive oxygen species (ROS). Increased ROS production has been linked to VSMC hypertrophy via a cell signaling pathway that is not well understood. One goal was to investigate the potential of resveratrol in preventing hypertrophy of VSMC that occurs in response to a physiologically relevant stimulus, namely activation of PGF2α receptors. VSMC hypertrophy is associated with a number of vascular pathologies. It can have detrimental effects on vascular remodeling which can bring about changes in circulation and an increase in the metabolic demands that are placed on the left ventricle. Ultimately, VSMC hypertrophy can lead to systolic hypertension, an alteration in coronary perfusion, and eventually hypertrophy of the left ventricle. In addition, it often contributes to mortality that is associated with end-stage renal failure. These important clinical considerations highlight the need to expand our knowledge and develop a more detailed understanding of the molecular mechanisms that underlie the onset of VSMC hypertrophy and to investigate ways in which responsible signaling mechanisms may be altered.

Incubation of A7r5 VSMCs with 1µM Fp resulted in a 35% increase (P0.05) in cell size in 48 hours as measured by flow cytometry. This Fp treatment was associated with markedly increased intracellular ROS levels as determined by incubation of cells with hydroethidium. However, when cells were pre-treated with 1 or 20 µM RV, FP-induced hypertrophy was completely attenuated. Resveratrol treatment at both levels was associated with a decline in intracellular ROS. The results of the present study suggest that very low levels of resveratrol (1µM) can completely attenuate hypertrophy that is induced by exposure to fluprostenol through a mechanism that involves suppression of the activation of ERK1/2 and ribosomal protein s6. On the other hand, resveratrol's reduction in hypertrophy in this study was associated with a slight increase, rather than the expected decrease, in Akt pathway activation. These results further our understanding of how resveratrol may exert its cardio-protective effects.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

MECHANISM OF ACTION OF STATINS ON ENDOTHELIAL CELL FUNCTION (0025)

TYPE: Research Subproject

%IDeA $: 5.000% **IDeA $:** 175,625

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Kreisberg, Robert PHD	Biology	West Liberty University, Wv Usa
Primerano, Donald A PHD	Biochemistry & Microbiology	

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

Ischemic heart disease is the generic designation for a group of closely related syndromes resulting from myocardial ischemia – an imbalance between the supply and demand for oxygenated blood. Until recently, the pathogenesis was attributed to reduction in coronary blood flow due to atherosclerotic coronary artery obstruction. The process is actually much more complicated. Recent data suggest the pathogenesis of atherosclerosis includes endothelial response to injury and compares to chronic inflammation of the vascular wall. Lipid-laden macrophages recruited to the vascular wall due to endothelial activation may play a role in the stability of the atherosclerotic plaque; rupture of the plaque due to release of matrix metalloproteinases from the macrophages causes exposure of the subintimal space resulting in thrombus formation and obstruction (1). Recently, experimental evidence suggests that 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGA-CoA) inhibitors, such as the statins used to treat hypercholesterolemia, also attenuate inflammation of the vascular wall independently of their lipid-lowering effects (2). The long-range goal of the proposed research is to uncover the molecular mechanisms of endothelial cell and macrophage activation by minimally-modified LDL (MM-LDL) and to determine whether statins inhibit this activation. The overall objective of this project is to identify the signaling processes affected by statin treatment.

SUBPROJECT PROGRESS

- Multiple abstract publications.
- Over 25 undergraduate students involved in the Cardiovascular Research Project.

o Greater than 90% matriculated into post-graduate education (Medical , Graduate, Dental, Pharmacy, and Physician Assistant Programs)

- Received over $600,000 in competitive WVEPSCoR funding to have a Summer Undergraduate Research Program (2005-2010) At West Liberty University. Over 90 students participated in this program.
- Fifteen different students presented their research results at both state and nationwide scientific conferences.
- The laboratory received a grant (Rosuvastatin) from AstraZeneca to look at compare Rosuvastatin to Lovastatin in its ability to inhibit IL8 production by Human Aortic Endothelial Cells.

o We have some very interesting results but unfortunately, I am unable to disclose at this time due to the confidentiality statement I signed.

- With the success of the Rosuvastatin experiments we are currently in serious discussions with AstraZeneca to use our current laboratory techniques to look at one of their new platelet drugs.
- Currently repeating some IL6 experiments (cleaning up data) so that a manuscript can be submitted for publication

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

Y 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

human cell lines, rDNA

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

WV-INBRE:APPALACHIAN CARDIOVASCULAR RESEARCH NETWORK (ACORN) (0006)

TYPE: Research Subproject

%IDeA $: 11.000% **IDeA $:** 406,206

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Primerano, Donald A PHD	Biochemistry & Microbiology	
Barr, Taura PHD, RN	Nursing	West Virginia University, Wv Usa
Boskovic, Goran PHD	Biochemistry & Microbiology	
Dementieva, Yulia PHD	Mathematics	Emmanuel College, Ma Usa
Denvir, James PHD	Biochemistry And Microbiology	
Egleton, Richard BS, PHD	Pharmacology, Physiology & Tox	
Fan, Jun BS, PHD	Biochemistry & Microbiology	
Kreisberg, Robert PHD	Biology	West Liberty University, Wv Usa
Mangiarua, Elsa PHD	Pharmacology, Physiology & Tox	
Neal, William MD	Pediatric Cardiology	West Virginia University , Wv Usa
Santanam, Nalini PHD	Pharmacology, Physiology & Tox	

Total # human subjects expected for entire study:	35
Total # human subjects enrolled to date:	25

SUBPROJECT DESCRIPTION

Overview and Current ACoRN Projects:

Cardiovascular disease (CAD) and one of its contributing causes, obesity, are significant health problems in West Virginia (WV). West Virginia ranks 6th highest in the rate of heart disease deaths among the 50 states and the District of Columbia (Kaiser Family State Health Facts (http://www.statehealthfacts.org/)) and has the highest rate of obesity in the United States. Understanding the genetic and dietary factors that lead to these two diseases is central to Appalachian Cardiovascular Research Network (ACoRN) research projects.

ACoRN is designed to explore the genetic and environmental causes of cardiovascular disease as well as the mechanism(s) of action of therapeutic agents. Our investigators are currently guiding four cardiovascular disease research studies and providing mentorship to two undergraduate project investigators at West Liberty University. Under WV-INBRE II, we have broadened the base of cardiovascular research, taken measures to improve human subject recruitment and increased the participation of undergraduate students in ACoRN supported research. Empowerment of individual project investigators with appropriate mentoring has broadened the base of ACoRN research and increased the potential of making significant findings on the pathogenesis and treatment of cardiovascular disease.

SUBPROJECT PROGRESS

ACoRN Y11 Progress Report

1. Current ACoRN Projects:

Progress reports for three of the five ACoRN projects are given under separate subproject IDs (SPIDs) in the APR or as part of this report as follows:

Project #1: The Molecular Actions of Atorvastatin on Progression of the Atheromatous Plaques (SPID #25). Project Investigator. Robert Kreisberg PhD, West Liberty University. Mentor: N. Santanam, Marshall University.

Project #2: The Genetic Basis of Familial Combined Hyperlipidemia (SPID #26). Project Director D. Primerano, PhD. Marshall University

Project #3: Epicardial Fat Biomarkers in Patients with Coronary Artery Disease in the Appalachian Region (SPID #36). Project Director N. Santanam PhD. Marshall University.

Progress Reports for Projects #4 and #5 are given below in sections 5 and 6.

Project #4: Monitoring gene expression post-stroke to predict stroke outcome. Project Director: Taura Barr PhD RN School of Nursing and Center for Neuroscience, West Virginia University).

Project #5: Regulation of brain endothelial phenotype and function by diabetic plasma Project Director: Richard Egleton, PhD, Department of Pharmacology, Physiology and Toxicology, Marshall University).

A table providing information on protection against research risks for Projects #4 and #5 is provided at the end of this document.

2. ACoRN Management Plan

ACoRN will consider applications from investigators at both lead and undergraduate institutions for new ACoRN research projects. Applications will be reviewed initially by WVU or MU faculty members, then by the WV-INBRE External Advisory Committee (EAC), and finally by our NCRR/NIGMS program officer. Approval by the EAC and program officer will be required in order for funding to be committed to a project. We anticipate issuing a request for applications in cardiovascular research and offering pilot grant awards in Y12 and Y13.

3. Revision to Genetic Basis of Familial Combined Hyperlipidemia

We have modified our proposal to adopt an approach based on sequencing the exomes of related affected individuals. Whole exome sequencing has successfully been used to identify causal variants for about 15 Mendelian disorders (e.g. Miller Syndrome, metachondromatosis and Kabuki Syndrome. Our immediate and long-range goals remain unchanged. We will improve our understanding of the molecular pathology of cholesterol and triglyceride regulation by identifying genes and variants that predispose to FCH.

The new experimental design involves several stages: (1) selection of FCH families with multiple affected individuals who are known to not have deleterious alleles in the LDLR gene, (2) removal of common variants from whole exome sequences by comparison to existing SNP databases such as HapMap and dbSNP and reduction of coincident variants by selection of variants shared by multiple affected individuals, and (3) confirmation of the presence of deleterious alleles in other affected family members and their absence in phenotypically normal individuals. Through this filtering process, we expect to obtain a small number of candidate genes that confer susceptibility to FCH.

We have completed sequencing of LDLR exons in 19 of 21 FCH families and have excluded four families based on our analysis. We have completed whole exome sequencing on six families and

begun the filtration process. A complete description of the modified analysis and initial data analyses are provided under SPID #26.

FCH Patient Recruitment and Re-consent

Charleston Area Medical Center (CAMC) and the University of Kentucky (UK) continued to recruit FCH families throughout Y11. These two centers and WVU completed re-consent of many of the originally enrolled FCH family members so that whole exome can be submitted to dbGAP.

4. Additional ACoRN Research Projects

In Y11, we added two new projects entitled (1) “Monitoring gene expression post-stroke to predict stroke outcome” (Taura Barr PhD RN School of Nursing and Center for Neuroscience, West Virginia University) and (2) “Regulation of brain endothelial phenotype and function by diabetic plasma” (Richard Egleton, PhD, Department of Pharmacology, Physiology and Toxicology, Marshall University). These projects are making good progress toward their research goals. Both investigators have presented their work as poster or platform presentations. Dr. Barr has submitted a manuscript entitled “Identification of a genomic profile following ischemic stroke that may mediate stroke recovery” that has been submitted to Science Translational Medicine. Dr. Barr’s proposal involves human subjects, and its IRB approval has been included in the appendix of the 2590 report. Dr. Egleton’s proposal involves animal studies and the corresponding IACUC approval has been included in the 2590 appendix. Inclusion of these projects as part of ACoRN was approved by the WV-INBRE, EAC and Dr. Krishan Arora at the NCRR early in 2011.

5. Project #4: Monitoring gene expression post-stroke to predict stroke outcome

Primary Investigator: Taura Barr PhD RN Nursing and Center for Neuroscience
Co-Investigators: Laurie Gutmann MD, Neurology; Todd Crocco MD Emergency Medicine; Stephen Davis MS Emergency Medicine; Reyna VanGilder PhD, Stephanie Rellick PhD Nursing and Center for Neuroscience; Jason Huber PhD Basic Pharmaceutical Sciences; Charles Rosen MD PhD Neurosurgery; Dongquan Chen PhD Bioinformatics; James Denvir PhD Biostatistics

Introduction: The physiologic response to ischemic stroke is complex, meditated by input from genetic factors and the environment. An emerging concept in human stroke recovery is the interrelationship between these factors and the processes associated with inflammation and immunity. A strong clinical observation is the phenomena of post-stroke immune suppression (SIDS), associated with increased mortality and morbidity. This has been characterized by the presence of lymphocytopenia, inactivation of macrophages, increased serum cytokine concentrations and decreased T-cell proliferation. Literature supports an inflammation triggered immune dysfunction post-stroke; however, the signaling mechanisms leading to human post-stroke immune-depression have not been well-defined.

Hypothesis: In an earlier study, we identified a panel of genes that differentiated ischemic stroke patients from control subjects. Many of these genes were involved in innate and adaptive immune responses. We hypothesized that the change in gene expression following stroke will reflect molecular pathways involved in brain recovery and may result in the identification of novel targets for stroke therapeutics.

Specific Aims: The objective of this study was to examine the relationship between the change in the peripheral blood gene expression over time with outcome. This was accomplished by identifying genes and other markers that predicted outcome.

Methods: Peripheral blood samples were collected from diagnosed ischemic stroke patients within 24 hours from last known normal and 24-48 hours later.Total RNA was extracted from whole blood stabilized in PAXgene RNA tubes, amplified, and hybridized to Illumina HumanRef-8v2 bead chips. Gene expression was compared in a univariate manner between stroke patients at both time points and outcome using t-test in GeneSpring. Inflation of type one error was corrected by Bonferroni and Ingenuity Systems Pathway analysis (IPA) was performed. A validation cohort of n=10 ischemic stroke patients was recruited from WVU Ruby Memorial Hospital. Following cDNA amplification of whole blood RNA, real-time PCR was completed using TaqMan probes specific for Arg1, CA4, CCR-7, CSPG2, IQGAP, LY96, MMP-9, Orm1, S100A12, TLR2 or TLR4 and β-Actin was used as a housekeeping control. In addition, a retrospective study of n=122 patients who underwent endovascular therapy for acute ischemic stroke (AIS) from 2008-2011 was conducted to identify the relationship between the neutrophil-lymphocyte stress factor (NLSF) and 90 day stroke recovery. An additional component of this project is the measurement of gene expression at 5 days post-stroke; recruitment is ongoing for this part of the project and is scheduled to be completed by June 2012.

Results: There were 21 genes with 1.5 fold difference in expression (Bonferroni corrected p2 fold (LY96, IL8, and SDPR). Pathway analysis revealed cytotoxic t-lymphocyte antigen 4 (CTLA4) and dopamine signaling as highly significant pathways present in the peripheral whole blood of IS patients 24-48 hrs post onset of symptoms. Seven genes were significantly associated with 30 day MRS, with Arginase 1 (ARG1) (p=8.58E-06) being the most significant. Follow up expression of ARG1 was significantly correlated with MRS (Pearson 0.72; p=0.000) and NIHSS (Pearson 0.54; p=0.001); however there was no relationship with age (Pearson 0.22; p=0.21). Follow up expression of ARG1 remained significantly associated with MRS after controlling for hypertension (p=0.062). Interestingly there was a significant association between baseline ARG1 expression and a positive history of hypertension (t=-2.43; p=0.021). Validation in a separate cohort of subjects recruited from WVU Ruby Memorial Hospital confirms these findings. LY96, IL8 and SDPR remained increased at 24 hours in this population; however these patients were younger with more severe strokes. TLR2 expression was decreased at the 24 hour time point (2 fold) and TLR4 was minimally changed (1.2 fold increase). ARG1 was 1.5 fold higher in patients with worse clinical outcomes at 30 or 90 days. In the retrospective study, there was a significant relationship between the NLSF and 90 day MRS (t=-2.38; p=0.019) that remained when controlling for infarct volume and age (p=0.048). Higher PMN and lower lymphocyte count predicted death and worse stroke recovery.

Discussion: We demonstrate that ischemic brain injury produces an immune response that can be observed in the peripheral blood following stroke. This was confirmed in a separate cohort of ischemic stroke patients, as well as in a retrospective study. This suggests clinicians may use the NLSF to stratify risk of death for stroke patients as well as closely monitor for post-stroke immune suppression. The phenomenon of Stroke induced immune suppression (SIDS) is analogous to immune suppression observed in other scenarios of major organ trauma such as myocardial infarction, severe burns, traumatic brain injury etc. Theories suggest that immune depression is a self-preserving mechanism originating from innate immune cells to reduce immune reactivity to self-antigens within adaptive immune cells. Dysregulation of SIDS could have profound implications for stroke recovery. In this study, we have identified immune pathways changing over time as well as a novel relationship between arginase 1 (ARG1) and stroke outcome. ARG1 expression is associated with chronic inflammatory stress and is representative of aberrant lipid metabolism related to atherosclerotic plaque. It has been suggested that enhanced arginase activity may limit L-arginine bioavailability for nitric oxide production through endothelial nitric oxide synthase. Our findings suggest that mechanisms linking immune dysfunction and stroke are closely linked to cardiovascular risk factors, which may impair cerebral blood flow following stroke. In addition to playing a role in atherosclerosis, arginase I has been implicated as an anti-inflammatory immune

regulator within alternative macrophages (M2). Arginase mediated signaling decreases T lymphocyte proliferation resulting in immune suppression. Markers of immune dysfunction in the early post-stroke phase, such as the NLSF, TLR signals, arginase, and T-cell activity may prove useful for identifying patients with increased risk of secondary complications. The mechanisms of post-stroke immune suppression will need further study and taken into account when developing stroke treatments impacting inflammatory and immune pathways.

Submitted Manuscript:
T. L. Barr, R. VanGilder, S. Rellick, Y. Conley, J. Ding, D. Chen, J. Denvir, A. Dillman, Steven Warach, Andrew Singleton, M. Matarin. Identification of a genomic profile following ischemic stroke that may mediate stroke recovery. Submitted to Science Translational Medicine.

6. Project #5:
Regulation of brain endothelial phenotype and function by diabetic plasma

We have previously identified that in the streptozotocin (STZ) model of diabetes there is a change in the barrier properties of the brain endothelial cells that regulate both the physical and transport properties of the blood brain barrier (BBB). These changes in BBB properties are prolonged and eventually become resistant to insulin therapy. Similar functional changes at the BBB have been reported clinically. During diabetes there are a number of changes that occur in the blood. Several factors that change clinically are known regulators of BBB function, though their role in regulation of the diabetic BBB is unknown. Our current working hypothesis is that diabetes induces changes in signaling molecules within the blood that can in turn regulate both the phenotype and function of the brain cerebromicrovasculature. Thus, the aim of this study is to investigate what role constituents of blood play in the regulation of BBB function in an animal model of diabetes.
Specific Aim 1: Investigate changes in gene expression in brain microvasculature endothelial cells in an animal model of diabetes.
In our earlier ACORN report, we reported on our studies investigating the effects of STZ on barrier function of cerebral microvasculature. Our focus in this study was investigating the changes in the expression of mRNA and proteins involved in VEGF and its signaling, at the blood brain barrier. We found increases in the mRNA of most of the genes in the VEGF signaling pathway. However we did not see a comparable increase in the levels of protein. Subsequent studies indicated that this may be due to regulation of translation via circulating microRNA. In this report we have two follow up studies:
1. Are the changes in VEGF similar in other barrier vascular beds of the Brain?
Our studies to date have concentrated on the BBB in the cortex of rats. During our studies we routinely collect the Choroid plexus, another brain barrier system that is heavily regulated by VEGF. Our hypothesis is that if circulating miRNA is indeed involved in regulating barrier VEGF then we should see similar changes at the choroid plexus.
Table 1: Comparison of protein changes by Western blot at the BBB and at the choroid plexus from STZ treated rats. [NC, no change]

	Cortex BBB	Choroid Plexus
VEGF	NC	NC
Flt-1	NC	NC
Flk-1	↑40%	↑45%

From table 1, it is apparent that for the three proteins we have looked at so far (VEGF and its two main receptors), that the changes are similar at the choroid plexus to those seen at the BBB, indicating that there may be similar regulation of both barrier systems.
2. Is there a change in VEGF receptor activation?
Changes in expression though important do not necessarily represent functional changes. Thus

we have investigated the activation of the VEGF receptor Flk-1. There are three tyrosines on Flk-1 that play an important role in regulating the downstream signaling. Phosphorylation profiles of these three sites dictate the signaling cascades activated by the receptor. Initially we have investigated the phosphorylation of Flk-1 Tyr-1175 in both brain and choroid plexus vasculature. Though the levels of expression for the Flk-1 were similar (increased by 40 and 45% in BBB and choroid plexus respectively), we saw a marked difference in activation. In the BBB phosphorylation of Tyr-1175 was reduced by 40% compared to control BBB. In contrast the choroid plexus Flk-1 Tyr-1175 was increased by 35% compared to non-diabetic animals. These tissues were isolated from the same animals. This indicates that there is a differential activation of the receptors in these two barrier systems of the brain. Tyr-1175 phosphorylation is involved in VEGFs activation of PLC, PI3K and FAK, key signaling cascades related to angiogenesis and cell survival.
For this specific aim we are also still working on producing high quality RNA for the gene analysis studies. We have discussed protocols with other BBB researchers and hope to have high quality micro vessel RNA preparations in the near future.

Specific Aim 2: Investigate the role of plasma signaling molecules in brain microvascular changes. We have held off on these studies as we would like to use cells that are treated with the plasma from the animals that we carry out the array studies on.

Potential WVU interactions: Based on our studies with the ACORN grant we have started to discuss collaborations with two investigators at WVU, Taura Barr (another ACORN investigator) and Jason Huber. This collaboration would not have been possible without the INBRE or ACORN grants.

7. Student Participation in ACoRN Projects:

During Y11, undergraduate students worked on ACoRN research projects in labs at West Liberty University (WLU) and Marshall University. Two undergraduate students (Stan Guertal and Ethan Kobe) worked in Dr. Robert Kreisberg's lab at WLU on Project #1 (SPID #26). Three undergraduate students (Logan Efaw, Melissa Massie and Courtney Crain) worked in Dr. Nalini Santanam's lab at MU on Project #3 (SPID #36).

8. Protection against research risks

	Project #5	Project #4
1.) Will human subjects be involved next year?	No	Yes
A. IRB #	N/A	H-22566
B. Date of IRB approval?	N/A	9/20/2010
C. IRB approval & Human subject education certification enclosed?	N/A	Yes

2.) Will vertebrate animals be used next year?	Yes	No
A. Date of Institutional Animal Care and Use Committee (IACUC) approval, enclosed?	09/16/2010,	N/A
B. IACUC #	459	N/A
C. If no approval date, please explain:	N/A	N/A
D. Is this IACUC approval date different from the date reported last year?	N/A	N/A
3.) Will recombinant DNA experiment(s) be conducted next year?	No	No
A. Date of Office of Recombinant DNA Activities (ORDA)?	N/A	N/A
B. EXEMPT?	N/A	N/A

4.) Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? No No

A. If yes, identify. N/A N/A

5.) Will any of the research-risk categories, not involved next year, be involved in future years? No No

A. If yes, identify. N/A N/A

PROTECTION AGAINST RESEARCH RISKS

Y 1. Will human subjects be involved next year?
If yes, provide complete the above Targeted/Planned Enrollment Table and the Inclusion Enrollment Report. Provide the date of IRB approval and enclose with transmittal.
09/20/2010

Y 2. Will vertebrate animals be used next year?
If yes, provide the date of Institutional Animal Care and Use Committee (IACUC) approval and enclose with transmittal.
09/16/2010
If no approval date, please explain.
Is this IACUC approval date different from the date reported last year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

GENETIC BASIS FOR FAMILIAL COMBINED HYPERLIPIDEMIA (FCHL) (0026)

TYPE: Research Subproject

%IDeA $: 1.000% **IDeA $:** 22,500

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Primerano, Donald A PHD	Biochemistry & Microbiology	
Baria, Kim RN	Health Research And Education	Charleston Area Medical Center, Wv Usa
Dementieva, Yulia PHD	Mathematics	Emmanuel College, Ma Usa
Denvir, James PHD	Biochemistry And Microbiology	
Fan, Jun BS, PHD	Biochemistry & Microbiology	
Henderson, Angela RN	Clinical Trials	Charleston Area Medical Center, Wv Usa
Neal, William MD	Pediatric Cardiology	West Virginia University , Wv Usa
Omar, Hatim FAAP, MD	Pediatrics	University Of Kentucky, Ky Usa

Total # human subjects expected for entire study: 500

Total # human subjects enrolled to date: 363

SUBPROJECT DESCRIPTION

Familial Combined Hyperlipidemia (FCHL) is generally defined by elevation of LDL cholesterol and triglycerides. FCHL affects 0.5-2% of the population of Westernized societies and is one of the most common genetic lipid disorders in patients with coronary artery disease. Recent segregation analyses and linkage studies suggest an oligogenic mode of inheritance. Our long-range goal is to gain an understanding of the molecular and cellular events that lead to disregulation of cholesterol and triglyceride levels. The overall objective of this project is to identify gene(s) that predispose to FCHL using family-based linkage analysis and association methods. By studying juvenile-based families we hope to identify a form of the disease with higher heritability. We will also study families that are based on affected adults. The central hypotheses are that there are specific genes that confer susceptibility to FCHL and that novel gene defects contribute to juvenile FCHL. The rationale is that once these susceptibility genes have been identified, we will better understand the molecular pathogenesis in individuals and be able to provide rational, personalized treatments or preventative measures can be implemented and tailored to the actual genetic defect. We plan to accomplish the objective of this application by pursuing the following two specific aims:

1. Identify novel FCHL loci by linkage analysis on a genome-wide set of markers.
2. Perform genetic fine mapping on the susceptibility loci in order to narrow the region of interest and identify variants that encode defective products.

The proposed work is innovative because we will analyze Appalachian families based on affected juvenile patients. These patients have greater genetic risk of developing CVD than adult FCHL patients. It is our expectation that this analysis will uncover novel loci and specific genes at those loci that contribute to the development of FCHL. Our findings will be significant because they will further our understanding of the pathogenesis of vascular disease and because these susceptibility genes represent new targets for preventative and therapeutic interventions.

SUBPROJECT PROGRESS

FCH Progress Report Y11

1. Introduction

Familial Combined Hyperlipidemia (FCH) is a complex genetic disease in which affected family

members exhibit elevated LDL cholesterol, triglycerides or both. With a prevalence of 0.5-2% in the US population, FCH accounts for 10-20% of premature cardiovascular disease (CVD). FCH was recognized as a genetic disorder in the early 70s and was thought to be inherited as an autosomal dominant disorder. More recent studies including segregation analyses indicate a polygenic mode of inheritance. Several whole-genome scans and candidate gene studies from different populations have identified a number of potential FCH susceptibility loci. We have compelling reasons for continuing our study of FCH families in the Appalachian population. (1) Based on previous linkage analyses, there is evidence that more than one gene confers susceptibility to this disorder. (2) Since West Virginia has the 4nd highest rate of CVD in the US, determination of FCH susceptibility gene(s) could help to understand the underlying pathology and ultimately reduce health disparities in this understudied population and shed light on the molecular basis of FCH heterogeneity. (3) We have identified families that are predicated on affected juvenile probands. Given that juvenile onset of dyslipidemia is now thought to predispose to cardiovascular disease, these families could represent a novel form of FCH in which individuals are at increased risk for developing premature cardiovascular disease.

2. Background and Significance:

Cardiovascular disease (CVD) is the leading cause of death in the US and Western societies countries. According to the American Heart Association 2010 Statistical Update ~81.1 million US residents have one or more types of CVD [1]. The state of WV incurs 309.2 CVD deaths per 100,000 and has the 6th highest rate among the 50 states + DC and Puerto Rico. The overall US death rate is 262.5 per 100,000. The estimated total direct and indirect cost of CVD in 2010 is $503.2 billion [1]. Serum lipid levels such as LDL cholesterol, HDL cholesterol and triglycerides are known CVD risk factors. Three inherited dyslipidemias [familial hypercholesterolemia (FH), FCHL and familial hypertriglyceridemia (FHTG)] contribute to the spectrum of CAD risk factors. FCHL is generally defined by elevation of LDL cholesterol and triglycerides and is one of the most common familial dyslipidemias [2, 3, 4]. With a prevalence of 0.5-2% in the US population, FCHL accounts for 10-20% of premature CVD [5]. FCHL was recognized as a genetic disorder in the early 70s and was thought to be inherited as an autosomal dominant disorder [6, 7]. More recent studies including segregation analyses indicate a polygenic mode of inheritance [8, 9]. Several whole-genome scans and candidate gene studies from different population have identified a number of potential susceptibility loci.

Most recent studies suggest that FCH is transmitted as either an oligogenic (caused by a small number) or multifactorial (genes and environment) disease [8, 10, 11, 12, 13, 14]. FCH and FHTG are usually expressed in late adolescence [15, 16], but at least one study indicates when FCH is present in young children the penetrance of the disease is nearly 100% [15]. Seven genes have been shown to be associated with FCH but only three of these (Upstream Stimulating Factor (USF1) on 1q21, apolipoprotein A1/C3/A4/A5 cluster (designated apoA1 in this report) on chromosome 11 and LPL have been replicated [8, 17, 18]. USF1 is a basic helix loop helix transcription factor that is a key regulator of numerous genes in lipid and glucose metabolism [18]. USF1 SNP usf1s2 is significantly associated with FCH and is located within an evolutionarily conserved 20 base pair region of intron 7. Electrophoresis mobility shift assays indicated that the region bound specifically to nuclear proteins from HeLa extracts [19]. SNP rs3737787 (usf1s1) is located in the untranslated region of exon 11 and captures most of the disease associated signal [8]. We also tested for linkage and association with coding SNP rs10825269 in the PCDH15 gene which encodes a protocadherin recently showed to be associated with FCH and deafness [20].

On chromosome 1q21 where USF1 is located, there is no evidence of genetic heterogeneity in Finnish families, whereas the proportion of Mexican, German and Chinese families contributing to linkage ranged from 22% to 71% [reviewed in 8]. Pajukanta et al. [10] combined data from genome-wide screens performed in Finnish and Dutch populations. The pooled data analysis identified 3 chromosomal regions, on 2p25.1, 9p23, and 16q24.1, where Logarithm of the Odds (LOD) scores were greater than 2.0. These observations taken together suggest that other genetic

loci confer susceptibility to FCH.

The process of atherosclerotic plaque formation begins in childhood [21] and the American Association of Pediatrics now recommends juvenile lipid screening with a positive family history of dyslipidemia or premature heart disease [22]. Given the increased levels of cholesterol and triglycerides at an early age, FCH juveniles could be at increased risk for cardiovascular disease. Although several groups have identified and characterized FCH in juveniles [16, 23], there are no studies on the FCH susceptibility loci in these families.

3. Goals and Revised Method of Analysis for Genetic Basis of Familial Combined Hyperlipidemia

The goal of the original FCH proposal was to identify candidate susceptibility genes by performing genome-wide linkage analysis. We planned to accomplish this goal by pursuing the following specific aims:

1. Identify novel FCH loci by linkage analysis on a genome-wide set of markers.
2. Perform genetic fine mapping on the susceptibility loci in order to narrow the region of interest. We would then sequence genes in that region in order to identify causal variants.

FCH is probably genetically heterogeneous, that is, the FCH phenotype can arise by the action of different genes acting individually. Consequently, linkage analysis would require fairly large numbers of families or large individual families in order to identify significant regions. In light of the difficulties in recruiting sufficient numbers of FCH families needed for genome-wide linkage analysis, we propose to adopt an approach based on sequencing the exomes of related affected individuals. Whole exome sequencing has successfully been used to identify causal variants for about ten Mendelian disorders (e.g. Miller Syndrome [24, 25, 26]. A number of investigators are now applying this method to the analysis of the families with polygenic and multifactorial disorders [27 and G. Jarvik personal communication). By focusing first on the discovery of susceptibility variants in individual families, investigators may circumvent problems associated with data pooled from genetically heterogeneous families. FCH is also thought to be an oligogenic disorder, i.e. the disease may arise from the combined actions of several genes. If the cause is in fact oligogenic, there should be sets of genes (with variants) that are shared among affected family members.

In Y10, we received approval to modify our method of identifying FCH susceptibility. We now using a strategy based in part on whole exome sequencing of individuals in selected FCH families. Our immediate and long-range goals remain unchanged. We will improve our understanding of the molecular pathology of cholesterol and triglyceride regulation by identifying genes and variants that predispose to FCH. The new experimental design will involve several stages: (1) selection of FCH families with multiple affected individuals who are known to not have deleterious alleles in the LDLR gene, (2) removal of common variants from whole exome sequences by comparison to existing SNP databases such as HapMap and dbSNP and reduction of coincident variants by selection of variants shared by multiple affected individuals, and (3) confirmation of the presence of deleterious alleles in other affected family members and their absence in phenotypically normal individuals.

Stage 1. We will begin by selecting families that have at least three affected individuals including the proband. Since about 20% of FCH affected individuals in one study had significant defects in the LDLR gene [28, 29], we will perform conventional DNA sequencing on all LDLR exons on two affected members in each of the “3+ affected” families. We will amplify proximal exons into three long-range PCR products and sequence the exons using primers and methods described in Van Leuven et al [30].

Stage 2. To obtain an initial collection of potential FCH variants, we will perform whole exome

sequencing on affected FCH family members and apply a stepwise filtering process to identify variants that are most likely to confer susceptibility to the disease. From those families that have no deleterious LDLR mutations in affected members, we will then select affected individuals for whole exome sequencing. We will select families with the largest numbers of affected individuals and with multiple generations of affected members. This would allow for substantial reduction in the number of coincident variants and for a comparison of variants between the families as means of detecting genetic heterogeneity.

(2a) We will first select pathogenic variants (nonsynonymous SNPs, splice site mutations and coding regions indels) by comparison to control genomes (e.g. HapMap and dbSNP reference genomes) as described in [2]. This will remove common variants, i.e. those with allele frequencies greater than 5%. Given that the frequency of FCH affected individuals in general populations is ~1%, the combined frequency of susceptibility alleles would be ~0.5% under a simple autosomal dominant model. However, since FCH is probably genetically heterogeneous, the actual allelic frequency of individual susceptibility variants may be much less than 0.5%. Given this, it is unlikely that exclusion of the common variants will eliminate FCH susceptibility variants.

(2b) We anticipate that step 2a will reduce the number of shared variant-containing genes to 5000-10,000 based on prior Mendelian studies [24,25]. We will further reduce the number of variants by selecting only those variants that are shared by affected members within the same family. By selecting more distantly related affected members in a pedigree (e.g. 2° and 3° relatives), we can more efficiently reduce the number of coincident variants that are identical by descent. This filtering process is based on the study of Ng et al [24,25]). In their study on Miller Syndrome (under an autosomal dominant model), Ng et al [25] were able to reduce the number of candidate genes to 83 by filtering for pathogenic variants and for variants shared between two affected siblings; by filtering only for sharing among three affected members, the number of shared genes was reduced to between 10-26 depending on the compared affected individuals. By using 2° and 3° relatives and 3 or more affected members per family, we anticipate being able to reduce the number of candidates to about 10-20. Since the mode of inheritance for FCH is unknown, we will conduct filtering under both autosomal dominant and recessive models. We will assess FCH phenotype using our standard algorithm based on age, sex and race adjusted cutoff values for cholesterol and triglycerides.

(2c) We will use the PolyPhen-2 program to predict variants that will be more deleterious to protein function. PolyPhen is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using physical and comparative considerations (http://genetics.bwh.harvard.edu/pph/). PolyPhen predictions may reduce the candidate genes by ruling in variants that are most likely to have an effect on protein function. Ng et al (2000b) used Polyphen in one of their filtering processes and found that it eliminated DHODH, their top candidate gene. They advocated use of alternative filtering strategies including + Polyphen and + unrelated affected individuals

Stage 3. Resequencing of candidate genes in affected and normal relatives. At the completion of all steps in stage 2, the total number of genes with shared variants should be reduced to between 10 and 20. We will confirm the significance of these variants by showing that these variants are present in other affected family members and that they are largely absent from unaffected relatives. This confirmatory sequencing will be limited to the variant sequence and ~200bp of flanking sequence and can therefore be effectively conducted on all affected and normal persons from our FCH families; currently there are 80 affected individuals and 92 normals based on our phenotype algorithm. In this fashion, we will eliminate coincident variants and focus on the remainder for biological relevance to FCH. Also, by expanding sequencing to unrelated affected members we can test for allelic and locus heterogeneity.

We note that a small fraction of affected members may not share a candidate variant because these individuals may be phenocopies (individuals who have the disease because of environmental but not genetic causes), or have inherited an alternative susceptibility variant from a married-in individual. Further, some of the unaffected relatives could have a candidate variant because they are nonpenetrant carriers.

Outcomes: FCH is thought to be an oligogenic disorder. By sequencing the exomes of three or more 2° or 3° individuals in a given family, we should be able to identify the major susceptibility genes. Further by sequencing the exomes of several unrelated families, we may find alternative or overlapping sets of susceptibility genes.

Confirmation of causality: Identification of one or more FCH candidate susceptibility genes by this filtering process should be viewed with some caution. Some initial confirmation can be achieved by review of the candidate gene's metabolic function and its relevance to the dyslipidemic phenotype. Long term studies should include replication of the FCH phenotype in animal models containing the appropriate variant transgenes. Funds for the animal studies are not budgeted and would need to be part of a future proposal.

Alternatives to whole exome sequencing approach

Whole exome sequencing is an efficient strategy for discovering the genetic causes of Mendelian disorders given that (1) most known variants disorders disrupt protein coding sequences, (2) a large fraction of rare variants are predicted to be deleterious and (3) there are now ten Mendelian disorders that have been solved by exome sequencing [8, 9 and J. Shendure, Illumina Webinar 2010). Although a number of investigators now view whole exome approaches as viable means of finding susceptibility in polygenic and multifactorial disorders, it is possible that we will not find appropriate candidates because (1) no variants are identified that are shared by a preponderance of the affected members or (2) we are not able to reduce the number of candidates to an appropriate size. In the former case, the failure to identify shared genes may stem from the presence of susceptibility variants in promoters or regulatory sequencers. Whole genome sequencing could be used to identify those variants but the cost would be prohibitive (at least at this time). Potential solutions would include seeking additional funding or finding a collaborator who is willing to absorb the cost. In the latter case, we may employ linkage analysis on single families to identify candidates of interest. We may also take steps to merge our exome data with other studies in an effort to reduce the pool of variants to a manageable number.

4. Materials and Methods:

Human Subject Enrollment and FCH Phenotypes:

We recruited FCH families who met adult inclusion criteria established by previous investigations or our criteria for FCH families with a juvenile proband. Human subjects were enrolled according to approved Institutional Review Board (IRB) protocols from the following WV hospitals or clinics: Charleston Area Medical Center, the Department of Adolescent Medicine at the University of Kentucky (UK), WVU Department of Pediatric Cardiology and Valley Health Systems. FCH families were recruited by two primary mechanisms: (1) The Coronary Artery Risk Detection In Appalachian Communities (CARDIAC) Project is a population-based and high-risk individualized program designed to raise awareness of CAD risk factors in the community and identify individuals at high risk for premature CAD. Dr. William Neal, CARDIAC program director, contacted adults from prospective families through the WV lipid clinics to initiate the enrollment process. This recruiting mechanism mainly provided families with juvenile probands. (2) We also recruited FCH families through Charleston Area Medical Center (CAMC), UK and Valley Health System (VHS). Prospective FCH probands were identified by chart review or by physician referral. This process provided both adult and juvenile FCH families.

FCH families were ascertained by identifying probands with one of two phenotypes.

Phenotype #1 (adults)
(1) fasting plasma total cholesterol ≥95th percentile for age, sex and race
(2) fasting plasma triglycerides ≥ 90th percentile for age, sex and race
(3) at least one first degree relative with a different hyperlipidemic phenotype
(4) a positive history of premature CVD (e.g. myocardial infarction) before age 60

Exclusion criteria for probands included (1) diabetes, (2) obesity (BMI 28), (3) tendon xanthomas, (4) evidence of defect in LDL receptor gene, and (5) type III hyperlipidemia (certain apoE2/E2 homozygotes). Related family members were designated as affected if they have either elevated total cholesterol, elevated triglycerides or positive history of CVD.

Phenotype #2 (juveniles)
(1) fasting total cholesterol ≥95th percentile for age, sex and race
(2) fasting triglycerides ≥90th percentile for age, sex and race

Similar exclusion criteria were used for this phenotype

Re-consent process and database submission

In Y10, we were advised by Dr. Krishan Arora (our NCRR program officer) that we would be required to submit all exome sequence and related clinical data to the database of Genotype and Phenotype (dbGAP). This necessitated revision of existing IRB protocols and consent forms and re-consent of already enrolled family members. Modification of IRB protocols at Marshall University, University of Kentucky (UK), West Virginia University (WVU) and Charleston Area Medical Center (CAMC) was completed late in Y10. Re-consent of participants began late in Y10 and continued through Y11. We selected families for whole exome sequencing based on completion of the re-consent and absence of pathogenic mutations in the LDLR gene. CAMC and UK collaborators continued to recruit FCH families in Y11.

Sequencing Logistics

LDLR exon sequencing was performed on an ABI3130 Genetic Analyzer by Dr. Jun Fan by the MU Genomics Core Facility. We received and installed an Illumina HiSeq1000 next generation sequencer in March 2011. We received training in the construction of libraries and high throughput sequencing in April 2011. Bar-coded whole exome libraries were prepared using the Illumina TruSeq Exome Enrichment Kits. Whole exomes were sequenced in either 2 x 100 or 2 x 125 paired end strategy. In our initial sequencing run, we obtained 3 to 9 million bases per exome (Table 1). Quality scores indicate sequencing error rates at ~1 in 10,000 bases and correspond to the expected error rates for Illumina sequencers. Variants were called by comparison to the human reference genome (hg19) using Casava 1.8 software.

Table 1. Sequence data per exome from families 115001, 115021, 115027

Family member	Total bases (Mbases)	% Align (PF)	Mean Quality Score (PF)	Avg fold coverage
115-001-001	3,732	87.81	36.07	18.29
115-001-002	6,242	88.03	35.87	28.22
115-001-003	5,920	88.17	35.92	30.54
115-001-005	8,958	88.01	36.00	38.05
115-001-006	5,508	86.16	35.48	28.60
115-001-022	4,868	86.86	35.60	23.78
115-001-023	6,462	87.11	35.78	28.84
115-001-024	5,232	87.95	35.78	22.96
115-001-025	3,008	87.30	35.88	8.13
115-021-001	5,324	87.81	36.12	24.25
115-021-002	7,022	88.17	36.09	35.55

115-021-005	7,080	88.88	36.16	43.39
115-021-006	7,730	88.36	35.82	44.68
115-027-001	6,478	87.99	36.09	37.79
115-027-006	3,728	87.71	36.09	16.99
115-027-007	6,056	87.68	35.91	31.43
115-027-009	5,630	86.38	35.43	31.81
115-027-012	4,990	88.25	36.10	33.57
115-027-015	5,036	88.92	36.19	32.70
115-027-027	5,964	88.68	36.12	43.34
115-027-032	5,264	88.70	35.93	37.30
115-027-033	7,856	88.00	35.70	61.58
115-027-034	7,258	88.28	35.87	56.77
total bases	135,346			

5. Results:

We enrolled FCH families where the proband was either an affected juvenile or adult member. We identified candidate genes for FCH susceptibility by applying a series of filtration steps. For our whole exome sequencing approach, we focused our efforts on the 22 largest families (15 with a juvenile proband and 7 with an adult proband). These 22 FCH families met the criterion for having at least one normal + two affected members. 18/22 families have 3 affected. At least 2 members of the 22 families had completed the re-consent process.

LDLR mutations: Since ~20% of FCH patients have been shown to have mutations in LDLR (and therefore have a form of FH), we sequenced all LDLR exons in at least two affected and one normal person in 20 of the 22 “3+ affected” families, via long-range PCR. We successfully sequenced 100% of all 18 exons in 10 families and ~90% of the exons in 8 families. Two family’s DNA (116004 and 117008) failed to amplify in long-range PCR. In our analysis of LDLR variants, we excluded all synonymous variants and those missense variants which had allele frequencies greater than 10% since they were unlikely to result in nonfunctional LDL receptor. Of the 18 families with LDLR data, 4 families carried rare, deleterious mutations in an LDLR exon:

(1) Affected members of Family 115006 had a known cysteine to serine at amino acid 56 (C56S) in exon 3 (adult proband)
(2) Affected members of Family 116001 had a novel splice donor SNP in exon 9 (juvenile proband)
(3) Affected members of Family 116002 had a known splice donor SNP in exon 3 (juvenile proband)
(4) Affected members of Family 117001 had a known 11 bp duplication in exon 4 (adult proband).
Figure 1 illustrates the effect of the “frameshift” insertion in initial sequence readout beginning at base 563.

Figure 1. Sequence of LDLR exon before and after the point in insertion. Notice that after base 563, there are two base calls at each position resulting from heterozygosity in the participant.

All mutations were present in phenotypically affected and absent in phenotypically normal members (minimum of four individuals). Three of the four mutations had been identified in prior studies. It is not clear why these “LDLR-defective” individuals have elevated triglycerides.

Whole exome sequencing and analysis of Family 115027
Based on LDLR analysis, 14 families were qualified to move onto whole exome sequencing (that is there were no demonstrable deleterious mutations in LDLR in these families). Because we multiplexed samples in the paired-end sequencing, we were able to economically sequence the whole exomes of 41 individuals from six FCH families as shown in Table 2.

Family	Affected/normal members
115001	8 affected + 1 normal
115021	3 affected + 1 normal
115027	9 affected + 1 normal
115028	4 affected + 2 normal
116003	5 affected + 2 normal
117005	4 affected + 1 normal

Table 2. Numbers of individuals for whom exomes have been sequenced. *Some normals are relatives and others are married in.

To date, we have performed discrete filtering on family 115027. We focused our efforts on this family because of the large numbers of affected members. Our filtering process considered total SNPs and indels and contained the following stages:

(1) Inclusion of all variants present in an exome but absent from the reference human genome (hg19)
(2) Inclusion of all variants present in all affected members and exclusion of all variants present in one normal relative and one unrelated normal.
(3) Exclusion of all variants with allele frequencies greater than 5% or 15%. (Given an autosomal dominant mode of inheritance, an affected genotype frequency of 5% (AA and Aa) and Hardy Weinberg equilibrium, the mutant allele frequency would be ~2.5%.)

In the filration process, we assumed that (1) the trait was transmitted as an autosomal dominant with complete penetrance, (2) all affected members have carry the mutation, and (3) the hg19 reference genome does not have the FCH mutation. After stage (1), we found an average of 231,213 SNPs and 12,273 indels per family member. Retention of SNPs present in all affected members and removal of SNPs present in the two normals reduced the number of SNPs to 624 and indels to 47 (stage 2). When we eliminate SNPs whose allele frequency is less than 5% or 15%, total SNPS are reduced to 29 or 70 SNPS, respectively (stage 3). (Indel allele frequencies have been obtained at this time. Table 3 shows the number of shared variants after each stage

Variants per stage	Filter	SNPS	Indels
Stage 1	Total (mean per subject)	231,213	12,273
Stage 2	Filtered by affected-normal	624	47
Stage 3	Filtered by MAF = 15%	70	NA

Table 3. Variants present after each filtration stage.

In order to identify pathogenic SNPs, we categorized the variants as shown in Table 4. Many of the variants were located in intronic regions or unknown (uncharacterized) loci. At the 15% minor allele frequency cutoff, two missense variants mapped to uncharacterized open reading frames on chromosomes 1 and 17.

Variant Type	Minor Allele Frequency	
	5%	15%
synonymous coding SNPs	1	4
intronic	8	30 (1 in ACLY in splice site)
missense	0	2 (uncharacterized ORFs)
Near 3' end of gene	1	2
Near 5' end of gene	1	1
Unknown (1 on Y chromosome)	11	13
3' untranslated region	2	13

5' untranslated region	2	2
novel SNPs	3	3
total SNPs	29	70

Table 4. Types of shared variants with allele frequencies 5% or 15%. SNP counts include those for which an allele frequency has not been determined. Novel SNPs are those that do not appear in the dbSNP database.

Examination of the post stage 2 set of variants showed that none of the 625 variants mapped to genes that are known to cause either FH or FCH (Table 5).

Gene	Chromosome	location (bps)	Family 115027 Variants Present in Gene Interval?
FH			
LDLR	19	11,200,038-11,244,508	NO
apoB	2	21,224,301-21,266,945	NO
PCSK9	1	55,505,149-55,530,526	NO
FCH			
USF1	1	161,009,041-161,015,757	NO
LPL	8	19,796,582-19,824,778	NO
apoA1	11	116,706,469-116,708,338	NO

Table 5. Presence of Variants in Known FH and FCH Causal Genes.

6. Discussion:

We have pursued a whole exome strategy to identify genes that confer susceptibility to FCH. We have successfully sequenced the whole exomes of 41 individuals and tested a variant discovery strategy on one FCH Family (1150027). Our strategy at a 15% allele frequency cutoff reduced the number of possible variants to 70 which 45 correspond to known genes. We have reviewed the functions of genes from variants which result in missense or possible changes in conserved splice sites. The missense mutations correspond to uncharacterized open reading frames on chromosomes 1 and 17. Severity of these missense mutations and potential biological function of these ORFs must be assessed. One variant in the ATP-citrate lyase (ACLY) gene is located near a splice donor site. This enzyme catalyzes the formation of acetyl-CoA and oxaloacetate from citrate and CoA with a concomitant hydrolysis of ATP to ADP and phosphate. The product, acetyl-CoA, serves several important biosynthetic pathways, including lipogenesis and cholesterogenesis. ACLY expression and activity is known to be suppressed by exogenous lipids (GeneCard database). Although effect of this mutation on ACLY mRNA splicing is unknown, ACLY may play a role in regulating the abundance of serum cholesterol and triglycerides.

No SNP or indel variants mapped to the 3 known FH and 3 known FCH gene. This reduces the likelihood that defects in these genes could be the cause of FCH in Family 11502 but does not eliminate it completely since deleterious mutations may be located in non-exonic regions of these genes. A more rigorous analysis of variants from all FCH families is under way.

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PROTECTION AGAINST RESEARCH RISKS

Y 1. Will human subjects be involved next year?
If yes, provide complete the above Targeted/Planned Enrollment Table and the Inclusion Enrollment Report. Provide the date of IRB approval and enclose with transmittal.
03/25/2011;01/25/2012

N 2. Will vertebrate animals be used next year?

Y 3. Will recombinant DNA experiment(s) be conducted next year?
If yes, provide the date of Office of Recombinant DNA Activities (ORDA), NIH approval:
EXEMPT

Y 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:
Bloodborne pathogens, human cell lines

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

EPICARDIAL FAT BIOMARKERS IN PATIENTS WITH CORONARY ARTERY DISEASE IN APPALACHIA (0036)

TYPE: Research Subproject

%IDeA $: **IDeA $:** 0

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Santanam, Nalini PHD	Pharmacology, Physiology & Tox	
Cushman, Kenneth PHD	Biology	West Liberty University, Wv Usa

Total # human subjects expected for entire study: 100

Total # human subjects enrolled to date: 60

SUBPROJECT DESCRIPTION

Obesity is highly prevalent in United States, especially in the Appalachian Region. Obesity increases risk to cardiovascular disease and diabetes. An understanding of factors that are altered during obesity will help in its prevention. Recent studies speculate that changes in epicardial fat – (EF, the fat that surrounds the heart) derived mediators will alter cardiac function. The present study will attempt to identify unique fat biomarkers from EF that could be used as predictors of cardiovascular risk or as potential therapeutic targets in patients with or without coronary artery disease (CAD). EF and subcutaneous fat (SF) from consented WV patients with and without CAD undergoing either coronary artery bypass graft surgery or valve surgery at St. Mary's Heart Center, Huntington, WV will be obtained. Fat derived biomarkers will be measured in the samples that are collected. In Aim 1: Circulating and fat derived biomarkers will be identified using multiplex ELISAs and real time PCR. Aim 2: Expression profiling of genes in the human genome will be performed on paired EF and subcutaneous fat obtained from all patients In Aim 3: the relative abundance of human microRNA (the noncoding RNAs that are master regulators of mRNA) in EF and subcutaneous fat will be determined via microarray-based profiling. The biochemical findings will be correlated to the clinical findings obtained from the Society of Thoracic Surgery database. This translational type of grant will address four out of the five major goals of the parent WVINBRE award. This grant involves interactions between investigators and clinicians at Marshall University (lead institution) and West Liberty University (partner institute). It will give an opportunity for undergraduate and medical students in translational research training and help with hiring or retaining WV residents.

SUBPROJECT PROGRESS

Introduction/Background: The long term goal of this translational project is to better understand the role of epicardial fat specific molecular markers in cardiovascular risk in patients with coronary artery disease in the Appalachian region. The immediate goal of this project is to identify unique fat biomarkers in epicardial fat obtained from West Virginia patients with and without coronary artery disease (CAD) undergoing thoracic surgery.

Specific Aims: The following specific aims will be performed using the samples obtained from these patients:

Specific Aim 1: Identify differences in expression of circulating and fat biomarkers in WV patients with CAD and non-CAD: Fat specific biomarkers will be measured in the EF and SF obtained from both CAD and non-CAD WV-patients to identify unique biomarkers between the paired fat depots.

Specific Aim 2: Expression profiling of EF and SF to identify unique fat biomarkers in WV patients with CAD and non-CAD: Expression profiling using Agilent Whole Human Genome 4 x 44 K microarrays will be performed on EF and SF samples obtained from CAD and non-CAD WV-patients to identify unique genes in epicardial fat that are altered during coronary artery disease.

Specific Aim 3: Identify differences in microRNAs in EF and SF
obtained from WV patients with CAD and non-CAD: Microarray for microRNAs (small noncoding RNAs that regulate gene expression pathways by degrading mRNA) will be performed on EF and SF obtained from patients with CAD and non-CAD to identify unique microRNAs as potential biomarkers or targets for therapy.

Methods/ Study Design: With help of Dr. Christopher Adams, Dr. Paulette Wehner and Dr. Todd Gress (Dept. Cardiovascular Services) and Dr. Chowdhury (Dept. Thoracic Surgery) we have successfully recruited 60 subjects (30 men and 30 women) undergoing coronary artery bypass graft surgery (CABG) at the St. Mary's Heart Center, Huntington, WV (IRB approved study). We are in the process of recruiting control patients (no coronary artery disease) undergoing valve type surgery. We collected blood, epicardial/perivascular fat and subcutaneous fat from all subjects at the time of the surgery.

Blood: Blood was centrifuged and plasma was separated immediately and frozen at -800C for further analysis. Circulating levels of adipokines, [adiponectin (high molecular weight, low molecular weight and total adiponectin), human TNF alpha, IL-6 and insulin] using ELISA assay was performed. We also performed a cytokine-chemokine array using Millipore Multiplexing assay that measures 39 cytokines simultaneously in a Luminex 100 system.
Epicardial and Subcutaneous fat: Total mRNA and miRNA were isolated from epicardial and subcutaneous fat using Tri reagent. Gene expression levels of pro-inflammatory markers (fractalkine, MDC and TARC, IL-6) and anti-inflammatory markers (adiponectin and PPAR gamma) were determined in the isolated mRNA from both the fat tissues using Bio-Rad MyiQ real time PCR system.
Superarray RT2 miRNA array (SA Biosciences) consisting of whole genome human miRNAs (800 miRNAs) are performed to assess miRNA changes between the Epicardial and subcutaneous fat. In addition, circulating miRNA isolated from the blood of a sub-set of patients will be performed in the future. The correlation between the circulating miRNA and tissue miRNA will be performed. Target mRNA for the significantly altered miRNAs will also be determined in the tissues.

Statistics and Bio-informatics: Dr. Todd Gress (Department of Medicine, MUSOM) helped with statistical analysis of the data and helped perform correlation studies between laboratory biochemical data to clinical parameters (data obtained from the Society of Thoracic Surgery database) of each patient.

RESULTS:
Aims 1 and 2: Our preliminary results indicated sex differences in both circulating protein levels of adiponectin (total and high molecular weight-HMW) and tumor necrosis factor-α and tissue mRNA levels of adiponectin, peroxisome-proliferator activated receptor γ and interleukin-6 in epicardial fat compared to subcutaneous fat. Correlation analysis of biochemical findings to clinical outcomes (using Society of Thoracic Surgeon's database) showed an inverse correlation between circulating adiponectin levels (both total and HMW) to previous myocardial infarction or congestive heart failure and serum creatinine and a positive correlation with high density lipoprotein. The patients who underwent urgent CABG procedure had lower adiponectin levels compared to an elective procedure.

The multiplex analysis (39 chemokines/cytokine array) of the plasma samples obtained from CAD patients revealed that several cytokines/chemokines were altered in a sex specific manner. In particular, we observed differences in the circulating protein levels of the linked chromosome16q13 chemokines (fractalkine, macrophage derived chemokine (MDC)) in patients with CAD. Similar changes in the tissue mRNA levels of 16q13 chemokines in the epicardial fat compared to subcutaneous fat using real time PCR were obtained which correlated with the circulating levels of

these chemokines. Clinical correlations revealed a positive association with the increase in chemokines to increased presence of congestive heart failure.

More samples are being analyzed to validate and confirm our findings. Validation of the biomarkers will also be performed using cell culture studies.

Aim 3: MiRNAs are 18-25 nucleotides long and regulate gene expression. These miRNAs influence the expression of a gene by either degrading or repressing the target mRNA. This aim investigates the genome wide differences in miRNA profile in EF and compares them to subcutaneous fat (SF) from all patients recruited. We will also investigate if there were sex specific changes in miRNA profile in the epicardial fat. As a preliminary study, the human miRNA microarray consisting of over 88 miRNAs relevant to humans was performed on the RNA isolated from EF and SF obtained from patients (n=8/sex). Threshold values were then used to compare miRNA expression in the EF of each patient. The SF samples from the respective patients were used as the control group when determining miRNA expression. Upon completion and initial review of the data, we found several interesting findings. Females had no up-regulated expression while males had 27 up-regulated miRNAs in EF. Females had 13 while males had 16 down-regulated miRNAs. MiR 122, MiR 196-b, MiR 302c, and MiR 210 all showed decreased expression in both males and females and remains a cause for further study. Statistical validations of our initial findings are still to be performed.

Whole genome miRNA arrays (800 miRNAs in humans) have been performed in approximately 8 patients. miRNAs were isolated from both epicardial and subcutaneous fat and whole genome miRNA was performed using the Roche 480 system. Data analysis will be performed with the assistance of the Bio-informatics core.

3. Human Subjects targeted/planned enrollment and an inclusion enrollment report is attached (see attached excel file)

4. No other funds are available for this project.

5. Abstracts Presented:

I. 22nd Annual Research Day, JCESOM, Marshall University, March 31st, 2010.
II. Arteriosclerosis, Thrombosis and Vascular Biology Annual Conference 2010, San Francisco, April 2010.
III. National IdeA Symposium of Biomedical Research Excellence (NISBRE) Washington, DC June, 2010
IV. This data was also presented at the Annual INBRE PI meeting held at NIH in Oct 2010.
V. 11th International Congress on Obesity, Stockholm, Sweden, July 2010
VI. Advancement in Free Radical Science & 10th Annual Meeting of the Society for Free Radical Research, Chennai, India, Jan 9th-11th, 2011
VII. American Heart Association Nutrition, Physical Activity and Metabolism / Cardiovascular Disease Epidemiology and Prevention 2011 Scientific Sessions, Atlanta, GA, March 23rd – 25th 2011
VIII. The 20th South Eastern Lipid Research Conference (SELRC), Callaway Gardens, Pine Mountain, GA, Oct 6th -8th 2011
IX. WV Chapter of the American College of Cardiology, Nov 22nd 2011. [Christopher Adams, Winner of best poster award and selected to present at the National American College of Cardiology conference]
X. National American College of Cardiology Conference, Las Vegas, Jan 11th 2012 [Christopher Adams, winner of best poster award at the National American College of Cardiology]

6. Students associated with the study:

Christopher Adams (Cardiology Fellow)
Kevin Johnson (1st year medical student)
Caitlin Kocher (Marshall Undergraduate)
Courtney Crain (Marshall Undergraduate)
Logan Efaw (Marshall Undergraduate)
Melissa Massie (Marshall Undergraduate)
Ashley Carroll (West Liberty State undergraduate)
Briana Cowan (West Liberty State undergraduate)

PROTECTION AGAINST RESEARCH RISKS

Y 1. Will human subjects be involved next year?
If yes, provide complete the above Targeted/Planned Enrollment Table and the Inclusion Enrollment Report. Provide the date of IRB approval and enclose with transmittal.
06/08/2011

N 2. Will vertebrate animals be used next year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

HOMER2 AS A SUPPRESSOR OF CELL INVASION AND PODOSOME FORMATION (0027)

TYPE: Research Subproject

%IDeA $: 5.000% **IDeA $:** 168,125

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Shurina, Robert PHD	Biology	Wheeling Jesuit University, Wv Usa
Weed, Scott PHD	Neurobiology And Anatomy	West Virginia University, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

Podosomes are actin-rich projections of the cell membrane that are associated with cancer cell migration and metastases. Their formation requires rearrangements of the actin cytoskeleton, which are mediated through the interaction of a host of actin-binding proteins. Tumor-promoting phorbol esters cause actin cytoskeletal rearrangements that result in podosome formation in a variety of cell types; including vascular smooth muscle cells, osteoclasts, macrophages, endothelial cells, neural cells myoblasts and transformed fibroblasts. Tumor-promoting phorbol esters function by activating one or more PKC isoforms, resulting in the reorganization of the actin cytoskeleton and src activation AFAP-110, an adaptor protein that cross-links actin filaments, activates Src in response to phosphorylation by PKC-□□□ and is involved in podosome formation. Increased levels of AFAP-110 are correlated with increases in podosome lifetime in A7r5 tumor cells and prostate cancer. Although the role of AFAP-110 as a Src activator is well established, the mechanism by which activated AFAP-110 is itself regulated remains incompletely characterized. Scaffolding proteins, such as Tks5, recruit AFAP-110 and other signaling proteins to podosomes. Homer2 is a scaffolding protein that interacts with both actin filaments and activated Rho GTPases in mouse cerebellar cells and can prevent podosome formation in cdc42-activated HeLa cells. Homer2 has been identified as a binding partner for AFAP-110 in two independent yeast two-hybrid studies. We hypothesize that Homer2 is a binding partner and regulator for AFAP-110. In this proposal we will address the mechanism by which Homer2 abrogates podosome formation.

SUBPROJECT PROGRESS

Specific Aim #1: Identify protein binding partners that regulate AFAP-110 activity. We have shown that Homer2 is expressed in a wide variety of human cancer cell lines, including HEK-293T cell line; the ovarian cancer CaOV3 cell line; the prostate cancer PC3 cell line; the neuroblastoma SY5Y cell line; and the epithelial cancer UMSSC-1 cell line.

We have also demonstrated that Homer2 and AFAP-110 co-localize to lamellipodia in A7r5 tumor cell lines that are stimulated with phorbol myristate acetate (PMA).

Other progress

Presentations: Richards, T.D. and R.D. Shurina (2011). Homer2 binds AFAP-110 and localizes to the cortical actin cytoskeleton in lamellipodia. Presented at the 10th Annual WV-INBRE Summer Research Symposium, Huntington, WV, July 2011.

Student research mentor: Eight undergraduate student researchers were supported by this award. Six of these students were awarded scholarships through the NASA-West Virginia Space Grant Consortium

New research collaborations: Thanks to the expertise that I gained from support through the WV-INBRE research network, my lab is in the process of forming a research collaboration with Dr. Gregory Merrick at the Schiffler Cancer Center of the Wheeling Hospital (Wheeling, WV) to investigate whether proteins involved in the AFAP-110/Src signaling pathway can be used as prognostic indicators of prostate cancer.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

N 2. Will vertebrate animals be used next year?

Y 3. Will recombinant DNA experiment(s) be conducted next year?
If yes, provide the date of Office of Recombinant DNA Activities (ORDA), NIH approval:
EXEMPT

Y 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:
Carcinogen

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

RESVERATROL MODULATES CISPLATIN OXIDATIVE STRESS (0037)

TYPE: Research Subproject

%IDeA $: 0.000% **IDeA $:** 0

INVESTIGATOR, DEGREE	DEPARTMENT	NON-HOST INSTITUTION: STATE, COUNTRY
Valentovic, Monica PHD	Pharmacology, Physiology & Tox	
Hardman, Wanda Elaine PHD	Biochemistry And Microbiology	
Troyer, Timothy PHD	Chemistry	West Virginia Wesleyan College, Wv Usa

Total # human subjects expected for entire study:	0
Total # human subjects enrolled to date:	0

SUBPROJECT DESCRIPTION

The West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) has successfully established a research experience for undergraduate students and established a research network between the lead instititon and partner institutions. This ARRA funded supplemental translational research proposal will further grow and establish the primary goals of the WV-INBRRE parent award. The supplement will further increase translational biomedical research opportunities/activities for undergraduate students and faculty at a partner institution (West Virginia Wesleyan College). The supplement will also foster interactions between WV-INBRE and another NCRR Program (COBRE). Additional individuals will be hired which will increase the workforce and tempo of scientific research conducted by American undergraduates, faculty and technical staff in WV. The scientific focus will be on cancer, which is one of the mutlidisciplinary research themes of the parent WV-INBRE award. Cancer is a major health concern and is the second leading cause of death in the U.S. Breast cancer afflicts 1 in 8 women in the U.S., and one of the cancer chemotherapeutic agents used to treat breast cancer is cisplatin. Unfortunately, cisplatin induces irreversible renal damage is some patients. Dr. Valentovic, a faculty member of the Marshall University School of Medicine, has established an in vitro model in which resveratrol (RES) reduces cisplatin renal toxicity. The research goal of this supplement is to establish the mechanism for RES attenuation of cisplatin renal toxicity. This project will focus on whether RES enhances renal excretion of cisplatin or reduces renal accumulation as part of its protective mechanism. This proposal will further examine whether reduction of oxidative stress is part of the cellular mechanism of RES protection for cisplatin toxicity. RES has been shown to inhibit tumor growth for some cancer cells, and the final aim of this translational project will be the first study to examine whether the combination of RES and cisplatin is more effective than cisplatin alone to slow breast cancer cell growth. Administration of an agent such as RES to reduce cisplatin toxicity and possibly improve tumor kill would be of immediate human health benefit to cancer chemotherapy patients.

SUBPROJECT PROGRESS

We have established collaborations with faculty at West Virginia Wesleyan University; Dr. Tim Troyer in the Department of Chemistry is collaborating with us on analysis of Pt for cisplatin pharmacokinetic studies. This grant also allows for the hiring of 2 part-time undergraduate students to work with Dr. Troyer on this research. Finally we have established a collaboration with a COBRE faculty member, Dr. Elaine Hardman. The focus of the collaboration with Dr. Hardman examines potential cancer chemo preventative effects of resveratrol when combined with cisplatin on breast cancer and is research that is translational.

In vitro renal studies: A major component of this research is to examine whether resveratrol (RES) reduced cisplatin renal toxicity. Initial studies were conducted using in vitro exposure of renal cortical slices to resveratrol (RES) and cisplatin. In vitro renal cortical slices exposure was used in order to eliminate any confounding factors of biotransformation and physiological parameters such as renal blood flow. Cisplatin renal toxicity was evident as indicated by an increase in Lactate

dehydrogenase (LDH) leakage within 120 min exposure. RES totally prevents LDH leakage by cisplatin and establishes that RES prevents cisplatin renal cytotoxicity.

Changes occurring in pathways prior to LDH leakage would suggest a role in the mechanism of toxicity. RES does prevent cisplatin mediated depletion of Manganese Superoxide Dismutase (MnSOD) activity. Maintenance of Mn SOD by RES would allow for continued detoxification of superoxide anions (O2-) and diminished oxidative stress that is associated with cisplatin exposure of renal tissue. New methods were established in our laboratory to examine Cu-Zn SOD and catalase enzyme activity. We have also helped Dr. Hardman's laboratory establish these assays for studies conducted by one of her graduate students. The results from our studies show that catalase enzyme activity is diminished by cisplatin. However, renal tissue in the presence of RES exposure to cisplatin did not demonstrate as extensive a decline in catalase activity.

Oxidative stress is a relative balance of antioxidant enzymes. A decline in MnSOD can result in accumulation of O2- and increased oxidative stress. However, if cisplatin induced a greater decline in catalase then oxidative stress can be more magnified as the product of MnSOD action, H2O2 will not be detoxified and can induce cytotoxicity. Our result suggests that RES decreases oxidative stress by cisplatin by maintaining a more favorable balance between MnSOD and catalase.

Our in vitro studies have shown that depletion of glutathione by cisplatin is not prevented by RES. Therefore, the mechanism for RES attenuation does not involve prevention of glutathione depletion. Initial studies examining mitochondrial swelling also suggest that RES does not act by preventing mitochondrial swelling induced by cisplatin. Further studies need to evaluate longer periods of time with mitochondrial exposure. A time dependent study has shown extensive modulation by RES of oxidative stress induced by cisplatin. RES prevents cisplatin oxidative stress 60 min prior to induction of LDH leakage. These studies suggest a RES greatly diminishes oxidative stress. More importantly, the decline in oxidative stress occurs prior to LDH leakage.

In vivo studies: We have conducted in vivo cisplatin renal toxicity studies between 6-72 h post cisplatin. Renal function will be analyzed by urinary protein excretion as a function of time. Qualitative analysis has shown no increase in urinary glucose and therefore urinary glucose excretion will not be analyzed. Cisplatin renal toxicity is assessed by kidney to body weight ratios, blood urea nitrogen (BUN) levels and histological evaluation by light microscopy. Our laboratory has established a new method for BUN measurement using a microtiter assay. This method is now established in our laboratory and can be used by other investigators.

Pharmacokinetic studies: RES may reduce cisplatin renal toxicity by: a) increasing cisplatin renal clearance or b) diminishing cisplatin renal tissue accumulation. Experiments have been completed to evaluate the pharmacokinetic interaction of RES and cisplatin. Male F344 rats were placed in metabolism cages. Parameters evaluated in metabolism cages include food and water intake, body weight, urine volume and urine protein. Rats were randomly divided into the following groups: Vehicle (DMSO and water), RES, Cisplatin (5 mg/kg, ip) and RES+Cisplatin. RES was injected 30 min prior to cisplatin. Plasma and renal tissue was collected 6, 24, 48 and 72 h after cisplatin injection. Urine was collected on ice Pt analysis at the following time points: 0-6 h, 6-24 h, 24-48 h. Pt is analyzed instead of cisplatin for all published pharmacokinetic studies. Samples will be acid digested and then analyzed by Dr. Troyer. The Pt analysis should be completed by July 2012.

Personnel

Two undergraduate students work part-time in my laboratory as research student assistants. Their names are Michael Wright and Bekkah Brown and these students work a maximum of 20 hours per week. A Master's level graduate student, Jacob Wolfe was hired in January 2012 and he works a maximum of20 hours per week. All of these students have become proficient at HPLC sample

extraction, sample injection and running the HPLC for ATP analysis. These students are becoming more proficient every day at conducting Western blot analysis. Michael Wright is independently able to conduct our protein analysis, gel preparation and antibody applications for western analysis.
We are currently accepting applications for the full-time technician position in the laboratory of Dr. Valentovic. Currently there are 8 applicants have applied for the position. It is hoped that interview and hiring can be completed by March 1.

PROTECTION AGAINST RESEARCH RISKS

N 1. Will human subjects be involved next year?

Y 2. Will vertebrate animals be used next year?

If yes, provide the date of Institutional Animal Care and Use Committee (IACUC) approval and enclose with transmittal.

02/06/2009

If no approval date, please explain.

N Is this IACUC approval date different from the date reported last year?

N 3. Will recombinant DNA experiment(s) be conducted next year?

N 4. Are there potential hazards to laboratory workers (carcinogens, pathogens, ionizing radiation, etc.) involved in the proposed research for next year? If yes, identify:

N 5. Will any of the research-risk categories,not involved next year, be involved future years? If yes, identify:

SHARED FACILITIES

WVU Flow Cytometry Core Facility

The WVU Flow Cytometry Core Facility provides instrumentation and scientific support for cell analysis and sorting. The facility routinely performs analysis of both eukaryotic and prokaryotic cells for expression of intracellular and extracellular proteins, cell cycle, cell quantitation, cytokine production, and cell sorting based on antigen expression or cell cycle. The facility is currently equipped with two cytometers, a Becton Dickinson (BD) FACSAria and a BD FACSCalibur. The FACSAria is a 15 parameter (13 fluorescent markers and two scatter parameters) high-speed bench top sorter capable of sorting cells into 4 different populations. Using a grant from the WV-INBRE, the Facility will purchase and install a biological safety cabinet (BSC) that is specially designed for the sorter prior to April 30, 2012. The BSC will replace the current aerosol containment system and enable the Facility to sort infectious agents or cells of human origin under Biosafety Level-2 conditions, which has become the recommended standard for these activities. The dual laser FACSCalibur is capable of 4-color analysis and is equipped with a sample autoloader. In addition to the two cytometers, the facility has a Miltenyi Biotec AutoMACS and tissue culture hood for sterile magnetic cell sorting. The facility has auxiliary laboratory space with appropriate equipment to train personnel and support the needs of researchers. The Flow Cytometry Core has several software packages including FCS Express, CellQuest Pro, ModfitLT and BD Diva software for data acquisition and analysis. It also has two separate analysis workstations with 2 print quality color laser printers. The facility is managed by the technical director Kathleen Brundage, PhD who has training from BD biosciences and many years of experience with flow cytometry. She oversees the day-to-day operations of the facility, operates the FACSAria for cell sorting, provides necessary instrument and software training, and consults on experimental design and data analysis. The facility is house in an approximately 750 sq ft lab space located on the 2nd floor of the Robert C. Byrd Health Sciences Center North building.

Users	Unique #	Fees Charged	Fees Paid by Center
Faculty/Post Doctorate/Staff		Sometimes	No
Graduate Students		No	No
Undergraduate Students		No	No

WVU Genomics Core

The WVU Genomics Core Facility offers full-service gene expression profiling and DNA sequencing for all investigators in West Virginia. For researchers without expertise in these laboratory-based techniques, the core provides full gene expression services, including RNA purification from provided tissue samples, assessment of RNA quality, amplification (if necessary), probe synthesis and labeling, array hybridization, and multi-level bioinformatics analysis. This breadth of service provides accessibility to gene expression profiling for many different types of investigators. For investigators more adept at molecular characterizations, a limited subset of these assays could be performed by the core facility personnel. Similar varied service levels are offered for DNA sequencing. The Director and Laboratory Manager for Microarray/Gene Expression are available prior to the start of experiments to ensure that appropriate design measures are included for statistically significant microarray findings. The Co-Director and Laboratory Manager for DNA Sequencing provide technical and bioinformatics consulting for users of those services. Interactions between investigators and the core staff through every step of the design and execution of the research study will facilitate better and faster scientific discovery and make these core services far more convenient and effective than commercial vendors. The core facility also assists investigators in placing datasets into public databases and maintains these datasets on Genomics Core Facility servers for access by the scientific community at large.

Users	Unique #	Fees Charged	Fees Paid by Center
Faculty/Post Doctorate/Staff		No	No
Graduate Students		No	No
Undergraduate Students		No	No

WVU Transgenic Animal Core Facility

The Transgenic Animal Core Facility (TACF) at West Virginia University is a fee-for-service facility that is housed within the specific pathogen-free (SPF) barrier in the animal quarters at the WVU Health Sciences Center. Our services within the TACF are available to investigators at WVU, the National Institute of Occupational Safety and Health and the Blanchette Rockefeller Neurosciences Institute. The services currently include 1) pronuclear injection of DNA fragments into mouse embryos for producing transgenic animals; 2) mouse embryonic stem cell injections into developing blastocysts for chimera production; 3) rederivation of infected strains; 4) cryopreservation of embryos and sperm; 5) resuscitation of frozen strains; and 6) speed congenic colony management to change background strains.

The barrier facility became operational in January 2004 for housing SPF animals and producing genetically modified animals. The current space occupies approximately 2500 square feet of space, including three animal holding rooms (two mouse and one rat), one procedure room, a transgenic production lab facility, a locker room/changing area, and a general operations/autoclave area. We have the capacity to house upwards of 1850 ventilated cages in the barrier facility. Major equipment includes a stereomicroscope for animal surgery and embryo manipulation, inverted microscope with Nomarski optics for DNA and ES cell injection, Sutter micropipette puller, Isoflurane anesthesia delivery system, CO2 incubators, laminar flow hood, gel electrophoresis apparatus, controlled-rate freezer, liquid nitrogen cell storage tanks, Eppendorf micromanipulators and a FemtoJet pressure injection system. We anticipate moving the TACF into the new barrier space of the Animal Facility Annex upon its completion in 2013.

The current investigators utilizing TACF services are faculty in the Departments of Biochemistry, Exercise Physiology, Microbiology, Immunology, and Cell Biology, Neurobiology and Anatomy, Ophthalmology, and Otolaryngology. These investigators also represent the Cancer Center, the Sensory Neuroscience Research Center and the Center for Cardiovascular and Respiratory Research. Based on a recent survey of interested investigators and known transgenic/barrier users, we have identified at least 18 investigators at WVU who express a strong to moderate interest in either the transgenic core facility and/or the SPF barrier.

Users	Unique #	Fees Charged	Fees Paid by Center
Faculty/Post Doctorate/Staff		Yes	No
Graduate Students		No	No
Undergraduate Students		No	No

WVU Animal Models and Imaging Facility

The Animal Models and Imaging Facility (AMIF) offers state-of-the-art small animal imaging to West Virginia University researchers and their collaborators. Conveniently located within the OLAR Animal Facility in the WVU Health Sciences Center, the AMIF currently performs acute and longitudinal studies on mice using an IVIS Lumina II for optical (fluorescence and bioluminescence) imaging. This system is often used to non-invasively follow tumor formation and metastasis, and has recently been used to study the growth of biofilms using bioluminescent bacteria. The facility also has a new VisualSonics Vevo 2100 for real-time, micro-ultrasound imaging. This system is being used to measure cardiac function, coronary artery flow and 3D tumor volumes. We have recently received training for ultrasound contrast applications. In addition, the ultrasound is being used for image-guided injections into mouse embryos. Another new addition is a clinical DEXA scanner that will be available for bone density and body composition analysis. The facility staff provides additional services, such as cell injections or tissue collections, to assist investigators with their animal experiments. The facility staff will maintains compliance with approved animal protocols, in addition to our own approved standard operating procedures, to ensure the health and welfare of the animals in our studies. The facility is dedicated to providing all the support and services needed for imaging in animal models of human disease. The animal imaging facility has been supported by the Mary Babb Randolph Cancer Center and NIH grants P20 RR016440, P30 RR032138/ GM103488 and S10 RR026378.

Users	Unique #	Fees Charged	Fees Paid by Center
Faculty/Post Doctorate/Staff		Yes	No

Graduate Students	No	No
Undergraduate Students	No	No

WVU Microscope Imaging Facility

The Microscope Imaging Facility provides resources for light microscopy image acquisition, as well as image processing and analysis, on a fee-per-use basis. This shared resource is available to all researchers across the university and the state. The facility currently has eight microscopes, two of which were added during the previous year. One is a new fully automated Zeiss AxioImager that is equipped for epifluorescence, brightfield, darkfield and DIC imaging. This system was purchased with funds provided by the WV-INBRE grant (P20 RR016477). The second new system is a Zeiss Axiovert 40 CFL tissue culture microscope for widefield fluorescence and phase contrast imaging. The facility also has two laser scanning confocal systems, an inverted Zeiss LSM 510 confocal system with 3 lasers (488, 514, 543 and 633nm) and an upright Zeiss LSM 510 confocal with four lasers (405, 458, 477, 488, 514, 543 and 633nm). These systems are used for experiments such as multi-color fluorescent imaging, 3D rendering and FRAP. An upright Olympus AX70 brightfield/epifluorescent microscope equipped with the MicroBrightField Neurolucida and Stereo Investigator software packages is available for color histology records, slide scanning and serial section reconstruction. For microdissection, the facility has a Zeiss PALM MicroBeam system for non-contact microdissection of single cells or groups of cells. The isolated cells can then be used for DNA or RNA analysis, proteomic analysis or for further cultivation. For live-cell imaging, the facility has a Nikon TE2000S epifluorescent microscope with Prior filter wheels and a Photometrics Coolsnap HQ CCD camera. This microscope also has an Eppendorf FemtoJet microinjection system and the MetaMorph and MetaFluor software packages. This system is used for multi-color time-lapse, FRET and calcium ratio image acquisition. For high-end live-cell imaging, the facility has a new Nikon Swept-Field Confocal system with 3 solid-state lasers (491, 561 and 638nm). This system uses a Photometrics QuantEM CCD camera with on-chip gain to maximize signal and to increase the rate of acquisition. This system is also equipped for epifluorescence, DIC, phase contrast and laser TIRF acquisition using a high resolution Photometrics Cool-Snap HQ2 CCD camera. A Prior ProScan II motorized stage supports multipoint acquisition, which has increased throughput from single experiments. Environmental control (OKO Labs Stage Top Incubator with temperature, humidity and CO2 control, a Bioptechs Delta T4 dish heater and objective heaters and a micro-perfusion system) and a fully equipped tissue culture facility including an incubator are available to maintain live cell cultures. Two off-line image analysis workstations are available to the facility users for image processing and data analysis. Software packages include AutoQuant (deconvolution), NIS-Elements (2D tracking, ratio and FRET analysis and 3D rendering), Zeiss AxioVision, Photoshop and Image J. The facility staff are dedicated to providing ongoing training and support to ensure the success of imaging projects. The Microscope Imaging Facility has been supported by the Mary Babb Randolph Cancer Center and NIH grants P20 RR016440, P30 RR032138/GM103488 and P20 RR016477.

Users	Unique #	Fees Charged	Fees Paid by Center
Faculty/Post Doctorate/Staff		Sometimes	No
Graduate Students		No	No
Undergraduate Students		No	No

MU Genomics Core Facility

Marshall University Genomics Core Facility:

Next generation sequencing and microarray experimentation require sophisticated methods, expensive instrumentation and reagents, and intense data management and analysis. These tasks are most efficiently executed in a core facility environment. The primary objective of the MU Genomics Core Facility is to enable the genomic research goals of the INBRE, COBRE and other research projects at WV universities and colleges. The Genomics Core Facility currently provides the following services: (1) high throughput next generation sequencing (NGS), (2) microarray-based gene expression profiling and statistical support, (3) automated capillary DNA sequencing and access to DNA sequence analysis software (4) access to real-time thermal cyclers for quantitative PCR and to Agilent 2100 Bioanalyzers for DNA/RNA quantitation and quality assessment, and (5) purification and banking of patient genomic DNA.

Core Staff: The Genomics Core provides centralized services that would not be available to smaller labs and training in genomic methods of analysis. These services position research project investigators to efficiently gather critical data and analyze it with the most up to date statistical tools. Dr. Donald Primerano oversees all services of the Genomics Core Facility, guides new protocol development and discuss proposed projects with individual investigators. Dr. Goran Boskovic performs all microarray analyses and next generation sequencing (NGS) and library construction; he also provides training in the use of Ingenuity Pathway Analysis software. Dr. James Denvir advises on experimental design and performs microarray and NGS data analyses. Conventional DNA sequencing, genotyping and genomic DNA banking are carried out by Dr. Jun Fan. Dr. Ivana Yang at the University of Colorado serves as an external advisor to the Genomics Core.

Equipment and Lab Space: The Genomics Core is equipped with (1) an Illumina HiSeq1000 next generation sequencer with 300 Gigabase/run throughput (acquired in March 2011), (2) an Agilent Microarray Scanner with 2 micron resolution, (3) an Agilent Hybridization Station, (4) 2 Model 7000 Sequence Detection Systems (real-time PCR), (5) a Step One Plus Sequence Detection System, (6) two Agilent Model 2100 BioAnalyzers, (7) two NanoDrop spectrophotometers, (8) an Applied Biosystems 3130 Genetic Analyzer, and (9) a Luminex 100 protein detection system. All services are available to all WV-INBRE, COBRE, MU and WVU investigators. Real-time thermal cyclers and spectrophotometers are available on a sign-up basis. The HiSeq1000 will support whole genome, whole exome, whole transcriptome (RNA-Seq) and chromatin (Chip-Seq) studies.

In support of the NGS service, we have also acquired a Linux Server for data analysis and storage (40TB), a 3rd Agilent BioAnalyzer for DNA/RNA quality control, an Invitrogen E-gel System for DNA/RNA purification, and a Covaris DNA Shearing System for genomic library preparation. Investigators may learn about the MU Genomics services through its web site (http://musom.marshall.edu/genomics/). The Genomics Core is located in the Robert C. Byrd Biotechnology Science Center and has sufficient space (1200 sq ft) to provide all services.

Administrative Issues: (i) All investigators must meet with the Genomics Core Staff to discuss NGS or microarray experimental design. Issues such as expected data, methods of analysis, biological replicates, and cost will also be discussed. (ii) Prioritization of NGS and microarray requests. We will accommodate peaks in microarray and NGS demand by handling requests from INBRE and CTSA investigators on a first-come first-serve basis. The Genomics Core will have discretion in determining the order of completion of experiments.

Users	**Unique #**	**Fees Charged**	**Fees Paid by Center**
Faculty/Post Doctorate/Staff	8	Yes	Yes
Graduate Students	12	Yes	Yes
Undergraduate Students	1	Yes	Yes

STUDENT ACTIVITY PARTICIPATION

ETHNICITY REPORTS

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Primerano, Donald A

Study Title: WV-INBRE:APPALACHIAN CARDIOVASCULAR RESEARCH NETWORK (ACORN) (0006)

TARGETED/PLANNED ENROLLMENT: Number of Subjects

Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Not Hispanic or Latino	19	16	0	35
Ethnic Category Total of all Subjects*	19	16	0	35
Racial Categories	**Females**	**Males**	**Unknown**	**Total**
Black or African American	1	0	0	1
White	18	16	0	34
Racial Categories: Total of all Subjects*	19	16	0	35

*These totals must agree.

**These totals must agree.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Primerano, Donald A

Study Title: GENETIC BASIS FOR FAMILIAL COMBINED HYPERLIPIDEMIA (FCHL) (0026)

TARGETED/PLANNED ENROLLMENT: Number of Subjects

Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Hispanic or Latino	3	2	0	5
Not Hispanic or Latino	247	248	0	495
Ethnic Category Total of all Subjects*	250	250	0	500
Rac al Categories	**Females**	**Males**	**Unknown**	**Total**
American Indian/Alaska Native	1	1	0	2
Asian	2	3	0	5
Native Hawaiian or Other Pacific Islander	1	0	0	1
Black or African American	8	8	0	16
White	238	238	0	476
Racial Categories: Total of all Subjects*	250	250	0	500

*These totals must agree.
**These totals must agree.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Primerano, Donald A

Study Title: GENOMICS CORE (0033)

TARGETED/PLANNED ENROLLMENT: Number of Subjects

Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Not Hispanic or Latino	5	5	0	10
Ethnic Category Total of all Subjects*	5	5	0	10
Racial Categories	**Females**	**Males**	**Unknown**	**Total**
Black or African American	1	0	0	1
White	4	5	0	9
Racial Categories: Total of all Subjects*	5	5	0	10

*These totals must agree.

**These totals must agree.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Santanam, Nalini

Study Title: EPICARDIAL FAT BIOMARKERS IN PATIENTS WITH CORONARY ARTERY DISEASE IN APPALACHIA (0036)

TARGETED/PLANNED ENROLLMENT: Number of Subjects

Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Hispanic or Latino	3	3	0	6
Not Hispanic or Latino	47	47	0	94
Ethnic Category Total of all Subjects*	50	50	0	100
Racial Categories	**Females**	**Males**	**Unknown**	**Total**
Asian	3	3	0	6
Black or African American	10	10	0	20
White	37	37	0	74
Racial Categories: Total of all Subjects*	50	50	0	100

*These totals must agree.

**These totals must agree.

Inclusion Enrollment Report Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Primerano, Donald A

Study Title: WV-INBRE:APPALACHIAN CARDIOVASCULAR RESEARCH NETWORK (ACORN) (0006)

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Hispanic or Latino**	1	0	0	1
Not Hispanic or Latino	13	11	0	24
Ethnic Category Total of all Subjects*	14	11	0	25
Racial Categories	**Females**	**Males**	**Unknown**	**Total**
White	14	11	0	25
Racial Categories: Total of all Subjects*	14	11	0	25

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	**Females**	**Males**	**Unknown**	**Total**
White	1	0	0	1
Racial Categories: Total of Hispanics or Lati	1	0	0	1

*These totals must agree.

**These totals must agree.

Inclusion Enrollment Report Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Primerano, Donald A

Study Title: GENETIC BASIS FOR FAMILIAL COMBINED HYPERLIPIDEMIA (FCHL) (0026)

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Unknown	Total
Hispanic or Latino**	1	0	0	1
Not Hispanic or Latino	202	146	1	349
Unknown (individuals not reporting ethnicity)	7	6	0	13
Ethnic Category Total of all Subjects*	210	152	1	363
Rac al Categories	**Females**	**Males**	**Unknown**	**Total**
American Indian/Alaska Native	0	3	0	3
Black or African American	0	2	0	2
White	210	145	1	356
Unknown or not reported	0	2	0	2
Racial Categories: Total of all Subjects*	210	152	1	363

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Unknown	Total
White	1	0	0	1
Racial Categories: Total of Hispanics or Lati	1	0	0	1

*These totals must agree.

**These totals must agree.

Inclusion Enrollment Report Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Primerano, Donald A

Study Title: GENOMICS CORE (0033)

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Eth ıic Category	**Females**	**Males**	**Unknown**	**Total**
Not Hispanic or Latino	3	1	0	4
Unknown (individuals not reporting ethnicity	0	0	6	6
Ethnic Category Total of all Subjects*	3	1	6	10
Rac al Categories	**Females**	**Males**	**Unknown**	**Total**
Black or African American	1	0	0	1
White	2	1	0	3
Unknown or not reported	0	0	6	6
Racial Categories: Total of all Subjects*	3	1	6	10

*These totals must agree.

**These totals must agree.

Inclusion Enrollment Report Table

This report format should NOT be used for data collection from study participants

Prinicpal Investigator: Santanam, Nalini

Study Title: EPICARDIAL FAT BIOMARKERS IN PATIENTS WITH CORONARY ARTERY DISEASE IN APPALACHIA (0036)

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Not Hispanic or Latino	30	30	0	60
Ethnic Category Total of all Subjects*	30	30	0	60
Racial Categories	**Females**	**Males**	**Unknown**	**Total**
White	30	30	0	60
Racial Categories: Total of all Subjects*	30	30	0	60

*These totals must agree.
**These totals must agree.

PUBLISHED: ABSTRACTS, BOOKS, & JOURNALS

‡ Grant Cited, * Grant Personnel

Abstracts

Reference	SPIDs
‡ *Cowen SJ McLaughlin S Coad J Hobbs G and Vona-Davis L. Effects of high-fat diet feeding on mammary tumor growth and metastasis in an ER-negative mouse model. AACR Metabolism and Cancer Special Conference Baltimore MD October16-19 2011.	0009
‡ *Luo H. Rankin G.O. DePriest L. and Chen Y.C. 2011 Kaempferol induces apoptosis in ovarian cancer cells through intrinsic pathway. American Association for Cancer Research (AACR) Meeting in Orlando FL April 2-6 2011.	0032
‡ *Rankin GO *Palmer D Sweeney A Schuetz C Kraynie A and Anestis D. Comparative in vitro aminophenol and aminochlorophenol-induced nephrotoxicity. Presented at the Annual Experimental Biology Meeting Washington D.C. April 9-13 2011. FASEB*J. 25:1087.4.	0009
‡ *Roberts KA Audet GN *Olfert IM. The effect of physical deconditioning on skeletal muscle expression of vascular endothelial growth factor American College of Sports Medicine Annual Meeting May 30- June 4 2011 Denver CO.	0009
‡ *Sine RK LaSalla PR Svensson AM Mohammad AA *Perrotta PL. Predicting antibiotic resistance through rapid glucose sensing. 11th General Meeting of the American Society of Microbiology New Orleans LA May 21-24 2011.	0009
‡ *Smurthwaite CB Baksi S Ferguson T Schuetz C Anestis D and *Rankin GO. In vitro 3,5-dichloroaniline nephrotoxicity in freshly isolated rat renal cortical cells. Annual Society of Toxicology Meeting March 5-9 2011 Washington D.C. Toxicologist 120 (2) 159 2011.	0009
*Valentovic M.A. Ball J.G. and Brown J.M. Cisplatin Mediated Alterations in Oxidative Stress Enzymes are Modulated by Resveratrol. Presented at Annual Experimental Biology Meeting Washington D.C. April 9-13 2011. FASEB J. 25;1087.14	0037
‡ *Valentovic MA Ball JG and Brown JM. "Modulation of Cisplatin Mediated Oxidative Stress Biomarkers By Resveratrol". Annual Society of Toxicology Meeting Washington D.C. March 5-10 2011. Toxicologist 120 (2) 160 2011.	0037
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0009, 0023 Wilson, AM, Wyatt, JD, Kreinbihl, DR, Phair, BS, Saling, JR, *Gannett, PM, & *Aguilar, JS. Expression and Purification of Cytochrome P450 2C9. WV-INBRE Summer Symposium at Marshall University, 2011.

0007, 0009 Gipson, D. & *Kim, SY. Petri Net-Based Modeling of Human Systems: Towards Drug Trial Modeling and Simulation - Effects of Chloroquine on Malaria. WV-INBRE Summer Symposium at Marshall University, 2011.

0007, 0009 Lloyd, JD, *Crick, DW, & Craddock, RD. Cytotoxicity and Antimicrobial Activity of Euphorbia corollata. WV-INBRE Summer Symposium at Marshall University, 2011.

0006 *Egleton R. Metabolic Syndrome and the Blood Brain Barrier. Penn State University Symposium on "Prevention of Metabolic Syndrome by Dietary Phytochemicals." State College, PA, September 2011.

0006 Ellison S. Rellick S.L., Ph.D. VanGilder R., Ph.D. *Barr T.L., RN, Ph.D. Gene Expression: A Potential Clinical Diagnostic for Ischemic Stroke. Summer Student Seminar WVU Health Sciences Center, March 10, 2011.

0002 Cook A, Williams H, Gao X, Boyd J. "Impact of Energy Metabolism on the Design of Kinase-Targeted Therapeutics". SOT Annual Meeting in Washington, DC. 2011.

0002 Stueckle TA, Wang H, Elbaz H, Pongrakhananon V, O G, *Rojanasakul Y. 2010. Digitoxin synthetic analogs: A new hope for anti-cancer therapeutics? West Virginia University Van Liere Research Symposium, April 21, Morgantown, WV.

0022 Johnson, A.J. *Harris R.T. *Hankins G.R. *Turner D.L. Vascular Smooth Muscle Cell Structure and Function are Altered by Resveratrol. 16th Biennial National Land Grant Research Symposium.

0009 Furby, R, Shah, P, Kocher, C, Cook, C, Marcello, A, *Egleton, R, & *Santanam, N. MicroRNA Involvement in Type 1 Diabetic Rat Models. WV-INBRE Summer Symposium at Marshall University, 2011.

0009, 0022 Karunathilake, A, Carte, K, *Blough, E, & *Harris, R. Expression of Titin in Smooth Muscle is Altered by Mechanical Stretch. WV-INBRE Summer Symposium at Marshall University, 2011.

0009, 0022 Niraj, N, Wu, M, *Harris, R, & *Blough, ER. Effect of Trichostatin A intervention on hindlimb unloading-induced skeletal muscle atrophy. WV-INBRE Summer Symposium at Marshall Univsersity, 2011.

0007, 0009 Tague, J, Mancheril, B, Yagodzinski, M, & *Linger, R. Mutagenesis, Overespression and Purification of E. coli Guanosine Monophosphate Synthetase. WV-INBRE Summer Symposium at Marshall University, 2011.

0009, 0023 Phair, BS, Wyatt, JD, Wilson, AM, Kreinbihl, DR, Saling, JR, *Gannett, PM, & *Aguilar, JS. Optimization of Expression Parameters for Cytochrome P450 2C9 Mutated Protein. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 Cavender, H, Cooke, J, & Grover, L. Effects of antidepressant drugs on hippocampal long-term potentiation (LTP) and n-Methyl-D-Aspartate (NMDA) glutamate receptors. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 Levin-Nielson, E, Marcelo, A, & *Egleton, R. Regulation of Vascular Endothelial Growth Factor Signaling (VEGF) in the Choroid Plexus (CP) in Diabetes. WV-INBRE Summer Symposium at Marshall University, 2011.

0001 Rankin GO. WV-INBRE Update. Annual West Virginia IDeA Meeting, October 21-22, 2011. Morgantown, WV

0007 Rollyson W.D. Racine C.R. Seidler M.E. Moore D.L. *Harris R.T. Wu Q. Simon J.E. Chin K.L. and *Hankins G.R. Effects of Hibiscus sabdariffa Extracts and Compounds on Tumor and Vascular Smooth Muscle Cells. 16th Biennial 1890 Research Symposium. Atlanta, GA, April 10-11, 2011.

0002 Ware, J; Kulkarni, Y.; Klinke, D. J.; Identifying biochemical cues secreted by malignant melanocytes that promote escape from immunoediting 2011 AIChE Mid-Atlantic Regional Conference, Penn State University, PA, April 2011.

0002 Klinke, D. J.; Timescale Analysis of Rule-based Biochemical Reaction Networks q-bio Conference - Rule-based Modeling Workshop, Santa Fe, NM, August 2011.

0002 Boyd JW. "In Vitro Predictions of Mixtures Toxicity". Invited Seminar for West Virginia University Health Sciences Center, Department of Biochemistry. Morgantown, WV. 2011.

0009, 0039 Jones, E, Martin, E, & *Belay, T. Norepinephrine and the Acquisition of Iron in Actinobacillus pleuropneumoniae. WV-INBRE Summer Symposium at Marshall University, 2011.

0009, 0010 Lamyaithong, AB, Brown, JM, Van Meter, S, Ball, JG, Mills, A, Wright, M, & *Valentovic, M. A. Novel Protective Mechanisms for Acetaminophen (APAP) Hepatic Toxicity by SAMe (S-Adenosyl-L-Methionine). WV-INBRE Summer Symposium at Marshall University, 2011.

0009, 0025 McGill, K, Guertal, S, Kobe, E, Dvoracek, LA, Kreisberg, JI, McKinney, J, Detrick, MS, *Kreisberg, R. OxPAPC Stimulated IL-6 Production is Dependent on Geranylgeranylated Proteins. WV-INBRE Summer Symposium at Marshall University, 2011.

0007 *Mai D and *Kaushal G. The Study of Solvents in the Formulation of Transdermal Delivery System of D-Cycloserine. Undergraduate Research Day at the Capitol, Charleston, WV, January 26, 2012.

0009, 0039 Tesfay, N, Mori, S, Martin, E, & *Belay, T. Characterizations of pigmentation lose in Pseudomonas aeruginosa during long-term starvation in sterile water. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 Thomas, RP, Patterson, WL, Hall, JA, & Georgel, PT. Histone post-translational modifications modulate binding of MeCP2. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 Thorpe, AD, O'Leary, HA, James, ML, Vejandla, H, Kothur, A, Fournier, SB, & Brock, RW. Effect of Remote Ischemic Preconditioning on Liver Microvascular Function. WV-INBRE Summer Symposium at Marshall University, 2011.

0009, 0038 Baldwin, J, Katrib, A, & *Hankins, GR. Response of Genes that are Dys-regulated in Meningioma Cells In Vitro. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 Dunn, C, Percifield, R, & Stoilov, P. Determining the Functional Role of Alternative Splicing in Cancer. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 LaFata, GL, Nichols, CE, Baseler, WA, Thapa, D, Knuckles, TL, Nurkiewicz, TR, & Hollander, J. M. Coal dust exposure increases markers of mitochondrially-driven apoptosis in rat cardiac tissue. WV-INBRE Summer Symposium at Marshall University, 2011.

0027 Pierson E.H. Novotny M.J. *Shurina R.D. Bridge D.R. and Olson J.C. Comparative Immunofluorescent Analysis of Tumor Cell Leading Edge Properties and Susceptibility to Pseudomonas Type lll Secretion. 11th Annual Wheeling Jesuit Student Research and Scholarship Symposium, Wheeling, WV April 2011.

0027 Hydeman L. and *Shurina R.D. 2011. Effect of C-peptide on Complex I Subunit Expression and Oxidative Metabolism of Glucose. 12th Annual Student Research Scholarship and Symposium, Wheeling Jesuit University, Wheeling WV. April 2011.

0027 Pierson E.H. Bridge D.R. Novotny M.J. Olson J.C. and *Shurina R.D. 2011. Comparative Immunofluorescent Analysis of Tumor Cell Leading Edge Properties and Susceptability to Pseudomonas Type lll Secretion. 12th Annual Student Research Scholarship and Symposium, Wheeling Jesuit University, Wheeling WV. April 2011.

0039 *Belay T. Differential Production of Cytokines and Chemokines in a Chlamydia trachomatis Infected Stress Mouse Model. Southeast Regional IDeA Meeting. New Orleans, LA. September 22-24, 2011.

0038 Katrib A. Responses of Cyclin Dependent Kinase Inhibitors to Female Steroid Hormones in Meningioma. Summer Undergraduate Research Experience Symposium, West Virginia State University, July 29, 2011.

0022 Smith T. Clifton T. Karunathilake A. and *Harris R. Changes in Gene Expression in Resveratrol Treated Smooth Muscle Cells. Third Annual West Virginia State University Summer Research Symposium, July 2011.

0002 Klinke, D. J.; "Signaling cartoons to model-based inference: A contemporary view towards understanding how cells make decisions" Biochemical and Molecular Engineering XVII: Emerging Frontiers, Seattle, WA, June 2011.

0006 *Barr T. Genomic Characterization of Ischemic Stroke and Clinical Translation. WVU Stroke Conference, Flatwoods WV May 2011.

0037 *Valentovic M.A. Ball J.G. Brown J.M. Wright M. and Van Meter S. Resveratrol reduction in Cisplatin Nephrotoxicity: Changes in Oxidative Stress Enzyme Activity and Oxidative Stress Biomarkers. Gordon Conference on Cellular Mechanisms of Toxicity. Proctor Academy, NH. August 2011.

0002 *N. Santanam, C. Kocher# C. Cook, B. Dawley. Pain Sensitive miRNAs in women with endometriosis. Accepted as a Plenary Presentation at the World Congress on Endometriosis, Montpellier, France, Sept 5-7th 2011.

0002, 0036 *N. Santanam, (Invited Speaker). "Fat biomarkers from the WV-Appalachian Heart Study". The 20th South Eastern Lipid Research Conference (SELRC), Callaway Gardens, Pine Mountain, GA, Oct 6th -8th 2011.

0002 Preeya Shah, Rebecca Furby, Aileen Marcello, Carla Cook, Richard Egleton and *Nalini Santanam. Circulating miRNA "regulating angiogenesis signaling in Type 1 diabetic Rat model. Annual Biomedical Research Conference for Minority Students (ABRCMS), St. Louis, MO, Nov 9th -12th 2011.

0007, 0009 Mai, D, Sirbu, C, & *Kaushal, G. The Effects of Solvent Systems in the In Vitro Transdermal Permeation of D-Cycloserine. WV-INBRE Summer Symposium at Marshall University, 2011.

0009, 0037 Mills, AM, *Van Meter, S, Brown, JM, Wright, M, Ball, JG, Lamyaithong, AB, & *Valentovic, MA. The Natural Product Resveratol (RES) Reduces Cisplatin Mediated Oxidative Modifications of Proteins in Renal Tissue as well as Renal Mitochondrial and Cytosolic Fractions. WV-INBRE Summer Symposium at Marshall University, 2011.

0007, 0009 Norman, R. & *Reardon, D. In vitro combinatorial therapy with somatostatin and taxol: modulation of tumor cell growth and metastic potential. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 Oney, K. & Schaller, M. Small Molecule Inhibitor Reistant FAK. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 *Roberts, KA, Olenich, S, Audet, GN, & Olfert, IM. Effects of physical deconditioning on skeletal muscle microvessel regulation. Undergraduate Research Day at the Capitol, Washington DC to be held Spring 2012. Sponsored by Council on Undergraduate Research (CUR).

0009, 0039 Richmond, S, Bailey, S, & *Belay, T. Decreased Production of Immune Cell Stimulatory Cytokines and Chemokines in Chlamydia trachomatis Infected Stress Mouse Model. WV-INBRE Summer Symposium at Marshall University, 2011.

0009 Smith, M, Withers, TR, Southerland, M, & *Yu, H. Activiation of Alginate Production in Pseudomonas aeruginosa with Wild-Type MucA. WV-INBRE Summer Symposium at Marshall University, 2011.

0007, 0009 Smith, S, Kessler, T, *Kim, SY, & Warburton, R. Petir Nets-based Modeling of Human Systems: Towards Drug Trial Modeling and Simulation. WV-INBRE Summer Symposium at Marshall University, 2011.

0039 *Belay T. Effect of Stress on resistance to Chlamydia trachomatis infection and immune responses in a mouse model. Department of Entomology, Virginia Tech, Blacksburg, VA. March 24, 2011.

0010, 0037 Brown, J.M. Ball J.G. and *Valentovic M.A. S-Adenosyl-L-methionine (SAMe) Attenuation of Lipid Peroxidation Biomarkers of Acetaminophen Toxicity. The Lipid Biology and Lipotoxicity Symposium in Kerry, Ireland May 2011.

0002 Kinzer C, Williams H, Rubenstein N, Cook A, Strawbridge S, Vrana J, Boyd J. "Alterations of Signaling Protein Interactions to Increasing Dose". SOT Annual Meeting in Washington, DC. 2011.

0002 Rebecca Furby, Preeya Shah, Aileen Marcello, Carla Cook, Richard Egleton and *Nalini Santanam. Circulating miRNA regulating insulin signaling in Type 1 diabetic Rat model. Annual Biomedical Research Conference for Minority Students (ABRCMS), Nov 9th -12th 2011.

Presented This Year

Presentations 117

SOURCE OF INVESTIGATORS' SUPPORT

NON-FEDERAL

FOUNDATION

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
Hardman, Wanda Elaine			
Aicr	MG07A001	$29,957	0009, 0037
Ivanov, Alexey			
Susan G Komen		$150,000	0033
Kim, Jung Han			
Aha	0855300E	$42,250	0009
Li, Bingyun			
Acs	RSG-08-034-01-CSM	$144,000	0009
Maher, John			
Univ Of Ky Res Foundation	3048107920-11-2751UL1	$136,347	
Salisbury, Travis			
Phrma		$60,000	0013
Yu, Hongwei			
Cystic Fibrosis Foundation	YU11G0	$97,200	0039

INDUSTRY

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
Blough, Eric			
Mcneil Consumer & Specialty		$56,953	0022
Novartis Pharma Ag	NOV-2011-110	$49,000	0022
Bristol Myers Squibb	CV181-173	$85,050	0022
Li, Bingyun			
Urs Corp		$123,514	0009
Urs Corp		$29,815	0009
Urs Corp		$70,013	0009
O'Donnell, James			
Tetra Discovery Partners, Llc		$80,000	0009

PVAS

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
Hollander, John M			
American Heart Association/Great River Affiliate	10PRE3420006	$46,000	0009
Niles, Richard			
Faseb		$12,698	0013
Olfert, Mark			
Aha	10BGIA3630002	$132,000	0009

PVAS

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
Schaller, Michael			
Aha	0855360E	$82,500	0009

OTHER NON FEDERAL

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
Blough, Eric			
Wvhepc	EPS08-05	$296,140	0022
Gannett, Peter			
Wvhepc	HEPC.DSR.09.013	$100,000	0023
Univ Of Minnestota		$170,390	0023
Univ Of Kentucky Res Fdn		$170,390	0023
Harris, Rob			
Wvsu		$5,500	0022
Li, Bingyun			
Thomas Jefferson Univ	080-04000-S00601	$124,985	0009
Wvhepc	HEPC.DSR.12.07	$5,000	0009
Maher, John			
Wvhepc	HEPC.DSR.11.07	$154,960	
Wvhepc	HEPC.DSR.10.02	$10,000	
Niles, Richard			
Wvhepc	HEPC.DSR.09.014	$199,977	0013
Salisbury, Travis			
Cddc		$20,000	0013
Valentovic, Monica			
Univ. Physc. Surg.		$10,000	0037
Yu, Hongwei			
Cddc		$10,000	0039

FEDERAL

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
FEDERAL - NON PHS			
Barr, Taura			
Dhhs	HHSN263201100872P	$67,340	0006
Belay, Tesfaye			
Nasa	WVEPSCOR	$10,000	0007, 0039
Nasa	WVEPSCOR	$20,000	0007, 0039
Doe	THURGOOD MARSHALL FUND	$40,000	0007, 0039
Blough, Eric			
Nasa	PO50048247	$173,224	0022
Chen, Yi C			
Nsf	WVEPSCOR	$50,000	0032
Collier, Simon			
Nasa	WVEPSCOR	$12,000	0009
Nsf	IOS-0843028	$140,000	0009
Egleton, Richard			
Nasa	WVEPSCOR	$12,000	0006, 0009
Georgel, Philippe			
Dod	W81XWH-10-1-0698	$160,375	0009
Hankins, Gerald			
Usda	2010-38821-21574	$100,000	0038
Usda	2008-38814-04772	$64,350	0038
Nasa	WVEPSCOR	$1,600	0038
Hardman, Wanda Elaine			
Nasa	WVEPSCOR	$12,000	0009, 0037
Dod	W81XWH-10-1-0697	$230,125	0009, 0037
Harris, Rob			
Nsf	WVEPSCOR	$119,432	0022
Nasa	WVEPSCOR	$20,000	0022
Nasa	WVSGC	$12,000	0022
Maher, John			
Nsf	WVEPSCOR	$144,000	
Nsf	EPS-1003907	$108,128	
Nsf	EPS-0918949	$355,091	
Doc	01-66-14102	$100,000	
Ed	P116Z090115	$85,667	
Hud	B03SPWV0868	$35,000	
Dhhs	1D1ARH10426-01-00	$497,700	
Serrat, Maria			
Nasa	WVEPSCOR	$20,000	0009
Yu, Hongwei			
Doe	79064-001-09	$17,500	0039

FEDERAL

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
Nasa	WVEPSCOR	$12,000	0039
Nasa	WVEPSCOR	$20,000	0039
Nasa	WVEPSCOR	$10,000	0039
Nasa	WVEPSCOR	$12,000	0039
	FEDERAL - NON PHS	$2,661,532	
FEDERAL - PHS			
Brock, Robert			
NIH	5R01DK067582-06	$246,247	0009
Cuff, Christopher F			
NIH	5R21AI083424-02	$219,750	0033
Gannett, Peter			
NIH	7R01GM086891-03	$362,141	0023
Gibson, Laura F			
NIH	2R01HL056888-14A1	$370,000	0009
NIH	5R01CA134573-04	$263,553	0009
NIH	3R01CA134573-03S1	$18,865	0009
NIH	1P30RR032138-01	$1,110,000	0009
NIH	5P20RR016440-10	$2,084,768	0009
Hardman, Wanda Elaine			
NIH	5R01CA114018-04	$266,000	0009, 0037
Hollander, John M			
NIH	3DP2DK083095-01S2	$2,198	0009
NIH	5DP2DK083095-04	$439,500	0009
Huber, Jason			
NIH	3R01NS061954-03S1	$73,500	0009
NIH	5R01NS061954-04	$314,059	0009
Kim, Jung Han			
NIH	5R01DK077202-04	$278,348	0009, 0033
Lukomski, Slawomir			
NIH	5R21AI083683-02	$219,780	0009
Niles, Richard			
NIH	5P20RR020180-05	$592,467	0013
NIH	3P20RR020180-05S1	$490,224	0013
O'Donnell, James			
NIH	5RC1MH088480-02	$468,313	0009
NIH	5T32GM081741-04	$157,184	0009
NIH	2R56MH040694-23	$366,250	0009
Rojanasakul, Yon			
NIH	5R01HL095579-02	$369,792	0032
NIH	5R01HL076340-04	$355,629	0032
NIH	3R01HL076340-04S1	$136,515	0032

FEDERAL

INVESTIGATOR ORGANIZATION	GRANT/CONTRACT	TOTAL FUNDING	SPID
Santanam, Nalini			
NIH	5R01HL074239-05	$331,863	0001, 0006, 0036
Valentovic, Monica			
NIH	5R21CA133701-02	$149,793	0037
	FEDERAL - PHS	$9,686,739	

RESOURCE SUMMARY: SUBPROJECTS

Table includes information entered elsewhere in your APR which is associated with subprojects.

	Admin. Core	Infra.	Pilot	Research	Career Dev.	Ed. & Train.	Total*
Number of Subprojects	2	4	1	11	3	1	22
Number of Investigators	11	21	11	30	21	28	69
Number of Published	0	12	8	17	0	17	49
Number of In Press	0	7	0	6	0	4	16
%Non-AIDS Dollars	20.000	20.000	3.000	46.000	4.000	7.000	100.000
% Total Dollars	20.000	20.000	3.000	46.000	4.000	7.000	100.000

*Excludes Duplicates

RESOURCE SUMMARY: ADMINISTRATIVE

PERSONNEL

	On Subprojects	Not On Subprojects
Host Personnel	69	11

GEOGRAPHICAL USAGE BY INVESTIGATORS AT NON-HOST INSTITUTIONS

USA Investigators by State

KY	2
MA	1
WV	50

INVESTIGATORS BY NON-HOST INSTITUTIONS

ALDERSON-BROADDUS COLLEGE	2
BETHANY COLLEGE	1
BLUEFIELD STATE COLLEGE	1
CHARLESTON AREA MEDICAL CENTER	2
CONCORD UNIVERSITY	1
DAVIS & ELKINS COLLEGE	1
EMMANUEL COLLEGE	1
GLENVILLE STATE COLLEGE	1
SHEPHERD UNIVERSITY	1
UNIVERSITY OF KENTUCKY	1
UNIVERSITY OF CHARLESTON	3
UNIVERSITY OF KENTUCKY	1
WEST LIBERTY UNIVERSITY	3
WEST VIRGINIA STATE UNIVERSITY	3
WEST VIRGINIA UNIVERSITY	27
WEST VIRGINIA WESLEYAN COLLEGE	3
WHEELING JESUIT UNIVERSITY	1

RESOURCE SUMMARY: PUBLICATION/PRESENTATION/SUPPORT

Publications

	Published		In Press		Public Access			
	Cited	Total	Cited	Total	PMCID	MSID	PMC Journal	Policy Not Applicable
Abstracts	26	29	6	9				
Books	0	0	1	1				
Journals	18	20	4	6	20	0	0	0
Total	44	49	11	16				

Presentations

Presentations	117

Investigator Support

NON-FEDERAL		
		$1,277,342
FOUNDATION		$659,754
INDUSTRY		$494,345
PVAS		$273,198
	NON-FEDERAL	**2,704,639**
FEDERAL		
NON-PHS		
DHHS		$565,040
DOC		$100,000
DOD		$390,500
DOE		$57,500
ED		$85,667
HUD		$35,000
NASA		$346,824
NSF		$916,651
USDA		$164,350
	NON-PHS	**2,661,532**
PHS		
AI		$439,530
CA		$698,211
DK		$966,293
GM		$519,325
HL		$1,563,799
MH		$834,563
NS		$387,559
RR		$4,277,459
	PHS	**9,686,739**
	TOTAL SUPPORT	**$15,052,910**

PROFILE SUMMARY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Subm	# Awards	Total Award $
All Sources	527	588	68,975,540
NIH Faculty	193	80	21,101,012
NIH - INBRE	45	31	9,633,037

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	1,303
Faculty - Newly Hired	140
Administrative - Total Employed	527

Faculty Served on Peer Review Groups	Number
Within this Institution	123
Outside this Institution	66

Research Space	Square Feet
Total	399,240
Newly Constructed or Renovated	25,283

BRIN Junior Investigators	Number
Total on Roster this Reporting Period	12
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	1
Still Junior Investigator at Reporting Period End	11

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	1,342	10,615	1,327	2,414
Degrees Awarded	295	1,452	455	490

	Yes	No
Faculty Release Time	3	10
Central Grants Administration	8	5

PROGRAM SUMMARY AND EVALUATION UPDATE

Administrative Core (AC)
The AC, led by PI Dr. Gary Rankin, Marshall University (MU), has been involved in many activities during the current grant year (Y11). One of those activities was conducting a competition among partner institution (PUI) faculty for new major PUI research awards. The overall scores and critiques were reviewed by the AC and submitted to External Advisory Committee (EAC) members for their review and recommendation. Based on EAC recommendations, three applications were selected for funding. These projects were selected to be funded starting in May, 2012 and will be conducted by Dr. Yi Chen, Alderson-Broaddus College (competitive renewal), Dr. Joseph Horzempa, West Liberty University, and Dr. Qing Wang, Shepherd University. Two of the currently funded major PUI investigators (Drs. Aguilar and Kreisberg) were not renewed based on productivity issues. The AC is also working to replace EAC member, Dr. Kenneth Ramos who resigned. The AC and EAC are discussing a replacement for Dr. Ramos. In addition, the AC also coordinated the very successful Summer Research Program during the summer of 2011, which culminated with the Summer Research Symposium held at Marshall University on July 28, 2011.

Dr. Sheil published a WV-INBRE newsletter for the fall of 2011. Faculty at the partner institutions competed for Faculty Research Development Awards (FRDAs) to begin in Y11. Four applications totaling $90,000 were selected for funding with awards being provided to Alderson-Broaddus College (1), Shepherd University (1), and the University of Charleston (2). Based on the Y10 competition for major PUI research awards, Dr. Tesfaye Belay, Bluefield State College was selected to receive a major PUI research award for Y11 for his project entitled “Effect of stress on pathogenesis of Chlamydia trachomatis and immune responses in a mouse model”. Dr. Belay is a previous FRDA recipient. In addition, carryover funds were obtained to purchase equipment to upgrade cores at MU and West Virginia University (WVU) and to provide new equipment at eight PUIs.

In Y09, WV-INBRE PI Gary Rankin was awarded four ARRA supplements (3P20RR016477-09S1-S4). These supplements supported summer research experiences for PUI students and middle and high school science educators (S1), advanced translation research (S2 and S4) and enhanced the research opportunities for Health Sciences and Technology Academy (HSTA) graduates at the PUIs and HSTA high school science teachers (S3). During Y10, S1 was completed, while S2 (see SPID 0036) and S4 (see SPID 0037) are active research projects, and S3 has actively recruited HSTA graduates to work on WV-INBRE funded research projects during the academic year and recruited HSTA high school science teachers to work on biomedical research projects during the summer. S3 ended during Y11, while S2 and S4 were awarded no cost extensions for one year.

Recently, WV-INBRE was given permission to establish a Center for Natural Products Research as part of the PUI research plan. The Center will coordinate natural products research in the area of finding and developing chemotherapeutic agents. An organizational meeting is planned for early April.

Steering Committee and External Advisory Committee Meetings: A Steering Committee meeting was held on July 27, 2011 at MU and attended by three External Advisory Committee (EAC) members (Tew [Chair of the EAC], Cutler and Yang). Dr. Fornsaglio, who could not attend, reviewed all meeting materials and contributed to the report. Their EAC report is attached. A second meeting is being planned for early April, 2012.

Additional Meetings and AC Presentations: (1) Drs. Rankin, Sheil and Primerano attended the IDeA Networks of Biomedical Research Excellence (INBRE) PIs and PCs meeting at NIH on October 4, 2011. (2) Dr. Rankin also met with other Southeast Region PIs on September 21, 2011 in New Orleans, LA to discuss best practices in each INBRE in our region. (3) Dr. Rankin attended the Southeast Regional IDeA Meeting in New Orleans, LA on September 22-24, 2011. Dr. Rankin also co-chaired an oral session entitled Cancer I. (4) Drs. Rankin, Sheil and Primerano helped the WVU COBRE PI, Dr. Laura Gibson, to plan and host the 2011 West Virginia IDeA Meeting. The meeting was held on October 21-22, 2011 at Waterfront Place Hotel in Morgantown, WV. Dr. Rankin presented an update on WV-INBRE, while Dr. Primerano

discussed genomics and next generation sequencing. Dr. Santanam gave an oral presentation entitled "Epicardial fat biomarkers in patients with coronary artery disease: WV-Appalachian Heart Study". (5) Dr. Rankin also prepared a presentation entitled "Update on NIH EPSCOR-Like Programs" for the West Virginia Science and Research Council meeting on January 26, 2012.

AC and HSTA: The AC is continuing to develop the links between WV-INBRE and the WVU Health Sciences and Technology Academy (HSTA) program headed by PI, Dr. Ann Chester. The WV-INBRE/HSTA Coordinator, Valerie Watson (WVU) has established interactive connections with the HSTA regional clubs throughout the state and representatives of the WV-INBRE partner institutions. Ms. Watson coordinated talks by INBRE undergraduate research interns and high school science educator research interns at some HSTA club meetings. Informational brochures were sent to HSTA Field Site Coordinators in 18 West Virginia counties. In addition, a WV-INBRE-HSTA Task Force has been formed to explore ways to enhance the WV-INBRE and HSTA interactions. WV-INBRE also utilized one of its four supplements (3P20RR016477-09S3) to enhance WV-INBRE HSTA activities. Funds from this supplement were used to place eight undergraduates in WV-INBRE funded research labs at the five partner institutions during the academic year of 2010-2011 and two students have been placed in two laboratories in 2011-2012 to date. Seven WV high school science educators were placed in biomedical research labs at Marshall University, Shepherd University, University of Charleston, and West Virginia State University to conduct summer research projects in 2011.

Bioinformatics Core (BC)
BC members provided assistance with experimental design, statistical analysis and use of Ingenuity Pathways Analysis (IPA) tools in the interpretation of results have resulted in 6 publications in peer reviewed journals, with an additional paper in press, and 16 presentations at national or regional meetings. BC resources have supported 18 grants, including 7 funded NIH grants, 2 pending NIH grants, a grant pending for DARPA, 2 grants funded by local sources, and 5 grants that were not funded (NIH, NSF, IARPA and a private foundation). In partnership with the COBRE-funded WVU Center for Neuroscience Genomics Core and the WV Clinical Translational Science Institute, the WV-INBRE has established and equipped a computational facility to support analyses of large or complex datasets using bioinformatic or statistical approaches. The facility also functions as a classroom lab, and is in use for teaching of bioinformatics to graduate students. WVU is in the process of hiring a new bioinformaticist/biostatistician. This position is being funded by COBREs at WVU (Neuroscience and Cancer), WV-INBRE, and the emerging School of Public Health. The pool of candidates includes many individuals with experience analyzing high dimensional data from expression or NexGen datasets, and using various algorithms for developing new methods for extracting meaningful relationships from these datasets. The BC is currently using Partek for NextGeneration Sequence analysis. Dr. Denvir is additionally using CASAVA to analyze data generated by the Illumina HiSeq 1000 at MU, and MACS to analyze Chip-seq data. The BC is currently initiating a month-long trial of CLC Genomics Server and Workbench with a view to purchase and install before the beginning of the next budget year. We anticipate this software will enable the BC to centralize access to and analysis of Next Generation Sequencing data.

Participation in Program of Excellence: Dr. James Denvir, Bioinformatics Core Co-Director, is participating as a potential mentor for the Southeast INBRE Region Program of Distinction Bioinformatics Internships for Undergraduate Student. If the project is selected by undergraduate student(s), Dr. Denvir, collaborating with a faculty researcher, will mentor the student intern in bioinformatics analysis of biological data.

Genomics Core (GC)
The GC continues to support the research of investigators within the WV-INBRE network. The GC provided the following services in Y11: (1) next generation sequencing (NGS), (2) microarray-based gene expression profiling and pathway analysis, (3) automated DNA sequencing and access to DNA/RNA sequence analysis software, (4) access to real-time PCR instrumentation, NanoDrop spectrophotometers and Agilent BioAnalyzer for DNA/RNA analysis and (5) access to the Luminex 100 for multiplex protein detection. Dr.

James Denvir was hired as an Assistant Professor in the Department of Biochemistry and Microbiology at Marshall University in July 2011. Previously, he was at WVU as part of the GC. He performs analysis of NGS datasets and assist in downstream pathway analyses. He is well acquainted with Casava, the software suite provided by Illumina for immediate data processing from the HiSeq 1000, and has attended training sessions in the use of Tuxedo Suite software and CLC Genomics Workbench. The latter is currently being trialed by the Genomics Core, and the intention is to install and support use of this software in the near future. Dr. Denvir also has expertise in MACS for analysis of Chip-Seq data and with IGV for the visualization of analyzed data. Genomics Core staff members have authored three publications and the GC acknowledged in two publications in the past year. The GC will also be an important resource for two grant submitted applications: (1) a COBRE application on Nutrition and Cancer (MU) and (2) West Virginia Cancer Genomics Network (MU and WVU) to the WV Division of Science and Technology.

Dr. Ivana Yang, an established microarray investigator at the University of Colorado in Denvir and a member of the WV-INBRE External Advisory Committee, served as the external advisor on experimental design and interpretation of data. She also advised in the selection of the Illumina NGS system and participated in the review of NGS pilot grant applications (see below). In order to stay current with microarray technology and methods of data analysis, core staff members are required to attend workshops or national meetings in their specific areas. Drs. Boskovic, Dr. Fan and Primerano attended the Association of Biomolecular Resource Facilities (ABRF) in February 2011. Drs. Denvir, Boskovic, Fan, Primerano and J.H. Kim will present a poster entitled “Identification of Potential Susceptibility Variants for Obesity and Type 2 Diabetes in the TALLYHO Mouse” at the ABRF in March 2012.

In Y10 and Y11, the WV-INBRE program funded six biomedical research pilot grants (4 at MU and 2 at WVU) that required the use of NGS technologies as part of the experimental design. These technologies include whole genome sequencing, whole exome sequencing, RNA-Seq, Chip-Seq, Methyl-Seq, microbiome studies and related high throughput methods. The primary purpose of these awards was to allow investigators to gather preliminary data for investigator-initiated grant applications in the biomedical sciences. This solicitation for applications was open to applications from investigators at West Virginia University, Marshall University and WV undergraduate institutions that are part of the WV-INBRE network. Funds ($12,000 per awardee) were used for the acquisition of NGS supplies or services. The projects funded and the PIs are: (1) Oral health disparities among elders with and without cognitive impairment: microbiome analysis, Chris Cuff (WVU), (2)Epigenetic modulations of breast cancer by omega-3 fatty acids mediated by changes in histone post-translational modifications, Philippe Georgel and Elaine Hardman (MU), (3) Identification of genomic binding sites of transcriptional repressors Snail and ZEB in epithelial cells undergoing epithelial-mesenchymal transition, Alexey Ivanov (WVU), (4) Identification of potential susceptibility variants for obesity and non-insulin dependent diabetes in the TALLYHO mouse, Jun Han Kim (MU), (5) Characterizing the AHR cistrome in human MCF-7 breast cancer cells, Travis Salisbury (MU), and (6) Global analysis of DNase I hypersensitivity sites in Treg cells , Wei-ping Zeng (MU).

Appalachian Cardiovascular Research Network (ACoRN)
The Appalachian Cardiovascular Research Network (ACoRN) was designed to explore the role of genetics in cardiovascular disease. The primary project for ACoRN has been the study of the role of genetics in familial combined hyperlipidemia (FCH, Dr. Primerano Project Director, SPID 26). Additional projects have been examining the molecular actions of statin drugs on the progression of atheromatous plaques (See progress for Dr. Kreisberg’s project below; and SPID 25), and exploring epicardical fat biomarkers in coronary artery disease in the Appalachian region (See progress for Dr. Santanam’s subproject below for 3P20RR016477-09S2 and SPID 36) and their progress will not be discussed here.

For the FCH project, Dr. Primerano sought to identify candidate susceptibility genes by performing genome-wide linkage analysis. Recently, he has modified his approach to begin to identify FCH susceptibility in part by whole exome sequencing of individuals in selected FCH families. He will begin by selecting families that have at least three affected individuals including the proband. To obtain an initial collection of potential FCH variants, he will perform whole exome sequencing on affected FCH family

members and apply a stepwise filtering process to identify variants that are most likely to confer susceptibility to the disease. From those families that have no deleterious LDLR mutations in affected members, he will then select affected individuals for whole exome sequencing. Once this stage is completed, Dr. Primerano will confirm the significance of these variants by showing that these variants are present in other affected family members and that they are largely absent from unaffected relatives. This confirmatory sequencing will be limited to the variant sequence and ~200bp of flanking sequence and can therefore be effectively conducted on all affected and normal persons from the FCH families; currently there are 80 affected individuals and 92 normals based on the phenotype algorithm. In this fashion, he will eliminate coincident variants and focus on the remainder for biological relevance to FCH. Also, by expanding sequencing to unrelated affected members he can test for allelic and locus heterogeneity.

To date, Dr. Primerano has successfully sequenced the whole exomes of 41 individuals and tested a variant discovery strategy on one FCH Family (1150027). His strategy at a 15% allele frequency cutoff reduced the number of possible variants to 70 which 45 correspond to known genes. He has reviewed the functions of genes from variants which result in missense or possible changes in conserved splice sites. The missense mutations correspond to uncharacterized open reading frames on chromosomes 1 and 17. Severity of these missense mutations and potential biological function of these ORFs must be assessed. One variant in the ATP-citrate lyase (ACLY) gene is located near a splice donor site. This enzyme catalyzes the formation of acetyl-CoA and oxaloacetate from citrate and CoA with a concomitant hydrolysis of ATP to ADP and phosphate. The product, acetyl-CoA, serves several important biosynthetic pathways, including lipogenesis and cholesterogenesis. ACLY expression and activity is known to be suppressed by exogenous lipids (GeneCard database). Although effect of this mutation on ACLY mRNA splicing is unknown, ACLY may play a role in regulating the abundance of serum cholesterol and triglycerides. No
SNP or indel variants mapped to the 3 known Familial Hypercholesterolemia and 3 known FCH genes. This reduces the likelihood that defects in these genes could be the cause of FCH in Family 11502 but does not eliminate it completely since deleterious mutations may be located in non-exonic regions of these genes. A more rigorous analysis of variants from all FCH families is under way.

In Y11, two additional ACoRN pilot projects were funded. One project was entitled “Monitoring gene expression post-stroke to predict stroke outcome” (Taura Barr PhD RN School of Nursing and Center for Neuroscience, West Virginia University) and the second project is “Regulation of brain endothelial phenotype and function by diabetic plasma” (Richard Egleton, PhD, Department of Pharmacology, Physiology and Toxicology, Marshall University). These projects are making good progress toward their research goals. Both investigators have presented their work as poster or platform presentations. Dr. Barr has submitted a manuscript entitled “Identification of a genomic profile following ischemic stroke that may mediate stroke recovery” that has been submitted to Science Translational Medicine. Dr. Barr’s proposal involves human subjects, and its IRB approval has been included in the appendix of the 2590 report. Dr. Egleton’s proposal involves animal studies and the corresponding IACUC approval has been included in the 2590 appendix. Inclusion of these projects as part of ACoRN was approved by the WV-INBRE, EAC and Dr. Krishan Arora at the NCRR early in 2011.

Summer Research Program
Sixty-eight students from the PUIs applied for the Summer Research Program in Y11. Twenty-two students were accepted with eleven conducting research at MU and eleven at WVU under the mentorship of faculty at the lead institutions. The program was concluded on July 28, 2011 with a Summer Research Symposium held at MU. Dr. Terrence J. Monks, Professor and Chair of Pharmacology and Toxicology, College of Pharmacy, University of Arizona Health Sciences Center was the keynote speaker. Students and faculty from the Summer Program, PIs holding major PUI research awards, F RDA recipients, and students and high school teachers supported by the WV-INBRE--HSTA initiative made a total of 56 poster and 6 oral presentations. In the fall of 2011, the 2012 Summer Research Program was promoted at all WV-INBRE partner institutions through presentations or through announcements mailed to the institutions. For the 2012 program, a Mentors Directory listing prospective research mentors and available projects for the summer research program, as well as application forms for the summer student internship and faculty

fellowship programs, were developed and placed on the WV-INBRE website. Electronic submission of applications was available. Three summer interns made presentations at the 9th Annual Undergraduate Research Day at the Capitol in Charleston, WV on January 26, 2012. Summer interns presented their summer research at the Orthopaedic Research Society (ORS) Annual Meeting in San Francisco, CA (Feb 2012), the American College of Sports Medicine (ACSM) Annual Meeting in Denver, CO (June 2011), the American Association for Cancer Research 102nd Annual Meeting in Orlando, FL (April 2011), and the American Association for Cancer Research Metabolism and Cancer Special Conference in Baltimore, MD (October 2011).

Two faculty members from the partner institutions, Dr. Kimberly Fisher from Bethany College and Dr. Gary Morris from Glenville State University, participated as 2011 summer fellows. For 2012, there will be two faculty members participating as summer fellows. Dr. Jennifer Franko from Bethany College will work at WVU with Dr. Rosana Schafer. Dr. Gary Morris from Glenville State College will return to MU to work in the lab of Dr. Travis Salisbury. Both fellows will present their research at the 2012 Summer Research Symposium which will be held on July 26th at WVU.

PUI Research Projects

Dr. Robert Harris, West Virginia State University, “Mechanotransduction, intracellular signaling and vascular biology”

Dr. Harris’ project focuses on the effects of shear stress on vascular muscle biology.

(1) Effects of stretch on titin: Titin forms filaments in cardiac and skeletal muscle that provide elasticity in relaxed cells, but the function of smooth muscle (SM) titin remains unclear. In this study, titin expression was determined (qRT-PCR) in A7r5 cells which were exposed to cyclic (dynamic) or static (step) stretch. There was an early decrease in expression of titin in response to 1hr (19%) and 2hrs (77%) of 15% unidirectional cyclic stretch (UCS). At 6hrs of UCS, when the cytoskeleton had remodeled to a lower stretch orientation, the decline in titin mRNA expression was less than the 2hr value (38% vs 77%). In contrast to UCS, cells responded to 1hr of 15% static stretch with an increase in titin expression (35%). In cells treated with cytochalasin D to block polymerization of actin stress fibers, there was a marked increase in titin expression in unstretched cells (335%) which increased further after 6hrs of UCS (530%). Together, these data suggest that SM cells may alter expression of titin in order to modulate internal stiffness. We believe that SM titin may play a sensory role for the cell.

(2) Resveratrol (RV) effects on vascular smoot muscle cells: Dr. Harris investigated the potential of RV to inhibit the growth of VSMC following stimulation with the PGF2α analog fluprostenol (Fp). It has been suggested that PGF2α is a mediator of vascular cell growth through a process that may involve the production of intracellular reactive oxygen species (ROS). Increased ROS production has been linked to VSMC hypertrophy via a cell signaling pathway that is not well understood. Incubation of A7r5 VSMCs with 1µM Fp resulted in a 35% increase (P0.05) in cell size in 48 hours as measured by flow cytometry. Fp treatment was associated with markedly increased intracellular ROS levels as determined by incubation of cells with hydroethidium. However, when cells were pre-treated with 1 or 20 µM RV, FP-induced hypertrophy was completely attenuated. RV treatment at both levels was associated with a decline in intracellular ROS. The results of the present study suggest that very low levels of RV (1µM) can completely attenuate hypertrophy that is induced by exposure to Fp through a mechanism that involves suppression of the activation of ERK1/2 and ribosomal protein s6. On the other hand, RV’s reduction in hypertrophy in this study was associated with a slight increase, rather than the expected decrease, in Akt pathway activation. These results further our understanding of how RV may exert its cardio-protective effects.

Dr. Jarrett Aguilar, West Liberty University, “Kinetic and molecular dynamics: correlations in cytochrome P450 2C9 mutants”

Dr. Aguilar’s project focuses on the potential drug-drug interactions at CYP2C9 using a molecular modeling approach. He reported issues with proposed site-directed mutagenesis studies related to plasmid creation. The problem was eventually resolved and site directed mutagenesis has been used successfully to generate all of the mutants that are presented in this proposal. Problems also arose in expressing the

CYP2C9 mutants. Dr. Aguilar has recently taken steps to use chaperones to assist in protein folding as to minimize the toxicity of the mutated proteins, and has been able to express several of the mutations and is working on getting the chaperones into those that do not express. The current list of proteins that have been expressed are N474I, T304A and S209A. He is working on E300V currently and has expressed R307L which has an impact on this project.

The purification of the expressed protein is being carried out according to protocol received from the lab of Dr. Timothy S. Tracy. Dr. Aguilar has successfully purified the wild type form of the protein and two of the expressed mutated forms. Purification is a week long process that takes approximately 8 hours of steady work to reach a stopping point and he is considering hiring a part time technician. With the success of expression and now purification he is in the process of beginning the kinetic analysis.

The molecular modeling part of the experiment has been completed in terms of looking at correlations of distances and hydrogen bonding. He is still trying to determine a viable way to address stacking. MM-PBSA has been run for the majority of the mutations in silico. He has been successful and an additional centrifuge, obtained from a WV-INBRE equipment grant, will allow him to speed up the processes and finish the kinetics and put out publications. He plans to submit an AREA grant to the NIH before the February 25th deadline.

Dr. Robert Kreisberg, West Liberty University, “Mechanism of action of statins on endothelial cell function”
Dr. Kreisberg’s project is designed to uncover the molecular mechanisms of endothelial cell and macrophage activation by minimally-modified LDL (MM-LDL) and to determine whether statins inhibit this activation. He has been very active in working with undergraduate students over the years with over 25 undergraduate students involved in his project. His laboratory received a grant (Rosuvastatin) from AstraZeneca to look at compare rosuvastatin to lovastatin in its ability to inhibit IL8 production by Human Aortic Endothelial Cells. He has indicated that has some very interesting results but unfortunately, he is unable to disclose progress at this time due to the confidentiality statement he signed. With the success of the rosuvastatin experiments, he is currently in discussions with AstraZeneca to use his current laboratory techniques to look at one of their new platelet drugs. He is also repeating some IL6 experiments (cleaning up data) so that a manuscript can be submitted for publication.

Dr. Robert Shurina, Wheeling Jesuit University, “Homer2 as a suppressor of cell invasion and podosome formation”
Dr. Shurina’s project is designed to address the mechanism by which Homer2 abrogates podosome formation. He has shown that Homer2 is expressed in a wide variety of human cancer cell lines, including HEK-293T cell line; the ovarian cancer CaOV3 cell line; the prostate cancer PC3 cell line; the neuroblastoma SY5Y cell line; and the epithelial cancer UMSSC-1 cell line. He has also demonstrated that Homer2 and AFAP-110 co-localize to lamellipodia in A7r5 tumor cell lines that are stimulated with phorbol myristate acetate (PMA). He has recently served as mentor for eight undergraduate students that were awarded scholarships through the NASA-West Virginia Space Grant Consortium. He also recently formed a research collaboration with Dr. Gregory Merrick at the Schiffler Cancer Center of the Wheeling Hospital (Wheeling, WV) to investigate whether proteins involved in the AFAP-110/Src signaling pathway can be used as prognostic indicators of prostate cancer.

Dr. Yi Charlie Chen, Alderson-Broaddus College, “PI3K, AKT, and ERR-alpha pathways in flavonoid-inhibiting tumorigenesis”
Dr. Chen has been examining the role of specific signaling pathways as a mechanistic approach to determining how a flavonoid, kaempferol, can inhibit the growth of ovarian cancer cells. He has successfully completed the specific aims proposed in his previous proposal. (1) Identify the roles and molecular mechanisms of flavonoids in inhibiting EGFR, PI3K, AKT, and ERRα in ovarian cancer cells. He found that the flavonoid kaempferol inhibited PI3K/AKT, and ERRα regulated VEGF expression in ovarian cancer cells. He has published these results. (2) Determine flavonoid-inhibiting signaling pathways that affect ovarian tumor growth. He found that both HIF dependent (PI3K/AKT) pathway and HIF independent

(ERRα and cMyc) pathways were involved in the ovarian tumor growth inhibition. He has published these results. (3) Determine whether kaempferol inhibit ovarian tumor angiogenesis, and identify which signaling molecules mediate kaempferol-inhibiting tumor angiogenesis. He found that kaempferol inhibits ovarian tumor angiogenesis through inhibiting HIF-1α, VEGF, cMyc and ERRα signaling molecules. He also published these results in peer-reviewed scientific journals. He has published three manuscripts and has one in press since the last APR. He has also submitted an R15 application that was not funded. He has revised and resubmitted his application. Overall, Dr. Chen has been the most productive of the PUI investigators to date.

Dr. Gerald Hankins, West Virginia State University, " Sex steroid hormones and epigenetics in meningiomas"

Dr. Hankin's central hypotheses are: (1) that meningioma tumorigenesis is driven in part by actions of female steroid hormones and (2) that the tumorigenesis may be mediated in part by progesterone and estrogen receptor containing chromatin-modifying complexes. This past year one graduate student and an undergraduate student have focused on fibroblast growth factors and their receptors and have demonstrated that both fibroblast growth factors and their receptors are expressed by meningioma cells. Further, growth factor message levels are modulated by steroid hormones. Treatment of meningioma cells with antagonists of FGF receptors decreases the proliferation of the cells and the phosphorylation of ERK1 and 2. Together, these results provide more evidence that steroid hormones may affect the expression of fibroblast growth factors (FGF2 and FGF9) in meningiomas and also that an FGF autocrine loop plays a role in meningioma cell proliferation, partially by signaling through ERK1/2.

He has also found that the expression of the cyclin dependent kinase p27 kip1 (CDKN1B) is modulated in meningioma cells by both the progesterone antagonist Mifepristone and the histone deacetylase inhibitor butyric acid. The transcriptional repressor KLF10 (Krueppel-like factor 10 or TIEG-1) was down regulated by a factor of 32 by the estrogen receptor antagonist, ZK164015 in more aggressive lines of meningioma, while Mifepristone had no effect. Since PR is typically higher in lower grades of meningioma while ER increases in higher grades, he is examining this in cells from lower grade tumors.

Although SLC20A2 (GLVR-2, Pit2) was not highlighted in the grant, he found that its expression was significantly down regulated by Mifepristone. Expression was also modulated by HDAC inhibitor treatment. The work of one graduate student who graduated during the past year was published during the summer (Manohar S et al. Cell Cycle 10 (15): 2529-2539, 2011). Dr. Hankin's mentor Dr. Mayion Park has left MU and Dr. Travis Salisbury has replaced her as his mentor. However, he is still collaborating with her on studies on chromatin modifying proteins. Dr. Salisbury's research on the aryl hydrocarbon receptor complements the grant research given the parallels and crosstalk between AHR and steroid receptors.

Dr. Tesfaye Belay, Bluefield State College, "Effect of stress on pathogenesis and immunity during chlamydia genital infection"

Dr. Belay's hypothesis is that cold-stress increases the severity of chlamydia genital infection and development of complications by modulating the immune response against Chlamydia.To assess the influence of stress on distribution pattern of immune cells in different regions of the genital tract during Chlamydia trachomatis infection, Dr. Belay has used the mouse stress model and flow cytometry analysis. In general, the total number of immune cells in stressed mice was reduced; however, no statistically significant difference between stressed and non-stressed was obtained. Increased infiltration of leukocytes into the genital tract of stressed or non-stressed infected mice was obtained. Further flow cytometry experiments are underway to localize neutrophils, lymphocytes and dendritic cells or adhesion molecules in the regions of the genital tract of stressed mice during chlamydia infection.

Dr. Belay has begun to assess the effect of cold-stress on Chlamydia-induced infertility in the mouse model. His initial results suggest that stress may increase complications of immunopathogenesis resulting from C. trachomatis genital infection and infertility. Further experimentation is underway to determine the effect and mechanism of stress in modulation of fertility in mice. (Abstract submitted to the General Meeting of American Society for Microbiology, San Francisco, CA, June 2012).

Dr. Belay is also determining the effect of cold-induced stress on the histopathological changes of the

genital tract during Chlamydia infection in the mouse model. Preliminary data of the cervical regions of mice representing stressed and non-stressed mice showed no significant difference of hispathology. However, gross examination revealed that stressed and infected mice had fluid-filled and heavily extended uterus unlike non-stressed infected mice.

Lastly, Dr. Belay is working to elucidate the mechanism(s) by which stress increases susceptibility to Chlamydia trachomatis genital infection in the mouse model. Work is just beginning in this area.

ARRA Supplement 3P20RR016477-09S2: Dr. Nalini Satanam, Project Director, MU, "Epicardial fat biomarkers in patients with coronary artery disease in Appalachia"
The immediate goal of this project is to identify unique fat biomarkers in epicardial fat obtained from West Virginia patients with and without coronary artery disease (CAD) undergoing thoracic surgery. To date, with the help of Dr. Christopher Adams, Dr. Paulette Wehner and Dr. Todd Gress (Dept. Cardiovascular Services) and Dr. Chowdhury (Dept. Thoracic Surgery) 60 subjects (30 men and 30 women) undergoing coronary artery bypass graft surgery (CABG) at the St. Mary's Heart Center, Huntington, WV (IRB approved study) have successfully recruited. They are in the process of recruiting control patients (no coronary artery disease) undergoing valve type surgery. Blood, epicardial/perivascular fat and subcutaneous fat from all subjects have been collected at the time of the surgery. The multiplex analysis (39 chemokines/cytokine array) of the plasma samples obtained from CAD patients revealed that several cytokines/chemokines were altered in a sex specific manner. Clinical correlations revealed a positive association with the increase in chemokines to increased presence of congestive heart failure. As a preliminary study, the human miRNA microarray consisting of over 88 miRNAs relevant to humans was performed on the RNA isolated from epicardial fat and subcutaneous fat obtained from patients (n=8/sex). Females had no up-regulated expression while males had 27 up-regulated miRNAs in EF. Females had 13 while males had 16 down-regulated miRNAs. MiR 122, MiR 196-b, MiR 302c, and MiR 210 all showed decreased expression in both males and females and remains a cause for further study.Whole genome miRNA arrays (800 miRNAs in humans) have been performed in approximately 8 patients. miRNAs were isolated from both epicardial and subcutaneous fat and whole genome miRNA was performed using the Roche 480 system. Data analysis will be performed with the assistance of the Bioinformatics Core. Since the last APR five presentations have been made. Dr. Christopher Adams won the "Best Poster" at the WV Chapter of the American College of Cardiology meeting and was selected to present the poster at the national meeting. One cardiology fellow (Dr. Adams), one medical student and six undergraduates have participated in this research.

ARRA Supplement 3P20RR016477-09S3 and HSTA initiatives
The goal of this supplement is to provide PUI undergraduates the opportunity to work on WV-INBRE funded research projects and provide HSTA high school science teachers the opportunity to participate in the summer research program to gain skills and experience in biomedical research. During the 2010-2011 academic year, 8 undergraduates were placed in the WV-INBRE funded research labs at Bluefield State College (2), Concord University (1), West Virginia State University (1), West Liberty University (2), and West Virginia Wesleyan College (2) with S3. Five high school science teachers were funded from the S3 supplement for 9-week summer research internships that began on June 13, 2011. Wendy Lee from Musselman High School and Denise Gipson from Jefferson High School were mentored by Dr. Seung-yun Kim at Shepherd University. Timothy Clifton from Herbert Hoover High School and Tiffani Smith from Huntington High School were mentored by Dr. Robert Harris at West Virginia State University. Rene Norman from Sissonville High School was mentored by Dr. Dean Reardon at University of Charleston. Two additional teachers were funded from the prime award for internships this summer: Johnathan Baldwin from Scott High School was mentored by Dr. Gerald Hankins at West Virginia State University and Brian McNeel from Cabell Midland High School was mentored by Dr. Richard Egleton at MU. During the 2011-2012 academic year, 2 undergraduates were placed in the WV-INBRE funded research labs: Bluefield State College (1) and West Liberty University (1).

Other WV-INBRE - HSTA activities

HSTA coordinator (Valerie Watson) and four other WV-INBRE AC representatives spoke at a number of HSTA symposia during April-May 2011. Talks were given at the following symposia: Braxton/Webster counties, Marion/Monongalia counties, Tucker/Barbour counties, Taylor County, Preston County, Greenbrier/Fayette counties, Eastern Panhandle Region, and Kanawha County. WV-INBRE representatives also acted as judges for the science projects. During the month of July, 2011, the following WV-INBRE representatives participated in HSTA Summer Institutes at WVU, MU, and West Virginia State University: Ashley Gerard, Dr. Gerald Hankins and Dr. Robert Harris of West Virginia State University, Dr. Elsa Mangiarua of MU, and Valerie Watson, and 2011 INBRE summer interns: John Baldwin, Hannah Cavender, Timothy Clifton, Carissa Dunn, Ryan Johnson, Brian McNeel, Tiffani Smith and John Phillip Thomas. INBRE/HSTA task force group was created in March 2011 and meets on a monthly basis during the academic school year to better coordinate the initiatives of INBRE and HSTA programs. INBRE members include: Dr. James Sheil, Valerie Watson, Vickie Sanders, Dr. Elsa Mangiarua, Dr. Andrew Shiemke, Jim Denvir and HSTA members include: Dr. Ann Chester, Kas Kasten, Cathy Morton-McSwain, Sara Hanks, Merge McMillion, and Summer Kuhn .

ARRA Supplement 3P20RR016477-09S4, Dr. Monica Valentovic, Project Director, MU, “Resveratrol modulates cisplatin oxidative stress”

The goals of this translational project are to further explore the protective effects of resveratrol (RES) against cisplatin-induced nephrotoxicity and establish the mechanism for RES attenuation of cisplatin renal toxicity. Dr. Valentovic has established a collaboration with Dr. Tim Troyer in the Department of Chemistry at West Virginia Wesleyan University; who is collaborating with us on analysis of Pt for cisplatin pharmacokinetic studies, and a collaboration with Dr. Elaine Hardman, MU. The focus of the collaboration with Dr. Hardman examines potential cancer chemo preventative effects of resveratrol when combined with cisplatin on breast cancer and is research that is translational. Since the last APR, five abstracts are either published or in press, along with one book chapter in press.

In vitro renal studies: Initial studies were conducted using in vitro exposure of renal cortical slices to resveratrol (RES) and cisplatin. Cisplatin renal toxicity was evident as indicated by an increase in Lactate dehydrogenase (LDH) leakage within 120 min exposure. RES totally prevents LDH leakage by cisplatin and establishes that RES prevents cisplatin renal cytotoxicity. RES does prevent cisplatin mediated depletion of Manganese Superoxide Dismutase (MnSOD) activity. Maintenance of Mn SOD by RES would allow for continued detoxification of superoxide anions (O2-) and diminished oxidative stress that is associated with cisplatin exposure of renal tissue. New methods were established in Dr. Valentovic’s laboratory to examine Cu-Zn SOD and catalase enzyme activity. The results from her studies show that catalase enzyme activity is diminished by cisplatin. However, renal tissue in the presence of RES exposure to cisplatin did not demonstrate as extensive a decline in catalase activity. She has also found that RES decreases oxidative stress by cisplatin by maintaining a more favorable balance between MnSOD and catalase. She has also shown that depletion of glutathione by cisplatin is not prevented by RES. Initial studies examining mitochondrial swelling also suggest that RES does not act by preventing mitochondrial swelling induced by cisplatin. A time dependent study has shown extensive modulation by RES of oxidative stress induced by cisplatin. RES prevents cisplatin oxidative stress 60 min prior to induction of LDH leakage. These studies suggest a RES greatly diminishes oxidative stress. More importantly, the decline in oxidative stress occurs prior to LDH leakage.

Pharmacokinetic studies: RES may reduce cisplatin renal toxicity by: a) increasing cisplatin renal clearance or b) diminishing cisplatin renal tissue accumulation. Experiments have been completed to evaluate the pharmacokinetic interaction of RES and cisplatin. Male F344 rats were placed in metabolism cages. Parameters evaluated in metabolism cages include food and water intake, body weight, urine volume and urine protein. Rats were randomly divided into the following groups: Vehicle (DMSO and water), RES, Cisplatin (5 mg/kg, ip) and RES+Cisplatin. RES was injected 30 min prior to cisplatin. Plasma and renal tissue was collected 6, 24, 48 and 72 h after cisplatin injection. Urine was collected on ice for Pt analysis at the following time points: 0-6 h, 6-24 h, 24-48 h. Pt is analyzed instead of cisplatin for all published

pharmacokinetic studies. Samples will be acid digested and then analyzed by Dr. Troyer. The Pt analysis should be completed by July 2012.

Additional Publications
Overall, WV-INBRE network participants published 29 abstracts and 29 journal articles (20 with PMC numbers) and have in press nine abstracts, five journal articles and one book chapter. Two of the published journal articles did not have PMC numbers last year, but do this year and have been added to the published list in the appropriate APR section. In addition, 117 presentations at state, national and international meetings were made. The nine published journal articles without PMC numbers at this time are:

Bell P, Wang L, Gao G, Haskins ME, Tarantal AF, McCarter RJ, Zhu Y, Yu H,Wilson JM. Inverse zonation of hepatocyte transduction with AAV vectors between mice andnon-human primates. Mol Genet Metab. 2011 Nov;104(3):395-403. Epub 2011 Jun 12. PMID21778099 (SPID 0009)

Bhullar J, Sollars VE. YBX1 expression and function in early hematopoiesis and leukemic cells. Immunogenetics. 2011 Jun;63(6):337-50. Epub 2011 Mar 3. PMID21369783 (SPID 0033)

Huang K, Huang Y, Frankel J, Addis C, Jaswani L, Wehner PS, Mangiarua EI,McCumbee WD. The short-term consumption of a moderately high-fat diet alters nitric oxidebioavailability in lean female Zucker rats. Can J Physiol Pharmacol. 2011 Apr;89(4):245-57. Epub 2011 Apr 13. PMID21539468 (SPID (0009)

The Appalachian Cardiovascular Research Network. Identification of Genes Contributing to Cardiovascular Disease in Overweight and Obese Individuals from West Virginia. West Virginia Medical Journal 108:23-30, 2012. (SPID 0006)

Vongsutilers V, Phillips DJ, Train BC, McKelvey GR, Thomsen NM, ShaughnessyKH, Lewis JP, Gannett PM. The conformational effect of para-substituted C8-arylguanine adducts on theB/Z-DNA equilibrium. Biophys Chem. 2011 Feb;154(1):41-8. Epub 2010 Dec 30. PMID21255902 (SPID 0023)

Kaushal G, Ramirez R, Alambo, D, Taupradist W, Choski K, Sirbu C. Initial characterization for D-cycloserine for future formulation development for anxiety disorders. Drug Develop Ther 2011 5(5): 253-260. (SPID 0007)

McCartney M, Keesee C, Flecther L, Stover S. Effects of sprint training on glutathione expression in liver and skeletal muscle. J Exercise Physiol online Oct 2011 14(5): 66-74.(SPID 0007;0010)

Boyd J, Saksena A, Patrone JB, Williams HN, Boggs N, Le H, Theodore M. Exploring the boundaries of additivity: mixtures of NADH: quinine oxidoreductase inhibitors. Chem Res Toxicol 2011 24(8): 1242-9. (SPID 0009).

Zeng WP, Sollars VE, Belelcazar Adel P. Domain requirements for the diverse immune regulatory functions of foxp3. Mol Immunol 2011 48 (15-16): 1932-9. (SPID 0033)

All journal articles but one cited support from WV-INBRE.

Center for Natural Products Research
Researchers from Alderson-Broaddus College, Concord University, West Virginia State University, West Virginia Wesleyan College, Marshall University and West Virginia University have expressed an interest in being part of the Center. Equipment has been purchased for researchers at Alderson-Broaddus College, Concord University and West Virginia State University to facilitate natural products research projects at these PUIs. A full organizational meeting of interested investigators will be held in late March or early April and projects selected for funding in Y12.

Evaluation

Dr. Santanam, Evaluation Coordinator, conducted an internal WV-INBRE evaluation meeting on November 10, 2011. A decision was made to update the way the WV-INBRE database was maintained and Dr. James Denvir was appointed to make the necessary changes and updates. An external review of WV-INBRE is scheduled for late March to evaluate interactions between the AC and the PUIs.

Reports from the Internal Program Evaluation Meeting held November 2011:

Surveys were sent out to nine network institutions that are part of the WV-INBRE network. The data obtained from the surveys indicated that, this past year there were twelve papers published, one published abstract and twenty five presentations at national and regional meetings by network investigators. A total of $9,333,299 of extramural funding was obtained this past year by the various network institutes. Funding sources included NSF and NASA-EPSCOR. Several of the network institutions have either provided matching funds for research or provided improved infrastructure for WV-INBRE investigators. There has been an increase in research partnerships between the two lead institutions and network PUI institutions but not between the WV-INBRE network investigators, which needs to be addressed. Such partnerships will strengthen the research base in West Virginia. All PUI PIs' progress was evaluated and Dr. Santanam has sent critiques to each investigator.

Online Resources in progress: With the assistance from the information technology expert, Mr. Brian Patton and the Biostatistician Dr. James Denvir, we have started establishing an updated student database that will list all the students who have been associated with WV-INBRE since its inception. This database will help in tracking the progress made by WV-INBRE associated students over the years. We are also in plans of setting up online evaluation surveys accessible to all WV-INBRE funded investigators. We anticipate that user-friendly online surveys will help improve responses from the investigators and help gather complete data.

External Evaluation March 30-31, 2012: WV-INBRE is planning on conducting the next external evaluation meeting on March 30-31, 2012. The evaluation core is currently in the process of planning for this meeting. With the recommendation from the AC, the topic to be evaluated this year is "The Interactions between the Administrative Core and PUI investigators". The evaluation core has already identified, Dr. David Essig, Professor, Department of Biology, Geneva College, Pennsylvania, to be the chair of the external evaluation team. A three member review team will help with the evaluation.

RESEARCH HIGHLIGHTS

RESEARCH HIGHLIGHTS

SPID(s): 0006, 0007, 0009, 0013, 0022, 0023, 0025, 0026, 0027, 0032, 0034, 0036, 0037, 0038, 0039, 0040

ADMINISTRATIVE INFORMATION

AWARDS, HONORS, SPECIAL RECOGNITION

5P20RR016477-11 WV-INBRE
Honors, Awards and Special Recognition

Dr. Tesfay Belay, Bluefield State College was awarded a Certificate of Appreciation from the American Society for Microbiology for his contributions to the Annual Biomedical Research Conference for Minority Students, St. Louis, MO, November 9-13, 2011.

Dr. Y.C. Chen, Alderson-Broaddus College, was a finalist for the West Virginia 2011 Professor of the Year Award.

Laura Hyderman, a student in Dr. Robert Shurina's laboratory at Wheeling Jesuit University, was the recipient of the WJU Haig Award for the graduating science major whose research embodies the concept of "individual excellence for public usefulness".

Dr. Bingyun Li, West Virginia University, was awarded the Berton Rahn Prize from the international orthopedic foundation in July, 2011 for his work in nanotechnology.

Dr. Li was also an invited guest presenter at the AO Foundation Board of Trustees Meeting in Berlin, Germany in July, 2011 and at the Osteosynthesis & Trauma Care Foundation meeting in Madrid, Spain in June , 2011.

Dr. Gary Rankin, WV-INBRE PI, was a finalist for the PhaRMA Researcher of the Year Award.

Dr. Rankin also serves on the West Virginia IDeA Research Council and the Science and Research Advisory Council for the state of West Virginia.

Dr. Rankin was a member of a Special Emphasis Panel for NIDDK, 2011. He also is a member of the NISBRE Steering Committee for the 2012 meeting.

Dr. Monica Valentovic was Co-Chair of NIH Study Section NIDDK ZRG1 DKUS-E (10)B, March 13-14, 2011.

Dr. Monica Valentovic was Chair of NIH Study Section NIDDK ZRG1 DKUS-E (10)B, July 19-20, 2011.

Dr. Monica Valentovic was elected as Secretary/Treasurer of the Division of Toxicology, American Society of Pharmacology and Experimental therapeutics.

Dr. Maria Serrat was selected to participate in the Association of American Medical Colleges Early Career Development Seminar in Washington, D.C. in July, 2011.

Dr. Melanie Sal, West Virginia Wesleyan College received the 201102012 Recognition for Exemplary Service Award for exceptional contributions and dedication to WVWC.

Dr. Christopher Adams, winner of best poster award at the National American College of Cardiology, National American College of Cardiology Conference, Las Vegas, Jan 11th 2012.

COMMITTEE MEMBERS

*Committee Chair

EXTERNAL ADVISORY COMMITTEE (External)

Name: Degrees	Department Institution: State, Country	Area of Expertise	Voting
Cutler, Stephen	Medicinal Chemistry - University Of Mississippi:MS, USA	Drug Development	Y
Fornsaglio, Jamie L	Biology - Seton Hill University:PA, USA	Cellular Biology	Y
*Tew, Kenneth D	Pharmacology - Medical University Of South Carolina:SC, USA	Cancer And Cell Signaling	Y
Yang, Ivana V	Medicine - National Jewish Medical And Research Center:CO, USA	Genomics	Y

*Committee Chair

STEERING COMMITTEE (External)

Name: Degrees	Department Institution: State, Country	Area of Expertise	Voting
Aguilar, Jarrett	Natural Science & Mathematics - West Liberty University:WV, USA	Molecular Modeling	Y
Chen, Yi C	Biology - Alderson-Broaddus College:WV, USA	Cancer Biology	Y
Davis, Mary E	Physiology & Pharmacology - West Virginia University:WV, USA	Bioinformatics	Y
Ehni, Peter	Physics - Wheeling Jesuit:WV, USA	Physics	Y
Griffith, Robert	Basic Pharmaceutical Sciences - West Virginia University:WV, USA	Medicinal Chemistry	Y
Harper, Kathy	Biology - West Virginia State University:WV, USA	Biology, Genetics	Y
Harris, Rob	Biology - West Virginia State University:WV, USA	Cell Physiology	Y
Kreisberg, Robert	Biology - West Liberty University:WV, USA	Cardiovascular Disease	Y
Maher, John	Vp For Research - :,	Research Administration	Y
Mangiarua, Elsa	Pharmacology, Physiology & Tox - :,	Cardivovascular Physiology	Y
Primerano, Donald A	Biochemistry & Microbiology - :,	Genetic Basis Of Disease	Y
Propst, Joan	Academic Affairs - Alderson-Broaddus College:WV, USA	Administration	Y
*Rankin, Gary O	Pharmacology, Physiology & Tox - :,	Toxicology	Y
Sheil, James M	Micro, Immun & Cell Bio - West Virginia University:WV, USA	Immunology	Y
Shurina, Robert	Biology - Wheeling Jesuit University:WV, USA	Cancer Biology	Y
Watson, Valerie	Micro, Immun & Cell Bio - West Virginia University:WV, USA	Microbiology	Y

MEMBER INSTITUTIONS

Institution	Role	*Minority Serving	Most-advanced Degree	BRIN$ Allocated
Alderson-Broaddus College	Network Outreach Institution	Y	Masters	191,200
Bethany College	Partner Institution	N	Bachelors	0
Bluefield State College	Network Outreach Institution	Y	Masters	214,366
Concord University	Network Outreach Institution	Y	Bachelors	17,483
Fairmont State University	Network Research Institution Marshall	Y	Masters	0
University	Lead Institution	Y	Doctorate	1,222,180
Shepherd University	Network Outreach Institution	Y	Bachelors	54,725
University Of Charleston	Network Outreach Institution	Y	Bachelors	23,200
West Liberty University	Network Research Institution	Y	Bachelors	398,670
West Virginia State University	Network Research Institution	Y	Masters	379,663
West Virginia University	Lead Institution	Y	Doctorate	870,282
West Virginia Wesleyan College	Network Outreach Institution	N	Bachelors	30,000
Wheeling Jesuit University	Network Research Institution	Y	Doctorate	168,125
				3,569,894

*A Minority-serving institution is one with an enrollment of more than 50% minority/ethnic students (African Americans, Hispanics, American Indians, Native Hawaiians, and Pacific Islanders.

PROFILE

ALDERSON-BROADDUS COLLEGE

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	1	1	125,000
NIH Faculty	1	1	125,000
NIH - INBRE	1	1	125,000

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	2
Faculty - Newly Hired	1
Administrative - Total Employed	1

Faculty Served on Peer Review Groups	Number
Within this Institution	1
Outside this Institution	0

Research Space	Square Feet
Total	1,150
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	1
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	1

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	28	160	80	0
Degrees Awarded	25	142	66	0

Faculty Release Time	N
Central Grants Administration	Y

BETHANY COLLEGE

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	1	1	12,000
NIH Faculty	1	1	12,000
NIH - INBRE	1	1	12,000

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	11
Faculty - Newly Hired	2
Administrative - Total Employed	0

Faculty Served on Peer Review Groups	Number
Within this Institution	4
Outside this Institution	1

Research Space	Square Feet
Total	3,590
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	1
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	1
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	0	182	0	0
Degrees Awarded	0	33	0	0

Faculty Release Time	N
Central Grants Administration	N

BLUEFIELD STATE COLLEGE

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	8	4	362,522
NIH Faculty	6	3	277,522
NIH - INBRE	2	2	204,246

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	74
Faculty - Newly Hired	5
Administrative - Total Employed	127

Faculty Served on Peer Review Groups	Number
Within this Institution	0
Outside this Institution	1

Research Space	Square Feet
Total	5,000
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	0
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	653	1,276	0	0
Degrees Awarded	82	242	0	0

Faculty Release Time	N
Central Grants Administration	Y

CONCORD UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	2	1	14,861
NIH Faculty	1	0	0
NIH - INBRE	1	0	0

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	9
Faculty - Newly Hired	1
Administrative - Total Employed	1

Faculty Served on Peer Review Groups	Number
Within this Institution	0
Outside this Institution	0

Research Space	Square Feet
Total	5,686
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	0
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	0	373	0	0
Degrees Awarded	0	40	0	0

Faculty Release Time	Y
Central Grants Administration	Y

FAIRMONT STATE UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	2	0	0
NIH Faculty	1	0	0
NIH - INBRE	1	0	0

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	33
Faculty - Newly Hired	0
Administrative - Total Employed	4

Faculty Served on Peer Review Groups	Number
Within this Institution	2
Outside this Institution	1

Research Space	Square Feet
Total	1,843
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	0
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	250	137	0	0
Degrees Awarded	80	43	0	0

Faculty Release Time	N
Central Grants Administration	N

MARSHALL UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	93	87	32,354,235
NIH Faculty	28	11	6,592,798
NIH - INBRE	15	7	5,821,004

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	123
Faculty - Newly Hired	6
Administrative - Total Employed	19

Faculty Served on Peer Review Groups	Number
Within this Institution	68
Outside this Institution	22

Research Space	Square Feet
Total	182,507
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	0
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	258	2,655	511	400
Degrees Awarded	91	320	142	79

Faculty Release Time	N
Central Grants Administration	Y

SHEPHERD UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	15	9	157,620
NIH Faculty	3	2	54,725
NIH - INBRE	3	2	54,725

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	37
Faculty - Newly Hired	5
Administrative - Total Employed	6

Faculty Served on Peer Review Groups	Number
Within this Institution	2
Outside this Institution	1

Research Space	Square Feet
Total	0
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	3
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	3

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	0	816	0	0
Degrees Awarded	0	93	0	0

Faculty Release Time	N
Central Grants Administration	N

UNIVERSITY OF CHARLESTON

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	7	4	45,200
NIH Faculty	4	3	41,200
NIH - INBRE	4	3	41,200

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	11
Faculty - Newly Hired	0
Administrative - Total Employed	2

Faculty Served on Peer Review Groups	Number
Within this Institution	3
Outside this Institution	0

Research Space	Square Feet
Total	2,400
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	0
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	92	1,238	149	385
Degrees Awarded	17	216	53	74

Faculty Release Time	N
Central Grants Administration	Y

WEST LIBERTY UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	8	8	363,000
NIH Faculty	2	2	260,000
NIH - INBRE	2	2	260,000

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	4
Faculty - Newly Hired	1
Administrative - Total Employed	1

Faculty Served on Peer Review Groups	Number
Within this Institution	0
Outside this Institution	0

Research Space	Square Feet
Total	2,200
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	3
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	3

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	61	621	0	0
Degrees Awarded	0	103	0	0

Faculty Release Time	N
Central Grants Administration	Y

WEST VIRGINIA STATE UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	3	2	423,063
NIH Faculty	3	2	410,423
NIH - INBRE	3	2	410,423

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	23
Faculty - Newly Hired	0
Administrative - Total Employed	3

Faculty Served on Peer Review Groups	Number
Within this Institution	1
Outside this Institution	2

Research Space	Square Feet
Total	12,360
Newly Constructed or Renovated	1,205

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	3
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	3

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	0	202	21	0
Degrees Awarded	0	18	7	0

Faculty Release Time	Y
Central Grants Administration	Y

WEST VIRGINIA UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	380	468	33,633,235
NIH Faculty	136	52	11,842,540
NIH - INBRE	6	9	2,522,587

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	911
Faculty - Newly Hired	108
Administrative - Total Employed	355

Faculty Served on Peer Review Groups	Number
Within this Institution	40
Outside this Institution	37

Research Space	Square Feet
Total	169,028
Newly Constructed or Renovated	24,078

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	0
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	0	2,033	410	1,538
Degrees Awarded	0	49	151	301

Faculty Release Time	N
Central Grants Administration	N

WEST VIRGINIA WESLEYAN COLLEGE

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	4	0	0
NIH Faculty	4	0	0
NIH - INBRE	4	0	0

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	25
Faculty - Newly Hired	3
Administrative - Total Employed	1

Faculty Served on Peer Review Groups	Number
Within this Institution	2
Outside this Institution	1

Research Space	Square Feet
Total	8,776
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	1
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	1

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	0	550	21	0
Degrees Awarded	0	96	3	0

Faculty Release Time	N
Central Grants Administration	N

WHEELING JESUIT UNIVERSITY

Biomedical & Behavioral Research Grants & Contracts

Funding Sources	Applications Submitted	# Awards	Total Award $
All Sources	3	3	1,484,804
NIH Faculty	3	3	1,484,804
NIH - INBRE	2	2	181,852

Science & Health-Related Research Personnel	Number
Faculty - Total Employed	40
Faculty - Newly Hired	8
Administrative - Total Employed	7

Faculty Served on Peer Review Groups	Number
Within this Institution	0
Outside this Institution	0

Research Space	Square Feet
Total	4,700
Newly Constructed or Renovated	0

INBRE Junior Investigators	Number
Total on Roster this Reporting Period	0
Independent Status Achieved	0
with Research or Program Project Grant	0
without Research or Program Project Grant	0
No Longer Participating	0
Still Junior Investigator at Reporting Period End	0

Junior Investigators Achieving Independent Status

Science & Health Related	Associates	Bachelors	Masters	Doctorate
Degrees Being Pursued	0	372	135	91
Degrees Awarded	0	57	33	36

Faculty Release Time	Y
Central Grants Administration	Y

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