

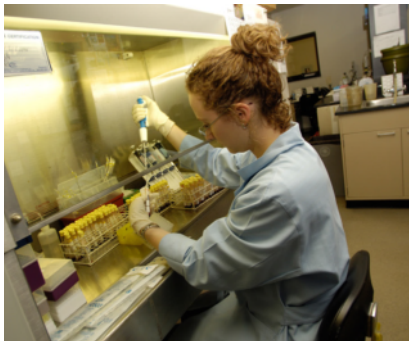

In this Issue

Explore Coriell 2008

Coriell Institute For Medical Research

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Michael F. Christman, Ph.D.

President and CEO, Coriell Institute for Medical Research

Michael F. Christman, Ph.D., is the President and Chief Executive Officer of the Coriell Institute for Medical Research. Dr. Christman is an expert in genetics and genomics and most recently served as professor and founding chair of the Department of Genetics and Genomics for Boston University School of Medicine. In that position, he led an international team of scientists in one of the first genome-wide scans associated with human genetic variation in disease using samples from the Framingham Heart Study. Dr. Christman received his bachelor's degree in chemistry with honors from the University of North Carolina, Chapel Hill, in 1981 and a doctorate in biochemistry from the University of California, Berkeley, in 1985. He was awarded a Jane Coffin Childs postdoctoral fellow at the Massachusetts Institute of Technology in 1986. Dr. Christman is a member of the Genetics Society of America, the New Jersey Technology Council Board of Directors, and the NIH Drug Discovery and Experimental Pharmacology Study Section.

Coriell Personalized Medicine Collaborative: Evidence-Based Research

The Coriell Personalized Medicine Collaborative (CPMC) is a research study that employs an evidence-based approach to determine the utility of using personal genome information in health management and clinical decision-making. The CPMC also aims to build a cohort with rich genotypic and phenotypic data with which to discover genetic variants that affect drug toxicity and efficacy, as well as to discover presently unknown gene variants that elevate a person's risk of cancer and other complex diseases.

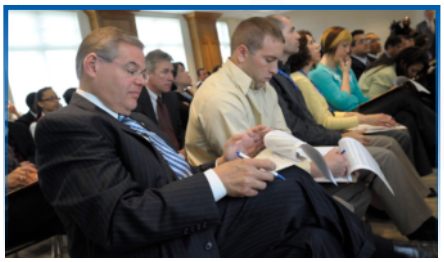
This forward-looking, collaborative effort involves physicians, scientists, ethicists, genetic counselors, volunteer study participants and information technology experts. Its goal is to better understand the impact of personalized, or genome-informed, medicine and guide its ethical, legal and responsible implementation. Coriell understands the importance of engaging medical professionals to develop successful strategies for integrating complex genetic information into the current medical paradigm. Coriell also appreciates the commonality of cancer in society and the enormous potential for cancer research and cancer care to be impacted by personalized medicine. Thus, we have established relationships with neighboring healthcare partners for the CPMC study and are engaging these individuals in the CPMC both as collaborators and participants.

Far exceeding our initial goal of enrolling 1,000 participants into the study by the end of the summer, the CPMC currently has more than 3,000 participants and dozens of upcoming enrollment opportunities scheduled. The study aims to enroll 10,000 individuals by the end of 2009 with an ultimate goal of 100,000 participants. There is no charge to study participants.

The study has received a great deal of media coverage and has had such figures as U.S. Senator Robert Menendez (D-NJ), Representative Robert E. Andrews (D-NJ), Assemblyman Louis D. Greenwald (D-NJ), and New Jersey Senator Diane Allen (R-NJ) enroll into the study as examples to the Delaware Valley community.

In January 2008, Coriell hosted top-ranking officials from the Federal Department of Health and Human Services (HHS) to discuss the CPMC. Billed as the "Challenges and Solutions in Personalized Medicine," the meeting brought together HHS leaders with the specific responsibility for and oversight of personalized medicine with our scientists and executives, as well as individuals from our hospital partners.

Following the HHS visit, Coriell was commissioned by Secretary Leavitt's Personalized Health Care Initiative to prepare a white paper describing important aspects of our research study. This paper is included in the HHS end-of-year report entitled "Pioneers, Pathways, and Partnerships: Towards a Personalized Health Care System," which focuses on the challenges of integrating personalized approaches into healthcare during the next several years. Additionally, we were in attendance at the invitation-only 2008 Personalized Health Care Summit, which brought together top executives and leaders from the public, private and academic sectors to create strategies to accelerate the integration of personalized healthcare into clinical practice and healthcare delivery.



From top to bottom: Senator Robert Menendez (D-NJ), Counselor Richard Campanelli, Representative Robert E. Andrews (D-NJ), each with Coriell personnel.

Genetic Counseling and the CPMC

The CPMC will deliver genetic results to study participants via the secure web portal in the coming months. While this is an exciting step forward in our research study aimed at assessing the impact of personalized genetic results on participant health and behavior, the task of delivering complex results in a clear and understandable fashion is a challenging one.

Earlier this year, we hired two board-certified genetic counselors, Erynn Gordon, MS, CGC, and Tara Schmidlen, MS, CGC, to develop educational materials for study participants and health professionals and to provide free genetic counseling to study participants and their physicians by phone, email and/or in person.

The CPMC genetic counselors will be available to participants for both pre-results and post-results genetic counseling. Genetic counselors can help participants understand complex genetic information and the implications of the CPMC results for themselves and their family members, and can work with participants to incorporate family and medical history information to estimate the risk of developing a particular disease. In addition, the CPMC genetic counselors may also provide participants with information about testing, treatment and prevention options that may be available for diseases for which they are at risk. Genetic counselors provide emotional support for people having a hard time adjusting to their risk for getting a disease or to their potential to pass on a genetic risk of disease to their children. Genetic counselors are not physicians and will not prescribe a treatment plan or tell study participants what to do about their genetic risk; instead, the CPMC genetic counselors will work to help participants understand their genetic results and inform them of options that are available for addressing that risk. However, based on the outcome of the risk assessment, the CPMC genetic counselors may recommend that participants see their primary care physician for further evaluation.

The CPMC leadership was quick to recognize the need for genetic counselors as medical professionals who will be instrumental in the effective communication of genomic information to study participants.

Erynn Gordon, MS, CGC

Senior Genetic Counselor, Coriell Personalized Medicine Collaborative

Erynn Gordon, MS, CGC, is a board-certified genetic counselor recruited from the University of Maryland School of Medicine to join the Coriell Institute's Coriell Personalized Medicine Collaborative as the Senior Genetic Counselor. Ms. Gordon earned a Master of Science degree in genetic counseling from the University of Pittsburgh in 2001 and was certified by the American Board of Genetic Counseling in 2002. Ms. Gordon previously worked as the genetic counselor for the Muscular Dystrophy Association Clinic at Children's National Medical Center in Washington, D.C., providing genetic counseling to families affected by neuromuscular diseases. While at Children's, Ms. Gordon also served as the laboratory genetic counselor for the Research Center for Genetic Medicine, conducting research on the psychosocial impact of non-disease genetic testing. She then moved to the University Of Maryland School of Medicine, where she was the genetic counselor for the Huntington's disease clinic and the adult genetics clinic. While at the University of Maryland, Ms. Gordon also provided cancer genetic counseling services at Baltimore Washington Medical Center.

Tara Schmidlen, MS, CGC

Genetic Counselor, Coriell Personalized Medicine Collaborative

Tara Schmidlen, MS, CGC, earned a Bachelor of Science degree in biobehavioral health from Pennsylvania State University in 2002, a Master of Science degree in genetic counseling from Arcadia University in 2006, and certification by the American Board of Genetic Counseling in 2007. Prior to joining the Coriell Personalized Medicine Collaborative, Ms. Schmidlen worked as a laboratory genetic counselor providing telephone-based genetic counseling for common complex diseases at Kimball Genetics in Denver, Colorado, and as a genetic coordinator facilitating testing and case management for reproductive and pharmacogenetic testing at Genzyme Genetics in Westborough, Massachusetts.

Josefina Nash

Director, Information Systems, Coriell Institute for Medical Research

Josefina Nash is Director of Coriell's Information Systems Department. As such, Ms. Nash manages the software development, databases and overall Information Technology (IT) infrastructure of Coriell. She serves as the IT liaison to Coriell's Principal Investigators for its various National Institutes of Health contracts, as well as a liaison to external IT collaborators such as dbSNP, dbGAP and Harvard Partners Center for Genetics and Genomics. Her department is currently focusing on such tasks as the development and maintenance of the Coriell Personalized Medicine Collaborative's (CPMC) web portal, the QUEUE Repository Information Management System and the Coriell Cell Repositories web catalog. Ms. Nash joined the Coriell Institute for Medical Research in May 1997 from BIOSIS, a non-profit organization in Philadelphia that produced an indexed, searchable bibliographic database of biological scientific literature. At BIOSIS, Ms. Nash served as a supervisor in the indexing department and worked on the development of a bibliographic index database system.

The CPMC Web Portal – Effectively Communicating Complex Genetic Information in a User-Friendly Format

Personalized medicine offers a new paradigm for healthcare and the practice of medicine. Effective implementation of personalized medicine into day-to-day medical care, however, will require robust decision support systems built upon electronic health records that contain not merely physiological data, such as a patient's blood pressure, temperature and blood chemistry results, but also the patient's own genetic and genomic data. By understanding illnesses on the molecular level, including gene variations linked to disease or drug response, doctors may be able to make more precise diagnoses and tailor treatment decisions. Similarly, drug makers can work to develop more targeted treatment therapies and identify potential clinical trial participants more effectively.

The Coriell Personalized Medicine Collaborative (CPMC) is an evidence-based research study designed to determine the utility of using genome information in clinical decision-making. A comprehensive information system has been developed to advance the objectives of the CPMC and to bring together the various constituents that will interact in the study. The system brings together, in a web portal, participant information obtained from medical history questionnaires and medical records, genetic results and a genetic variant knowledge base. As the central information hub of the study, the web portal contains tools for participants to update their consent options, share their data with physicians, request appointments with the CPMC genetic counselors and complete questionnaires that assess the utility of the genetic information that is presented to them. The web portal also provides extensive educational material available to study participants, medical professionals and the general public.

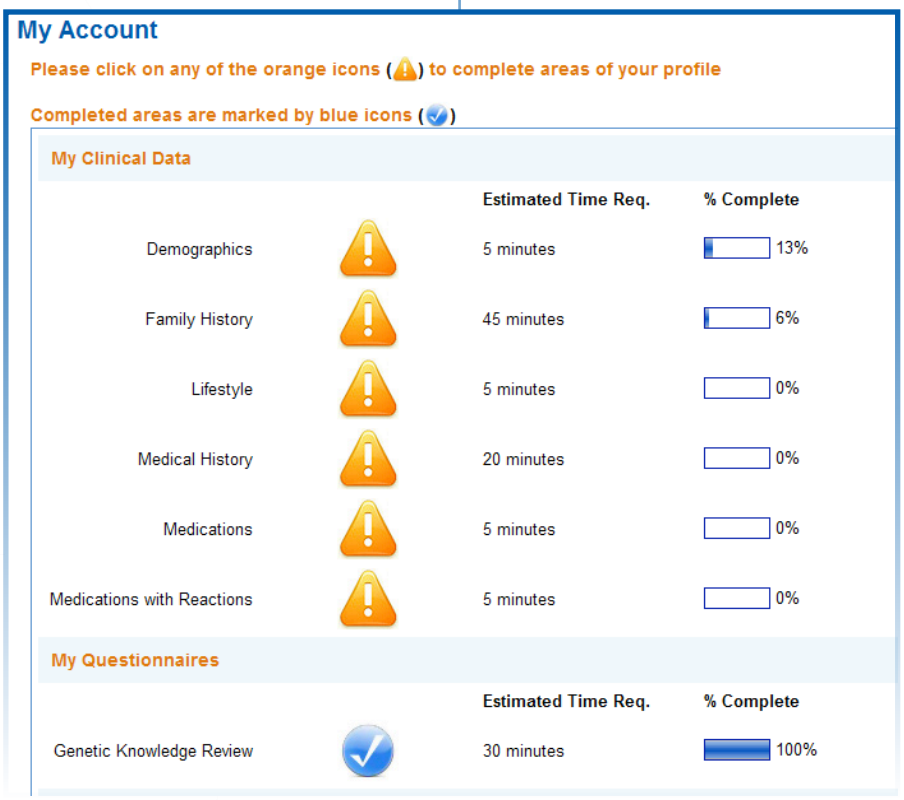
The web portal will contain a secure "My Account" area that includes account management, health information management and genetic results-reporting interfaces. Participants will be required to complete medical history, family history and lifestyle questionnaires. The portal will allow participants to complete this during several sessions, tracking their progress toward completion with a graphic for each portion of the questionnaire. Completion of these questionnaires is mandatory and a prerequisite to the release of personal genetic variant information. Participants will be asked to review and update their medical and family history information annually and will be able to update contact information at anytime through their account manager. The longitudinal nature of the study will capture medical and family history data over time, allowing tracking of changes in both health and wellness criteria.

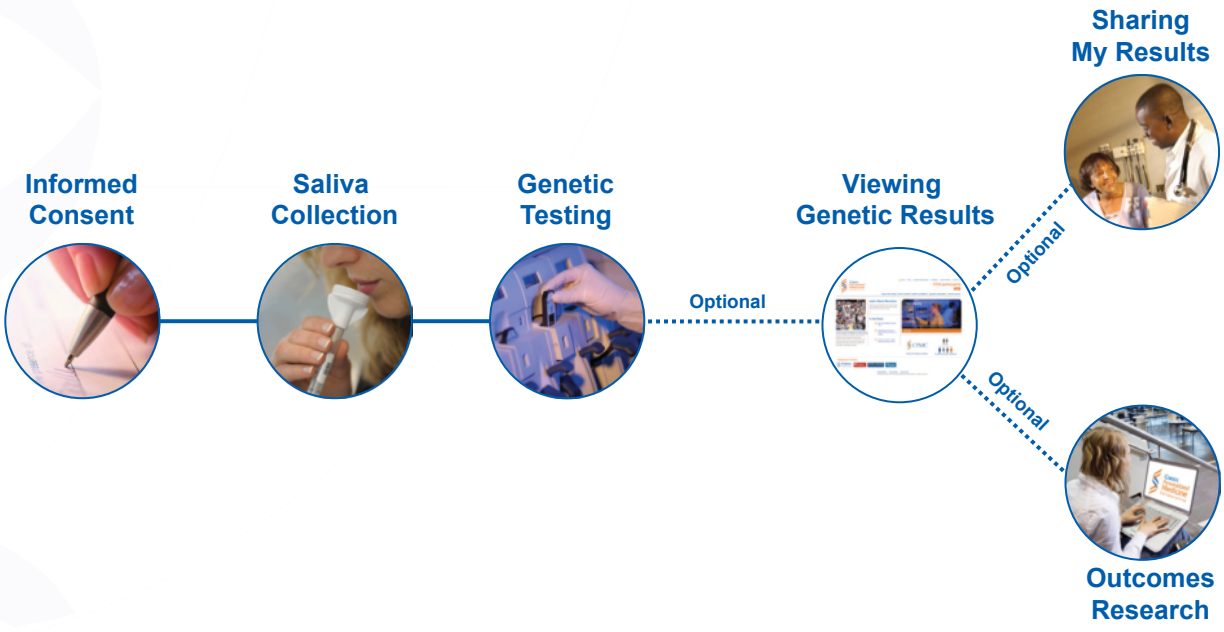
Participants will access their genetic results through the secure "My Account" portion of the web portal. Within this secure area of the site, they can view a list of genetic risk variants. Before their risk variant status (their genotype) can be viewed, educational material describing the disease or condition influenced by the variant will be provided, including a short video by a genetic counselor supplying anticipatory guidance. The participant can then view a report that indicates their risk status based on their personal health information, family history and genetic variants, as well as additional information that explains the risk conferred by the variant, information associated with the condition and inheritance.

After viewing genetic results, participants will be asked to complete web-based outcomes surveys to assess health outcomes. These survey questions will pertain to the conditions for which participants have variants known to increase disease risk and will query participants regarding lifestyle changes, prevention and screening strategies, and medical intervention sought or implemented based on the genetic variant results provided.

Underlying the web portal is a set of databases that manage the consent, questionnaire, phenotypic and genotypic data utilized in the study. These databases are logically integrated into a "User Participation System," which provides a unified mechanism to capture various types of data entered by participants and to generate genetic testing results. The visible interface to the "User Participation System" is a set of dashboards in the web portal that organize and facilitate the entry of the various data sets requested of the participants in the study.

Genetic- and genomic-based clinical decision support is the end goal of personalized medicine. Decision support may involve direct propagation of results from genetic variant databases to clinicians. More likely, however, clinical decision support will involve the combination of genetic or genomic data with the phenotypic data that exist in a patient's medical record. The informatics infrastructure being developed for the CPMC study assembles some of the critical components that will be required for a sustainable and scaleable genetics-aware clinical decision support system. The genetic/genomic knowledge repository and genetic and phenotypic databases underlying the CPMC web portal are the building blocks that will be needed to develop clinical decision support systems in the future.





Norman P. Gerry, Ph.D.

Director, Coriell Genotyping and Microarray Center
Associate Professor, Coriell Institute for Medical Research

Norman Gerry, Ph.D., is a genetics scientist recruited from the Department of Genetics and Genomics at Boston University to join Dr. Christman in the Coriell Personalized Medicine Collaborative. Dr. Gerry, former director of Boston University's Genotyping and Microarray Resource, established and directs Coriell's Genotyping and Microarray Center – the facility that performs the genome analyses for the CPMC. He has been involved in microarray and DNA testing for several years and has successfully processed thousands of single nucleotide polymorphism (SNP) and gene expression arrays, including all of the genotyping from the first genome scan of the Framingham Heart Study [Herbert et al. (2006), Science; Herbert et al. (2007), Nature Genetics]. He has also authored or co-authored numerous peer-reviewed scientific publications.

Genotyping and Microarray Center – A High-Throughput Resource

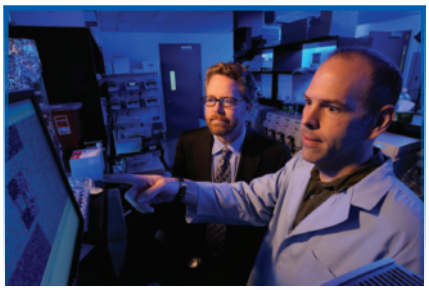
The Coriell Institute Genotyping and Microarray Center (GMAC) was established in August 2007. The mission of our facility is to provide investigators with the tools they need to fulfill their research goals. The widespread use of microarrays has led to a paradigm shift in detecting sequence variations and gene expression on a genomic scale. This is just the tip of the iceberg in terms of possibilities. There are continuing developments in the areas of pathogen detection, mapping of DNA protein binding sites (ChIP on chip), micro- and non-coding RNA expression, analysis of recombination, and determination of copy number variations (CNVs).

Genotyping and Microarray services fall into two main categories. The first is sample preparation and data analysis for Affymetrix expression and SNP profiling GeneChips. The second is assistance in the design and analysis of arrays for non-model organism expression profiling or unique applications such as ChIP on chip or resequencing. For these experiments, the CPMC, along with Affymetrix or other third-party vendors, will work with investigators to produce an array as well as prepare samples for hybridization.

The GMAC is a high-capacity facility, consisting of twelve FS450 Affymetrix fluidics stations and three state-of-the-art GCS3000 scanners. Each scanner is equipped with an autoloader. The facility can process up to 3,200 DNA or RNA samples per month. Optional assistance with data interpretation, both statistical and bioinformatics, is also available.

During the past year, the GMAC obtained CLIA certification for processing samples on the Affymetrix SNP 6.0 array. In addition, GMAC researchers have processed samples for several research projects, including:

- Genotyping 2,000 participants of the Howard University Family Study (HUFS) for a genome-wide association study of cardiac-related phenotypes.
- Genotyping 400 samples from the HapMap Diversity Panels for distribution to the research community.
- Genotyping 600 Samples from the General Medicine Repository for CNV and molecular karyotyping analysis.



Alan Herbert, MB.ChB., Ph.D.

Consultant, Coriell Institute for Medical Research

Associate Professor, Department of Genetics and Genomics, Boston University

Alan Herbert, MB.ChB., Ph.D., was recruited by Dr. Christman from Boston University to join the Coriell Personalized Medicine Collaborative team and serves as a consultant to the Institute. Dr. Herbert is also an associate professor at Boston University. He received his Bachelor of Science degree in human biology from the University of Auckland in 1976 before continuing on to earn his MB.ChB. in medicine and his Ph.D. in immunology. Dr. Herbert completed his post-doctorate for molecular immunology at the Massachusetts Institute of Technology studying alternative DNA conformations and its effects on editing of genetic readout. Recently, Dr. Herbert, along with Drs. Christman and Gerry, completed a whole genome scan of families from a community-based population in Framingham, MA, which involved the genotyping of 100,000 single nucleotide polymorphisms per individual. This scan was the first of its kind and allowed the researchers to identify a common variant that increases risk for obesity.

Genotyping of an African-American Cohort

Early this year the Coriell Institute for Medical Research received a major grant from the W.W. Smith Charitable Trust to fund the research project, "Genetic Risk Factors for Heart Disease in African-Americans." This study is the first genome scan of an African-American cohort and involves a close collaboration with Dr. Charles Rotimi, Director of the NIH Intramural Center for Genomics and Health Disparities (NICGHD).

Studies of genetic factors that are known to increase the burden of disease in specific populations are desperately needed. African-Americans are disproportionately predisposed to heart disease, obesity, type 2 diabetes and hypertension. Collectively, these diseases explain more than 80 percent of the health disparity between Americans of African descent and Americans of Western European descent. The high level of heritability for these diseases indicates that genetic factors and/or gene environment interactions contribute to these negative outcomes.

To identify genetic risk factors, Coriell is working with Dr. Rotimi and his group to study participants from the Howard University Family Study (HUFS) based in Washington, D.C.

The research study was designed with the aim of genotyping 2,000 African-Americans for 1 million single nucleotide polymorphisms (SNPs) and preparing a phenotypic database for analysis. A second aim of the study is to analyze the resulting data in order to associate genetic variation with quantitative heart disease-related traits, including hypertension and type 2 diabetes.

To date, Dr. Rotimi's group has reviewed medical information and phenotypic measurements from each individual in the HUFS collection; these data include measures of obesity and diabetic and hypertensive status collected from a single exam visit. The information was checked for accuracy and consistency. Furthermore, pedigree structure was confirmed using genotyping results. The participant dataset is now frozen and ready for analysis. Researchers in Coriell's Genotyping and Microarray Center (GMAC) genotyped 763 males and 1,213 females using the Affymetrix Human 6.0 GeneChip. Subsequent to the removal of SNPs with low allele frequency, low call rate and those outside of Hardy Weinberg equilibrium, 841,156 genetic variations resulted and are available for analysis for each individual.

We are now searching results for genetic variants that are associated with obesity and hypertension using approaches based on 755 nuclear families within the HUFS population, either separately or in combination with the 956 unrelated individuals. We are examining association of SNPs with disease outcomes

and are determining the impact of copy-number variants on phenotypic variation.

A variety of analytical approaches are being implemented in Helixtree, pLINK, Partek, FBAT and R. In parallel, data are being analyzed from the Framingham Heart Study (FHS) to identify variants that show association with disease in both populations (HUFS and FHS). To replicate positive findings, we are also collaborating with the Scandinavian FUSION study of type 2 diabetics and the deCODE group studying Icelanders.

This work is expected to continue throughout the next year with a possibility that further targeted genotyping or deep resequencing of particular individual DNAs will lead to identification of functional variants. It is our hope that data collected through this study will be made accessible to other scientists through a mechanism such as NIH's dbGAP.

Margaret A. Keller, Ph.D.

Associate Professor, Coriell Institute for Medical Research

Associate Director, Cell Culture Laboratories, Coriell Cell Repositories

Margaret A. Keller, Ph.D., is a molecular geneticist recruited to direct the New Jersey Stem Cell Resource (NJSCR) in fall 2006. Dr. Keller came to Coriell from Thomas Jefferson University (TJU), where she was a research assistant professor in the Cardeza Foundation for Hematologic Research in the Division of Hematology in the Department of Medicine. Her research, in collaboration with Dr. Saul Surrey at TJU, has focused on lineage commitment of hematopoietic stem cells. Currently, in collaboration with Coriell and UMDNJ-SOM faculty, Dr. Keller is studying the dynamics of the pluripotency phenotype of mesenchymal stem cells isolated from umbilical cord blood (UCB) and expanded in the laboratory. In her role as Director of the NJSCR, Dr. Keller oversees isolation, characterization, banking and distribution of biomaterials derived from UCB for use in basic research. She is also involved in the design and implementation of the Coriell Personalized Medicine Collaborative (CPMC) research study. Dr. Keller is working with Coriell scientists, genetic counselors and information technologists, as well as with clinicians and researchers outside of Coriell in many aspects of the study. In addition, Dr. Keller is an adjunct professor at TJU and was recently appointed Assistant Professor of Medicine at UMDNJ.

Shannon Morgan, Ph.D.

Postdoctoral Fellow, New Jersey Stem Cell Resource Laboratory, Coriell Institute for Medical Research

Shannon Morgan, Ph.D., is a Postdoctoral Fellow in the New Jersey Stem Cell Resource Laboratory at the Coriell Institute. Dr. Morgan holds a Bachelor of Science degree in biology from The Catholic University of America, Washington, D.C. She earned her Doctorate in Microbiology and Immunology from Temple University, Philadelphia, PA.

Effects of Ex Vivo Expansion on Mesenchymal Stem Cell Phenotype

Mesenchymal stem cells (MSCs) are adult somatic stem cells that have enormous clinical potential. MSCs can be found in several tissue sources including bone marrow and umbilical cord blood (UCB) [1,2,3,4]. Because MSCs are stem cells, they are capable both of self renewal as well as differentiation into multiple cell types, two hallmarks of stem cells. MSCs have the capacity to differentiate toward mesodermal tissues, including cartilage, bone, fat, and muscle. Although MSCs can be found in multiple tissues of the body, they are present at very low frequency and therefore, extensive ex vivo expansion is used to generate sufficient numbers for their study or for clinical use.

Initially, MSCs grow quickly in culture, but exhibit a limited life span. Other stem cell types, including embryonic stem cells, show chromosomal instability under particular culture conditions [5]. Additionally, there is evidence that MSCs may lose some of their stem cell characteristics and enter into a state of cell senescence during attempts to expand the cells in culture [6,7]. Cellular changes that occur during ex vivo expansion may render MSCs, generated in this manner, unsuitable for use as a cellular therapeutic due to loss of the pluripotency phenotype. In addition, the clinical utility of MSCs will depend on the ability to perform ex vivo expansion while maintaining genomic stability. Therefore, a thorough understanding of how culture conditions and time in culture affect the pluripotency phenotype, genomic stability, proliferation, and differentiation capacities of MSCs is critical to developing safe protocols for their therapeutic use.

The aim of our recent work has been to determine the effects of ex vivo expansion on the capacity of UCB-MSCs to self renew, and on the expression of various stem cell regulators. MSCs from UCB were cultured until they reached senescence at approximately 40 population doublings. At four time points during this continuous culture, we examined self-renewal, cell proliferation kinetics and colony forming unit-fibroblast (CFU-F) ability, as well as expression of pluripotency genes. Additionally, we were able to examine the occupancy of acetylated histones on the promoters of several genes known to be important to maintenance of pluripotency.

First, we examined the cell proliferation kinetics of the MSCs (Figure 1). As expected, the MSCs grew quickly in culture after the initial isolation, with the cell rate slowing as the time in passage progressed. Eventually, the cell culture became senescent, which was defined as subcultivations that did not result in at least one cell population doubling after three weeks in culture.

Next, we looked at the CFU-F ability of the cells at various time points (TP) in culture (Figure 2). We chose to examine the cells at TP1 (passage 2, ~ 22 CPD), TP2 (passage 5, ~28 CPD), TP3 (passage 9, ~38 CPD) and TP4 (12 passages, ~40 CPD). At the first two

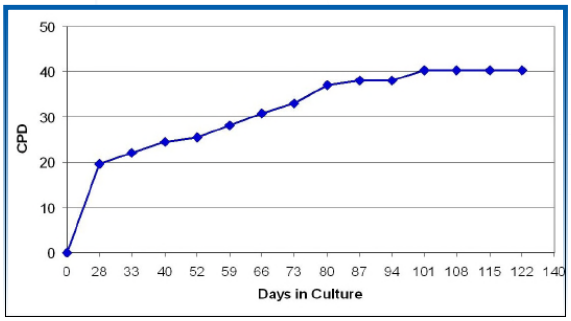


Figure 1: MSC Lifespan Analysis: UCB-MSC were seeded in T25 flasks at ~4x10^5 cells/flask in DMEM, 0.02M Hepes, and 15%FBS. Cells were grown for 7 days, at which time cell yield was counted and cells were used to seed another flask. Cumulative population doubling is calculated as [log10(NH)-log10(NI)]/log10(2)=x, where NH=cell harvest number, NI=inoculum number and x= population doublings. Cell cultures were considered senescent when sub-cultivations did not results in at least one cell population doubling after 3 weeks in culture.

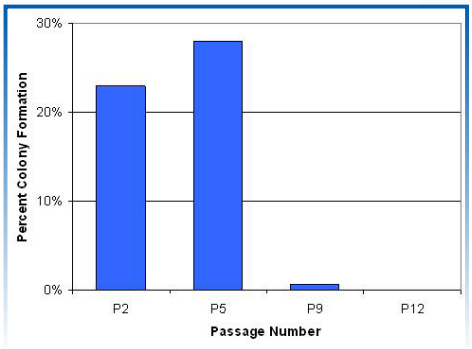


Figure 2: CFU-F Ability of MSC: UCB-MSC were seeded in triplicate in 3 T-25 flasks at a density of 3 cells/cm^2 in DMEM with high glucose and 10% FBS. After 14 days of incubation at 37°C with 5% CO2, the colonies were stained with 0.5% crystal violet in methanol for 10 minutes at room temperature. Purple colonies were counted to determine the percent CFU-F formation. Percent CFU was calculated as [(colonies counted/cells seeded)*100]. Average CFU was determined for each triplicate set.

time points, the MSCs showed 20 and 27 percent of the cells placed in culture were capable of generating colonies. However, at the later two time points, this ability for self-renewal was lost.

We examined mRNA expression during this time course using the TaqMan ® Low Density Array (TLDA) Pluripotency Panel (Applied Biosystems, Foster City, CA) which simultaneously monitors changes in expression of ~90 genes via real-time PCR. We assessed the expression state of genes involved in proliferation or stemness as well as those involved in differentiation at TP1 (Figure 3). Several genes known to be necessary for pluripotency were expressed in the UCB-MSCs at this early time point, including NANOG, SOX2, and OCT4.

Additionally, the TLDA panels were used to examine the relative change in gene expression during time in culture. We focused on genes that showed changes in expression when the two early time points were compared to the two later time points, since this would correspond to the loss of self-renewal capacity demonstrated by the CFU-F assay. We examined relative expression of five genes: NANOG, LIN28, SOX2, GATA6, and OCT4, as these genes are known to play a role in the pluripotency and differentiation capabilities of pluripotent stem cells [8,9,10,11] (Figure 4). NANOG, LIN28, and SOX2 showed decreases in expression comparing the TP1 and TP2 to TP3 and TP4. Conversely, GATA6, which is known to be expressed during the differentiation of stem cells into other cell types [12,13,14], showed increased expression at TP4 when compared to TP1.

Gene Function Category	Expressed	Not Expressed
Proliferation/Stemness	BRIX, CD9, COMMD3, CRABP2, DNMT3B, EBAF, FGF5, FOXD3, IFITM1, IFITM2, IL6ST, IMP2, KIT, LEFTB, LIN28, NANOG, NOG, NR6A1, PODXL, POU5F1, PTEN, REST, SEMA3A, SOX2, TDGF1, UTF1	FGF4, GBX2, GDF3, NODAL, NR5A2, TFCP2L1
Differentiation	CD34, CGB, COL1A1, DES, FLT1, FN1, GATA6, HBZ, LAMA1, LAMB1, LAMC1, NES, PECAM1, RUNX2, SERPINA1, SOX17, SYP	CDX2, FOXA2, GATA4, GCG, IAPP, IPF1, ISL1, KRT1, MYF5, MYOD1, NPPA, PAX4, PAX6, PTF1A, SST
Controls	18S, ACTB, RAF1, CTNNBA, GAPD, EEF1A1	

Figure 3: mRNA Expression Analysis of Early Passage MSC: mRNA expression analysis was done using TaqMan® Low-Density Arrays (TLDA, Applied Biosystems) containing pluripotency genes, differentiation genes, and endogenous controls. UCB-MSC was seeded in a T25 at $2x10^4$ cells/cm^2 in DMEM, 0.02M Hepes, and 15%FBS. Cells were grown until confluent, at which time total RNA was obtained using RNeasy Mini Kit (Qiagen). Total RNA (100 ng) was used for cDNA synthesis using the High Capacity RNA-to-cDNA Kit (ABI) and cDNA was combined with PCR master mix and loaded onto the microfluidics card. The card was spun to distribute the reaction mix to each well of the card and cycled in an ABI 7900 real-time instrument. Expression at the earliest passage (P2) is categorized as "not expressed" if the CT was undetermined.

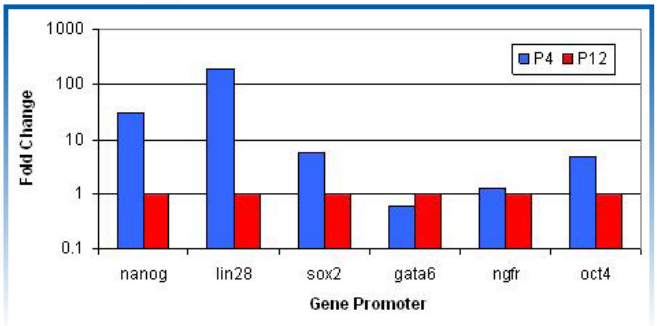


Figure 4: Gene Expression Changes During MSC Expansion: UCB-MSC was seeded in a T25 at $2x10^4$ cells/cm^2 in DMEM, 0.02M Hepes, and 15% FBS. Cells were grown until confluent, at which time total RNA was obtained using RNeasy Mini Kit (Qiagen). Total RNA (100 ng) was used for cDNA synthesis using the High Capacity RNA-to-cDNA Kit (ABI) and cDNA was combined with PCR master mix and loaded onto a TaqMan Low Density Array. The card was spun to distribute the reaction mix to each well of the card and cycled in an ABI 7900 real-time instrument. Relative expression was determined by using the ΔΔCT method and by comparing later passages (P4, P9, and P12) to the earliest passage (P2). Five genes that show changes between early (P2, P4) and late (P9, P12) in two replicate assays are shown.

Finally, we wanted to determine if the changes seen in gene expression correlated with changes in epigenetic regulation of these genes. Chromatin immunoprecipitation assays using an anti-histone H3 antibody were performed followed by quantitative PCR of the promoter regions of the genes. We compared TP2 to TP4 and were able to show that there was an increase in histone H3 occupancy on the promoters of NANOG, LIN28, SOX2, and OCT4 at TP2 when compared to TP4 (Figure 5). The promoter of GATA6 showed a decrease in histone H3 occupancy at TP2 when compared to TP4. Because histone H3 occupancy is often associated with regions of transcriptionally-active chromatin, this suggests that these genes may be under epigenetic control.

Together, these data demonstrate that as the cumulative population doublings of the UCB-MSCs increase during repeated ex vivo culturing, the pluripotency phenotype is lost. This includes the decrease in expression of regulators of stem cell proliferation as well as the loss of the MSCs capacity for self-renewal. This work highlights the need for vigilant monitoring of MSCs expanded ex vivo and further development of culturing conditions to preserve their stem phenotype and differentiation capacity for use in vivo.

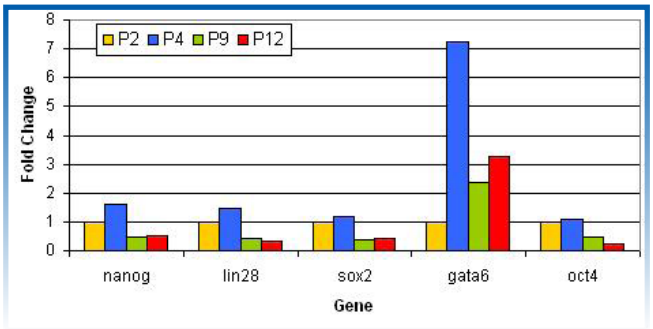


Figure 5: Occupancy of acH3 on Stemness Gene Promoters Changes During MSC Expansion: UCB-MSC was seeded in a T25 at $2x10^4$ cells/cm^2 in DMEM, 0.02M Hepes, and 15% FBS. Cells were grown until confluent, at which time proteins and DNA were crosslinked with 1% formaldehyde. Immunoprecipitation was carried out with anti-histone H3. Cross-links were reversed and DNA was purified by QiaQuick purification kit (Qiagen). qPCR was carried out on the recovered DNA in an ABI 7900 real-time instrument using primers specific for the promoters of the indicated genes. Relative expression was determined by using the ΔΔCT method and by comparing P4 to P12.

This work was done in collaboration with Dr. Biagio Saitta (Coriell Institute) and Robert Nagele (University of Medicine and Dentistry of New Jersey School of Osteopathic Medicine and Institute for Successful Aging) and was supported by a grant from the New Jersey Commission on Science and Technology.

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Biagio Saitta, Ph.D.

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Associate Professor, Coriell Institute for Medical Research

Biagio Saitta, Ph.D., is Associate Professor and Director of the Laboratory of Stem Cell and Matrix Biology at the Coriell Institute for Medical Research. He also has a faculty appointment in the Department of Medicine at UMDNJ/RWJMS. Dr. Saitta received his Ph.D. in Biological Sciences from the University of Messina in Italy. He then completed fellowships at the Pharmacology Institute Mario Negri of Milan and at the Developmental Biology Institute of CNR of Palermo, Italy. He was subsequently a postdoctoral fellow and junior faculty member at Thomas Jefferson University in Philadelphia. Dr. Saitta joined the Coriell Institute in 2002, and the focus of his laboratory research is the study of multipotent mesenchymal stem cell (MSC) populations isolated from human umbilical cord blood (UCB) and their differentiation potential into specific cell lineages, including myocytes and chondrocytes. Dr. Saitta's work has shown evidence that UCB-MSCs can be used as a complementary source of stem cells as in vitro models for basic research. He is also interested in studying the role of extracellular matrix proteins (ECM) involved in cell-specific lineage differentiation and analysis of ECM modulation during stem cell response to cardiac and skeletal muscle cell injury.

Regenerative Potential of Human Cord Blood Mesenchymal Stem Cells

Stem cells can be isolated from either adult or embryonic tissue and differ in their capacity to differentiate into multiple cell lineages [1]. The evolving field of "regenerative medicine" utilizes stem cells as a strategy to repair damaged tissue and preserve or regain function [2,3]. Adult stem cells or mesenchymal stem cells (MSCs) are multipotent, fibroblast-like cells that were first described in the mid-1970s and were found in bone marrow [4]. Cultured bone marrow MSCs have been transplanted in children with osteogenesis imperfecta (OI), a disease causing bone fractures and fragility. When MSCs were engrafted into the defective bone, reduced bone fractures and increased bone density resulted [5].

MSCs are attractive for a number of therapeutic applications, as they are known to migrate to some tissues, particularly when injured or under pathological conditions [2]. Our research team has focused on isolating and characterizing MSCs from low volumes of umbilical cord blood (UCB) samples (Figure 1).

We also demonstrated that MSCs can be efficiently propagated and differentiated into adipogenic, chondrogenic and osteogenic cell lineages and can serve as in vitro models for basic research [6,7]. We obtained UCB samples from the New Jersey Cord Blood Bank (NJCBB), a facility housed within the Coriell Institute where stem cell-rich cord blood is collected and distributed for transplantation and research. UCB is collected at birth from male and female infants from a variety of ethnic backgrounds, whose parents have provided informed consent prior to donation. UCB has been increasingly explored as an alternative source to bone marrow for multipotent MSCs and for use in regenerative medicine [8]. UCB is a widely accepted source of hematopoietic stem cells, and cord blood-derived stem cell transplants have been successfully performed for a number of hematologic and oncologic diseases [9].

Since MSCs can differentiate into several cell types and have less immune-related issues, these cells have been used in several preclinical and clinical trials (http://www.clinicaltrials.gov), as well as in trauma and myocardial infarction (MI) conditions.

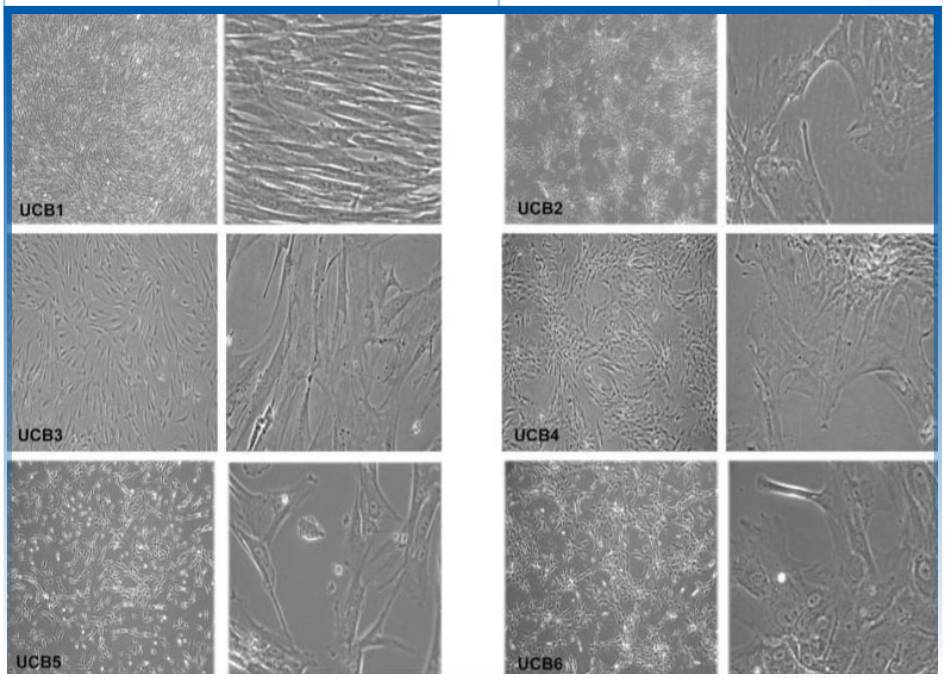

Figure 1. Distinct morphology of six different single isolated UCB-MSC colonies. By phase contrast microscopy, UCB1, UCB3 and UCB5 cells are spindle-shaped, small cells that grow to confluence, while UCB2, UCB4 and UCB6 cells are larger and grow in focal patches (passage 3 for all six MSCs). UCB1 and UCB2 were derived from the same donor (Markov et al, 2007), and UCB3 and UCB4 were also isolated from a single donor. UCB5 and UCB6 were obtained from two different donors. This morphology was maintained through all population doublings for all six UCB-MSCs. The magnifications shown are 40x and 200x.

Our laboratory of Stem Cell and Matrix Biology has focused on understanding molecular mechanisms involved in tissue repair of these adult stem cells. With support from the New Jersey Commission on Science and Technology (NJCST), we are investigating the ability of UCB-derived MSCs to respond to myocardial damage in an in vitro model. To simulate the effects of MI at the molecular level, we analyzed injured rat cardiac cells (myocytes and fibroblasts) for apoptosis, necrosis and viability. We also identified specific extracellular matrix (ECM) and angiogenic genes expressed by the UCB-MSCs that are modulated when exposed to the hypoxic adult rat cardiac cells. Of these specific genes, the matrix metalloproteinases (MMP-1, MMP-2) and tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2) are involved in remodeling of injured and fibrotic tissues. Other genes involved in vascular remodeling of injured and fibrotic tissues include: angiopoietins (ANGPT1 and ANGPT2), chemokines (CXCL1 and CXCL6), fibroblast growth factors (FGF1 and FGF2), and interleukins (IL6 and IL8). These genes encode critical ECM and angiogenic proteins that provide a substrate for cells to migrate, grow and differentiate. As such, the matrix is an integral regulator of cell and tissue function. Our preliminary data show increased expression of genes involved in the synthesis and remodeling of cardiac tissue and supports our aim of finding potential mechanisms involved in the process of cardiac repair. Through a collaborative effort with Drs. Steven Hollenberg and Joseph Parrillo, clinicians and cardiovascular researchers at Cooper Heart Institute, these studies are being extended to understand functional outcomes of our in vitro model. This approach will determine if MSCs can improve the contractility of the injured cardiac cells.

In addition, our laboratory has initiated several collaborative projects on both a national and international level aimed at strengthening the stem cell biology program at Coriell. Collaborative projects include: i) studying the engraftment of our UCB-MSCs into the ventricles of a rat model of MI. With Dr. Marisa Jaconi, University of Geneva, Switzerland, we demonstrated that the cells invade and engraft into the myocardium and ventricular wall in the region of infarct and form vessel-like structures at four weeks post-infarct. Markers indicated that these structures originated from the transplanted human UCB-MSCs (Figure 2), suggesting that MSCs differentiate toward endothelial lineages and may contribute to new blood vessels in the damaged tissue; ii) testing the potential of UCB-MSCs toward myogenic lineage differentiation in an in vivo model of skeletal muscle regeneration using a population of UCB-MSCs as a source for in vivo muscle repair. Our initial work, in collaboration with Drs. Pier Paolo Parnigotto and Maria Teresa Conconi, University of Padova, Italy, found muscular engraftment and myogenic differentiation (Figure 3), without immuno-rejection (Figure 4), of these stem cells after muscle injury; iii) investigations with Drs. Margaret Keller and Jay Leonard, Coriell Institute, and Dr. Robert Nagele, UMDNJ-SOM, to examine the safety of cord blood MSCs for cellular therapeutics by examining their stability after being grown in the laboratory.

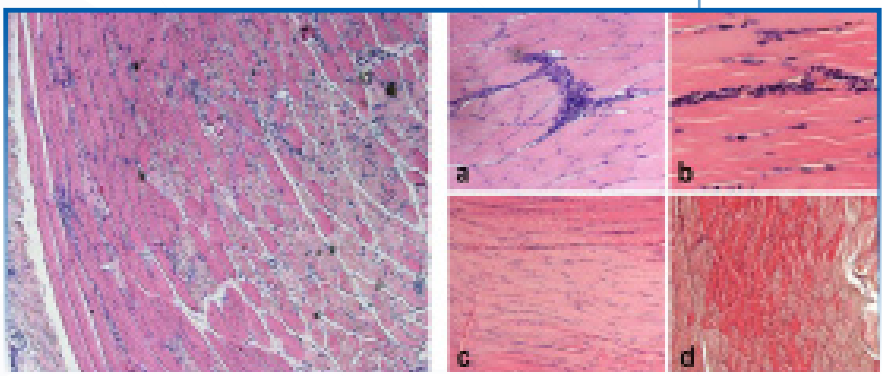

Figure 2. Engraftment of UCB-MSCs transplanted into the heart of a rat model of cardiac infarct. (A) Left panels (top) show UCB-MSC-containing biogel transplanted into the ventricle. Middle panels demonstrate engraftment of UCB-MSCs expressing green fluorescent protein (GFP) into ventricular wall (black arrows). Red arrowheads show vessel-like structures (100x magnification).

It is essential to define the biological mechanisms involved in the responses of stem cells and tissue-engineered products to injury at a molecular level. Our future goal is to combine stem cell technology with

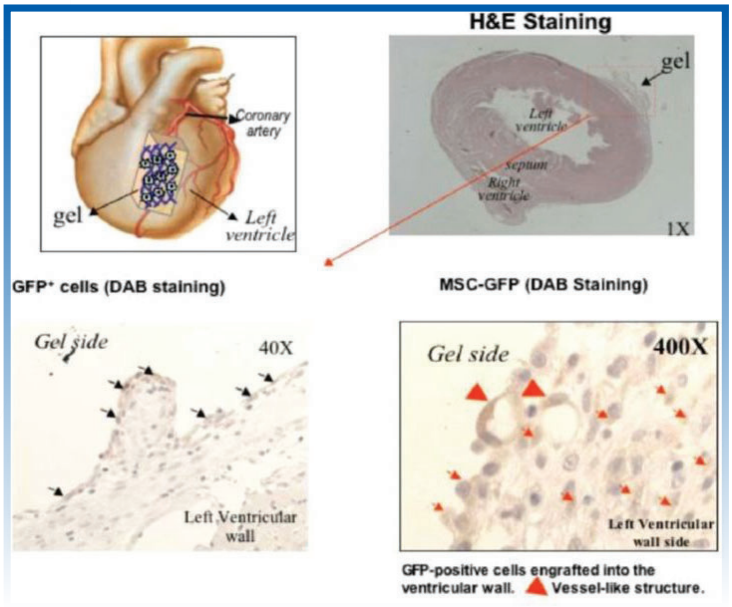

Figure 3. Hematoxylin-Eosin (H&E) stain of rat tibialis anterior (TA) shows muscle damage with Bupivicaine Hydrochloride solution. Left panel: 48 hours after bupivacaine, muscles had degenerative changes and an inflammatory response. Many muscle fibers showed necrosis (pale fibers) and internal nuclei reflected macrophage invasion (50x magnification). The right panels show H&E stain of TA skeletal muscle sections after injection of MSC suspension (a-c, treated) or saline solution (d, control). After 7 days (a, 100x and b, 200x) with UCB-MSCs, there was no sign of severe immunological response, with only occasional endomysial macrophage infiltration (a) and perivascular inflammation (b). The fibers showed normal size variation and were not pale. After 14 days (c, 50x) the muscle appeared intact with a "native" skeletal muscle appearance showing distinctive longitudinal myofibers and peripheral nuclei (dark points). After saline solution injection, necrotic areas began to disappear, but fibers remained pale (d, 100x).

tissue engineering approaches to develop tissue-specific extracellular matrix-based grafts for testing in injury repair, both for in vitro and in vivo models. This approach will lead to optimization of conditions and identification of factors that can be modulated to improve outcomes.

These studies will provide important new information on the mechanism of MSC action and new targets for therapeutic discovery.

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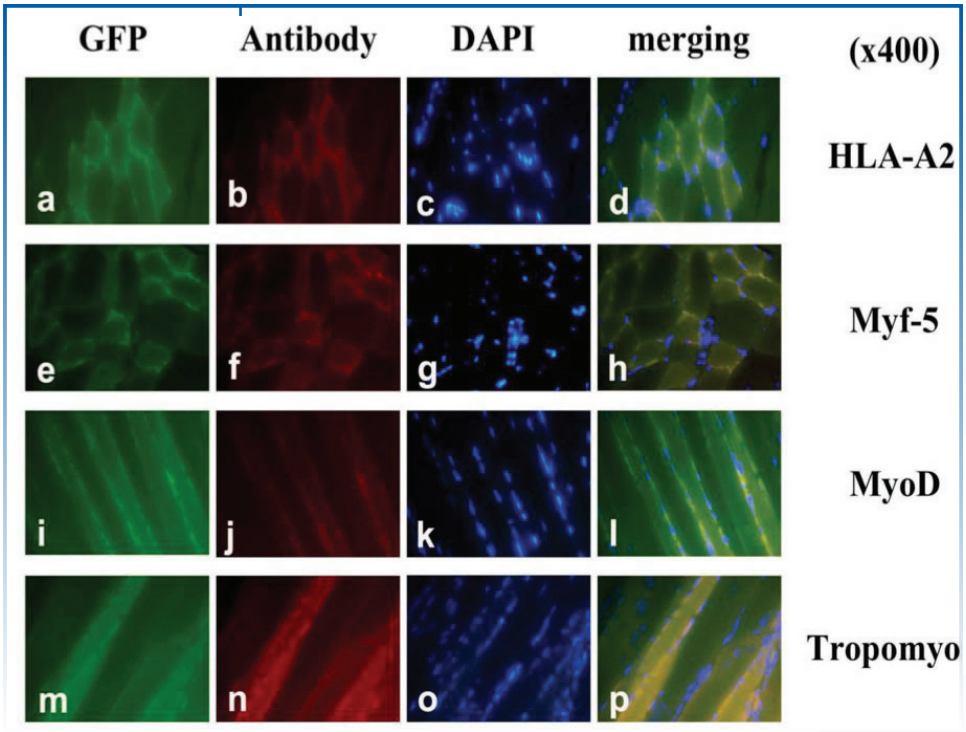

Figure 4. Immunofluorescence analysis of TA muscle sections. After 7 days (a-i) the GFP-labeled MSCs had engrafted into the TA muscle (Green Fluorescent Protein a, e, and i) and their human origin was confirmed by immunohistochemistry using HLA-A2 antibody (b). Fibers showed immunostaining for both the early myogenic markers Myf-5 (f) and MyoD (j), which are nuclear regulatory factors controlling proliferation and differentiation during embryogenesis and muscle growth and repair. At 14 days (m-p), GFP-labeled MSCs were detected in TA muscle that also stained for sarcomeric tropomyosin (p). (Green: GFP, Red: specific antibody, Blue: DAPI, Merged: fusion of three color channels. All images at 400x magnification).

Recent Innovations in Stem Cell Technology

In November 2007, research groups headed by James Thompson at the University of Wisconsin-Madison and Shinya Yamanaka at Kyoto University in Japan described methods to reprogram adult humansomatic cells into a pluripotent state [1,2]. Using viral vectors containing four transcription factors (Oct4, Sox2, and either Nanog and Lin28 or Klf4 and cMyc, respectively), human fibroblasts were genetically modified to behave like embryonic stem (ES) cells. These induced pluripotent stem (iPS) cell lines are similar to ES cells in morphology, proliferation, surface antigens, gene expression, telomerase activity and developmental potential. These studies utilized viral vectors that rely on integration of DNA sequences into the human genome, thus carrying the potential of tumor formation. In order to develop iPS cells amenable for in vivo use, researchers have developed non-viral means of generating iPS cells [3,4] and have explored methods that do not utilize the cMyc oncogene [3]. These advances would allow for the development of patient-specific iPS lines that would constitute an enormous step towards personalized medicine.

In June 2008, George Daley of Harvard Medical School and colleagues demonstrated that iPS cells could be established from a wide variety of disease-specific fibroblasts, which he obtained from the NIGMS Repository at Coriell [5]. Disease-specific iPS cells will be invaluable tools with which researchers can examine the development of disease tissues in culture. They have the potential to further our current understanding of the underlying mechanisms of disease development and to allow development of new treatments to slow or even stop the progression of a number of devastating diseases.

Following the publication of these reports describing the creation of iPS cells from fibroblasts from both normal individuals and individuals with inherited diseases, Coriell has distributed 146 NIGMS fibroblast cell lines, representing more than 50 different diagnoses, to researchers who have stated their intention of creating disease-specific iPS cell lines.

Coriell recently restructured its stem cell repository capabilities in order to offer researchers the opportunity to bank their iPS lines at the Repository. Further, Coriell will bring in-house the capabilities to establish, maintain, characterize, bank and distribute this important stem cell resource. Thus, Coriell is doing its part in providing the highest quality genetic resources to the biomedical research community.

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Staff Scientist, Coriell Cell Repositories, Cell Biology Research Laboratory
Coriell Institute for Medical Research

Karen Fecenko-Tacka, Ph.D., is a staff scientist for the Coriell Cell Repositories' Cell Biology Research Laboratory. Prior to her promotion and appointment earlier this year, Dr. Fecenko-Tacka served as a post-doctorate fellow and later a research associate at the Coriell Institute. She also served as a member of the science faculty responsible for organizing and teaching the Stem Cell Training Course at Coriell in January 2006. Dr. Fecenko-Tacka holds a Bachelor of Science degree with a major in biology and minor in neuroscience from Kings College in Wilkes-Barre, PA. She later earned her doctorate in neuroscience from State University of New York – Upstate Medical Center, Syracuse, NY.

Characterization of *Herpesvirus saimiri* Transformed T-lymphocytes

Transformation of B-cells by Epstein-Barr Virus (EBV) is used by the Coriell Cell Repositories (CCR) to produce renewable cell lines and DNA to further genomic and proteomic studies of inherited diseases. With the focus of science shifting to encompass whole-genome studies, specifically, genetic analysis for susceptibility to complex diseases, publicly available cell lines and DNA are becoming more widely sought. However, as much as two percent of clinical peripheral blood samples do not transform with EBV after repeated attempts due to either genetic disease, such as Bruton's agammaglobulinemia, or to transformation resistance [1,2]. Therefore, it is of interest to optimize alternative methods of transformation of human lymphocytes.

Alternative methods of lymphocyte transformation, such as fusion hybridomas and the use of the transforming virus, HTLV-1, have proven inefficient or unsuitable for large-scale laboratory usage or the study of genetics [3,4]. However, a method similar to EBV transformation has been developed using a T-lymphotrophic virus found in squirrel monkeys, *Herpesvirus saimiri* (*H. saimiri*). *H. saimiri* is a gamma-herpesvirus capable of producing stable transformation of T-cells in humans [5,6]. Although the transformed lymphocytes often remain IL-2 dependent, they do not require periodic restimulation with antigens or mitogens [5]. Like EBV-transformed B-lymphocytes, HVS-transformed human T-lymphocytes express a limited number of viral genes and infectious virus is not detectable [5,6], making this virus an ideal method to obtain a continuous cell line for donors who are not easily transformed by EBV. Therefore, a study was undertaken by CCR to compare the standard transformation protocol using EBV to an alternative transformation protocol using *H. saimiri*.

Samples were collected from healthy individuals and lymphocytes were isolated by centrifugation on Ficol-Histopaque gradients. Cells were plated at a density of $1.5x10^6$ – $2.5x10^6$ in a standard T25 flask containing RPMI 1640 medium, 2 mM glutamine, 20% FBS and 10 ng/ul phytohemaglutinin (PHA). EBV-transformed cell lines were incubated with EBV at an MOI of 10. T-lymphocyte cultures were stimulated with 20 U/ml IL-2 and immediately incubated with *H. saimiri* or stimulated with IL-2 for 24 hours prior to incubation with *H. saimiri*. A control flask was stimulated with growth factors but not infected with virus. Samples were maintained in RPMI, 2 mM glutamine, 20% FBS and observed daily for signs of transformation (clumps of small birefringent cells).

EBV-transformed cells were cryopreserved once cultures reached 1 x 10^8 cells. Since the growth characteristics of *H. saimiri*-transformed cultures are quite different than EBV-transformed cultures, the same criteria for evaluating transformation of the cells could not be used. During the first six to eight weeks of cell culture, normal growth of T-lympho-

Innoculation	*# Samples*	*% Transformed*	*Days to Freeze*	*% Transformed*
EBV	4	100%	32 days	100%
HVS Same day	5	60%	56 days	20%
HVS 24 hrs after isolation	9	66%	56 days	66%

Table 1: Chart depicts the transformation efficiency of EBV and *H. saimiri* using whole blood. Adult lymphocytes were isolated by centrifugation on Ficol-Histopaque gradients. Cells were plated at a density of $1.5x10^6$ – $2.5x10^6$ in a standard T25 flask. Lymphocyte cultures were grown in RPMI, 2 mM glutamine, 20% FBS and 10 ng/ul PHA (lectin). Flasks were either incubated with EBV to produce EBV transformed cell lines or was stimulated 20 U/ml IL-2 for 24 hours prior to incubation with *H. saimiri* to produce T-lymphocyte cultures. A control flask was stimulated with growth factors but left uninfected. For EBV infected cultures, transformation was assessed as continued proliferation to 1 x 10^8 cells and recovery from cryopreservation. For *H. saimiri* infected cultures, transformation was determined by continued proliferation as compared to uninfected control and recovery from cryopreservation.

cytes was observed and the cells were not considered transformed. Following this initial rapid proliferation, the cells went through a crisis period until a small outgrowth of virally-infected cells was observed. Transformed cells were cryopreserved once cultures reached 1 x 10^8 cells, 56 days for *H. saimiri* transformed lines and 32 days for EBV. Upon recovery from cryopreservation, cells were assessed for viability and continued cell growth. It was determined that EBV-transformed cell lines were obtained with an efficiency of 100%, while the efficiency for *H. saimiri* transformation was approximately 66% when infected 24 hours after stimulation with IL-2 (Table 1).

Following transformation using *H. saimiri* in 20 U/ml of IL-2, we wanted to determine whether we could maintain the cell lines in decreased concentrations of IL-2. Therefore, *H. saimiri* lymphocyte cultures were plated at a density of 5 x 10^5cells/well on 6-well dishes in five different IL-2 concentrations (0-20 U/ml). Proliferation was assessed at week 1 and week 2 by XTT cell proliferation assay according to manufacturer's protocol (Roche). As seen in Figure 1A, it was determined that cellular proliferation was not significantly diminished in concentrations of IL-2 as low as 2 U/ml when the proliferation of transformed cells ( ) was compared to uninfected control cells ( ), indicating that the concentration of IL-2 can be decreased following the establishment of the cell lines. Figure 1B depicts the average cell proliferation of five transformed cell lines during a period of three weeks in response to different IL-2 concentrations, further indicating that IL-2 concentration can be reduced to maintain the transformed cell lines without a significant decline in cell number.

EBV-transformed cell lines are used extensively in the study of genetics and the genetic basis of disease and thus, it is important to determine that genome stability is retained following transformation and culturing. It has been established that karyotypes of EBV-transformed cell lines show chromosomal stability up to high passages [7]. In order to assess the stability of lines produced by *H. saimiri* transformation, matched EBV and *H. saimiri* cell lines (same individual) were analyzed for chromosomal abnormalities by karyotyping (g-banding). In two of the three *H. saimiri* cultures, multiple chromosomal abnormalities were detected, suggesting that the lines may be somewhat chromosomally unstable (Table 2). Only one of the three EBV cell lines contained significant chromosomal abnormalities (Table 2).

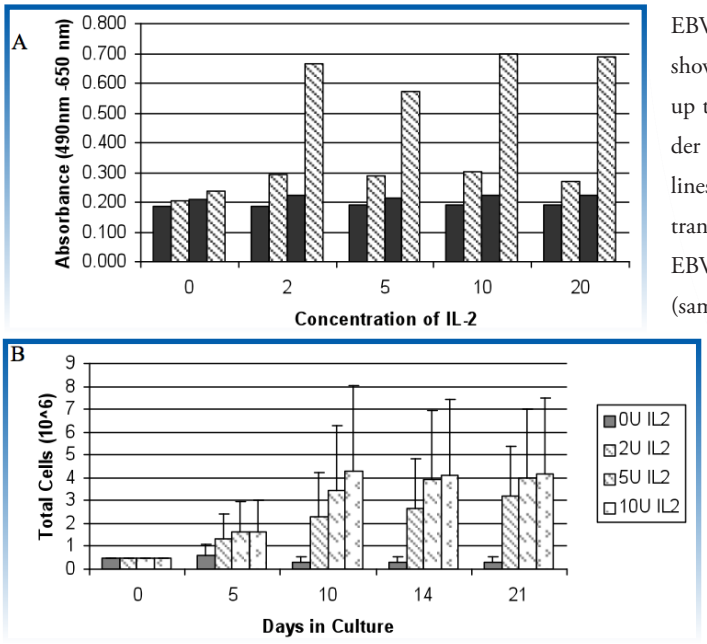

Figure 1: A) Histogram depicts cell proliferation of uninfected cells ( ) as compared to a transformed cell line( ) in response to IL-2 concentration. Lymphocyte cultures were either infected with *H. saimiri* or left uninfected and grown in RPMI supplemented with glutamine (350ug/0l), 10% fetal calf serum, and IL-2 (20U/ml). After 44 days in culture, 5 x 10^5cells/well were plated on 6-well dishes in 5 different IL-2 concentrations. Proliferation was assessed by XTT cell proliferation assay according to manufacturer's protocol (Roche). (B) Histogram depicts average cell proliferation of transformed cells over three weeks in response to different IL-2 concentrations. Cell counts were also performed at various time points to confirm the XTT results using Vi-Cell XR 2.03.

Sample	Karyotype	% Abnormalities
RE00475 EBV	46 XX	4%
RE00475 HVS	46 XX	4%
RE00476 EBV	46 XX	2%
RE00476 HVS	48 XXX +16/ 47 XXX	16%
RE00477 EBV	46 XY	12%
RE00477 HVS	46 XY i(10)(q10)/ 46 XY	12%

Table 2: Chart outlines the karyotype and percentage of abnormal cells within the cell population. Both EBV and HVS transformed cell lines were g-banded in accordance with our standard operating procedures.

Once it was determined that *H. saimiri* could produce continuously growing cell lines and chromosomal stability of the cells lines were assessed, it was important to determine whether the cell lines retained characteristics of their respective lineages. Lineage fidelity was assessed by cell surface antigen characterization and gene expression analysis. Characterization of cell surface antigens was performed to determine the subtypes within the EBV- and HVS-transformed populations. EBV-transformed cell lines were classified on the basis of CD45, CD19 and CD3. Surface antigen CD45 is present on all lymphocytes, while CD19 and CD3 are mutually exclusive to B-cells and T-cells, respectively. HVS-transformed T-cell lines were subtyped on the basis of CD8 or CD4 expression. Surface antigen CD8 is indicative of cytotoxic T-cells, while CD4 is expressed by T-helper cells; however, these antigens are not exclusive and may be simultaneously expressed by the same cell (double positive).

As expected, the EBV-transformed cell line mostly comprised CD19+ B-lymphocytes with a very small percentage of CD3+ T-lymphocytes (Figure 2B). All imaged cells were CD45+; however the intensity of the expression of CD19+ varied from cell to cell (Figure 2A). HVS-transformed cell lines contained both single positive CD8 (68.2%) and CD4 (3.07%), along with double positive populations (22.8%; Figure 2C-D).

Finally, Affymetrix expression arrays were analyzed in the Coriell Genotyping and Microarray Center to identify which genes were up- or down-regulated upon exposure to the transforming viruses. Based on our cell surface antigen classification, we expected EBV-transformed cell lines to express high levels of genes associated with B-cell differentiation

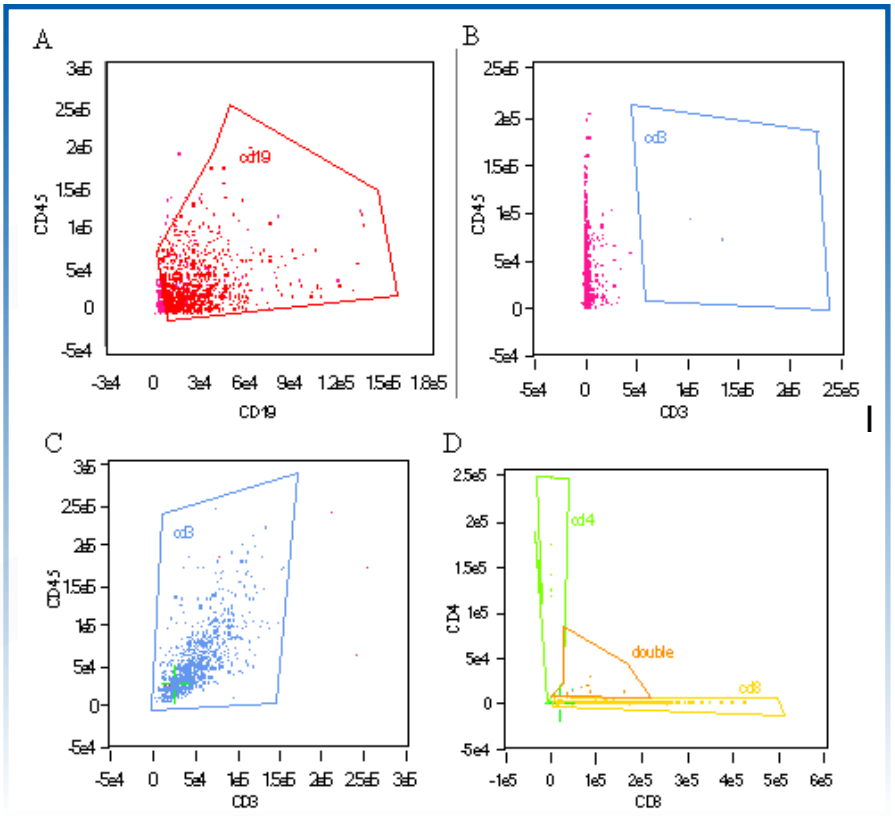

Figure 2: Surface Antigen Analysis of EBV transformed cells. (A) Dot plot showing the CD19+ population (red) of cells within the larger population of CD45+ (pink) lymphocytes (B) Dot plot showing the CD3+ population (blue) of cells within the larger population of CD45+ (pink) lymphocytes. Surface Antigen Expression of *H. saimiri* transformed cells. (C) Dot plot showing the CD3+ population (blue) of cells within the larger population of CD45+ (pink) lymphocytes (D) Dot plot showing the different subtypes of T cells that comprise the CD3+ cells, CD4+ population (green), CD8+ population (yellow) and CD4+,CD8+ population (orange).

and linage. On the other hand, *H. saimiri*-transformed cell lines should express higher levels of genes associated with T-cell differentiation and lineage. Figure 3A is a population comparative graph showing that EBV-transformed cell lines grouped together and were considered a separate population from HVS-transformed cell lines. Figure 3B is a heat map indicating that genes were differentially expressed in EBV-transformed cell lines as compared with HVS-transformed lines. Further elucidating genes that showed the greatest changes in gene expression between transformants, the volcano graph (Figure 3C) shows genes that were highly expressed (right upper quadrant) and significantly down-regulated (left upper quadrant) between populations. As expected, genes showing the greatest up-regulation in HVS-transformed cell lines were genes associated with T-cell signaling and differentiation, such as CD2, indicating the transformed cell lines retain characteristics of cell lineage.

In this study, we have evaluated the transformation process using *H. saimiri* in comparison to the traditional transformation protocol using EBV. We show data that T-lymphocytes transform following *H. saimiri* infection and that, although the T-cells remain IL-2 dependent, the concentration of IL-2 can be reduced 10-fold without a significant reduction in proliferation. Furthermore, we show that *H. saimiri*-transformed lymphocytes retain characteristics of T-lymphocytes, such as cell surface antigens and gene expression as assessed by microarray analysis. A larger study is currently underway to further investigate the use of *H. saimiri* as an alternative transformant to the traditional EBV-transformation protocol.

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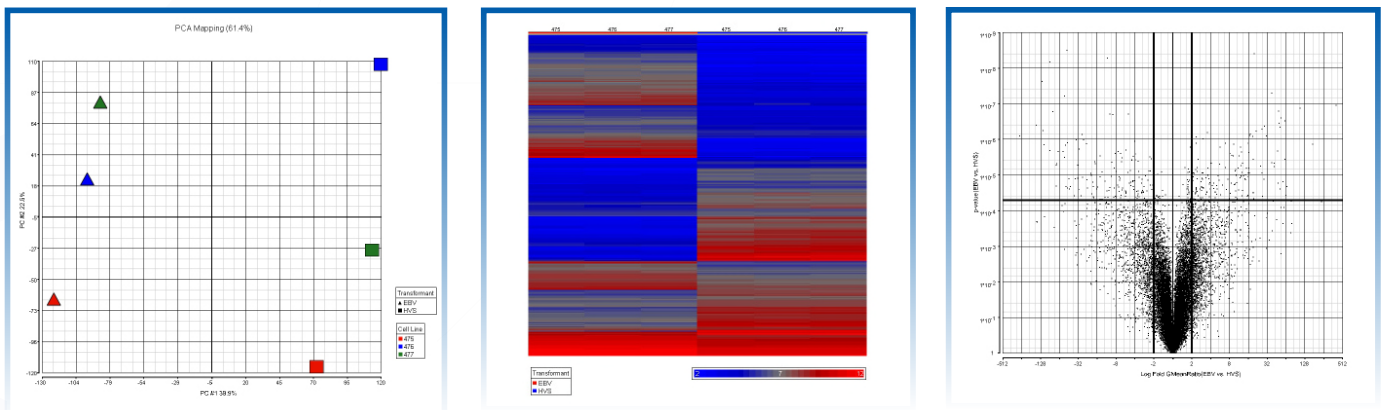

Figure 3: Gene Expression Analysis of RNA isolated from cell lines using Affimetrix Platform. (A) Principal Component Analysis (PCA) where triangles are EBV transformed cell lines and squares are *H. saimiri* transformed cell lines (B) Heat Map of differences in gene expression between EBV and *H. saimiri* transformed cell lines where down-regulation of gene expression is indicated by blue and up-regulation of gene expression is indicated by red (C) Volcano Graph of analyzed genes, points in the upper two quadrants show the greatest changes in gene expression between transformants (left is down regulation and right is upregulation)

Jay C. Leonard, Ph.D.

Associate Professor, Coriell Institute for Medical Research
Director, Cytogenetics Laboratory

Jay C. Leonard, Ph.D., joined the scientific staff of the Coriell Cell Repositories (CCR) in 1992 as director of its Cytogenetics Laboratory. He is also an associate professor for the Coriell Institute. Dr. Leonard and his staff conduct applied research to ensure that cytogenetic analyses for the CCR meet the best and most current standards of practice. They are also involved in mining the CCR's extensive set of cytogenetics data. Prior to joining the Institute, Dr. Leonard served as the Cytogenetics Laboratory Director of the Wilson Genetics Center, where his laboratory specialized in prenatal diagnosis. At that time, he was also an adjunct assistant professor in the Program for Genetics. In 1990, Dr. Leonard became a Diplomate of the American Board of Medical Genetics in Clinical Cytogenetics, and from 1991-1992, he directed the Cytogenetics Laboratory in the Pediatrics Department of the Albert Einstein Medical Center in Philadelphia, PA.

Using Copy Number Analysis as a Tool for Cytogenetics

Jay Leonard, Ph.D., Director of the Cytogenetics Laboratory, and Norman Gerry, Ph.D., Director of the Genotyping and Microarray Center, together with James Collins of Affymetrix, are evaluating the Affymetrix Human Genome-Wide SNP Array 6.0 for cytogenetic analysis.

Cytogeneticists advance the practice of clinical genetics by defining specific changes in the genome that are the basis of specific genetic diseases. Karyotype analysis, based on direct microscopic analysis of metaphase chromosomes, detects changes in chromosome number and structure in a single cell. Unbalanced structural changes involving gains and losses of chromosome segments of 5 to 10Mb or greater can be inferred from changes in chromosome banding patterns. Changes that do not involve gains or losses, but rather a reshuffling of chromosome segments, are also detected; however, breakpoint placement is more difficult.

Fluorescence in situ hybridization to metaphases detects the presence or absence of specific segments (commonly 10 to 100 Kb) and chromosome location. While this is a significant gain in sensitivity, data are only obtained for the segments that are probed and most laboratories hybridize only two or three probes at a time. The strength of these assays is detection of both balanced and unbalanced changes, even when only a small number of cells in a sample are affected.

Copy number analysis by microarray is an indirect method that measures imbalances between genomes through the relative hybridization intensities of fluorescent labeled DNAs to a series of oligonucleotide probes. Its inherent limitations are that it cannot detect balanced changes in chromosomal organization and that it is limited in the detection of low-frequency mosaic changes. However, since the majority of chromosomal abnormalities that underlie genetic disease involve gains and losses of chromosomal segments in all cells, copy number analysis promises to transform cytogenetics because of the greater sensitivity and resolution that it provides.

The Genome-Wide SNP Array 6.0 contains nearly one million single nucleotide polymorphism (SNP) probes and nearly one million copy number variant (CNV) probes. This results in marker spacing in the range of 7 Kb. The large number and regular spacing of both types of probes permit a cytogeneticist to define gains and losses of chromosomal segments with greater resolution and precision than standard cytogenetic techniques. Furthermore, loss of heterozygosity can be assessed, which is not possible with standard cytogenetic methods.

Our study includes more than five hundred cultures from the National Institute of General Medical Sciences (NIGMS) Chromosomal Aberrations Collection. Cultures selected for the study have gains and/or losses of chromosomal segments that were previously defined by G-band karyotype analysis or FISH analysis and, in some cases, by molecular analyses provided from the investigator who submitted the culture(s). These analyses have been reviewed by the NIGMS Repository staff and by two independent reviewers prior to inclusion in the NIGMS Repository catalog.

Sample preparation, hybridization and scanning were performed using GeneChip® Instrument System hardware according to manufacturer's specifications (Affymetrix, Santa Clara, CA). Analysis was performed using the Affymetrix Genotyping Console software. The samples met Affymetrix-recommended values for contrast QC (SNP) and MAPD QC (CNV). The intensities of both SNP and CNV probes were used to determine segments that varied in copy number. The segment report was restricted to regions of 100kb or greater with ten or more consecutive probes that differed significantly from the expected normalized diploid values. SNP intensities were normalized against the values for the

HapMap 270 samples. Copy number intensities for the group were normalized against themselves.

Complete Ascertainment of Large Unbalanced Rearrangements Ascertained by G-band Karyotype Analysis

Each sample previously judged to be unbalanced by traditional G-band analysis was confirmed to be unbalanced by SNP array 6.0 analysis. G-bands are alternating regions of light and dark staining along metaphase chromosomes of approximately 5 Mb in length. These are most commonly produced by controlled exposure to a proteolytic enzyme followed by Giemsa or Wright's stains. There was a high degree of consistency between the breakpoints involved in the abnormalities as called by the CNV data and by the G-band data, according to the National Center for Biotechnology Information (NCBI) and University of California Santa Clara Genome Browsers. In most cases, the calls coincided. For the other cases, the copy number data indicated the breakpoints occur in adjacent bands or sub-bands. This was not unexpected since small fractions of chromosome bands often are not visualized by microscopy.

Improved Resolution of Breakpoints

GM50122 was established from a patient with chromosome 18q deletion syndrome. Standard G-band analysis indicated 46,XY,del(18)(pter>q21.3:). FISH analysis with probes to the 18p and 18q subtelomeric region showed the deletion to be terminal. The karyotype in our catalog is therefore specified 46,XY,del(18)(pter>q21.3:).ish del(18)(VIJ-YRM2102+,18qtel11-). Copy number analysis refines the breakpoint to band 18q21.33 as seen in Figure 1.

Further analysis by Katz et al. [1] placed the breakpoint between SERPINB4 (SCCA2) and SERPINB3 (SCCA1). As seen in Figure 2, we found the breakpoint to be within SERPINB4. The difference between these calls was approximately 10 Kb.

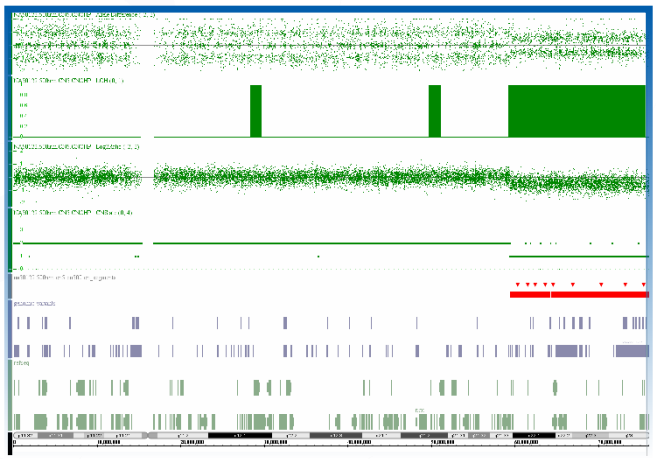

Figure 1.View of deletion 18q chromosome in GM50122. Track 1, Allele difference Track 2, LOH. Track 3, log2 ratio. Track 4, Hidden Markov Model calculation of Copy Number State. Copy number segment: deletion shown in red. Track 5, Toronto Database of Genomic Variants. Track 6, RefSeq genes. Track 7, chromosome bands. Track 8, Bp from pter.

Accurate Characterization of Contiguous Gene Syndromes: microscopic and submicroscopic

The Coriell/Affymetrix collaborative study included microscopic (>5 Mb) and submicroscopic (1 – 2 Mb) deletions and duplications. Microarray analysis consistently detected loss of critical regions to microdeletion syndromes, which had previously been FISH-confirmed by the Coriell Cytogenetics group. Successful application of copy number analysis to the characterization of FISH-verified microdeletion cell lines led us to use this microarray for other microduplication or microdeletions with molecular characterization by the submitter, but for which commercial FISH probes are not available. Our results to date show the Affymetrix GeneChip 6.0 is a reliable method for characterizing these disorders.

Syndrome	# cases
Azospermia Factor C	3
Charcot Marie Tooth 1A	1
Cri-du-chat	5
DiGeorge/Velocardiofacial	9
Duchenne and Becker DMD	10
Greig Cephalopolysyndactyly	2
Trichorhinophalangeal II	1
Miller Dieker	4
Muscular Dystrophy	7
Pelizaeus-Merzbacher	1
Prader Willi/Angelman	14
Retinoblastoma	4
Smith Magenis	8
Aniridia/WAGR/Wilms	5
Williams Beuren	8
Wolf Hirschhorn	1
X-linked Ichthyosis	6

Copy number analysis is particularly useful for reliable detection of small duplications which are not resolvable by metaphase FISH and it does so without the technical challenges inherent in fiber FISH or interphase FISH. Pelizaeus-Merzbacher disease is an X-linked recessive disorder resulting from dysmyelization of the central nervous system due to disruption of proteolipid protein-1 (PLP1). This has been related to mutation or duplication of PLP1. The PLP1 gene is included in the

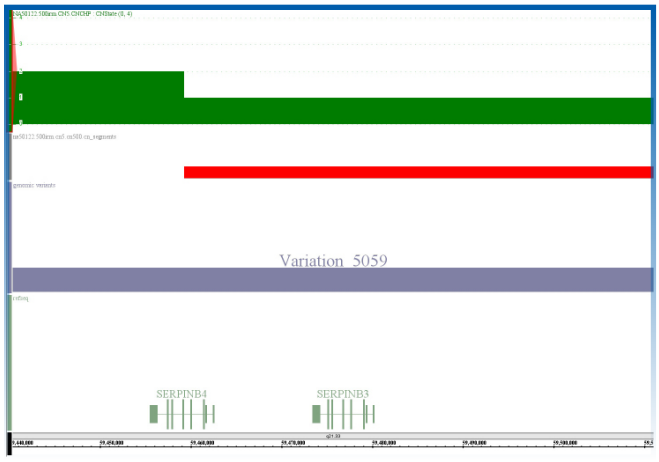

Figure 2. High Resolution View of the 18q breakpoint in GM50122. Track 1, copy number state calculated by HMM in dark green. Track 2, deleted segment in red. Track 3, Toronto Database of Genomic Variants in gray. Track 4, RefSeq genes in light green. The breakpoint for the deletion 18q is shown to be within SERPINB4 in 18q21.33. SERPINs are serine protease inhibitors. Clade B of the SERPIN multigene family is located in this area of chromosome 18; B4 and B3 are within the Copy Number Variant 5059.

Detection of Copy Number Changes Within Genes

Figure 4 displays copy number changes within the Dystrophin gene for cultures established from one carrier mother and ten males affected with Duchenne Muscular Dystrophy. Consistent results were found among relatives and between cultures established from different tissues from the same donor. The same deletion was seen in GM04100 and in GM04099, his unaffected carrier mother. Another deletion was seen consistently in two brothers, GM03181 and GM03182, and in GM3780, a lymphoblastoid cell line from the brother who was the donor of the fibroblast culture, GM03781. The submitter found no Dystrophin deletion of GM05126 by multiplex PCR. We have detected a duplication of DMD of approximately 750 Kb located within chrX:31224486-31977208.

The smallest deletion included in the study removes exon alpha of the SNRPN gene in cultures from an unaffected father and his son and daughter who are affected with Prader-Willi Syndrome [3]. This deletion was about 38 Kb in size (Figure 5).

Based on these results, copy number analysis is now a standard component of cytogenetic analysis performed by the NIGMS Human Genetic Cell Repository. We are currently in the process of posting descriptions of the disorder-specific copy number changes on our website to aid users in the selection of lines for use as reference materials.

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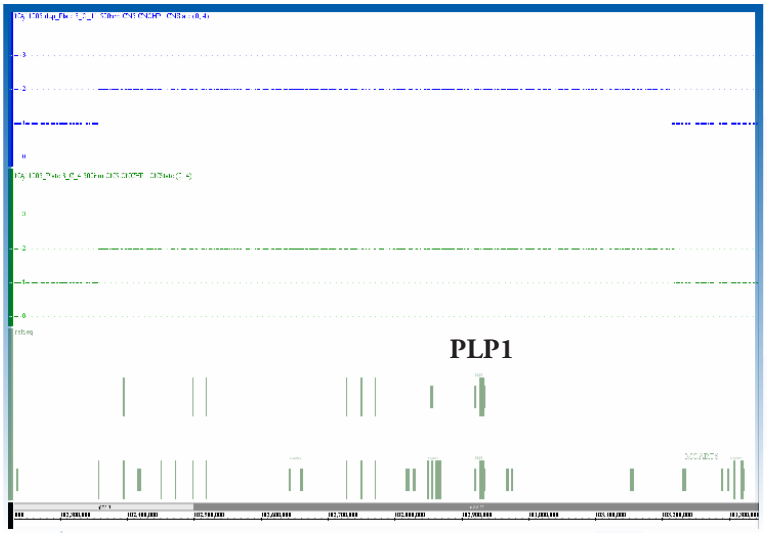


Figure 3. GM11005 a case of Pelizaeus-Merzbacher disease due to duplication of the proteolipid protein-1. Figure 3 is exported from Affymetrix Genotyping Console Browser. It shows a duplication of Xq22.1>Xq22.2 detected in two separate experiments (copy number state = red and green lines, calculated duplication = blue lines). Refseq genes, chromosome bands and base pairs from pter are given in the lower three tracks.

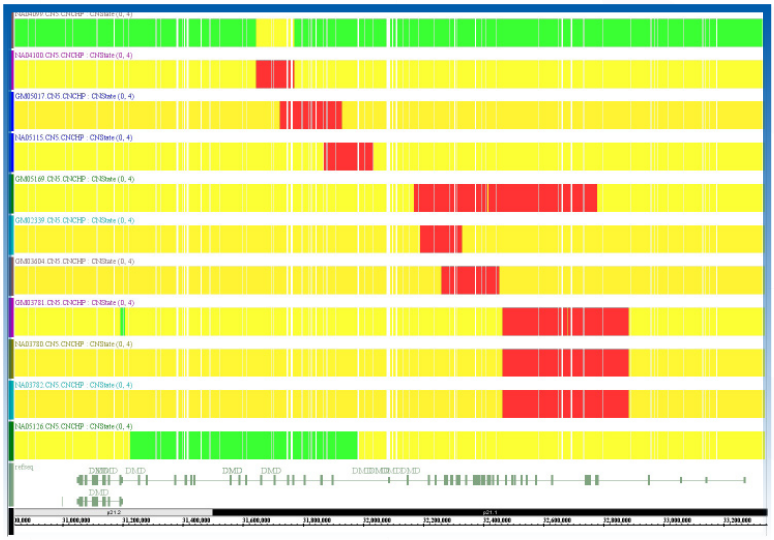


Figure 4. Consistent detection of Dystrophin deletions and in cultures from different tissues from the same person and in relatives. Figure 5 plots copy number changes in the Dystrophin genein the form of a heat map: 0 copies = red; 1 copy = yellow; 2 copies = green. Order from top to bottom: GM04099, GM04100, GM05107, GM05115, GM05169, GM02339, GM03064, GM03781, GM03780, GM03782, GM05126.

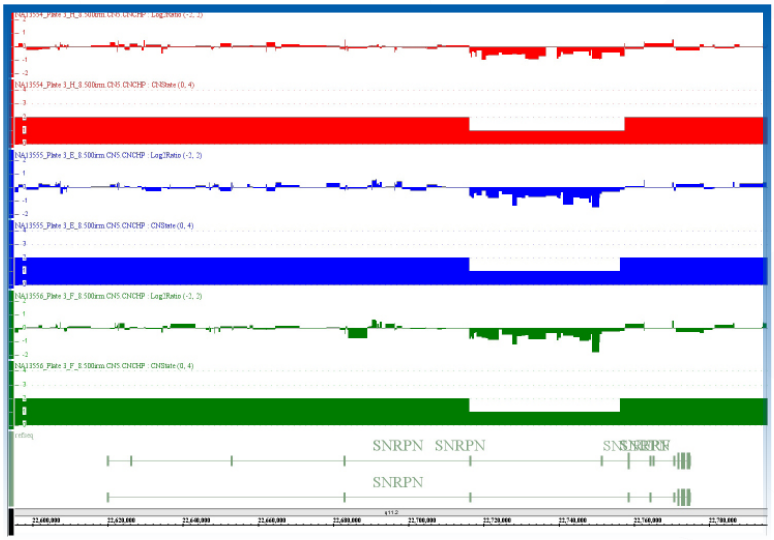


Figure 5. Consistent detection of a deletion of part of the SNRPN Gene. Three paired tracks show log2 ratio above and HMM calculated copy number state below. Color code: red GM13354 unaffected father, blue GM13355 proband PWS affected son, green GM13556 affected daughter. The Ref Seq gene track in light green shows the SNRPN gene mapped against chromosome band 15q11.2 and bp from pter band: 22.6 Mb to 22.78 Mb.

Roderick A. Corriveau, Ph.D.

Associate Professor and Scientific Program Manager,
Coriell Institute for Medical Research

Roderick A. Corriveau, Ph.D., was recruited to Coriell during the fall of 2006 as an Associate Professor and Scientific Program Manager. Dr. Corriveau spearheaded the recent successful competitive renewal of the NINDS Human Genetics Resource Center at Coriell. Also called the NINDS Repository, this international flagship project supports high throughput human genetic discovery, including by genome-wide genotype/phenotype studies. As Principal Investigator of the NINDS Repository, Dr. Corriveau works with NINDS, clinicians, clinician scientists, and the Coriell team to develop the tools necessary for discovering genetic causes and risk factors for neurodegenerative disease. In addition, Dr. Corriveau is the Coriell Principal Investigator for Huntington's disease research sponsored by CHDI, including the COHORT Repository and the Mitochondrial Stress Test initiative. He also manages two private ischemic stroke repositories, SWISS (Siblings With Ischemic Stroke) and ISGS (Ischemic Stroke Genetics Study).

The NINDS Repository for Neurological Disease: Coriell Awarded NINDS Repository Contract Through Competitive Renewal Process

Following a nationwide competition to determine the future home of the NINDS Human Genetics Resource Center, also known as the NINDS Repository, the Coriell Institute for Medical Research received a $16.3 million contract from the National Institutes of Health (NIH)/National Institute for Neurological Disorders and Stroke (NINDS). Coriell was first awarded this contract in 2002 and now, upon winning the competitive renewal, has the opportunity to continue its efforts in this important field.

Principal Investigator, Roderick A. Corriveau, Ph.D., Associate Professor at Coriell, is honored that the NINDS has entrusted this important project to Coriell: "We look forward to working closely with all participants, from private citizens who donate their samples and clinical data, to clinicians who care for patients with neurological disorders, to scientists who discover genetic risk factors for neurological and other diseases."

The NINDS Repository is an international flagship project with the stated goal of improving human health by accelerating discovery of genetic risk factors for neurological disease. The Repository does this by supporting innovative studies that use clinical data and genetic data from many thousands of de-identified study participants, a powerful approach that has been, in significant part, pioneered by the NINDS Repository and its participants.

During the first six years of the project, 23,785 individuals donated their samples and clinical data through the participation of 189 clinician scientists from nine different countries. This remarkable response resulted in more than 90 publications for discovery of genetic risk for ALS, epilepsy, Parkinson's disease, and stroke. Tourette syndrome was recently added to the collection, and NINDS plans to add other neurological disorders in the near future.

Starting on September 30, 2008, the NINDS Repository at Coriell began its work under the new contract with the goal of collecting and distributing 30,000 new and unique genetic samples.

Genetics of Common Neurodegenerative Disease Neurodegenerative

Disorders affect more than one in three people at some point during life. Most forms of neurodegenerative disease display a significant degree of heritability, and heritable human diseases are often genetically complex. Rather than being caused by mutation in a single gene, they are multifactorial, due to multiple genetic risk factors that act in combination

with one another and with environmental components (e.g. diet or airborne exposure to toxins). The complex path to neurodegenerative disease makes it challenging to discover genetic susceptibility, and thus slows the search for successful therapeutic intervention. At present, the best strategy available to solve genetically complex disease involves high-throughput genetic studies of large numbers of subjects.

Accomplishments and Goals of the NINDS Repository

The NINDS Repository was founded in 2002 to serve as a centralized national and international resource to support investigators in overcoming the challenges of managing thousands of biospecimens and associated clinical data. With samples from more than 23,000 individuals added since its inception, the NINDS collection is the fastest growing repository in the history of Coriell. During the first six years of the project, 16,737 DNA samples, 164 lymphoblastoid cell lines, and 200 DNA panels were distributed to researchers worldwide.

The NINDS Repository was among the first publicly available sources for biomaterials associated with phenotypic and genotypic data. Each sample in the NINDS collection is linked to clinical data including gender, race, age, diagnosis, and medical history for hypertension, heart disease, cancer, and neurological disorders. The NINDS Repository, playing a pioneering role in housing and distributing genotype/phenotype data, was a predecessor to dbGaP, the international resource that now serves this function through the National Center for Biotechnology Information (NCBI).

A series of samples are available from Coriell that have been characterized for mutations known to be associated with Parkinson's disease (e.g. a-synuclein triplication) and ALS (e.g. superoxide dismutase). Additionally, genome-wide single nucleotide polymorphism (SNP) data are available for many samples. In an effort to increase the rate of discovery, all of these data are freely and publicly available via the NINDS Repository catalog: *(http://ccr.coriell.org)*.

Samples and data in the NINDS Repository have been used for more than ninety studies published in peer-reviewed scientific journals including Science, American Journal of Human Genetics, and PLOS One. The pace of discovery is rapid, with reported results ranging from the discovery of disease-associated mutations (e.g. Simon-Sanchez et al, 2007) to genomics tool development (e.g. Purcell, Sham and colleagues, 2007). This state-of-the-art combination of biology and bioinformatics sets the stage for breakthroughs that will translate the public investment made by NINDS into improved diagnostic and prognostic tools, new targeted therapeutics and, ultimately, relief for millions that suffer consequences of neurodegenerative disease.

Selected publications utilizing resources from the NINDS Repository:

Paisán-Ruíz, C., Singleton, A.B., *et al.*, (2008) Comprehensive analysis of LRRK2 in publicly available Parkinson's disease cases and neurologically normal controls. *Human Mutation* 29:485-490.

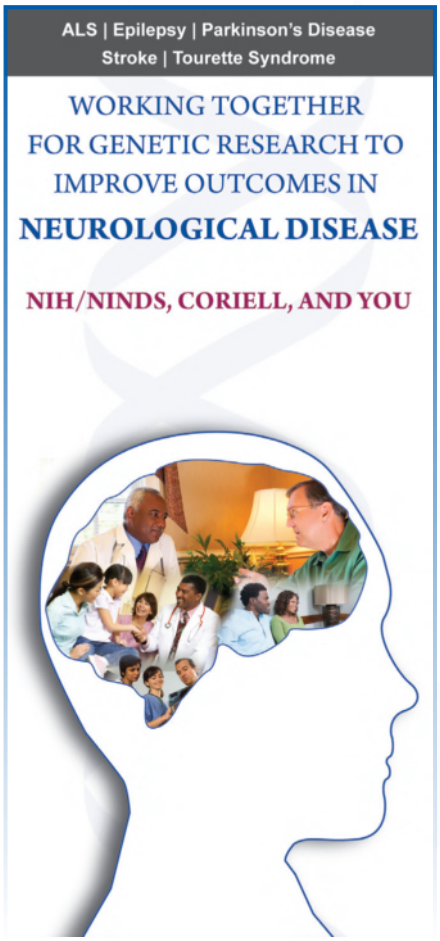
Lesnick, T.G., Maraganore, D.M., *et al.*, (2008) Beyond Parkinson disease: Amyotrophic lateral sclerosis and the axon guidance pathway. *PLOS One* 2(12): e1254.

Purcell, S., Sham, P.C., *et al.*, (2007) PLINK: a toolset for whole genome association and population-based linkage analyses. *American Journal of Human Genetics* 81(3):559-75.

Simon-Sanchez, J., Singleton, A., *et al.*, (2007) Genomewide SNP assay reveals mutations underlying Parkinson disease. *Human Mutation* 29:315-22.

Gwinn K., Corriveau, R.A., Keller, M.A., *et al.*, (2007) Amyotrophic lateral sclerosis: An emerging era of collaborative gene discovery. *PLOS One* 2(12): e1254.

For a full listing of publications that use NINDS Repository samples and data, please visit: *http://ccr.coriell.org/Sections/Collections/NINDS/Publns.aspx?PgId=490&coll=ND*



Collaborative For Discovery of Huntington Disease Markers: Call for Participants

Cooperative **H**untington's **O**bservational **R**esearch **T**rial (COHORT) is a large worldwide collaborative research effort by Huntington Study Group (HSG) research centers. The goal of this study is to discover phenotypic and biological markers of Huntington's disease (HD) that can be used to improve the treatment and prognosis of HD.

Advances in understanding the pathogenesis of HD have largely been limited by the lack of availability of suitable phenotypic data that are linked to biological markers for disease progression in individual patients. COHORT addresses this need by collecting biological samples and phenotypic data from consenting individuals who are affected by HD or who are part of an HD family. The large descriptive COHORT phenotypic database is used to study the natural history and progression of HD, while biological specimens are used to investigate relationships among HD genotypes and phenotypes. Biological specimens are provided to scientists for identification of useful biomarkers for HD.

Renewable biological resources for HD in the COHORT Repository, together with corresponding clinical data, are a rich resource designed to stimulate worldwide efforts for biomarker discovery in HD.

Who is eligible to participate in COHORT?

For individuals 18 years of age and older, the following may participate:

- Individuals who have HD or have tested positive for an HD gene expansion.
- Parents, grandparents, children, grandchildren, siblings, and spouses of individuals who have HD or have tested positive for an HD gene expansion. Family members who have tested negative for an HD gene expansion are still included.

For individuals under the age of 18, only those who have HD are eligible to participate.

How does one register to participate and provide a sample for inclusion in COHORT?

The Huntington Study Group (HSG) is an international association of more than 200 clinical investigators, coordinators, scientists, and staff from 55 participating hospitals and universities in North America, Europe, and Australia. Formed in 1993, the HSG strives to advance knowledge about the cause, process, and clinical impact of HD in order to develop and test promising therapeutic interventions.

If you are interested in learning more about this study contact the Huntington Study Group at (800) 487-7671 or **www.Huntington-Study-Group.org**, or visit the Huntington Project website at **www.huntingtonproject.org**. The HSG and the Huntington Project are supported by the Huntington's Disease Society of America, the Hereditary Disease Foundation, the Huntington Society of Canada, and the CHDI, Inc.

Donald L. Coppock, Ph.D.

Associate Professor, Coriell Institute for Medical Research
Assistant Director, Coriell Cell Repositories

Donald L. Coppock, Ph.D., is Associate Professor and the Assistant Director of the Coriell Cell Repositories (CCR). In this role, Dr. Coppock is the Principal Investigator of three NIH cell and DNA Repositories: the National Institute of General Medical Sciences' Human Genetic Cell Repository, the National Institute on Aging's Aged Cell Repository, and the National Human Genetic Research Institute's Human Genetic Sample Repository. The goal for all three is to provide the scientific community with high-quality cell lines and DNA samples from individuals with a wide range of inherited conditions, samples for investigation of aging at the cellular level and for the study of human genetic variation. Among several other projects, Dr. Coppock is developing a bioinformatic integration of the data from the CCR with the information from the other genetic databases. Previously, as the director of the Oncology Research Lab at Winthrop University Hospital in Mineola, NY, and as Associate Professor at State University of NY, Stony Brook, Dr. Coppock developed two programs in cancer research: one investigating how the malignant melanoma cell differs from the normal melanocyte in the regulation of cell growth and cell death, and the other investigating the mechanism by which normal cells arrest growth and how this differs from tumorigenic cells. In the future, his goal is to use the genetic and genomic information at Coriell to make an integrated resource for the study of human disease.

Finding a Needle in the Haystacks

One of the most powerful approaches to understanding a seemingly inherited disease is the examination of its occurrence throughout a large family. By tracking the disorder back several generations, the genetic basis for the disease may be revealed. The NIGMS Human Genetic Cell Repository has acquired many of these extended families specifically for this type of research study.

The largest of these families is an eleven-generation family of Old Order Amish from Eastern Pennsylvania with Bipolar Affective Disorder (BPAD or manic-depressive illness). These individuals are subjects of a long-term study by Dr. Janice Egeland of the University of Miami. BPAD usually onsets in the late teens or early adulthood and afflicts about one in every 100 people. It is characterized by episodes of mania interspersed with periods of depression. Dr. Egeland began her studies of four large Amish families with BPAD in 1976. The Amish have no more BAPD than any other population but have always viewed it as a medical condition that – "Siss im blut" – "it's in the blood." This longitudinal study has followed the disorder in the Amish community for more than 30 years and still continues today.

In many ways the Old Order Amish, originally founded by a small group of 20 or 30 couples coming from Switzerland in the 1700s, provide a natural laboratory for genetic research. A close-knit group that strongly discourages marriage outside the community, they have remained largely separate from the surrounding populations. Because of the genetic similarity and the insularity, they are a special population that provides a unique opportunity for the study of inherited disorders. In addition, the Amish have large families (seven children on average) and keep extensive genealogical records. As a result of these and other factors, the Amish also have a high frequency of many other inherited disorders besides BPAD, such as osteogenesis imperfecta (also known as brittle bone disease), macular degeneration, nemaline myopathy (a progressive wasting of the muscles), infantile epilepsy, cartilage-hair hyperplasia and Ellis-Van Creveld syndrome (both forms of skeletal dysplasia), and Byler disease (a fatal form of liver dysfunction).

Dr. Egeland, a Yale graduate and one of the founders of the study of genetics in the Old Order Amish, initiated her study when she first observed that BAPD was concentrated in only a few family lines. Not only does bipolar behavior contrast sharply with the community's quiet ways, making it easier to diagnose, but several possible confounding behaviors are absent or extremely rare: alcoholism, drug abuse, unemployment, divorce and violence. At the time Dr. Egeland initiated her studies in the 1960s and 1970s, there was very limited acceptance that one could find a genetic basis for a psychiatric disorder. The study was funded by the NIMH in 1976 and in 1987, Dr. Egeland published a major paper on a potential linkage to a marker for the cause of BAPD on chromosome 11 [1]. Later, as the study was extended to include additional family members and controls, the probability of this linkage

was reduced [2]. The hunt was now on using genome-wide linkage scans to identify genes that might prevent or modify the clinical manifestations of BPAD [3].

Dr. Egeland developed new methodologies of data collection that were needed for the study of a late-onset disorder. The diagnoses had to be "clinically pure" and included use of the psychiatric instrument the SADS-L (administered to subjects by trained staff), a detailed medical record, interviews with parents and neighbors, and finally, a review of the cases by a panel of experts who were not given the preliminary diagnosis or familial relationships. This approach led to a very high consensus on who was affected and a reanalysis of the data by a second group of experts confirmed the findings [4].

Dr. Janice Egeland (left) and her associate, Cleona Allen (right), shown reviewing the pedigree of the Amish family.

A pair of fortuitous connections led to the collection coming to Coriell. Early on, Dr. Egeland felt that it was important to preserve biological material for this study so that it could be analyzed with future technologies. In the 1970s, recombinant DNA technology was in its very early phase. Dr. Eliot Gershon of the NIMH suggested that she might consider placing the samples in the "Camden Repository," as the NIGMS Human Genetic Cell Repository was known at that time, and her collaborator, Dr. James Sussex, turned out to be a former medical school classmate and good friend of Dr. Lewis Coriell. This cemented the relationship and beginning in 1982, samples from the Amish bipolar study began arriving at the Coriell Institute. The family consists of four major branches: Pedigrees 110, 210, 310 and 410. The first study observations were made from the "core 110" portion of the family. The collection has most recently been extended by the addition of new generations and additional members in all families. The pedigree now reaches back eleven generations to one of the first founding families. There are 300 cell lines from 256 subjects currently available from Coriell and cell lines from the additional 227 subjects will become available in 2009.

Another critical aspect of this collection, which has now been in process for more than 30 years, is that Dr. Egeland has been in continuous contact with these Amish families and has collected longitudinal data on most of the members of each family. This is particularly important because of this disorder's late onset. To understand how the disease manifests itself before it is regarded clinically as BPAD, Dr. Egeland and her colleagues initiated an additional study on the origin of the disorder in children at risk: CARE – the Child and Adolescent Research Evaluation [5]. This ongoing, longitudinal study is looking for childhood behavioral patterns indicating a higher risk of BPAD in adulthood. All data thus far indicate that children with a higher risk of BPAD (a parent with the disorder) manifest certain features (hypersensitivity, hyper-alertness to the feelings of others, anxiety or worry, nervousness and hypersensitivity to color) at a higher frequency than study controls. Currently, Dr. Egeland and collaborators are using high-density genotyping to analyze DNA samples from the entire Amish bipolar collection.

To date, 300 cell lines are available from the Old Order Amish Bipolar Collection. All clinical data have been recently reviewed and updated. Additional cell lines from new family members will become available to the scientific community in 2009. This extended collection provides a rich resource for the advancement of bipolar affective disorder gene discovery.

Special thanks to Barbara Frederick, Project Manager, for her contribution to this article.

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David K. Moscatello, Ph.D.

Assistant Professor, Coriell Institute for Medical Research
Director, Differentiated Cell Culture Laboratory

David K. Moscatello, Ph.D., Assistant Professor and Director of Coriell's Differentiated Cell Culture Laboratory, joined the Institute in October 1999 to pursue his research interests and make differentiated cells more readily available to other biomedical researchers. The mission of the Differentiated Cell Laboratory is the isolation, culturing and characterization of differentiated cells; that is, cells other than fibroblasts and lymphoblasts. These include endothelial and smooth muscle cells, keratinocytes and epithelial cells from tissues such as skin, breast and prostate. Dr. Moscatello's current work includes the isolation, culture and characterization of adult tissue-derived stem cells. His laboratory has a particular focus on Adipose Stromal Cells (ASC), multipotent stem cells from fat that can differentiate into fat cells (adipocytes), cartilage (chondrocytes), and bone (osteoblasts). Prior to his work at Coriell, Dr. Moscatello was employed at Thomas Jefferson University in the laboratory of Dr. Albert Wong, where he was supported as an NIH post-doctoral research fellow studying the mechanisms by which a mutant epidermal growth factor receptor (EGFRvIII) results in the neoplastic transformation of cells. He was promoted to the position of research instructor, where he continued his research into the signal transduction pathways activated downstream of EGFRvIII, and the development of a therapeutic vaccine to target tumors which express this mutant protein.

Adipose Stromal Cells for Adult Stem Cell Research

It has been known for some time that there are precursor cells present in human adipose (fat) tissue, and that these small, fibroblast-like cells can be grown and differentiated into mature adipocytes (fat cells) in culture [1,2]. Thus, these cells have been generally referred to as "preadipocytes." Although they have not been routinely used as isolated populations in human transplantation, these cells may contribute significantly to the long-term survival of fresh adipose tissue grafted by injection, as they would be less susceptible than mature adipocytes to mechanical lysis during re-injection. However, a number of studies have shown "preadipocytes" to be multipotent, capable of differentiation not only into adipocytes, but also into chondrogenic, osteogenic and myogenic lineages (cartilage, bone and muscle, respectively [3,4,5]). Thus, such cells of the stromal-vascular fraction of adipose tissue are now variously referred to as processed lipoaspirate cells, adipose-tissue-derived stromal cells, adipose-derived adult stem (ADAS) cells, or adipose stromal/stem cells (ASCs). The differentiated cell types obtained from adipose stromal cells, to date, are the same as those reported for the more extensively studied mesenchymal stem cells (MSCs) from adult bone marrow, suggesting that ASCs and MSCs are quite similar. Therefore ASCs, readily isolated from "waste" fat obtained from tumescent liposuction procedures, may have the same potential therapeutic applications as MSCs, but can be much more readily harvested in large numbers. Furthermore, most patients in the United States could serve as their own donor, obviating any concerns about graft rejection or disease transmission. Thus, a potentially valuable resource awaits efficient mechanisms for harvesting, culture, differentiation and cryopreservation.

Isolation and Culture of Adipose Stromal Cells

ASCs are routinely isolated by a modification of the method of Strutt et al [6]. If intact surgical specimens are received, the fat is washed with a large volume of buffer, cleaned of obvious blood vessels or connective tissue and cut into small pieces with sterile scalpels. All of the ASC cultures currently available in the Coriell catalog were obtained from tumescent lipoaspirate waste, which eliminates the need for dissection. The cleaned fat is washed with saline and the floating, clean fat is recovered by centrifugation. The fat is then digested with collagenase to release the ASCs from the adipose tissue matrix. The ASCs are isolated by centrifugation after which the mature adipocytes float, whereas cells of the stromal-vascular fraction, including the ASCs, are found in the pellet. The cell pellet is further processed to remove debris and plated in fibronectin-coated flasks (1.5 µg/ cm2) in a low-serum medium developed at Coriell. Sufficient lipoaspirate is received to freeze the cells as primary or passage one cultures. The cells can then be tested for their capacity to differentiate into adipocytes, chondrocytes and osteoblasts in vitro using published protocols (Figure 1).

ASC preparations initiated and subcultured in standard cell culture media containing 10% FBS may have significant fractions of endothelial and smooth muscle cells 4 and exhibit inefficient differentiation in vitro. The concentrations of fetal bovine serum used in most tissue culture media blocks the efficient differentiation of ASCs into adipocytes and has been reported to change the cell surface phenotype and reduce the lifespan of MSCs relative to that obtained in reduced-serum media [7]. The media developed by Lennon et al. [8] for expansion of rat MSCs and the modification by Reyes et al. [7] for in vitro expansion of human "mesenchymal progenitor cells" do not support vigorous growth of human ASCs without the addition of fetal bovine serum.

The Differentiated Cell Laboratory at the Coriell Institute has developed very low serum (0.5%) and serum-free media for successful expansion of ASCs while maintaining stem

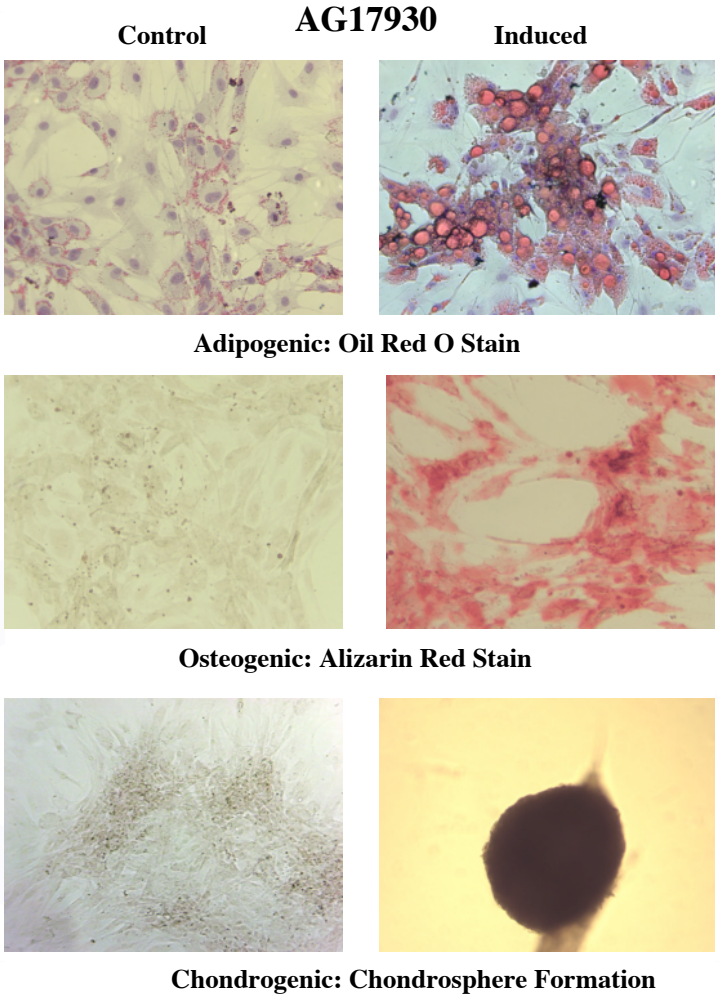

Figure 1. Multilineage Differentiation of AG17930 Human Adipose Stromal Cells. Cells were plated in 0.5% FBS ASC medium and grown to near confluence, then switched to control medium (1% human serum albumin in DMEM:Ham's F12 (1:1)) or the indicated adipogenic, osteogenic or chondrogenic differentiation media. Cultures were incubated for a further 14-21 days with twice weekly feedings, then fixed and stained as indicated. Chondrogenic differentiation was confirmed with Alcian blue staining, and osteogenic differentiation also confirmed using von Kossa staining. Left column: cells incubated in control medium. Right column: Cells incubated in the indicated differentiation media. All images at 200x magnification.

Catalog Ref	Loc_Id	Adipogenic Differentiation	Chondrogenic Differentiation	Osteogenic Differentiation
AG17870	ASC-13	+	+	w
AG17875	ASC-14	+	ND	ND
AG17902	ASC-16	+	w*	w
AG17907	ASC-17	++	+	w
AG17928	ASC-18	+	ND	ND
AG17929	ASC-19	+	ND	ND
AG17930	ASC-20	+	+	+
AG18928	ASC-31	++	ND	+
AG19304	ASC-32	++	+*	+
AG20367	ASC-7	+	+	w
AG20368	ASC-9	+	ND	ND
AG20369	ASC-12	++	+	++
AG20470	ASC-22	++	ND	+
AG20471	ASC-23	++	+	w

Table 1. Multilineage differentiation of human adipose stromal cells in the NIA catalog. - = negative differentiation; + = positive; ++ = strong positive; w = weak positive; ND = no data. *Spontaneous "chondrosphere" formation in control.

cell characteristics. Our goal is to develop a completely defined medium to permit expansion of ASCs in culture, maintaining their differentiation capacity without phenotypic alterations. The use of low-serum or serum-free media is necessary to retain differentiation potential in response to "physiological" inducers (i.e., growth factors, vitamins and other co-factors, without pharmacologic agents) [3,4,9]. The combination of a very low serum concentration and the absence of endothelial cell growth factors allows expansion of ASCs but not microvascular endothelial cells that are present in the initial stromal-vascular fraction.

Research Applications

The ability of ASCs to grow well in vitro, coupled with their multipotential differentiation capabilities, make them a valuable resource for both basic and applied research. The cells are useful for studies of differentiation into adipogenic, chondrogenic, myogenic, and osteogenic lineages, cellular physiology, and tissue engineering. The cells can also be tested for differentiation into other lineages with novel protocols devised from methods used for other stem cells. The ability to switch the cells from growth conditions to those favoring differentiation to a specific lineage enables studies of sequential changes in gene expression at the mRNA or protein level [10], as well as efforts to improve the differentiation efficiency. ASCs are also useful for comparative studies with other adult tissue-derived stem cells. A number of human adipose cell lines have been established at Coriell from lipoaspirate waste. In addition to our usual quality control assays for microbial and viral contamination, the ASC cultures are characterized with respect to culture lifespan and ability to undergo adipogenic differentiation. Since differentiation capacity of ASCs declines with increased passage (Moscatello et al., manuscript in preparation), it is important to note that all our human ASCs were frozen as primary or passage one cultures. A number of these cell lines, and the required culture media and methods, are currently available from the National Institute on Aging (NIA) Repository at Coriell. Some of these cell lines have also been shown to be capable of chondrogenic and osteogenic differentiation (Table 1). Additional cell lines will be added to the Coriell catalog in the near future.

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Patrick K. Bender, Ph.D.

Associate Professor, Coriell Institute for Medical Research
Director, Molecular Biology Laboratories
Associate Professor of Medicine, RWJ/UMDNJ Medical School

Patrick K. Bender, Ph.D., is an associate professor and Acting Director of Coriell's Molecular Biology Laboratories, as well as an associate professor of medicine at the RWJ/UMDNJ Medical School. Dr. Bender came to the Coriell Institute in 1996 to contribute to ongoing efforts within the scientific community to study human genetics and heritable disorders. The Molecular Biology Laboratories are responsible for the preparation of high-quality DNA and RNA from the repositories' biomaterials for distribution to the research community. Additional applied research efforts are in improving the quality control procedures and tests performed by the Institute, while basic research efforts focus on genetics and cell aging. Dr. Bender previously served as a research assistant professor in the department of biology at the University of Virginia, directing an independent research program in the study of gene regulation in muscle tissue, and later accepted a faculty position in the department of biochemistry at the Virginia Polytechnic Institute before accepting a position at the Coriell Institute.

Methylation Patterns at the Prader-Willi Locus in Culture Lymphoblasts

Epigenetics has been of increasing interest amongst the scientific community because of its important role in both normal chromosome organization and human disease [1]. One mechanism of epigenetic regulation known to be involved in human disease is the methylation of cytosine at CpG dinucleotides. This mechanism of DNA modification can result in gene repression and is the basis of imprinting. It is recognized as an important mechanism in the loss of imprinting in Prader-Willi/Angelman and Beckwith-Wiedemann syndromes, as well as for the X-linked disorder, Fragile X [2]. Recently, several investigators also reported that cytosine methylation patterns are aberrant in tumor cells as compared to normal cells [3]. Similar observations have been reported in immortalized cultured cells [4].

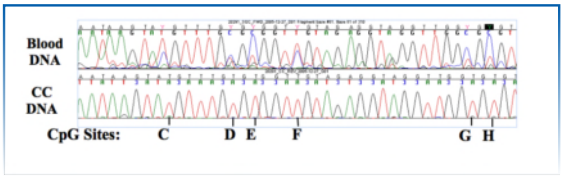

Figure 1. Region of the SNRPN CpG island sequenced after bisulfite treatment and revealing the state of cytosine methylation at the six sites labeled C through H. Six (C-H) of the 23 sites potentially methylated in the SNRP gene are illustrated. The presence of a blue and red peak at the same position indicates the occurrence of both a cytosine and thymidine, a result consistent with heterozygous methylation at that position. A red peak alone at the labeled positions indicates only thymidine was detected and that there was no methylation in the genomic DNA. Top trace is sequence from genomic DNA extracted from blood (Blood DNA). Bottom trace is sequence of genomic DNA extracted from Lymphoblast cell line (cc DNA).

Given the importance of cell lines in the study of human disease and the strength of the National Institute of General Medical Science's (NIGMS) collection at Coriell in providing such cells, we set out to investigate whether methylation patterns at the Prader-Willi locus were retained in EBV-transformed lymphoblast cell lines. For this pilot study, we analyzed the methylation of CpGs in the SNRPN gene at the Prader-Willi locus. The SNRPN gene has 23 CpG islands, labeled A through W, in an approximate 300 base pair region, including exon 1 and adjacent sequence [5].

Methods and Results

Methylation of cytosine was determined by treatment of DNA with bisulfite to convert unmethylated cytosine to uracil [6]. Methylated cytosines do not react with bisulfite and, thus, remain as cytosine. The bisulfite-treated DNA product was amplified by PCR and subjected to cycle sequencing (Big-Dye Cycle Sequencing Kit, Applied Biosystems). In the sequencing products, the bisulfite-treated DNA had thymidine nucleotides at the positions of unmethylated cytosine, whereas methylated cytosines remained cytosines. Because the CpG islands at the SNRPN gene are maternally imprinted in DNA from normal donors, the sequence pattern has both thymidine and cytosine residues. With respect to methyl cytosine, normal samples are heterozygous – one allele methylated and one allele unmethylated.

The 23 CpG dinucleotides in the SNRPN gene are normally heterozygous methylated [5]. The methylation status of this region was determined in 14 samples of DNA isolated from lymphoblast cell lines. Each of these cell lines had a matching sample of DNA isolated directed from the whole blood from which the cultures originated. Only one of the 14 samples from the cell lines exhibited a hypomethylation pattern at most of the 23 sites (Figure 1, "CC DNA"). The other 13 samples showed normal heterozygous methylation. We repeated the assay using the matching sample of DNA from whole blood. Results are shown in Figure 1 ("Blood DNA"). The sequence indicates the normal heterozygous methylation pattern at this gene.

We investigated the possibility that the loss of methylation results from a loss of chromosome 15. We used the DNA from the culture line without methylation for array CGH on bacterial artificial chromosome (BAC) arrays. The results demonstrated that this cell line is dip-

loid. However, the resolution of the BAC chip was not sufficient to rule out the possibility of a small deletion of the Prader-Will locus in the maternal chromosome 15.

Discussion

Initial results indicated that one out of the 14 samples tested lost imprinting methylation at the Prader-Willi locus as a result of EBV-transformation and/or culturing. Because there was little evidence of methylation at this locus, it is likely that the loss of methylation occurred early in the transformation process and is the dominant phenotype in this cell line. The other 13 cell lines exhibit a normal methylation pattern; however, we were only investigating one locus. If we had investigated multiple methylation loci, we may have found that aberrant methylation patterns are more common. We are continuing these studies by using methods that allow a more global assay of methylation patterns.

Special thanks to Lorraine Toji, Ph.D., for her contribution to this article.

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Joseph L. Mintzer

Executive Vice President and COO, Coriell Institute for Medical Research

Joseph L. Mintzer joined the Coriell Institute for Medical Research in June 1993 as Vice President and Chief Operating Officer. He was promoted to Executive Vice President, Chief Operating Officer in March 2005. In his role as EVP/COO, he is responsible for managing all of the administrative and financial operations of the Institute.

2007 – 2008: New Opportunities, Challenges and Achievements

The past year has been a year of achievement and of challenge, as Coriell – much like every other organization in the country – has had to push forward through the economic struggles of the current age. However, with the support of generous philanthropic donors and though the hard work of the Coriell Staff, the Institute has continued to flourish as a leader in the field of scientific research and education. In addition, several opportunities presented to the Institute during the current year offer the promise of a new and exciting period to come. On behalf of the Board of Trustees and Coriell Staff, I am pleased to share several highlights of the 2007/2008 year that exemplify Coriell's ongoing success in scientific research, in the banking and storage of cells and in the commitment to education.

Winter 2007

• In December 2007, Coriell Institute officially launched its personalized medicine research study at a Congressional briefing hosted by Rep. Robert Andrews (D-NJ) in Washington, D.C. The Coriell Personalized Medicine Collaborative (CPMC) is an evidence-based approach to determine the utility of genomic information in health management and clinical decision-making, and to discover variants that elevate risk of complex disease and those that affect drug toxicity and efficacy.

• Coriell received a $1 million grant from the William G. Rohrer Foundation to help establish the computing and information technology resources necessary for the storage, analysis and mining of the enormous amounts of information generated by the CPMC.

• Coriell received a three-year, $500,000 grant from the W.W. Smith Charitable Trust to fund the first genome scan of an African-American cohort.

• The Institute hosted top-ranking officials from the US Department of Health and Human Services (HHS) for a roundtable discussion about the CPMC and challenges of implementing personalized medicine.

• Coriell received approval from the Camden County Improvement Authority for the purchase of $6 million in tax-exempt financing for renovation of laboratory space.

Spring 2008

• Coriell and Cooper University Hospital officially launched their CPMC partnership with the goal of facilitating the education of medical professionals about genome-informed medicine.

• Coriell sponsored the 27th Annual Science Fair, where approximately 300 sixth to twelfth graders and 70+ judges participated.

• Coriell celebrated the enrollment of its 1,000th participant in the CPMC in April.

• National Geographic visited Coriell to film a documentary on the science of aging.

• US Senator Robert Menendez (D-NJ) visited Coriell and enrolled into the CPMC to promote the importance of preventative healthcare.

Summer 2008

• The CPMC Informed Cohort Oversight Board (ICOB) – the advisory board responsible for determining which genetic variants are "potentially medically actionable" and reported to CPMC participants – held its first meeting.

• CPMC enrolled its 2,000th participant.

• The RNR Foundation awarded Coriell a three-year, $600,000 grant to help support activities of the CPMC's ICOB.

Fall 2008

• Coriell's Genotyping and Microarray Center received Clinical Laboratory Improvement Act (CLIA) certification.

• Coriell won the competitive renewal of the five-year, $16.3 million NINDS Human Genetics Resource Center contract.

• Coriell attended the HHS invitation-only National Summit on Personalized Healthcare, where top executives and leaders from the public, private and academic sectors came together to create strategies to accelerate the integration of personalized healthcare into clinical practice and healthcare delivery.

• The CPMC reached the milestone of 3,000 participants, putting the study well on its way to reaching its goal of 10,000 participants by the end of 2009.

Contract Services from the Coriell Institute

The Coriell Institute for Medical Research offers a menu of services employing protocols that are continually refined and validated by Coriell's experienced scientific staff.

- **Biomaterials Banking**

Banking, cryopreservation and fail-safe storage of cell lines, DNA and other biomaterials

- **Cell Culture**

Establishment of Cell Cultures

Primary cultures:
Endothelial
Epithelial
Fibroblast
Smooth muscle
EBV transformed lymphoblast cultures

Maintenance of Seed and Distribution Stocks

Growth of cultures to expand cell stock for molecular and cytogenetic analyses

Immunofluorescence Characterization

Detection of Mycoplasma

PCR or microbiological assays
Identification of mycoplasma species

- **Genotyping and Microarray**

CLIA-Certified
Affymetrix and Illumina Platforms
Genome-Wide Genotyping
Targeted Genotyping
Expression Profiling
Copy Number Variation
Custom Genotyping Panels

- **Cytogenetics**

Karyotype Analysis

Human
Mouse (especially mouse embryonic stem cell lines)
Rat
Wide range of mammalian species
Wide range of cell types

Fluorescent In Situ Hybridization (FISH)

Transgene insertion site mapping

Copy Number Analysis and Molecular Karyotyping

Spectral Karyotype Analysis (SKY)

Human
Mouse
Rat

- **Molecular Biology and Nucleic Acids**

Isolation of High Molecular Weight DNA
Isolation of Polyadenylated (mRNA) and Total RNA
Whole Genome Amplification (WGA)
DNA Sequencing

- **Stem Cell Biomaterials Available by Custom Order**

Unprocessed cord blood
Cord blood mononuclear cells
CD34-enriched cells from cord blood
CD34-depleted cells from cord blood
Cord blood mesenchymal cells

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