Dear Friends and Family,

We apologize for the late arrival of this newsletter but with busy schedules and a complex article to write, it took some extra time to get the newsletter completed. We hope that you will find it worth waiting for there is a lot of ACD research information herein.

Late last year, the doctors at Baylor College of Medicine in Houston, Texas took some time out of their busy schedules and holiday plans, to give us a tour of their labs and ACD research. In the main article of this newsletter, we summarize their efforts. We are so grateful for their continuing efforts to find a cause and ultimately a cure for ACD. This past holiday season we gave thanks to all of you who made possible the $30,000 grant award to Baylor last year and to the doctors at Baylor.

Our visit to Baylor reminded us of the amount of financing required to answer all the questions we have about ACD. We encourage all of you to continue your efforts to raise money for our ACD Restricted Research Fund at NORD.

This newsletter also launches the “Learning Series,” a periodic overview of information to aid in understanding genetics and ACD research.

Steve and Donna Hanson
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**Research Team**
The research at Baylor is a collaboration between researchers of different disciplines. This team consists of Dr. Stockton (geneticist), Dr. Langston (pathologist) and Dr. Sen (molecular cell biologist) each having their own laboratories and support teams.

Dr. Stockton is Assistant Professor, Departments of Molecular and Human Genetics, Medicine, and Ophthalmology and Director, Kleberg Genotyping Center. He also works in clinical genetics. He provided an overview of all the possible genetic transmission methods for ACD that will be presented in a future ACDA newsletter. He has several areas of research focus and many publications. For more information see [http://imgen.bcm.tmc.edu/molgen/facultyaz/stockton.html](http://imgen.bcm.tmc.edu/molgen/facultyaz/stockton.html).

Dr. Langston is a professor specializing in pediatric lungs and has been at Texas Children’s Hospital/Baylor for 25 years. As a pathologist, she is an expert on ACD diagnosis from lung tissue and her opinion is sought out worldwide for confirmation on lung tissue samples. She is a past Secretary/Treasurer for the Society for Pediatric Pathology and named one of the “Best Woman Doctors” in Jan 2003. Dr. Langston is co-author and collaborator on many journal articles.

Dr. Sen is the director of the Child Health Research Center (CHRC) Molecular Core Laboratory. The laboratory provides DNA sequencing and DNA synthesis services to the CHRC awardees and their mentors and Baylor faculty at large.

Dr. Bejjani was the original geneticist on the ACD research team and was instrumental in receiving the initial ACD research funding. Dr. Bejjani is currently a research professor at Washington State University at Spokane and co-director of molecular diagnostics at Sacred Heart Medical Center. After his move to Washington, he continued collaboration with Baylor College of Medicine on the original ACD study grant. He is now heavily involved in genetic research on the human eye.

**ACD Research History**
The exact cause of ACD is not known, but many professionals suspect it is probably a genetic disease and that is where the known research has been focused. Baylor has received two different ACD research grants which will be discussed below:

- A National Institute of Health (NIH) grant in 2003 that has been completed
- A NORD research grant that was awarded in 2005 and is currently in progress

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NIH Grant (2003)
The first ACD related research grant that we are aware of was to the Baylor College of Medicine research team for a feasibility study on collecting and preparing DNA from ACD families. The specific aims of the research were:

- **Phase 1:** Identify affected individuals, evaluate clinically both patients and family members, analyze the pedigrees, obtain blood and tissue samples and isolate genomic DNA from families with ACD.
- **Phase 2:** Determine the genetic map position of the gene(s) responsible for ACD by conventional linkage analysis in out bred North American families.
- **Phase 3:** Identify the gene responsible for ACD by a combined positional/functional cloning approach and study its DNA sequence, pattern of expression as a prelude to elucidating its role in lung, heart, gut and kidney development.

Baylor received an initial grant for Phase 1 to find and collect tissue samples from ACD patients and blood samples from family members. Primarily through the ACDA member’s authorization for release of tissue samples, the research team has assembled the world’s largest collection of ACD tissue samples for study. It also has blood samples from many parents and siblings. Even though this research grant has now expired, Baylor and Dr. Bejjani continue to collect additional samples which are critical for ongoing research.

During Phase 1, the researchers demonstrated that they could gather the needed tissue and blood samples and they could amplify the DNA to obtain quantities necessary for research.

*DNA amplification is a technique where a short, well defined piece of DNA (not an entire DNA structure) is copied to make an exact duplicate of the original. The technique is repeated and the small piece of DNA is amplified many times, in an exponential manner (2 become 4, then 8 then 16 and so on into the millions). With more quantity of DNA available, analysis is made much easier.*

Based on this successful Phase 1 feasibility study, Baylor applied for a follow on research grant to continue the NIH study for the next phase. The application was not approved because NIH reviewers indicated that while the Baylor approach seemed plausible, it was very difficult research and they felt the chance for success was low. Baylor revised and resubmitted the application, but it was again denied.

National Organization of Rare Diseases (NORD) Research Grant (2005)
Each year, NORD awards research grants that provide seed money to academic scientists studying new treatments or diagnostics for rare diseases. Baylor submitted a research proposal to NORD last year and received a research grant for $30,000 in October 2005. This was one of 14 research grants made by NORD in 2005 and this particular funding was made available through the hard work and generous contributions of ACDA members, family and friends to the NORD Alveolar Capillary Dysplasia Restricted Research Fund.

Specifically, the grant is to Partha Sen, PhD, Baylor College of Medicine for "Recruitment of New Families and Histochemical Studies on the Lung Specimens of Patients with Alveolar Capillary Dysplasia".

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This grant is being used for histological screening on lung tissue samples. This involves comparing the ACD tissue samples to “normal” tissue samples to look for presence or absence of specific proteins that are known to affect lung development. Significant variations in proteins between the ACD and normal tissue samples could provide insight to which gene(s) might be involved and provide a direction for additional research.

*Genes are responsible for controlling the production of proteins, so variations in certain proteins would implicate problems the corresponding gene(s) that control them.*

Since genetics research is a very complex field where the possible combinations and interactions of variables that contribute to diseases is mind boggling, techniques are used to limit some variables. For example, in this study, Dr. Langston has a set of criteria for classifying tissue samples as ACD and each sample must meet all the criteria for it to be included in the study. Using this process increases the likelihood that each sample will contain the unique ACD genetic signature and not introduce “noise” into the study. The criteria are:

- misalignment of pulmonary veins
- under development of gas exchange
- deficient capillary number
- lymphatic deficiencies (in about 1/3 of cases)
- thickening of the pulmonary arteries

Another technique being used at Baylor to reduce variables in the comparative study of ACD tissue and “normal” lung tissue is that the “normal” tissue will be from babies that have undergone the same types of medical care (drugs, ventilation, etc.) as the ACD patients in order to minimize any affects from different medicines and treatments. The study will be looking for 6-8 proteins that are all known to be involved in lung development. Currently the tissue samples are being prepared and the testing solutions are being standardized.

What became apparent during conversations with the research team is the enormity and complexity of finding a specific gene(s) that causes a disease. However, with the “map” from the Human Genome project, sophisticated equipment and analytical methods, rigorous detective work, fast computers, statistics and sometimes a little luck – genetics puzzles are being solved.

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**Meet our New ACDA Families**

It is with mixed emotions that we introduce two new families that have contacted the ACDA since the last newsletter. Please take the time to introduce yourself, offer support and share the story of your child (children).
Safe Arrivals

Congratulations to the [redacted] family of New Jersey on the arrival of [redacted].
Born on [redacted].
(Sister of [redacted].)

Congratulations to the [redacted] family of The Netherlands on the arrival of [redacted].
Born on [redacted].
(Brother of [redacted].)

Congratulations to the [redacted] family of Canada on the arrival of [redacted].
Born on [redacted].
(Sister of [redacted].)
Memorial Garden

We are dedicated to remembering the birth dates of our family's babies who are not here to share our lives. Please pause to remember them.

Please let us know if we have inadvertently omitted your baby's name or if you do not wish to have your baby's name included in this section. You can email us at sdesj@verizon.net.

A Candle for Your Child

Today you will light a little white candle and say aloud your child's name.

For one fateful day, your life was changed.
The holidays will never be the same.

Today you will light a little white candle and hang an ornament on a special tree. Who would have thought you would be in this place? Sharing your child as a memory.

Today you will light a little white candle, a small gesture to some others. Here we share the pain of our loss, with Mothers, Fathers, Sisters and Brothers.

Today you will light a little white candle, and as you gaze into the flame, may comforting memories flood your mind, as you proudly say your child's name.

Today you will light a little white candle, With us your compassionate friends... For all of us know that though they're not here, our love for them NEVER ends.

Author Unknown

http://www.acd-association.com
LEARNING SERIES
This is a series of articles about the technical aspects of genetics, research methods and associated topics to provide a broader understanding of ACD related information. This Newsletter’s focus is to provide a more detailed review of the research that is being conducted at Baylor under the NORD research grant.

Baylor Research – What Are They Looking For, Why and How???

The following section is for those with a desire to understand more of the technical aspects of the Baylor research team’s approach. I will attempt to keep the following overview and information at a layperson level as much as possible, provide biology “refresher” information as needed to aid in your understanding of the information and define technical terms. However, I acknowledge that parts of the following sections will still be very technical and I apologize for that, but the overall subject matter is quite complex. I will try to convey some details that I hope will educate the reader and enable a better understanding of just what is involved in the research required to understand what causes ACD.

ACD is a lung disorder and the current Baylor study is focusing on proteins that are related to lung vasculature and overall lung development and their regulation. A general understanding of the lung structures and terminology will assist in a better understanding of the descriptions of the proteins that are being investigated in the study.

Alveolar Capillary Dysplasia involves development problems between the air sacs in the lungs (alveolus) and the blood vessels (capillaries) that prevent proper transfer of oxygen to the blood. Although one may think of ACD as a lung disorder it also involves the circulatory system that interacts with the lungs. To provide a better overall understanding of the biology involved, we will start with a brief overview of the mammalian circulation system, then “zoom” in on the lung and then down to the alveolar capillaries.

Circulatory System
- Oxygen is necessary for cells in the body to survive and the blood is how the oxygen is provided to the body. The lung’s role is to transfer oxygen into the blood, which is circulated around to the cells in the body by the heart. The lungs also transfer carbon dioxide out of the blood so it can be expelled when you exhale. Blood is moved through the body by the heart which is actually two pumps that work together to move the blood in your body as shown in the illustrations to the right. The right side of the heart (labeled 1 and 11) pumps deoxygenated blood (shown in blue) to the lungs (labeled 3) where it receives oxygen. The left side of the heart (labeled 4 and 5) then pumps the oxygenated blood (shown in red) from the lungs to the rest of the body. This describes the basic blood flow to the lungs, which will be discussed more in the section below about the lungs.
**Lungs** – When you breathe, air flows down your windpipe and branches out toward each lung through tubes called bronchi as shown in the illustration to the right. Each bronchi enters its lung and begins a series of branches, called the bronchial or respiratory tree. Each branch continues to branch into smaller and smaller bronchioles.

The smallest bronchioles are covered with thin-skinned air sacs that allow gasses to pass through them. These sacs, which contain alveoli, are called alveolar sacs. The lungs have many millions of alveoli, which provide a large surface area for gas exchange.

The alveoli are covered in interlinking capillaries through which blood flows. The alveoli and the capillary walls form the respiratory membrane. Capillaries have walls composed of a single layer of cells, the endothelium. This layer is so thin that molecules such as oxygen and water can pass through them by diffusion and enter the tissues.

A closer illustration of capillaries on an alveolar sac (shown in purple) where the oxygen inside the alveolar sac is transferred to the blood in the capillaries.

### Alveolar Capillaries

Alveolar-capillary contact surface area is estimated at from 50 to 400 square meters.
For maximum oxygenation efficiency, proteins control the amount of blood passing through a capillary on alveoli to match the amount of gas exchange. When there is not enough gas in that alveoli, certain pulmonary vessels tighten, slowing the flow of blood. When there is a lot of gas exchange occurring, those vessels widen, allowing for more blood to pass through. Proteins cause a similar process happen to bronchioles. When an alveoli has a lot of carbon dioxide in it, the bronchioles that connects it to the outside air widen, allowing it to leave more quickly.

At this point, the terms in the medical condition “Alveolar Capillary Dysplasia” hopefully begin to make more sense. It is abnormal development (Dysplasia) of the alveolus (the sacs that contain air) and the capillaries (minute blood vessels). This abnormality impedes the transfer of oxygen from the air sacs to the blood. This lack of oxygen transfer causes low blood oxygen levels and results in “blue baby” symptom that ACD babies encounter.

This understanding of basic lung biology and terminology should provide the foundation of knowledge needed to understand why the proteins discussed below are being studied by Baylor.

**What is Baylor looking for and Why?**

Proteins are required for the structure, function, and regulation of the body's cells, tissues, and organs, and each protein has unique functions (examples are hormones, enzymes and antibodies). The Baylor study will be looking for proteins that are known to be involved with lung related development, specifically those that are involved with the formation of blood vessels in the lungs. The proteins to be investigated are listed below along with a brief overview of why they are of interest. The study will be looking for the presence/absence (or significant variations) of these proteins between normal and ACD lung tissue.

**VEGF** (vascular endothelial growth factor) a substance made by cells that stimulates the formation of new blood vessels, a process called angiogenesis. VEGF also acts as a mitogen for vascular endothelial (vessel lining) cells, stimulating these cells to divide and multiply. There are several isoforms of VEGF that will be studied. **These proteins are responsible for stimulating growth of blood vessels (capillaries) in different types of tissue.**

**Flk-1** (Fetal liver kinase 1) one of the two VEGF receptors and is crucial for vascular development. **This protein receives the signal from VEGF to enable the blood vessels to grow.**
**eNos3** (endothelial cell nitric oxide synthase) Produces nitric oxide (NO) which is implicated in vascular smooth muscle relaxation. It mediates vascular endothelial growth factor (VEGF)-induced angiogenesis in coronary vessels and promotes blood clotting through the activation of platelets. May play a significant role in normal and abnormal limb development. 

This protein regulates the growth of new blood vessels that are stimulated to grow in response to VEGF.

NOTE: This protein produces Nitric Oxide which is the same gas that is sometimes used as a treatment on ACD babies. The purpose of this treatment is to relax the blood vessels in the babies lungs so they open and allow increased blood flow and transport of oxygen in the babies.

**HIF-1a** (hypoxia-inducible factor 1, alpha subunit) Regulates the physiological responses to low oxygen levels and is expressed when oxygen levels are below 6%. Several dozen HIF-1-regulated genes have been identified so far, including genes coding for proteins involved in angiogenesis, energy metabolism, erythropoiesis, cell proliferation and viability, vascular remodeling and vasomotor responses. These proteins are produced in response to low oxygen levels and can stimulate the growth of new blood vessels or cause existing ones to increase in size to allow more blood flow.

**pVHL** (von Hippel-Lindau protein) at least one of its jobs is to regulate which genes are "on" and "off" in a cell. Cells without normal pVHL behave as though they are deprived of oxygen even when they are not. Because these cells are “stuck” on they can not turn off the gene that produces VEGF (VEGF stimulates blood vessel formation). When adequate levels of oxygen are present, this protein turns off the gene that produces VEGF thereby stopping the growth of more blood vessels.

**TIE 2** (tyrosine kinase) important in angiogenesis, particularly for vascular network formation. It may regulate the endothelial cell proliferation, differentiation, and proper patterning. TIE appears to be acting downstream of VEGF receptors. This protein may control how the blood vessels grow and how they branch.

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Possible relationship of the proteins for this study. All proteins are related to various aspects of blood vessel growth.

- **VEGF** – sends signal to grow blood vessels
- **FLK-1** – receives signal to grow blood vessels
- **pVHL** – turns off the gene(s) that produce VEGF when there are adequate oxygen levels.
- **TIE 2** – controls the blood vessel growth pattern
- **eNOS3** – regulates the amount of blood vessel growth
- **HIF-1a** – stimulates growth of blood vessels and affects operation.

**Angiogenesis** – growth of new blood vessels.

**Coronary** – refers to vessels that carry blood and oxygen to the heart.

**Erythropoiesis** - is the development of mature red blood cells.

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**How are proteins found?**
With this high level understanding of which proteins are being investigated and why, the next section discusses how the investigation is being performed. Keep in mind that the study is comparing ACD tissue samples to those of “normal” tissue samples to look for differences in presence/absence of proteins. The technique for determining the presence of proteins in tissue samples is very interesting and is indicative of many of the sophisticated methods required for genetic research.

Proteins are too small to be seen without specialized microscopes because they are only a few nanometers in size. To complicate things, there are about 10,000 different proteins in each cell so it is impractical to look for proteins directly. Instead a “visual tagging” system is used which takes advantage of the ability of antibodies to selectively attach to a specific protein. The process used is:

- use an antibody that will attach to the specific protein of interest,
- alter the antibody so that it contains a fluorescent marker
- apply the antibody solution to the tissue samples and the fluorescent tagged antibodies will attach to any specific proteins that are present
- view the sample under a fluorescent light to find the desired proteins. The desired proteins are found indirectly by looking for the antibodies that glow under the fluorescent light.

**What’s an antibody?**

*Layman’s explanation of antibodies – When you get sick, your immune system goes on the offensive to fight off the infection that is making you sick. Your body produces what are called antibodies which attach to the infection and kill it or block its function so you can get well. The cells that generated the antibodies will reproduce in your system and always be available in the future to attack that same specific infection because they will recognize its “signature”. The vaccinations you receive take advantage of this process by introducing a crippled infection that your body will create antibodies for and this protects you from future exposure to this specific infection.*

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Nanometer - one billionth of a meter. For reference a human hair is approximately 100,000 nanometers across.

[Technical definition of Antibody]
A type of protein made by plasma cells (a type of white blood cell) in response to an antigen (foreign substance). Each antibody can bind to only one specific antigen. The purpose of this binding is to help destroy the antigen. Antibodies are part of the immune system.
How are the desired antibodies obtained?
A specific antibody is needed that will attach to each unique protein to be studied. Obtaining the antibody of interest typically begins by injecting the specific protein into a mouse or a rabbit. The animal’s immune system recognizes the protein as a foreign body and begins producing antibodies that will attach to and fight the protein. The cells that produce the desired antibody(s) are then removed from the animal and fused with a cancer cell (because they can live indefinitely in a laboratory culture). This fused cell is called a hybridoma and it will multiply rapidly and indefinitely (since it is a cancer cell) and produce the desired antibody(s). Many antibodies are commercially available from specialized companies.

What is the current status of the research at Baylor?
The tissue samples of ACD tissue samples and “normal” tissue samples are being prepared for the comparative testing. The dilution of the antibody solutions has been finalized by testing against standards for validation. The antibody solutions will then be applied to the tissue samples to begin the comparative tests.

What will the results of these tests reveal?
Hopefully, these tests will identify one or more differences in the proteins between the tissue samples. The most definitive results would be presence of a protein in one sample that is absent in the other. Significant differences in the amount of a protein between the samples could also be meaningful.

If a difference in a protein(s) is found, what is next?
Differences in protein(s) from this test would provide a starting point for a more rigorous genetic study. Recall that gene(s) are responsible for producing proteins in the body so any differences in proteins would point toward a problem in the gene(s) that produce that specific protein. The associated linkage between many proteins and the gene(s) that control them is known from other research. Therefore, assuming that differences in a protein can be found from this study then a different type of study would be needed to further investigate differences in the genes.

How do genes make proteins?  
Most genes contain the information needed to make functional molecules called proteins. The journey from gene to protein is complex and tightly controlled within each cell. It consists of two major steps: transcription and translation. Together, transcription and translation are known as gene expression.

During the process of transcription, the information stored in a gene's DNA is transferred to a similar molecule called RNA (ribonucleic acid) in the cell nucleus. Both RNA and DNA are made up of a chain of nucleotide bases, but they have slightly different chemical properties. The type of RNA that contains the information for making a protein is called messenger RNA (mRNA) because it carries the information, or message, from the DNA out of the nucleus into the cytoplasm.
Translation, the second step in getting from a gene to a protein, takes place in the cytoplasm. The mRNA interacts with a specialized complex called a ribosome, which "reads" the sequence of mRNA bases. Each sequence of three bases, called a codon, usually codes for one particular amino acid. (Amino acids are the building blocks of proteins.) A type of RNA called transfer RNA (tRNA) assembles the protein, one amino acid at a time. Protein assembly continues until the ribosome encounters a "stop" codon (a sequence of three bases that does not code for an amino acid).

The flow of information from DNA to RNA to proteins is one of the fundamental principles of molecular biology. It is so important that it is sometimes called the “central dogma.” More information on this will be provided in a future Newsletter.

**Has Baylor looked at any genes that could be causing ACD?**

Baylor has investigated 2 genes that “code for” (produce) proteins that are related to the structures affected in ACD. The genes were the Emap2 and bmpr2 genes. The reason these genes were reviewed:

**BMPR2**

11 (bone morphogenetic protein receptor, type II) Researchers have identified more than 60 BMPR2 mutations that can cause primary pulmonary hypertension (a key symptom of ACD is persistent pulmonary hypertension). About half of these mutations interrupt assembly of the BMPR2 protein, reducing the amount of this protein in cells. Other mutations prevent the BMPR2 protein from reaching the cell surface, or alter its structure so it cannot receive or transmit signals.

It remains unclear how BMPR2 mutations cause primary pulmonary hypertension. Researchers suggest that a mutation in this gene promotes cell proliferation or prevents cell death, resulting in an overgrowth of cells. Cell overgrowth in the smallest arteries in the lungs can narrow the diameter of the arteries and increase resistance to blood flow. When these small arteries are constricted, blood pressure increases to overcome the increased resistance, leading to the characteristic signs of primary pulmonary hypertension.

**EMAP2**

12 (endothelial monocyte activating polypeptide 2) a gene that plays a regulatory role for apoptosis. Research indicates that it is present at areas of tissue remodeling. During development, many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing many organs and tissues. EMAP2 recruits macrophages to sites of apoptosis.

**Summary**

The NORD research grant is being used by Baylor to compare samples of ACD lung tissue to normal lung tissue. The objective is to determine if there are differences in the proteins in the tissue samples with the focus on several proteins that are known to be involved in blood vessel formation. Any differences in proteins that are found would direct future research to the gene(s) that control that protein(s). Such a genetic study would investigate the gene sequences in the ACD tissue to try to determine which gene(s) are responsible for ACD (this is based on the assumption that the cause of ACD is genetic). If a genetic cause is eventually found and understood, then it would then be possible to develop a test to determine who carries the ACD gene(s).

Regardless of the outcome of this current study, additional time and funding is going to be required to understand ACD, so keep we all need to keep up fundraising activities in memory of our babies.  

Steve
I would like to give credit to various sources from which some of the information for this article was derived or adapted.