







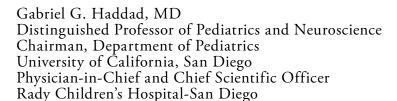
Dear friends and colleagues,

It is my privilege to present to you the inaugural Scientific Discoveries report from UC San Diego (UCSD), Department of Pediatrics and Rady Children's Hospital (RCH).

I am extremely proud of the achievements of our researchers and scientists. The work highlighted here is a just a summary and an illustration of what goes on in the research corridors pertaining to developmental diseases and conditions at both the School of Medicine at UCSD and RCH. This is indeed the result of the work of the spectacular faculty that we have in our Department.

This is an exciting time as we continue to grow and adapt to a changing world, remaining motivated, and responsive to improving clinical care through research and discoveries.

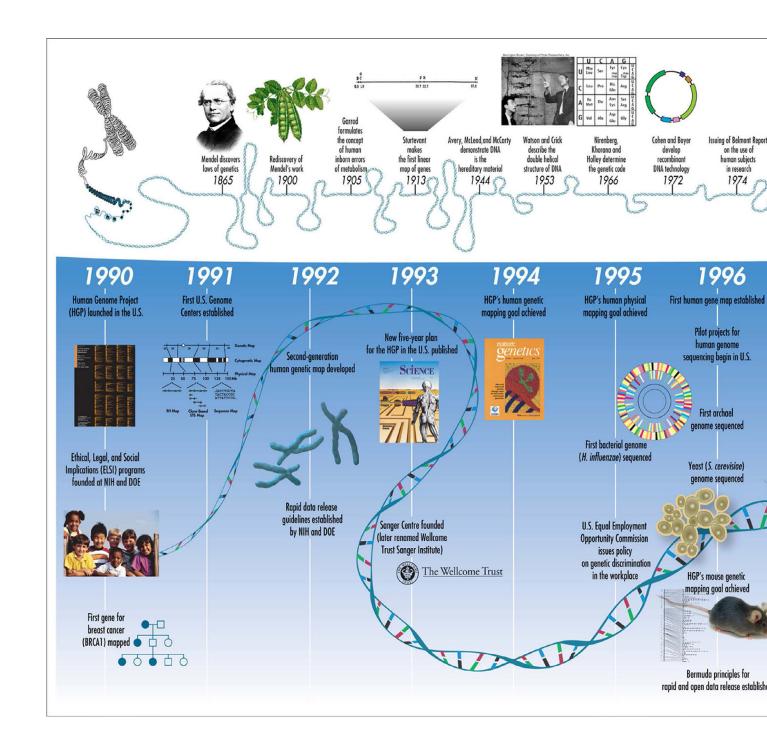
One major reason to be excited about putting together such a summary is the hope that this will open the doors a little wider to potential collaborations with faculty around the nation and the world. I trust that such collaborations can bring investigators closer to solving diseases of childhood and hence building a better society for the future.

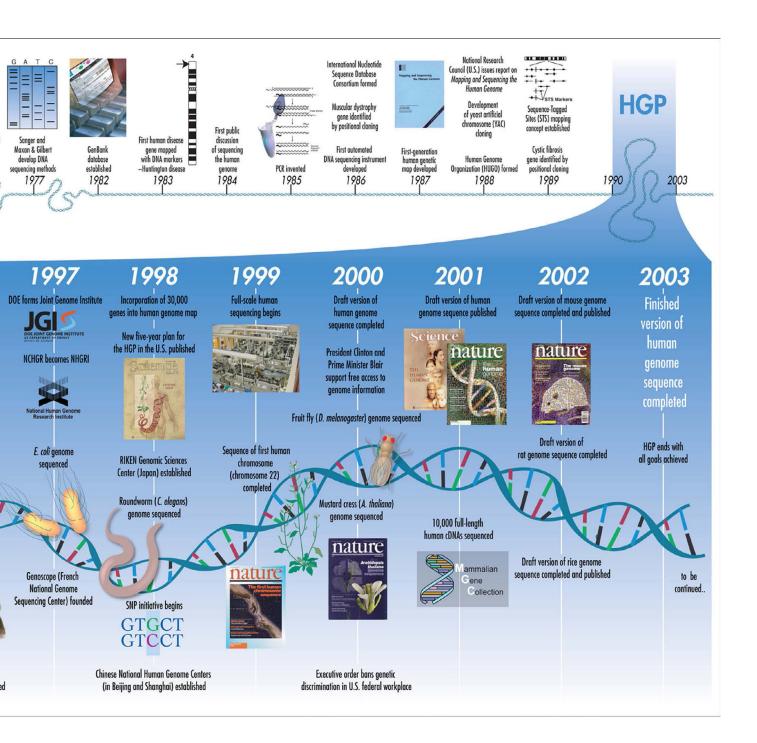




"Improving
Clinical Care
through
Research and
Discoveries"

An amazing two decades...





LOOKING FORWARD

TO A BRIGHT FUTURE...

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SCIENTIFIC DISCOVERIES

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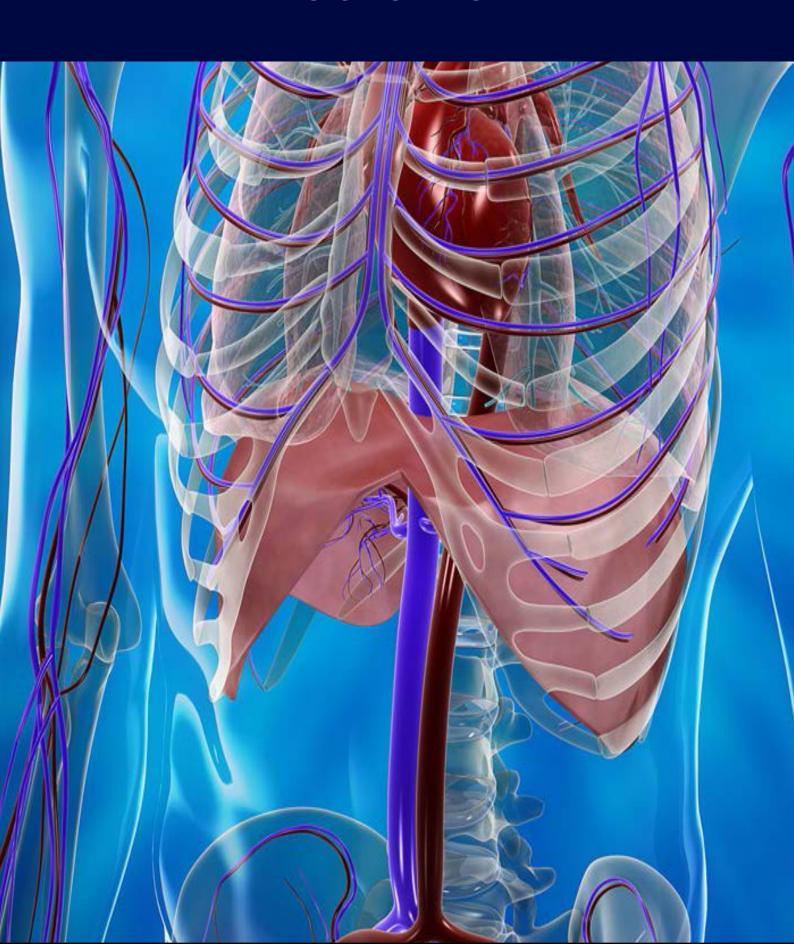
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James Hagood, M.D.
Division Chief, Respiratory Medicine
Professor of Pediatrics,
Respiratory Medicine

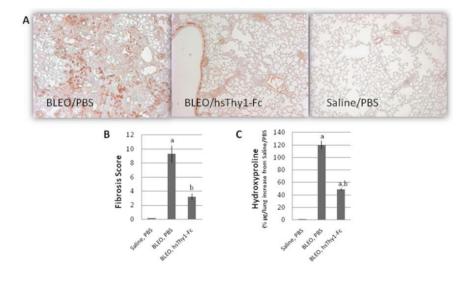
THY-1 PROMOTES RESOLUTION OF PULMONARY FIBROSIS

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease of unknown cause that usually results in death or lung transplantation, and has no effective treatment. Our lab has focused on understanding how lung fibroblasts (scar-forming cells) become activated in the context of pulmonary fibrosis. Previously we have shown that the cell surface protein Thy-1 inhibits the profibrotic differentiation in lung fibroblasts, and that Thy-1 expression is silenced in the fibrotic lesions in patients with IPF. In cultured fibroblasts, cells lacking Thy-1 have uninhibited activation of the profibrotic growth factor transforming growth factor-



beta (TGF-b). This activation can be inhibited by transfection of the Thy-1 gene, as well as addition of Thy-1 as a soluble mediator. These findings led us to speculate that soluble Thy-1 (sThy-1) may have a therapeutic effect in lung fibrosis. In mice in which pulmonary fibrosis is induced with the chemotherapeutic drug bleomycin, sThy-1 given intravenously as a single dose promotes near-complete resolution of fibrosis, even when given when fibrosis is well

established (see Figure). Furthermore sThy-1 reverses the profibrotic differentiation of fibroblasts in injured lungs, as indicated by expression of the contractile protein alpha smooth muscle actin (aSMA). These finding suggest that Thy-1, or a derivative, may be a useful therapy for idiopathic pulmonary fibrosis.



Recombinant human sThy1-Fc (1 mg/kg) or phosphate-buffered saline (PBS) was given, iv., in female C57BL/6 WT mice Day 14 after Bleomycin (BLEO, 5 U/kg) treatment by orotracheal intubation/MicroSprayer. At 21 days, lungs were harvested and processed for immunohistochemistry (IHC) using anti-@SMA (A), fibrosis score (B), and hydroxyproline, a measure of collagen deposition (C). ANOVA was calculated for each Mean (± SE, n=3-5/group). a, b p<0.05 compared to saline/PBS and BLEO/PBS, respectively.



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Distinguished Professor,
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Children's Hospital

Human High Altitude Genomic Studies and Discoveries

The hypoxic conditions at high altitudes present a challenge for survival, causing pressure for adaptation. The Haddad Lab has thus far studied two populations at high altitude: One in Ethiopia and the other in the Andes. Although it has been proposed that there are genetic factors that contribute to adaptation to high altitude, these have remained largely unproven. We report here on the first whole genome re-sequencing-based analysis identifying genes that can modulate adaptation of Ethiopian highlander as well as in the Andes. Interestingly, many high-altitude

denizens (particularly in the Andes) are maladapted, with a condition known as chronic mountain sickness (CMS) or Monge disease. To decode the genetic basis of this disease, the whole genomes of 20 Andean subjects (10 with CMS and 10 without) were sequenced and compared. It was discovered 11 regions genome-wide with significant differences in haplotype frequencies consistent with selective sweeps. In these regions, two genes (an erythropoiesis regulator, SENP1, and an oncogene, ANP32D) had a higher transcriptional response to hypoxia in individuals with CMS relative to those without. It was further found that down-regulating the orthologs of these genes in flies dramatically enhanced survival rates under hypoxia, demonstrating that suppression of SENP1 and ANP32D plays an essential role in hypoxia tolerance. Our study provides an unbiased framework to identify and validate the genetic basis of adaptation to high altitudes and identifies potentially targetable mechanisms for CMS treatment.

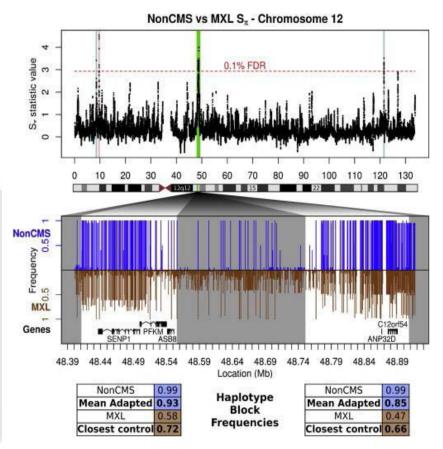
In the Ethiopian study, crosspopulation tests were used to identify regions with significant loss of diversity, indicative of a selection sweep, very similar also to our analytical approach for the Andean population. A single region on chromosome 19, containing 8 protein coding genes and 135 SNPs, was significant for all subjects (13 subjects). To validate the potential role of the candidate genes, we tested whether these genes played a role in hypoxia tolerance in Drosophila. There was 2 to 4 fold increase in survival rate when specific genes were down-regulated that include the Drosophila orthologs of CIC, LIPE and PAFAH1B3.

These studies show that whole genome sequencing of Ethiopian and Andean highlanders reveals the importance of evolutionary conserved genes that can modulate tolerance to low oxygen environments.

Zhou D, Udpa N, Ronen R, Stobdan T, Liang J, Appenzeller O, Zhao HW, Yin Y, Du Y, Guo L, Cao R, Wang Y, Jin X, Huang C, Jia W, Cao D, Guo G, Gamboa JL, Villafuerte F, Callacondo D, Xue J, Liu S, Frazer KA, Li Y, Bafna V, Haddad GG. Whole-genome sequencing uncovers the genetic basis of chronic mountain sickness in Andean highlanders. Am J Hum Genetics. 2013 Sep 5;93(3):452-62.

Zhou D, Haddad GG. <u>Genetic analysis</u> of hypoxia tolerance and susceptibility in <u>Drosophila and humans</u>. Annu Rev Genomics Hum Genetics. 2013;14:25-43.

HOW THE BODY ADAPTS TO HIGH ALTITUDE



Profile of genomic candidate regions. Profile of the 2 candidate regions that are significant in non-CMS versus CMS and non-CMS versus the MXL population, considered as control with similar genetic background. One of the statistics in which both of these regions are significant (Sp,MXL) is plotted across chromosome 12 (upper panel). Five distinct regions exceed the 0.1% FDR threshold—the two highlighted in light blue do not have a major frequency differential between the non-CMS and MXL populations, whereas the one highlighted in pink is similar in other controls. The remaining two are prioritized and highlighted in green. The SNP frequencies in the area encompassing these two, part of q13.11, are plotted in the middle (zoomed on). In this plot, the two prioritized regions are highlighted in white (lower panel), and other regions are shaded in gray. As can be seen, in both regions, there is an almost complete fixation (almost or reaching a frequency of 100%) of a haplotype in the non-CMS population that is at a much lower frequency in all lowlander and maladapted CMS subjects.

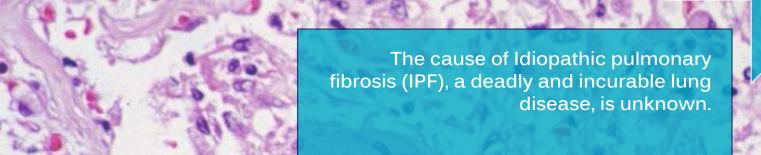
Epigenetic Modifications Change Expression of Relevant Genes in Pulmonary Fibrosis

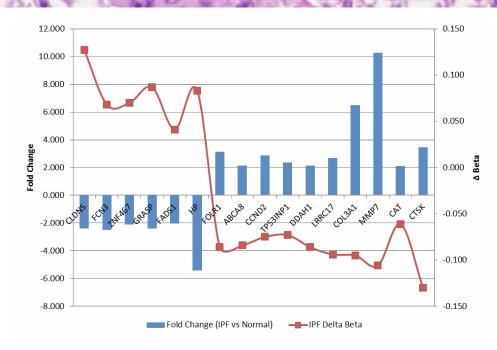
The cause of Idiopathic pulmonary fibrosis (IPF), a deadly and incurable lung disease, is unknown. Although a small number of genetic mutations have been identified, there is no single genetic abnormality that explains the majority of cases. Epigenetic changes modify gene function without alterations in gene sequence. DNA methylation is an important epigenetic mechanism, which often occurs in response to environmental stimuli and is crucial in regulating gene expression. This lab had previously identified silencing of the gene Thy-1 by DNA methylation in the lesional fibroblasts in IPF, associated with profibrotic differentiation of the cells. To determine the global DNA methylation changes in IPF and their effects on gene expression, we compared total DNA methylation and DNA methyltransferase expression in IPF and normal control lung tissues. IPF and normal tissues were subjected to comparative analysis of genomewide DNA methylation and RNA expression using Illumina BeadChip platforms. IPF samples demonstrated higher DNA methyltransferase expression without observed alterations in global DNA methylation. Genome-wide differences in DNA methylation status and RNA expression were demonstrated by array hybridization. Among the genes whose DNA methylation status and RNA expression were both significantly altered, 16 genes were hypermethylated in DNA associated with decreased mRNA expression or vice versa (see Figure). The majority of these genes were known to play a role in regulation of fibrosis in the lung and other tissues. Furthermore, we validated alteration in CLDN5, ZNF467, TP53INP1, and DDAH1 genes at the level of DNA methylation status, RNA, and protein-level expression.

Changes in DNA methylation correspond to altered mRNA expression of a number of genes, some with known and others with previously uncharacterized roles in IPF, suggesting that DNA methylation is important in the pathogenesis of IPF, and may represent a new target for development of therapies.

Sanders YY, Ambalavanan N, Halloran B, Zhang X, Liu H, Crossman DK, Bray M, Zhang K, Thannickal VJ, Hagood JS. Altered DNA methylation profile in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012 Sep 15;186(6):525-35.







Graphic representation of the 16 genes that were different in both data sets by P value. The columns represent fold changes in mRNA expression (idiopathic pulmonary fibrosis [IPF] vs. normal); the solid line represents the $\Delta\beta$ of DNA methylation status for IPF (vs. normal).



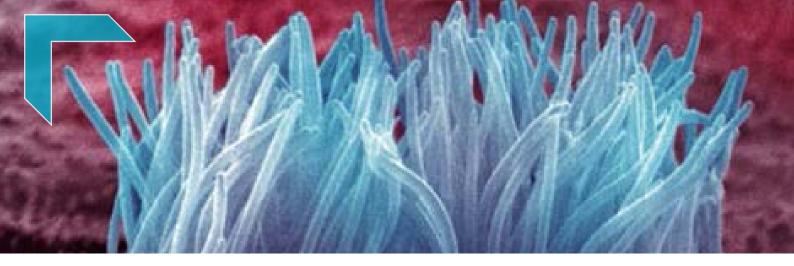
Paul Quinton Ph.D.
Professor of Pediatrics
Nancy Olmsted Chair, Pediatric
Pulmonary Research
Respiratory Medicine

More Than a Buffer

Everyone knows bicarbonate (HCO₃-) is the major extracellular buffer of body fluids. Physiologists hate it because it precipitates Ca++ from their solutions and alters pH unless it is bubbled constantly with CO₂. But we discovered that HCO3- has another previously obscure, but truly critical role in keeping us healthy. HCO₃ is essential for normal mucus formation. In fact, we found that the defect in HCO3- secretion in Cystic Fibrosis, known for its thick sticky mucus, is a major, if not the principal cause of the pathogenic mucus in this generally fatal genetic disease. To be perfectly straight, it was the defects in mucus and in HCO3 secretion in CF

that inspired us to look for an effect of HCO₃ on mucus. When we incubated lengths of small intestine in solutions with and without HCO₃, we observed that far less mucus was secreted in the absence of HCO₃. We also found that the viscosity of mucus secreted without HCO₃ was significantly higher than with HCO₃. Subsequently, we found the same phenomenon occurred in other mucus secreting systems such as the uterine cervix, airways, and cultured A549 cells.

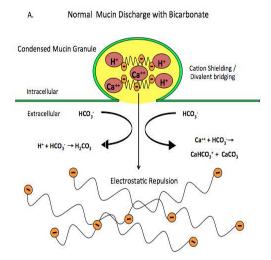
Moreover, a colleague has now shown that mucins secreted from CF intestines into high concentrations of HCO₃ are essentially normalized. We believe the most plausible explanation for this effect is due to the fact that gel forming mucins are extremely large, highly anionic molecules that are stored as compacted intracellular

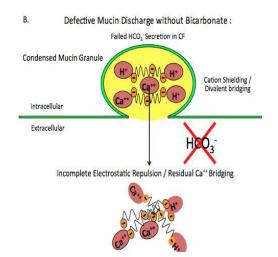


mucin granules. Within the granules, low pH and divalent Ca++ cations shield the fixed anions on the mucin molecules and prevent the enormous electrostatic forces on the fixed anions from violently expanding the mucin molecules, which would occur without cationic shielding. Upon release from the granule, Ca++ and H+ must be removed rapidly to allow the mucins to expand up to 1000x granule volume to form the tangled networks of mucin macromolecules that give mucus its vital properties that protect and clear epithelial surfaces. HCO₃ is perfect for the task of attracting Ca⁺⁺ and H+ away from the anions of the mucins and thereby allowing the negative electrostatic forces to explode the mucins into massive molecular strings to form mucus. Without HCO, Ca++ maintains intra- and intermolecular bridges that obviate mucus expansion as we see in Cystic Fibrosis mucus secretions. It seems likely given the crucial role of mucins

in protecting the intestines and airways, as well as numerous other delicate interfaces of the body, that alterations in HCO₃ probably result in pathologies other than CF. In CF it seems likely that maneuvers that would result in a timely secretion of increased amounts of HCO₃ will serve to ameliorate CF pathology.

Yang N, Garcia MA, Quinton PM. Normal mucus formation requires cAMP-dependent HCO3- secretion and Ca2+-mediated mucin exocytosis. J Physiol. 2013 Sep 15;591(Pt 18):4581-93.





WHY DO SMALL AIRWAYS LOOK THE WAY THEY DO?

There are few structures in the body for which the physiological function remains unknown.

So it may seem strange that there should still exist such a structure in our lungs. The surfaces of bronchi and bronchioles are characterized by accordion like folds and pleats that run parallel with the tubular axis of the airways (Fig. A). The teleological reason for this arrangement is that it allows the airway to expand and contract with inspiration and expi-

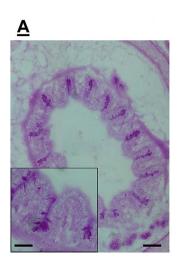
ration without stretching or contracting the epithelial lining, but we have discovered there is more to it than that. That is, every airway constantly faces a critical challenge to maintain just the right depth (volume) of fluid on its luminal surface. Too much fluid and we drown; too little fluid and we cannot clear debris and noxious materials from the airways and we die of obstruction from infections, inflammation, and accumulated mass.

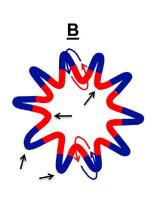


Until now, it was widely believed that this critical function was managed by the airway epithelium, which alternately absorbed fluid when fluid became excessive, and then, when fluid was inadequate, reverse its transport direction and begin to secrete fluid. This concept was unsettling for us because to our knowledge there is no example of any epithelium that physiologically reverses fluid transport direction; that is, an epithelium is destined to a lifetime of either secreting fluids or absorbing fluids. In contrast to this dogma, we discovered that the epithelial surface does both secrete and absorb, simultaneously. Clearly, no cell can go in both directions at once so that the only way concurrent secretion and absorption could occur would be for two types of cells, secretory and absorptive, to be active at once. Indeed, our results from transepithelial electrophysiological measurements showed that both activities occur concurrently, and are well corroborated by additional immunohistochemical results that show that the cells in the base of the pleats exhibit staining for a protein (NKCC1) characteristic of fluid secretory cells, which is essentially absent from the cells on the luminal folds. Fig. B shows the accordion like structure of the epithelia of the airways and indicates fluid secretion at the base of the pleats with absorption at the ends of the folds. This structure-function relation would allow management of surface fluid by control of the relative activities of absorption *vs.* secretion. It also provides for mobilizing mucus secreted at the base of the pleats (dark stained cells.)

We are tempted to surmise that under normal conditions, the respiratory cycle may be an integral part of airway fluid control. That is, when expiration shrinks airway diameter, the pleats come closer together and drive the secreted fluid within them into the lumen where it is readily absorbed by the continuously absorbing cells toward the ends of luminal folds. Inspiration reverses the process and withdraws luminal fluid down into the pleats away from the folds. With this cyclical activity, constant secretory activity wets the surface while constant absorptive activity prevents excessive accumulations. To wit, each local area of airway surface draws its own bath.

Shamsuddin AK, Quinton PM. Surface fluid absorption and secretion in small airways. J Physiol. 2012 Aug 1;590(Pt 15):3561-74. Yang N, Garcia MA, Quinton PM. Normal mucus formation requires cAMP-dependent HCO3- secretion and Ca2+-mediated mucin exocytosis. J Physiol. 2013 Sep 15;591(Pt 18):4581-93





Schematic model and structure of small airways. A, schematic model of an epithelium composed of cells that maintain the ASF layer by secreting (blue) fluid until the volume becomes excessive, whereupon the same cells reverse the direction of transport to absorb (red) fluid. When the volume depletes, the process reverts to secretion again. Appropriate fluid levels in this model would be maintained by continuous oscillations between absorption and secretion along the airway. (Blue represents secretory capacity; red represents absorptive capacity.) B, schematic model of separate groups of absorptive and secretory cells in airway epithelia that maintain the fluid layer covering the small airways. Cells located within the pleats of the epithelia secrete (blue) fluid while cells located around the folds in the epithelium concurrently absorb (red) secreted fluid. The volume of ASF is maintained by the relative activities of the two processes. C, cross section of small airway epithelium illustrating its arrangement into folds and pleats that run parallel to the longitudinal axis of the airway (perpendicular to the plane of the page). Mucous cells are generally found in clusters at the contraluminal bases of the pleats (intensely stained cells; inset scale bar = $50 \mu m$). This arrangement suggests a distribution of secretory cells toward the bottom of the pleats proximal to the mucus cells with absorptive cells located more distally toward and along the luminal folds in the epithelium. (Periodic acid Schiff staining, diameter \sim 1 mm; scale bar = 100 μ m.)

Cardiology





Paul Grossfeld, M.D.
Associate Clinical Professor
of Pediatric Cardiology
Director of Pediatric Cardiology
Fellowship Program

DELETION OF ETS-1, CAUSES A SPECTRUM OF NEURAL CREST DEFECTS IN MICE

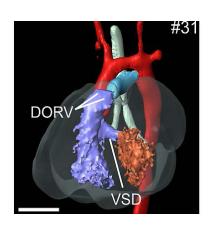
Ventricular septal defects (VSDs) are among the most common congenital heart defects (CHDs) in liveborn infants and often require surgical closure to ensure a normal life expectancy. To date, understanding the molecular and cellular causes of VSDs is limited because most animal models lack isolated VSDs that are associated with individual human

genes. It was discovered that deletion of the E26 avian leukemia 1, 5' domain (ETS-1) gene, a member of the ETS-domain transcription factor family is deleted in all patients with Jacobsen syndrome with congenital heart defects. Deletion of ETS-1 in mice causes VSDs and an overriding aorta (double outlet right ventricle) in a pure C57/B6 background, and additional structural heart defects in a mixed genetic background including tetralogy of Fallot and a single ventricle. Loss of ETS-1 in mice causes a cardiac neural crest cell (cNCC) migration defect, specifically a decrease in the number of migrating cNCCs. Interestingly, conditional deletion of ETS-1 in the neural crest did not result in the generation of heart defects, but did cause hydrocephalus, kyphosis and white color coat in a subset of mice.

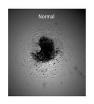
Taken together, our studies indicate that ETS-1 has a critical role in neural crest cell function, likely essential for cell fate determination for the cNCCs required for proximal outflow tract development, and via a cell-autonomous mechanism involving brain, spine, and melanocyte development. The results of these studies will provide important and novel insights into the function of the ETS-1 gene in multi-organ development, and in the pathogenesis of these neural crest-derived structures.

Ye M, Coldren C, Benson W, Goldmuntz E, Ostrowski M, Watson D, Perryman B, Grossfeld P. Deletion of ETS-1, a gene in the Jacobsen

> syndrome critical region, causes



Knockout of ETS-1 affects cardiac NCCs migration





Top 3-D cardiac MRI reconstruction of E15.5 ETS-1 KO heart. Center and right: Explanted neural crest tissue from WT and ETS-1 KO ED8.5 mouse embryos, showing decreased migration and numbers of explanted cardiac neural crest cells in ETS-1 KOs.

ventricular septal defects and abnormal ventricular morphology in mice. Human Molecular Genetics, 2010 Feb 15;19(4):648-56.

Kazumi Fukatsu, Lizhu Lin, Yan Yin, Maoqing Ye, Le Ann Sinha-Garrett, Michael Ostrowski, Marianne Bronner, Paul Grossfeld. Deletion of ETS-1, a gene in the Jacobsen syndrome cardiac critical region, causes congenital heart defects through a cardiac neural crest cell migration defect. Weinstein Meeting on Cardiovascular Development, May, 2013 Tucson (Poster)

Migration distances of cultured cardiac NCCs in normal and ETS-1 knockout mice, 24h post-explantation. Normal (n=8) ETS-1 KO (n=7) P<0.005 150-300 0-150 150-300 Migration distance from neural tube, 24 hours post-explantation (μm) Percentage of total cells migration distance, # of cells/ 24 hours post-explantation embryo' 0-150 um 150-300 um 300- um 290 Normal 42% 41% 17% 4% KO 63% 33% 187

Left: Conditional deletion of ETS-1 in neural crest cells, causing a pigmentation defect, kyphosis, and hydrocephalus (wildtype littermate control above).



Gabriel Haddad, M.D. Chair, Department of Pediatrics Distinguished Professor, Department of Pediatrics and Neurosciences Chief Scientific Officer & Physicianin-Chief Rady Children's Hospital

ATHEROSCLEROSIS IN THE PULMONARY ARTERIES OF MICE?

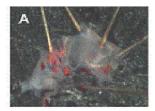
Patients with obstructive sleep apnea, who experience episodic hypoxia and hypercapnia during sleep, often demonstrate increased inflammation, oxidative stress, and lipid disturbances. Because of this and other findings in the literature, it was hypothesized that

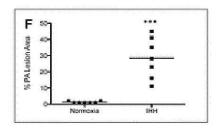
sleep apnea patients are predisposed to the development of atherosclerosis. To determine whether this is the case and in order to dissect the mechanisms involved, we developed an animal model in mice whereby these animals are exposed to intermittent low oxygen and high CO2 (IHH). Two- to threemonth-old low-density lipoprotein receptor deficient (Ldlr(-/-)) mice were fed a high-fat diet for 8 or 16 wk while being exposed to IHH for either 10 h/ day or 24 h/day. Plasma lipid levels, examination of pulmonary artery and aortic atherosclerotic lesions, and cardiac function were then assayed. Surprisingly, atherosclerosis in the aorta of mice exposed to IHH was similar compared with controls. However, in these experimental IHH mice, atherosclerosis was markedly increased in the trunk and proximal branches of the pulmonary artery, even though plasma cholesterol and triglycerides were lower than in controls. Hemodynamic analysis revealed that heart function was abnormal: right ventricular maximum pressure and isovolumic relaxation constant were significantly increased in IHH exposed mice and left ventricular % fractional shortening was reduced. In conclusion, 1) Intermittent hypoxia/hypercapnia remarkably accelerated atherosclerotic lesions in the pulmonary artery of Ldlr(-/-) mice and

2) increased lesion formation in the pulmonary artery was associated with right and left ventricular dysfunction.

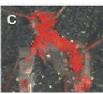
These findings raise the possibility that patients with obstructive sleep apnea may be susceptible to atherosclerotic disease in the pulmonary vasculature, an observation that has not been previously recognized.

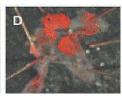








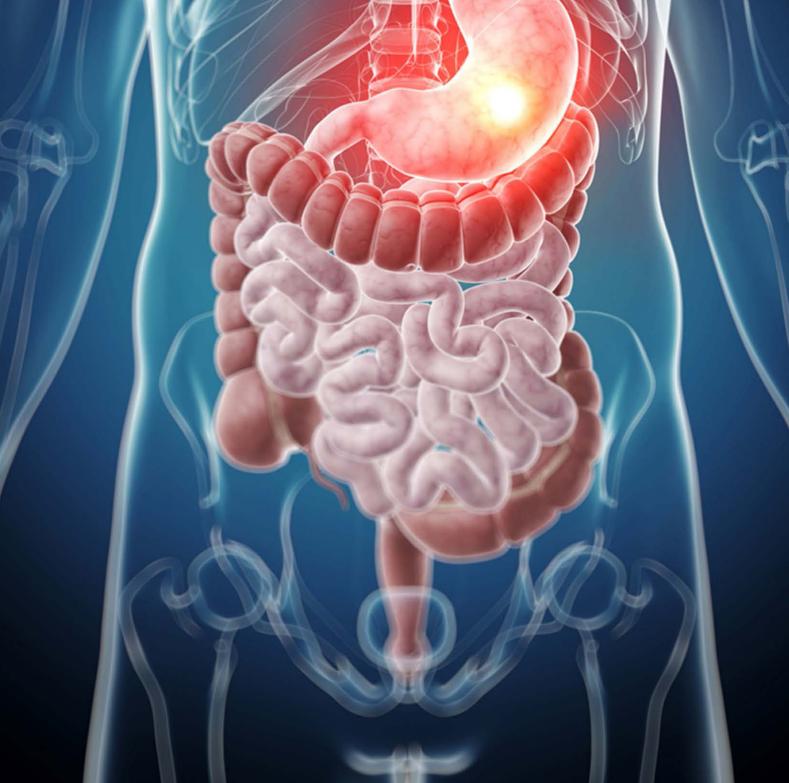






Left: Pulmonary artery lesions. Lesion development in the pulmonary arteries in mice exposed to normal oxygenation versus those exposed to IHH (see text) for 8 weeks. A-E show the appearance of the pulmonary arteries (PAs) after dissection and Sudan IV staining. A shows the control PA, while B-E show the dramatic lesions (in red) of PAs in mice exposed to IHH. F shows the highly significant change in percent of Sudan IV staining of the entire PA for all IHH mice and normoxic control.







Ariel Feldstein, M.D. Division Chief, Pediatric Gastroenterology, Professor of Pediatrics

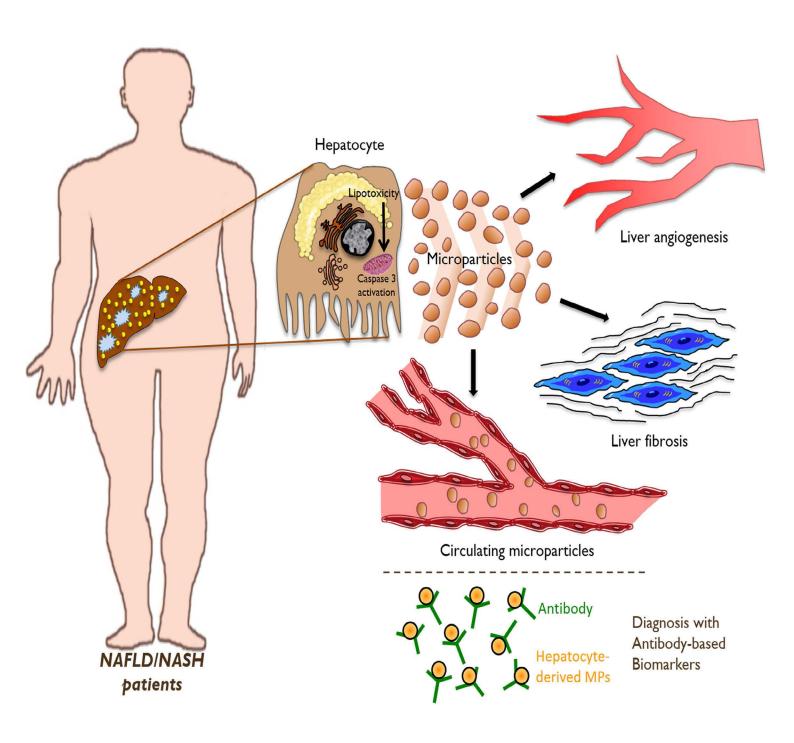
SENDING AN ANGIOGENIC Message

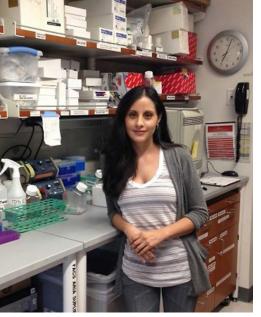
Excess amounts of saturated fatty acids are a potential dietary trigger for the fatty liver disease steatohepatitis, in which the liver develops fat deposits and inflammation. Progression of the disease to more serious forms, which can include scarring and other serious complications, is associated with the formation of new blood vessels, a process called angiogenesis, which requires endothelial cells to migrate and form tubular structures. Investigators in Dr. Feldstein lab found that hepatocytes exposed to excess amounts of saturated fatty acids released membranebound microparticles that induced angiogenesis when administered to mice. Microparticles from the blood of mice with diet-induced steatohepatitis originated from the liver and triggered migration and tubular structure formation when applied to endothelial cells. The angiogenic effects of microparticles generated by hepatocytes exposed to saturated fatty acids or of those from mice with diet-induced steatohepatitis involved the uptake of the microparticles by endothelial cells, a process that required Vanin-1, an enzyme located on the surface of the microparticles. Thus, the pathological angiogenesis that can occur in steatohepatitis could be reduced by preventing endothelial cells from internalizing Vanin-1-positive microparticles from hepatocytes.

These discoveries open new venues to develop liver- and

disease-specific blood biomarkers as well as novel therapeutic targets to treat what is now the most common form of chronic liver disease in both children and adults in the United States.

D. Povero, A. Eguchi, I. R. Niesman, N. Andronikou, X. de Mollerat du Jeu, A. Mulya, M. Berk, M. Lazic, S. Thapaliya, M. Parola, H. H. Patel, A. E. Feldstein, Lipid-Induced Toxicity Stimulates Hepatocytes to Release Angiogenic Microparticles That Require Vanin-1 for Uptake by Endothelial Cells. Science Signaling. 2013 Oct 8;6(296):ra88. doi: 10.1126/scisignal.2004512.





Paulina Ordonez, M.D. Assistant Professor of Pediatrics Pediatric Gastroenterology,

AUTOPHAGY AS A MEDIATOR OF SURVIVAL

Niemann-Pick type C1 (NPC1) is a progressive and incurable pediatric lysosomal storage disease caused by mutations of the cholesterol transporter NPC1. Children affected with the classic form of NPC1 present as toddlers, and gradually develop neurologic and liver disease leading to death in their early childhood to teenage years. Although rare, NPC1 imposes an emotional and economic burden in patients, families,

and society that

is disproportionate to its relative infrequency. Despite continued efforts, strategies using traditional animal models and human fibroblasts have not identified a clear therapeutic avenue for NPC1. Human induced pluripotent stem cell (hIPSC) technology is a powerful tool to model human disease in relevant cell populations. The use of hIPSC offers the unprecedented opportunity to manipulate and examine live, patient-derived cell types that cannot be easily accessed otherwise, such as neurons and hepatocytes. Using this technology, a team led by Dr. Ordonez discovered a key and unique cellular phenotype in neurons derived from NPC1 patients. NPC1 neurons exhibit substantial disruption of mitochondrial turnover by autophagy, which leads to accumulation of potentially neurotoxic mitochondrial fragments and mitochondrial dysfunction. Interestingly, Dr. Ordonez' new data suggest that autophagy may function as part of a "backup" cholesterol trafficking pathway that releases and distributes trapped cholesterol in NPC1 mutants and may also function in normal cells. Dr. Ordonez' team is now conducting targeted screening of compounds that rescue the mitochondrial turnover phenotype without reducing bulk autophagy, which may be necessary to maintain cell viability.

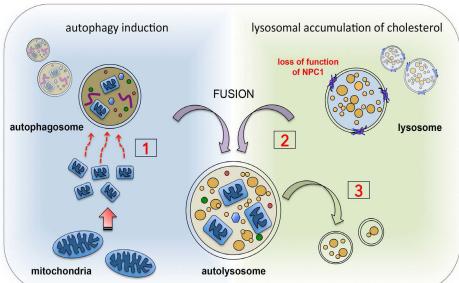
This work will have a potentially large impact on drug development

"New data suggest that autophagy may function as part of a "backup" cholesterol trafficking pathway that releases and distributes trapped cholesterol"

approaches for NPC1 and may substantially increase the likelihood of finding an effective therapy for NPC1 and related disorders

Ordonez MP, Roberts EA, Kidwell CU, Yuan SH, Plaisted WC, Goldstein LS. Disruption and Therapeutic Rescue of Autophagy in a Human Model of Niemann Pick type C1. Human Molecular Genetics. 2012 Jun 15; 21 (12):2651-62.

Ordonez MP. Defective mitophagy in human Niemann-Pick Type C1 is due to abnormal autophagy activation. Autophagy. 2012 May 31; 8 (7).



Autophagy is strongly induced in NPC1 as a aresult of relative cholesterol starvation in sites outside the lysosome. 1. As a result of persistent autophay induction, mitochondria are fragmented and targeted to teh autophagosome. 2. Autophagy progression is disrupted in NPC1, causing impaired turnover of mitochondria and accumulation of potentially toxic mitochondrial fragments. 3. Autophagy mediatres slow release of lysosomal cholesterol, allowing short-term survival of NPC1 cells.



Lars Bode, Ph.D.
Associate Professor of Pediatrics in Residence
Pediatric Gastroenterology,
Hepatology & Nutrition

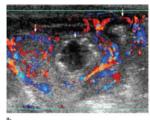
Oligosaccharides and Enterocolitis

Necrotizing Enterocolitis (NEC) is one of the most frequent and often fatal intestinal disorders in preterm infants. 5-10% of all very low birth weight infants develop NEC; one quarter of the affected babies will not survive. Therapies to meet the clinical needs for this special and highly vulnerable population are extremely limited. Help in developing new safe and effective therapies for NEC may be guided by epidemiological observations showing that breastfed infants are at a 6- to 10-fold lower risk to develop this devastating condition. What is it that makes human milk so powerfully protective against NEC? A team led by Dr. Lars Bode, an Associate Professor of Pediatrics with training in both nutrition and glycobiology, has recently shown that specific complex sugars called Human Milk Oligosaccharides (HMO) protect from NEC in a preclinical rodent model of the disease. Survival rates and intestinal pathology improved significantly when rodent pups received HMO with their diet.

In the following, the team was able to identify and characterize one specific structure out of the more than 150 known and structurally distinct HMO. The Bode lab now aims to determine whether results from the preclinical model translate to benefit the human preterm infant.

Jantscher-Krenn E, Zherebtsov M, Nissan C, Goth K, Guner YS, Naidu N, Choudhury BP, Grishin AV, Ford HR, <u>Bode L</u> (2012) The Human Milk Oligosaccharide Disialyllacto-Ntetraose prevents Necrotizing Enterocolitis in neonatal rats. GUT 61:1417-25

<u>Bode L</u> (2012) Human Milk Oligosaccharides: Every baby Needs a Sugar Mama. Glycobiology 22: 1147-62



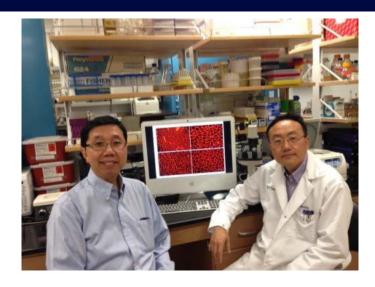


NEC and bowel necrosis in a neonate. (a) Color Doppler sonogram shows three bowel loops (arrows). The two outer loops (long arrows) are markedly hyperemic, with flow around the entire circumference of each loop and some prominent mesenteric vessels. The central loop has an irregular wall with focal thinning at one site (short arrow) and small hyperechoic foci suggestive of intramural gas. There is no flow in the wall, a finding highly suggestive of necrosis. (b) Intraoperative photograph of the bowel shows the necrotic bowel loop (arrow).

Chronic Renal Disease



The first publication on the pathophysiologic role of leptin and melanocortin signaling in CKD associated cachexia



Wilson Cheung, Ph.D. (left) Research Scientist, Nephrology

> Robert Mak, M.D., Ph.D. (right) Division Chief, Professor of Pediatrics Nephrology

Novel THERAPEUTIC TARGETS FOR WASTING IN RENAL DISEASE

The Mak Laboratory is focused on understanding the pathogenesis and developing novel therapy for cachexia in chronic kidney disease(CKD). We are the first to

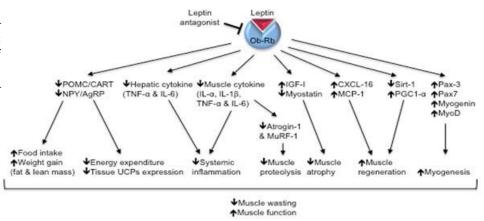
publish on the pathophysiologic role of leptin and melanocortin signaling in CKD associated cachexia. (Cheung W, Marks DL, Yu X, Cone R, Mak RH. Role of leptin and melanocortin signaling in uremia-associated cachexia. Journal of Clinical Investigation 2005;115:1659-1665.) In the past 2 years, we focused on the translational aspect of our earlier discovery, which led to the following 2 discoveries.

Leptin antagonist may be a novel therapy for cachexia in chronic kidney disease Elevated serum leptin levels correlate with inflammation and predict changes in lean body mass in patients with CKD, and activation of the melanocortin system by leptin signaling mediates the pathophysiology of CKD-associated cachexia. We tested whether treatment with a

pegylated leptin receptor antagonist (PLA) attenuates cachexia in mice with CKD. CKD and Sham mice received vehicle or PLA (2 or 7 mg/kg per day). At these doses, PLA did not influence serum leptin levels in mice. Treatment with 7 mg/kg per day PLA stimulated appetite and weight gain, improved lean mass and muscle function, reduced energy expenditure, and normalized the levels of hepatic TNF-α and IL-6 mRNA in mice with CKD.

Furthermore, treatment with 7 mg/kg per day PLA attenuated the CKD-associated increase in the transcriptional and protein abundance of uncoupling proteins that mediates thermogenesis, and it normalized the molecular signatures of processes associated with muscle wasting in CKD, including proteolysis, myogenesis and muscle regeneration, and expression of proinflammatory muscle cytokines, such as IL-1a, -1β, and -6 and TNF-α. Our results suggest that leptin antagonism may represent a viable therapeutic strategy for cachexia in CKD.

Cheung WW, Ding W, Gunta SS, Gu Y, Tabakman R, Klapper LN, Gertler A, Mak RHA Pegylated Leptin Antagonist Ameliorates CKD-Associated Cachexia in Mice. Journal of the American Society of Nephrology 2014; 25(1):119-28



The effect of the pegylated leptin antagonist on the various pathways in CKD-associated cachexia is summarized in the figure above.

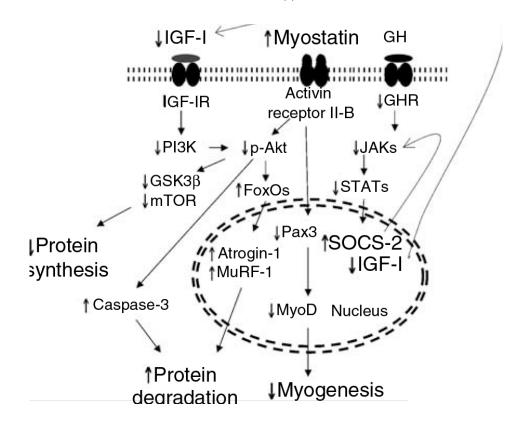
NOVEL THERAPEUTIC TARGETS FOR WASTING IN CHRONIC KIDNEY DISEASE

Aberrant melanocortin signaling has been implicated in the pathogenesis of wasting in chronic kidney disease (CKD). Previously, we demonstrated that agouti-related peptide (AgRP), a melanocortin-4 receptor antagonist, reduced CKD-associated cachexia in CKD mice. Our previous studies with AgRP utilized dual energy X-ray (DXA) densitometry to assess the body composition in mice (Cheung W, Kuo HJ, Markison S, Chen C, Foster AC, Marks DL, Mak RH. J Am Soc Nephrol 18: 2517-2524, 2007; Cheung W, Yu PX, Little BM, Cone RD, Marks DL, Mak RH. J Clin Invest 115: 1659-1665, 2005). DXA is unable to differentiate water content in mice, and fluid retention in CKD may lead to an overestimate of lean mass.

In this study, we employed quantitative magnetic resonance technique to evaluate body composition change following central administration of AgRP in a CKD mouse model. AgRP treatment improved energy expenditure, total body mass, fat mass, and lean body mass in CKD mouse. We also investigated the effect of CKD-associated cachexia on the signaling pathways leading to wasting in skeletal muscle, as well as whether these changes can be ameliorated by central administration of AgRP. AgRP treatment caused an overall decrease in proinflammatory cytokines, which may be one important mechanism of its effects.

Muscle wasting in CKD may be due to the activation of proteolytic pathways as well as inhibition of myogenesis and muscle regeneration processes. Our results suggest that melanocortin antagonists may represent a novel therapy for muscle wasting in CKD.

Cheung WW, Mak RH. Melanocortin antagonism ameliorates muscle wasting and inflammation in chronic kidney disease. American Journal of Physiol (Renal physiology) 2012, 303(9):F1315-1324.



The pathologic pathways associated with muscle wasting CKD are summarized in the figure.

Infectious Diseases and Pharmacological Science



Discoveries in fighting Antibiotic Resistance



Edmund Capparelli, PharmD
Professor of Clinical Pediatrics/Clinical
Pharmacy Pediatric Pharmacology & Drug Discovery

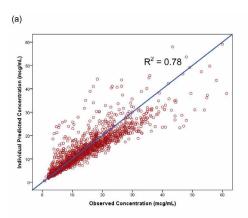
PHARMACODYNAMIC-BASED ANTIBIOTIC DOSE OPTIMIZATION

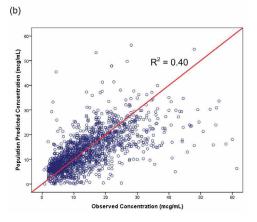
Dr. Capparelli led a project to improve the way that pediatricians dose vancomycin, one of the most common antibiotics administered intravenously in the hospital for serious infections such as those caused by methicillin-resistant Staphylococcus aureus.

Analyzing over 700 patients with a new computer model and thousands of "Monte Carlo" simulations of drug exposure, the team discovered that current dosing approaches were inadequate in most subjects. A new approach to achieve proper vancomycin levels using "area under curve"/MIC of the bacterial isolate rather than standard trough concentrations was devised.

These recommendations have the potential to reduce treatment failures and limit the selection for antibiotic resistant isolates among pediatric patients in the hospitals and intensive care units.

Le J, Bradley JS, Murray W, Romanowski GL, Tran TT, Nguyen N, Cho S, Natale S, Bui I, Tran TM, Capparelli EV. Improved vancomycin dosing in children using area under the curve exposure. Pediatr Infect Dis J. 2013;e155-63.





Observed versus Predicted Concentrations based on Individual (a) and Population Parameters (b)





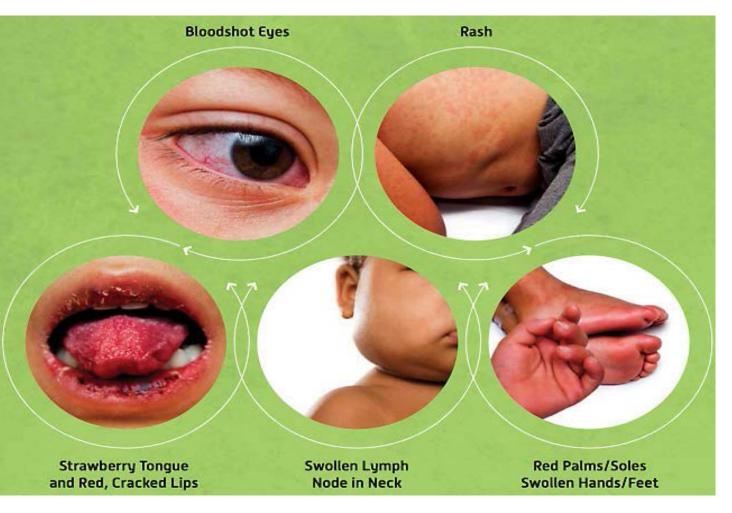
Adriana Tremoulet, M.D. Associate Adjunct Professor of Pediatrics Pediatric Pharmacology & Drug Discovery

INFLIXIMAB FOR PRIMARY THERAPY OF KAWASAKI DISEASE

Kawasaki Disease (KD) is a severe childhood disease that many parents, even some doctors, mistake for an inconsequential viral infection. If not diagnosed or treated in time, it can lead to irreversible heart damage. Dr. Tremoulet was lead author of a study at UCSD and RCHSD that looked at intensification of initial therapy for all children with KD in order to prevent IVIG-resistance and associated coronary artery abnormalities, by assessing the addition of the medication infliximab to current standard therapy. Infliximab is a monoclonal antibody that binds tumor necrosis factor alpha (TNF α), a cytokine that promotes inflammation in KD. Infliximab was well-tolerated, achieved a greater reduction in the size of the left coronary artery, and reduced the number of days of fever and laboratory markers of inflammation.

The authors concluded that use of infliximab is safe in infants and children and that early treatment could help children with KD that experience high levels of inflammation or early signs of coronary artery damage.

Tremoulet AH, Jain S, Jaggi P, Jimenez-Fernandez S, Pancheri JM, Sun X, Kanegaye JT, Kovalchin JP, Printz BF, Ramilo O, Burns JC. Infliximab for intensification of primary therapy for Kawasaki disease: a phase 3 randomised, double-blind, placebo-controlled trial. Lancet 2014 (in press.)



Frontiers in Pediatric AIDS Research

3.2 million children were living with HIV at the end of 2013, 91% of them in sub-Saharan Africa





Grant Campbell, Ph.D. (left) Assistant Project Scientist Infectious Diseases

Stephen Spector, M.D. (right)
Co-Chief, Division of Infectious Diseases
Distinguished Professor of Pediatrics
Infectious Diseases

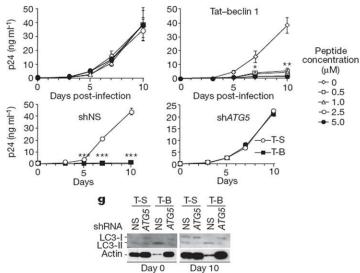
Exploiting HIV to Treat Infectious Diseases and Neurodegenerative Diseases

In collaboration with Dr. Beth Levine's laboratory at Southwestern Medical Center, the laboratory of Dr. Stephen Spector contributed to the identification of an autophagy-inducing peptide that has the potential to treat human diseases including infectious and neurodegenerative diseases.

Autophagy is a lysosomal degradation pathway that has a crucial role in the defense against infection, neurodegenerative disorders, cancer and aging. The Spector laboratory was the first to demonstrate that when HIV productively infects cells there is a down-regulation of autophagy. Subsequent studies showed that the HIV Nef protein interacts with the autophagy-inducing protein beclin 1 to inhibit autophagy. In this article, the Levine laboratory identified the region on beclin 1 that binds Nef and is critical for its autophagic function, and then designed a peptide composed of that region. In subsequent experiments, the Tat-beclin 1 peptide was shown to interact with GAPR-1 (also known as GLIPR2), a previously unrecognized negative regulator of autophagy. The peptide was linked to the HIV Tat protein to facilitate entry into cells. This Tat-beclin 1 peptide was shown to reduce mortality in mice infected with chikungunya or West Nile virus. Grant Campbell in the Spector lab showed that the Tat-beclin 1 peptide could inhibit HIV through the induction of autophagy.

This research demonstrates that autophagy-inducing peptides like the Tat-beclin 1 peptide may be useful in treating diseases including HIV and neurodegenerative disorders such as such as Huntington's disease, the spinocerebellar ataxias, and synucleinopathies and tauopathies

Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, Wilkins AD, Sun Q, Pallauf K, MacDuff D, Huerta C, Virgin HW, Helms JB, Eerland R, Tooze SA, Xavier R, Lenschow DJ, Yamamoto A, King D, Lichtarge O, Grishin NV, Spector SA, Kaloyanova DV, and Levine B. Identification of a candidate therapeutic autophagy-inducing peptide. Nature 2013; 494:201-206.



The Spector Laboratory has been able to show that Vitamin D has the potential to inhibit HIV replication globally

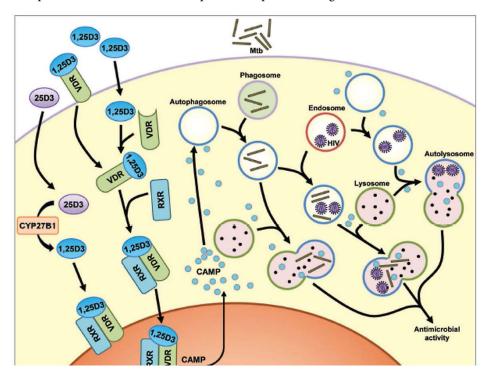
VITAMIN D BATTLES INFECTIOUS DISEASES

Vitamin D is important for cell growth, immunity and metabolism. Vitamin D deficiency has been implicated in bone diseases, autoimmune diseases, cancers (including colon, prostate and breast), myocardial infarction, diabetes, and inflammatory bowel disease. Vitamin D deficiency has also been associated with increased risk for tuberculosis and HIV infection, and leads to more rapid HIV-related disease progression.

The Spector laboratory has examined how vitamin D might affect HIV and Mycobacterium tuberculosis (M. Tuberculosis) infection. Campbell and Spector had previously shown 1a,25-dihydroxycholecalciferol (1,25D3), the active form of vitamin D, inhibits HIV replication in human macrophages through the induction of autophagy. In the current study, they reported that physiological concentrations of 1,25D3 (the active form of vitamin D) induces the production of the human cathelicidin microbial peptide (CAMP) and autophagic flux in HIV and M. Tuberculosis co-infected human macrophages which inhibits mycobacterial growth and the replication of HIV. Using RNA interference for Beclin-1 and the autophagy-related 5 homologue, combined with the chemical inhibitors of autophagic

flux, bafilomycin A1, an inhibitor of autophagosome-lysosome fusion and subsequent acidification, and SID 26681509 an inhibitor of the lysosome hydrolase cathepsin L, they show that the 1,25D3-mediated inhibition of HIV replication and mycobacterial growth during single infection or dual infection is dependent not only upon the induction of autophagy, but also on phagosomal maturation. Moreover, through the use of RNA interference for CAMP, they demonstrate that cathelicidin is essential for the 1,25D3 induced autophagic flux and inhibition of HIV replication and mycobacterial growth.

In further research, Campbell and Spector have identified an additional important role for vitamin D in pathogen recognition and innate immunity. Toll-like receptors (TLR) are important in recognizing microbial pathogens and triggering host innate immune responses, including autophagy, and in the mediation of immune activation during human immunodeficiency virus type-1 (HIV) infection. Campbell and Spector in a recent publication report that TLR8 activation in human macrophages induces the expression of the human cathelicidin microbial peptide (CAMP), the vitamin D receptor (VDR) and cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1), which 1α-hydroxylates the inactive form of vitamin D, 25-hydroxycholecalciferol, into its biologically active metabolite. Moreover, they demonstrate using RNA interference, pharamcologic inhibitors and vitamin



The role of autophagy and CAMP in 1,25D3-mediated inhibition of Mtb and HIV. HIV budding occurs into the multivesicular endosomes of macrophages. Mtb enters through phagocytosis. Cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) 1α-hydroxylates the inactive 25D3 into the active 1,25D3. 1,25D3 induces the expression of CAMP presumably through binding to the vitamin D (1,25D3) receptor (VDR), which het-erodimerizes with the retinoid X receptor (RXR) and directly regulates transcription by binding to the vitamin D response element (VDRE) consensus sequence located upstream of the Camp gene. The expression of CAMP is required both for autophagosome and phagolysosome biogenesis, which leads to killing of the microbial pathogens through autophagy. Adapted from Jo EK. Innate immunity to mycobacteria: vitamin D and autophagy. Cell Microbiol 2010; 12:1026–35; http://dx.doi. org/10.1111/j.1462-5822.2010.01491.x.

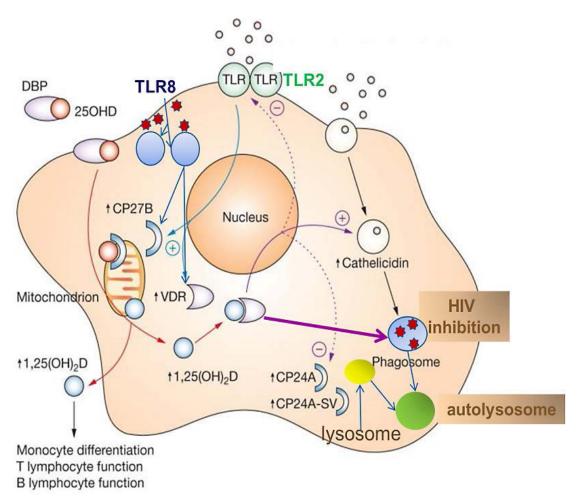
D deficient media that TLR8 agonists inhibit HIV through a vitamin D, and CAMP dependent autophagic mechanism.

In total, the findings of Campbell and Spector support an important role for vitamin D in the control of HIV infection, and provide a biological explanation for the benefits of vitamin D. These findings also provide new insights into potential novel targets to prevent and treat HIV infection and other opportunistic infections.

Campbell G, Spector SA. Vitamin D Inhibits Human Immunodeficiency Virus type 1 and Mycobacterium tuber-

culosis Infection in macrophages through the induction of autophagy. PLoS Pathogens 2012;8(5):e1002689.

Campbell G, Spector SA. Toll-Like Receptor 8 ligands activate a vitamin D mediated autophagic response that inhibits Human Immunodeficiency Virus Type 1. PLoS Pathogens 2012. epub Nov;8(11):e1003017



Model of link between TLR8 signaling and Vitamin D pathway





Ankita Garg, Ph.D. (left) Assistant Project Scientist Infectious Diseases

Stephen Spector, M.D.(right) Co-Chief, Division of Infectious Diseases Distinguised Professor of Pediatrics Infectious Diseases

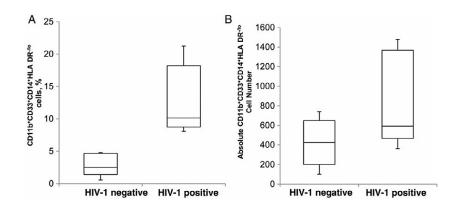
HIV Suppresses Immune **FUNCTION THROUGH** Induction of Myeloid DERIVED SUPPRESSOR CELLS

Although the hallmark of the immune deficiency associated with HIV is the depletion of CD4+ lymphocytes, the immunologic impairment associated with HIV infection occurs even with normal CD4+ cell numbers. Drs. Garg and Spector examined the role of myeloid-derived suppressor cells (MDSCs) during HIV infection. Peripheral blood mononuclear cells (PBMCs) were cultured with HIV gp120

and infectious or inactivated HIV, with or without antiinterleukin 6 (IL-6) antibody. CD33(+), CD4(+), and CD8(+) cells were isolated from PBMCs and co-cultured in the presence or absence of inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), and arginase 1 inhibitors. CD11b(+)CD33(+)CD14(+)HLA-DR(-/lo) MDSCs, phosphorylated STAT3 (pSTAT3), and CD4(+)CD25(+)FoxP3(+) cells were evaluated by flow cytometry. IL-6, interferon γ (IFN-γ), interleukin 10 (IL-10), and gp120 levels were quantified by an enzyme-linked immunosorbent assay. They found that MDSCs expanded when PBMCs were exposed to infectious or inactivated HIV. Exposure to HIV gp120 alone also led to MDSC expansion, with increases in IL-6 levels and pSTAT3 expression. Of note, anti-IL-6 abrogated MDSC expansion and pSTAT3 expression. HIV gp120-expanded CD33(+) MDSCs inhibited IFN-y release from autologous T cells, which was restored upon ROS and iNOS inhibition. HIV gp120-expanded CD33(+) MDSCs increased IL-10 and CD4(+)CD25(+)FoxP3(+) regulatory T-cell levels in CD4(+) T-cell cocultures. Finally, high frequencies of MDSCs were present in HIV-infected persons, compared with healthy controls.

The findings from this research demonstrate that HIV gp120 induces IL-6 and MDSC expansion, which contributes to immune suppression by modulating cytokine and cellular responses. These findings help to explain how HIV can cause broad immunosuppression and identify novel strategies designed to improve the immune function of HIV infected persons. Moreover, strategies designed to improve immune function have the potential to improve the response to HIV therapeutic vaccines.

Garg A, Spector SA. HIV type 1 gp120 induced expansion of myeloid derived suppressor cells is dependent on interleukin 6 and suppresses immunity. J Infect Dis 2014; 209: 441-451.



CD11b+CD33+CD14+HLA-DR-/lo cell counts are increased in human immunodeficiency virus type 1-infected patients. Whole blood specimens from 5 HIV-infected persons and 5 healthy controls were stained with antibody to CD11b, CD33, CD14, and HLA-DR. Flow cytometry was used to deter-mine percentages (A) and absolute counts (B) of CD11b+CD33+CD14+HLA-DR-/lo cells. The upper and lower limits of the boxes denote the interquartile ranges. The horizontal lines within the boxes denote median values. The upper and lower lines outside the boxes denote the highest and lowest values, re-spectively.



Fundamental Discovery May Lead to Safer Vaccine Against Strep Throat. "Group A Streptococcus (GAS) is one of the most important human pathogens, causing 700 million cases of "strep throat" annually" The Nizet Lab identified the long sought after genetic basis of GAC and its defining GlcNAc side chain, and generated

THE FIRST BACTERIAL MUTANTS FOR ANALYSIS OF ITS ROLE IN VIRULENCE.



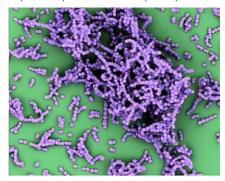
Victor Nizet, M.D.
Professor of Pediatrics & Pharmacy
Division Chief, Pediatric
Pharmacology
& Drug Discovery

Group A Streptococcus (GAS) is one of the most important human pathogens, causing 700 million cases of "strep throat" annually, can cause serious invasive infections such as necrotizing fasciitis ("flesh-eating disease"), and is the trigger for the immunological disease rheumatic fever, which damages the heart valves and is a leading cause of morbidity and mortality in many developing countries. GAS is defined as a species by a carbohydrate antigen called group A carbohydrate (GAC), which is the basis of all rapid diagnostic

testing for GAS infection, but its function in disease pathogenesis was not known. The GlcNAc of GAC has also been implicated as a possible trigger of cross-reactive antibodies in the pathogenesis of rheumatic heart disease, confounding its appeal as a universal vaccine antigen. The Nizet Lab identified the long sought after genetic basis of GAC and its defining GlcNAc side chain, and generated the first bacterial mutants for analysis of its role in virulence. The researchers found that a mutant strep strain lacking the human-like GlcNAc sugar on the GAC molecule exhibited normal bacterial growth and expressed key proteins known to be associated with strep virulence, but was easily killed when exposed to human white blood cells or serum. The mutant strep bacteria also lost the ability to produce severe disease in animal infection models. The researchers also identified a way to remove the problematic GlcNAc sugar so that a mutant form of the bacteria with only rhamnose-containing GAC could be purified and tested as a vaccine antigen. Antibodies produced against mutant GAC antigen helped human white blood cells kill the pathogen and protected mice from lethal strep infection. Because GAC is present in

all strep strains, this will represent a safer antigen for inclusion in a universal strep vaccine.

van Sorge NM, Cole JN, Kuipers K, Henningham A, Aziz RK, Kasirer-Friede A, Lin L, Berends



ETM, Davies MR, Dougan G, Zhang F, Dahesh S, Shaw L, Gin J, Cunningham M, Merriman JA, Hütter J, Lepenies B, Rooijakkers SHM, Malley R, Walker MJ, Shattil SJ, Schlievert PM, Choudhury B, Nizet V. The classical Lancefield antigen of group A streptococcus is a virulence determinant with implications for vaccine design. Cell Host Microbe 2014; 15:729-740.





34 WCSD Department of Pediatrics



Elizabeth Winzeler, M.D.
Professor of Pediatrics
Pediatric Pharmacology & Drug Discovery

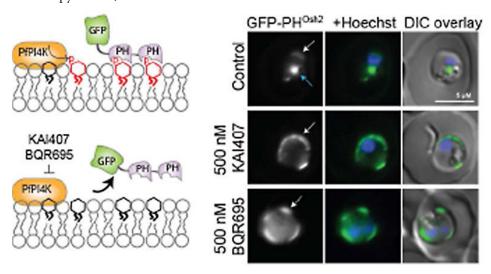
Phosphoinositol-4 kinase, a New Drug Target for Malaria Elimination

Achieving the goal of malaria elimination may depend on creating new sterilizing medicines that target pathways of the Plasmodium parasite essential across all life stages including the asymptomatic liver and transmission stages. An obstacle to this is the lack of any known drug target that is important for all both symptomatic and asymptomatic stages. Recently we identified a lipid kinase, phosphatidylinositol-4-OH kinase (PI(4)K), as the target of imidazopyrazines, a new antimalarial

compound class that inhibits the intracellular development of multiple Plasmodium species at each stage of infection in the vertebrate host. Imidazopyrazines demonstrate potent preventive, therapeutic, and transmission-blocking activity in rodent malaria models, are active against blood-stage field isolates of the major human pathogens P. falciparum and P. vivax, and inhibit liver-stage hypnozoites in the simian parasite P. cynomolgi. Imidazopyrazines (such KAI407 and BQR695) exert their effect through inhibitory interaction with the ATP-binding pocket of PI(4) K, altering the intracellular distribution of phosphatidylinositol-4-phosphate.

Collectively, these data define PI(4)K as a key Plasmodium vulnerability, opening up new avenues of target-based discovery to identify drugs with an ideal activity profile for the prevention, treatment and elimination of malaria.

McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Nagle A, Simon O, Yeung BK, Chatterjee AK, McCormack SL, Manary MJ, Zeeman AM, Dechering KJ, Kumar TR, Henrich PP, Gagaring K, Ibanez M, Kato N, Kuhen KL, Fischli C, Rottmann M, Plouffe DM, Bursulaya B, Meister S, Rameh L, Trappe J, Haasen D, Timmerman M, Sauerwein RW, Suwanarusk R, Russell B, Renia L, Nosten F, Tully DC, Kocken CH, Glynne RJ, Bodenreider C, Fidock DA, Diagana TT, Winzeler EA. Targeting Plasmodium PI(4)K to eliminate malaria. Nature 2013; 504:248-53.



Immunologic and Dermatologic Disorders







Andrew Krakowski, M.D. Assistant Clinical Professor of Pediatrics Pediatric and Adolescent Dermatology

FRACTIONATED CO2 LASER ABLATION MAY MINIMIZE SCARRING IN CHILDREN

Fractional photothermolysis (FP), first described by Manstein and colleagues in 2004, describes the controlled creation of discontinuous columns of cutaneous thermal injury over a fraction of the treatment area based on the heating of tissue water. FP may be ablative or non-ablative based on the laser wavelength and corresponding water absorption, with a tunable depth of injury proportional to the operator-selected energy density. Ablative lasers, such as the 10,600-nm carbon dioxide (CO2) laser, are characterized by avid water absorption and tissue ablation at standard

treatment settings. Uniqueness of the technology includes unprecedented treatment depths (up to 4 mm with current devices) combined with relatively large areas of adjacent tissue sparing, facilitating rapid re-epithelialization and providing ample viable cells to drive the subsequent tissue healing and remodeling response. Ablative fractional photothermolysis has been used for adult cosmetic conditions such as facial rhytides, hyperpigmentation, and lentigenes.

Adaption of the technology has allowed its utilization to decrease functional impact and minimize deformity associated with traumatic scars and scars from inflammatory conditions and vascular tumors. Fractionated carbon dioxide laser was effectively to non-invasively treat lymphangioma circumscriptum. A similar protocol was used to help repair dystrophic fingernails in a female patient with significant scarring from a scald injury. Most recently, AFP was utilized to treat a young female with intra-mammary residual cribriform scarring from hidradenitis suppurativa, a chronic, relapsing, inflammatory skin condition that can have a significant psychosocial impact.

Krakowski AC, Admani S, Uebelhoer N, Eichenfield LF, Shumaker P. Residual scarring from hidradenitis suppurativa: Fractionated CO2 laser as a novel and non-invasive approach. Pediatrics 2014;133(1):e248-51.

Krakowski AC, Admani S, Shumaker P, Uebelhoer N. Fractionated CO2 laser as a novel and non-invasive approach for burn scar-related nail dystrophy. Dermatologic Surgery 2013;71(12):1317-21.

Shumaker PR, Dela Rosa KM, Krakowski AC. Treatment of lymphangioma circumscriptum using fractional carbon dioxide laser ablation. Pediatric Dermatology 2013;12.







Sheila Fallon Friedlander, M.D.
Professor of Clinical Pediatrics
Pediatric and Adolescent Dermatology

Infantile Hemangiomas Occur in Approximately 5% of Births

The first American prospective study was conducted evaluating incidence and risk factors for hemangiomas which present during infancy. 578 pregnant mothers gave birth to 594 infants and these infant-mother pairs were followed for 9 months. The overall incidence of hemangiomas (congenital & infantile) was 4.8%. anomalies were noted in almost 35% of hemangioma-related pregnancies, approximately twice the incidence noted in pregnancies with unaffected infants (p=.025). Other risk factors included prematurity (p=.016) & low birth weight (p=.028). Predominantly truncal location and the occurrence of small abortive/telangiectatic lesions were novel findings not previously appreciated in prior studies. Only one hemangioma required intervention, a much lower incidence than previously reported in retrospective studies.

This large prospective study documents the incidence and demographics of hemangiomas in infants. It provides reassuring evidence that the incidence and risk of complications are lower than previously reported. A statistically significant association with placental anomalies was identified, supporting the hypothesis of hypoxia as a key component in the pathogenesis of these lesions.

Prospective study of infantile hemangiomas: Incidence, clinical characteristics and association with placental anom-



alies. Munden A, Butschek R, Tom W, Sanders Marshall J, Milbert Poeltler D, Krohne SE, Alió AB, Ritter M, Friedlander DF, Catanzarite V, Mendoza A, Smith L, Friedlander M, Friedlander SF. British Journal of Dermatology. February 2014. DOI: 10.1111/bjd.12804.

Infantile hemangiomas: an update on pathogenesis and therapy. Chen TS, Eichenfield LF, Friedlander SF. Pediatrics. 2013 Jan;131(1):99-108. doi: 10.1542/peds.2012-1128. Epub 2012 Dec 24. Review.

Beneficial effects of early pulsed dye laser therapy in individuals with infantile hemangiomas. Admani S, Krakowski AC, Nelson JS, Eichenfield LF, Friedlander SF. DermatolSurg. 2012 Oct;38(10):1732-8.



Lawrence Eichenfield, M.D.

Division Chief, Professor of Clinical Pediatrics

Pediatric and Adolescent Dermatology

PRE-ADOLESCENT ACNE CAN BE EFFECTIVELY TREATED

Acne is one of the most common skin conditions in teenagers. Increasingly acne in patients under 12 years of age is quite common. However, clinical studies of therapeutic agents have not previously been performed, and there has been a limited evidence basis to guide pediatric acne management. Dr. Eichenfield and colleagues have investigated acne in this age group, displaying efficacy and safety of topical retinoid and topical retinoid/benzyol peroxide combination therapies in 9 to 12 year olds.

This research has led to the first FDA-approval of an acne medication for children ages 9 and older, and expanded therapies. In addition, recognizing the lack of acknowledged guidelines for pediatric acne management, Dr. Eichenfield mobilized the American Acne

and Rosacea Society and the American Academy of Pediatrics to formulate and publish the first evidencebased recommendations for the diagnosis and treatment of pediatric acne.

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Friedlander SF, Levy M, Lucky A, Mancini AJ, Orlow SJ, Yan AC, Vaux KK, Webster G, Zaenglein AL, Thiboutot DM; American Acne and Rosacea Society. Evidence-based recommendations for the diagnosis and treatment of pediatric acne. Pediatrics. 2013 May;131 Suppl 3:S163-86. doi: 10.1542/peds.2013-0490B. PubMed PMID: 23637225.

Eichenfield LF, Draelos Z, Lucky AW, Hebert AA, Sugarman J, Stein Gold L, Rudisill D, Liu H, Manna V. Preadolescent moderate acne vulgaris: a randomized trial of the efficacy and safety of topical adapalene-benzoyl peroxides. J Drugs Dermatol. 2013 Jun 1;12(6):611-8. PubMed PMID: 23839175.





Alessandra Franco, Ph.D. Associate Adjunct Professor of Pediatrics Allergy, Immunology & Rheumatology

MECHANISM OF INTRAVENOUS IMMUNOGLOBULIN (IVIG) IN REGULATING VASCULAR INFLAMMATION

Intravenous immunoglobulin (IVIG) is the treatment of choice for many immune-mediated disorders, yet its mechanisms of action are incompletely understood. IVIG is an expensive and scarce resource and simply unavailable in many parts of the world creating an unmet medical need for new therapies. Regulatory T cells (Treg) play an important role in modulation of inflammation. Department of Pediatric researcher Alessandra Franco investigated the possibility that IVIG played a direct role in the expansion of Treg observed during recovery from Kawasaki disease (KD), a pediatric T cell-mediated vasculitis of the coronary arteries. For her paper soon to be published in Autoimmunity, she generated Treg clones from KD subjects and discovered that they recognize the heavy chain constant region of immunoglobulin G (Fc). These Fcspecific Treg clones secrete IL-10 and, surprisingly, small amounts of IL-4 in response to exogenous Fc fragments presented by conventional, autologous antigen-presenting cells or in response to autologous mature IgG+ B cells in the absence of exogenous Fc. These results support a new function of mature B cells in inducing Fc-specific Treg expansion. The activation of Fc-specific Treg is strongly associated with downregulation of vascular inflammation in children with KD in that patients with dilated coronary arteries or aneurysms despite IVIG treatment failed to expand this Treg population.

These results point to a novel mechafor the anti-inflammatory action and clinical benefit provided by IVIG therapy in patients with KD and, potentially, other vasculitides. Identification of immunodominant Fc peptides that elicit a strong expansion of Treg in a wide variety of Human Leucocytes Antigens (HLA) haplotypes will be excellent candidates for therapeutic development. With current peptide technology, it should be possible to develop a stable, peptide therapeutic for testing as a treatment for acute KD. By mapping T cell receptor contacts, it will be also possible to generate superior peptides with greater affinity and efficacy. These therapeutic

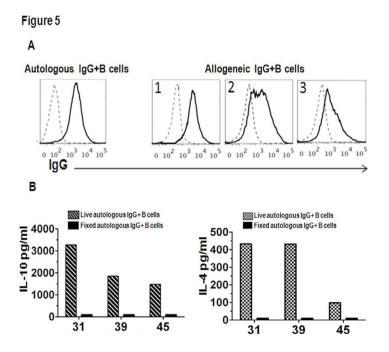
peptides may have applications other immune-mediated vasculopathies including atherosclerosis. Franco et al. Autoimmunity (in press)

Franco, A., Touma, R., Song, Y., Shimizu, C., Tremoulet, A.H., Kanegaye, J.T., Burns, J.C. Specificity of regulatory T cells that modulate vascular inflammation. Autoimmunity. 2014, in press (PMID:24490882).

Burns, J.C., Song, Y., Bujold, M., Shimizu, C., J.T. Kanegaye, Tremoulet, A.H., Franco, A. Immunemonitoring in Kawasaki disease children treated with infliximab and intravenous immunoglobulin. Clinical Experimental Immunology. 2013. 174:337-344. (PMID:23901839).

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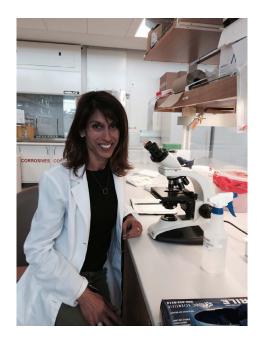
Franco, A., Shimizu, C., Tremoulet, A.H., Burns, J.C. Memory T cells and peripheral T cell clones in acute Kawasaki disease. Autoimmunity. 2010. 43: 317-324. (PMID 20166878).



Fc-specific Treg recognize the endogenous processed IgG presented by autologous B cells in a HLA-restricted, TcR-mediated fashion. Panel A:

Autologous and three allogeneic IgG+B cell lines used as APC in these experiments. Panel B: Live, but not paraformaldehyde-fixed, autologous EBV-transformed IgG+ B cells stimulated nTreg clones in the absence of exogenous Fc demonstrating that a) IgG needs to be processed for nTreg recognition; b) the nTreg response is not directed to self HLA molecules. Panel C left: Only autologous, but not allogeneic IgG+ B cells activated Fc-specific

Treg clone 39 (representative) demonstrating that the Treg response is MHC restricted.



Seema Aceves, M.D. Ph.D. Associate Professor of Pediatrics Allergy, Immunology & Rheumatology

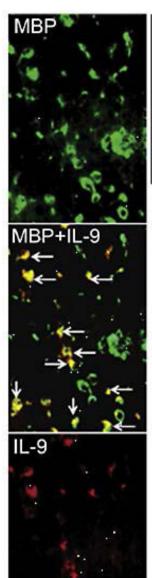
Expanding the Paradigm of Eosinophilic Esophagitis: Mast Cells and IL-9

Eosinophilic esophagitis (EoE) is a newly recognized antigen mediated chronic allergic esophageal disease that affects an increasing number of pediatric and adult subjects worldwide. Clinically, patients with EoE complain of dysphagia and children can have poor growth with vomiting and inadequate caloric intake. Complications of EoE include esophageal strictures and food impactions. The pathogenesis of these complications lies in tissue remodeling which causes esophageal fibrosis, rigidity, and dysmotility.

The Aceves lab was the first to describe the presence of and molecular mechanisms for remodeling in children with EoE. Both eosinophils and mast cells produce pro-fibrotic factors in EoE but the interactions between these cells are not clear. As a step in understanding this process, researchers in the departments of Pediatrics and Medicine, Otani and Aceves delineated the role of IL-9 in eosinophilic esophagitis in their 2013 publication in the Journal of Allergy and Clinical Immunology. Otani et al discovered that esophageal eosinophils in EoE produce the mast cell survival and chemotactic factor, IL-9. They further demonstrated that eosinophils and mast cells were found in closely paired cellular couplets. These cellular clusters were significantly diminished upon treatment with the biologic agent anti-IL-5 that reduces eosinophils. By evaluating cellular changes occurring in the presence of an antibody that specifically targets eosinophils, the researchers were able to discern that the eosinophil is essential for the instigation and/or propagation of esophageal mastocytosis. As this group of researchers had previously elucidated that mast cells were part of the molecular machinery that likely drive esophageal dysmotility in EoE. This study provided a novel potential therapeutic target in EoE that could improve patient symp-

Otani IM, Anilkumar AA, Newbury RO, Bhagat M, Beppu LY,

toms and esophageal complications.



Eosinophils produce II-9 in EoE. MBP (eosinophils, green) produce IL-9 (red, overlapping cells are yellow) in the esophagus of children with EoE

Dohil R, Broide DH, **Aceves SS**. Anti-IL-5 therapy reduces mast cell and IL-9 cell numbers in pediatric pateints with eosino-philic esophagitis. J Allergy Clin Immunol. 131:1576-82. 2013

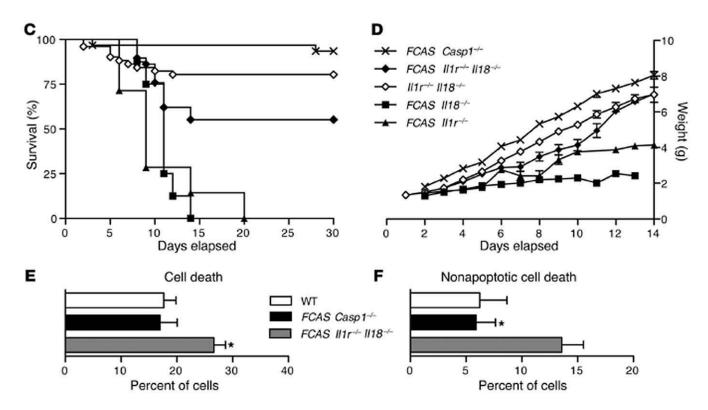


Lori Broderick M.D. Ph.D.
Postdoctoral Scholar
Allergy, Immunology & Rheumatology

Novel Therapeutic Targets for Autoinflammatory Diseases

Autoinflammatory diseases are a relatively new immune disorder classification encompassing rare inherited and

common inflammatory conditions that, in contrast to autoimmune and allergic disorders, are primarily dependent on dysregulated innate immune mechanisms. In 2001, we identified the gene for the cryopyrin associated periodic syndromes (CAPS), the most well-studied inherited autoinflammatory disease, and demonstrated that gain-of-function mutations in the gene that codes for cryopyrin result in hyperactive interleukin-1β (IL-1β) release and CAPS-associated systemic inflammatory symptoms. This led to effective, and now FDA-approved, IL-1 targeted therapy for patients with CAPS and other autoinflammatory disorders. Since that time, most research concerning the molecular basis of the autoinflammatory diseases has focused on pathways involving IL-1. To further elucidate autoinflammatory disorder pathophysiology, a team of Department of Pediatrics researchers, Broderick, Brydges, and Hoffman, examined the role of IL-18 in CAPS as described in a recently published article in the Journal of Clinical Investigation. We took a straightforward genetic approach to dissect the innate immune mechanisms involved in this inherited disease using human blood from CAPS patients and a mouse model of CAPS. It was demonstrated that human monocytes from CAPS patients produced elevated levels of IL-18, another mediator regulated by cryopyrin, and similarly, that IL-18 was elevated in serum and tissues from mutant CAPS mice. Dr. Broderick's group engineered CAPS associated mutations on IL-1 receptor (IL-1R) and IL-18 receptor (IL-18R) deficient



IL-1, IL-18, and cell death are all involved in CAPS disease pathogenesis. Caspase-1 induces inflammation in CAPS via cytokine mediators and pyroptosis. (C) Survival and (D) growth of of FCAS Casp1-/-, FCAS Il1r-/-Il18-/-, Il1r-/-Il18-/-, FCAS Il18-/-, and FCAS Il1r-/- mice (n = 7-51 for survival and 1-40 for growth). Weights are shown as mean ± SEM. Bone marrow cells were isolated from 16-week-old FCAS Casp1-/-, FCAS Il1r-/-Il18-/-, and WT mice and evaluated for (E) membrane asymmetry and (F) dyeexclusion by flow cytometry. Data are representative of 3 independent experiments (mean ± SEM shown). *P < 0.05, by Student's t test. This research was recently published in JCI. Figure from Brydges, Broderick et al. Journal of Clinical Investigation 123: 11: 4695-4705:

backgrounds to investigate the independent and collaborative role of these cytokines in CAPS pathogenesis.

The results showed that loss of IL-18R significantly improved survival and ameliorated inflammatory tissue phenotypes in young mice, but the inflammation reappeared in aged mice, suggesting that IL-18 is more important in the early phase of the disease. Surprisingly, mutant mice on a background deficient in both IL-18R and IL-1R still displayed a phenotype, suggesting other non-cytokine-mediated mechanisms, such as cell death, are also involved in this autoinflammatory disease. This study has important clinical implications for the treatment of patients with CAPS and other inflammatory diseases as clinicians are increasingly identifying patients resistant to IL-1 targeted therapy.

Brydges SD, Broderick L, McGeough MD, Pena CA, Mueller JL, Hoffman, HM. (2013) Divergence of IL-1, IL-18 and cell death in NLRP3 inflammasomopathies. J Clin Invest. 123: 11: 4695–4705

Brydges SD, Mueller JL, McGeough MD, Pena CA, Misaghi A, Gandhi C, Putnam CD, Boyle DL, Firestein GS, Horner AA, Soroosh P, Watford WT, O'Shea JJ, Kastner DL, Hoffman HM (2009). Animal Models of Inflammasomopathies Reveal Roles for Innate but not Adaptive Immunity. Immunity, 30: 6: 875-87.

Hoffman HM, Throne ML, Amar NJ, Sebai M, Kivitz AJ, Kavanaugh A, Weinstein SP, Belomestnov P, Yancopoulos GD, Stahl N, Mellis SJ (2008). Efficacy and safety of rilonacept (interleukin-1 trap) in patients with cryopyrin-associated periodic syndromes: Results from two sequential placebo-controlled studies. Arthritis Rheum, 30: 58(8): 2443-52

Hoffman HM, Rosengren S, Boyle D, Cho J, Nayar J, Mueller J, Anderson J, Wanderer A, Firestein G (2004). Prevention of cold-associated inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist prevents. Lancet, 364:9447:1779-85.

Hoffman HM, Mueller, JL, Broide, DH, Wanderer, AA, Kolodner, RD (2001). Mutation of a new gene encoding a putative pyrinlike protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. Nature Genetics, 29:3:301-305.





Jane C. Burns, M.D.
Professor of Pediatrics in Residence
Allergy, Immunology & Rheumatology

Primary Therapy for Kawasaki Disease

Kawasaki disease (KD) is the leading cause of acquired heart disease in developed countries for which intravenous immunoglobulin (IVIG) is an effective treatment that reduces the risk of aneurysms from 25% to 5%. However, approximately 10-20% of patients fail to become afebrile after a single IVIG infusion and these IVIG-resistant patients have a higher risk of aneurysms. Tumor necrosis factor (TNF)α plays an important role in the pathogenesis of KD. A single dose of the anti-TNFα monoclonal antibody, infliximab, was shown by our group to be safe in children with KD. It was assessed whether the addition of infliximab to standard intravenous immune globulin (IVIG) and aspirin therapy in acute KD reduces the rate of treatment resistance. Department of Pediatrics researchers, Tremoulet and Burns conducted a Phase III, randomized, double-blinded, placebo-controlled, two-center trial to evaluate the addition of infliximab (5 mg/kg) to standard therapy in children with acute KD as described in an upcoming article in The Lancet. Among 195 subjects who received study drug, the treatment resistance rate was 11% in both arms. Subjects treated with infliximab had fewer days of fever (1 vs. 2, P<0.0001), greater reductions in erythrocyte sedimentation rate (P=0.009) and Z score of the left anterior descending coronary artery (LAD) at week 2 (P=0.045), and greater reductions in C-reactive protein concentration (P<0.001) and absolute neutrophil count

(P<0.024) 24 hours after completion of the IVIG infusion than those treated with standard therapy. No IVIG infusion reactions occurred in subjects treated with infliximab compared to 13% of placebo-treated subjects (P<0.0001). No serious adverse events were directly attributable to infliximab infusion.

Therefore, although the addition of infliximab to primary treatment in acute KD did not reduce treatment resistance, it was safe and well-toler-

ated and reduced fever duration, markers of inflammation, LAD Z score, and IVIG reaction rates. Based on these data, Drs. Burns and Tremoulet advocate the use of infliximab for high-risk KD patients with clinically severe disease as evidenced by marked elevation of inflammatory parameters, a presentation with shock, or coronary artery Z scores >2.5 on the initial echocardiogram. *Tremoulet et al. Lancet (in press)*

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K, Keenan G, Ramilo O. Infliximab treatment of IVIG-resistant Kawasaki disease. J Pediatr 2008 153(6):833-8. (PMID: 18672254)

Son MB, Gauvreau K, Burns JC, Corinaldesi E, Tremoulet AH, Watson VE, Baker A, Fulton DR, Sundel RP, Newburger JW. Infliximab for IVIG resistance in Kawasaki disease: A retrospective two-center study. J Pediatr. 2011 Apr;158(4):644-649.e1. (PMID: 21129756)

Tremoulet AH, Jain S, Jaggi P, Jiminez-Fernandez S, Pancheri JM, Sun X, Kanegaye JT, Kovalchin JP, Printz B, Ramilo O, Burns JC. Phase III randomized, double-blind, placebo-controlled trial of infliximab for intensification of primary therapy for Kawasaki disease. In press, Lancet.

Burns JC, Song Y, Bujold M, Shimizu C, Kanegaye JT, Tremoulet AH, Franco A. Immune-monitoring in Kawasaki disease patients treated with infliximab and intravenous immunoglobulin. Clinical and Experimental Immunology. 2013 Jul 31. doi: 10.1111/cei.12182. [Epub ahead of print] (PMID:23901839)

	Infliximab	Placebo	P value
Treatment resistance, n (%)	11 (11.2)	11 (11.3)	NS
IVIG infusion reaction, n (%)	0 (0)	13 (13.4)	<0.0001
Days of hospitalization, n (IQR)	3 (3-4)	3 (3-4)	NS
Days of fever, (IQR)*	1 (1-2)	2 (1-2)	<0.0001
C-reactive protein @ 24h post-treatment, mg/dl	5.3	4.1	0.01
Erythrocyte sedimentation rate @ 2 weeks, mm/h	46	36	0.003
Change in left anterior descending artery Z score**	-0.61 (-0.81 to 0.40)	-0.31 (-0.51 to 0.11)	0.045

Table. Clinical outcomes in subjects by treatment group.

NS = not significant *Day of fever = Calendar day from
the day of enrollment with any temperature ≥ 38oC

IQR= interquartile range **Z score= standard deviation units from the mean normalized for body surface
area From Tremoulet et al. Lancet (in press)



Wynnis Tom, M.D. Assistant Clinical Professor of Pediatrics Pediatric and Adolescent Dermatology

SEVERE ECZEMA NEEDS IMMUNE SUPRESSION

Atopic dermatitis (eczema) is a chronic and very itchy inflammatory skin disorder that affects over 20% of children. While most can be well-managed with topical therapies, a small subset continues to have severe, uncontrolled disease that may need treatment with oral medications that suppress the immune system. While helpful, they do carry substantial risks and need careful dosing and monitoring. Dr. Tom has been investigating

these drugs to improve their use while trying to minimize side effects.

Dr. Tom's research led to the discovery that a protein that breaks down the drug azathioprine, called TPMT (thiopurine methyltransferase), may change its activity during treatment. This can affect how well the drug works, either positively or negatively, as well as alter how much drug should be given. Previously, TPMT activity was only checked before starting treatment, but her work has shown that repeat testing may be needed in some patients. Dr. Tom is continuing to extend her research to other drugs used to treat severe eczema, and she is collaborating with multiple large pediatric centers to provide evidence-based information.

Caufield M and Tom WL. Oral Azathioprine for Recalcitrant Pediatric Atopic Dermatitis: Clinical Response and Thiopurine Monitoring. Journal of the American Academy of Dermatology. 2013 Jan;68(1):29-35.

Dasher DA and Tom WL. Atopic Dermatitis. In Severe Skin Diseases in Children: Beyond Topical Therapy. Tom WL, ed. Berlin; New York: Springe



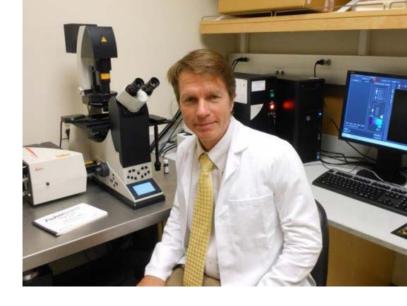


9 year-old with severe eczema and good response to azathioprine therapy

Lawrence (Lance)
Prince, M.D., Ph.D.
Division Chief, Associate
Professor of Pediatrics
Neonatology

How Inflammation Disrupts Normal Lung Development

The laboratory of Dr. Lawrence (Lance) Prince has discovered new insights into how infection and inflammation disrupt normal growth and development in preterm infants. The NF-κB signaling pathway has been known to signal an inflammatory response when cells are exposed to infectious organisms or specific pro-inflammatory cytokines. However, the molecular mechanisms linking this pathway to abnormal lung development in preterm infants that develop bronchopulmonary dysplasia was unknown. The Prince lab previously discovered that NF-kB activation inhibited expression of the key growth fac-

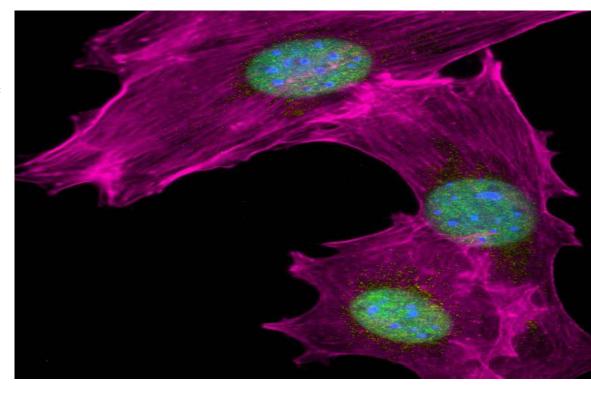


tor FGF-10. In a more recent study, they determined the molecular mechanisms linking NF- κ B with abnormal FGF-10 expression. When activated, NF- κ B interacts with the transcription factor Sp3, switching Sp3 from a gene activator to a repressor. This effect involves interactions between the RelA subunit of NF- κ B and previously uncharacterized regions in the amino-terminus of Sp3.

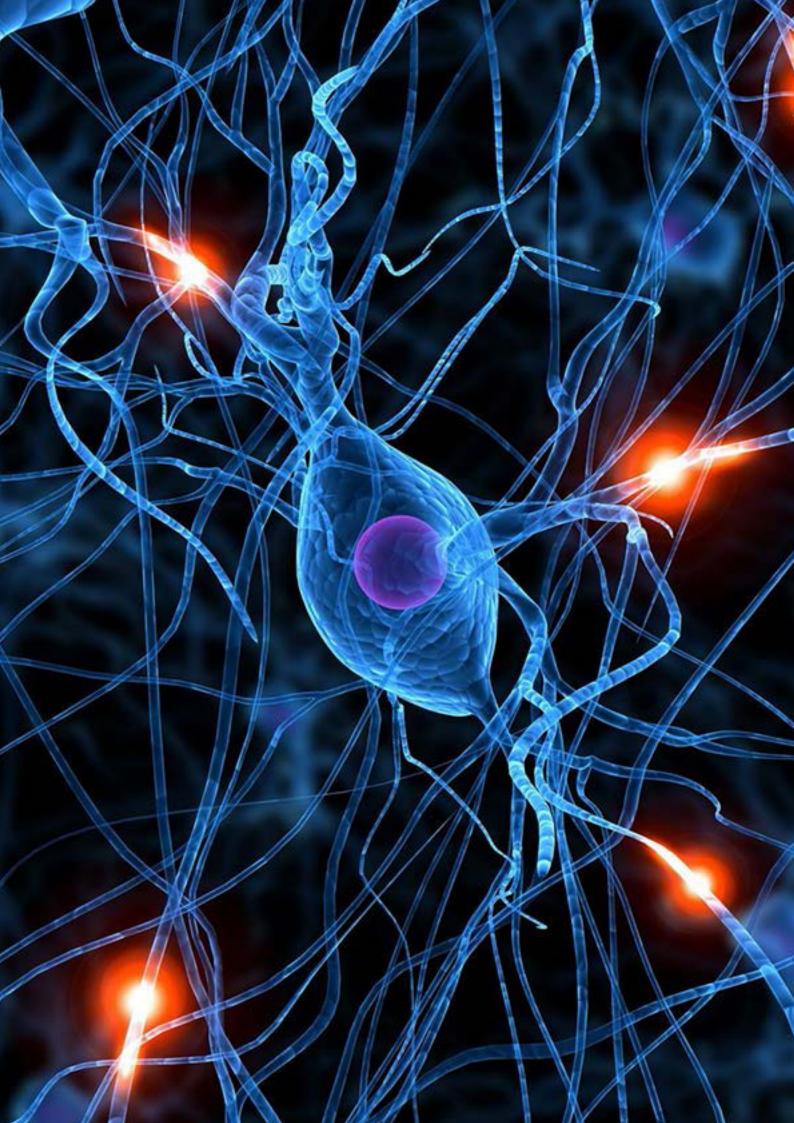
By uncovering this mechanism, the Prince lab has identified a novel mechanism that could be targeted for developing new pharmacologic agents to protect human development in the face of infection and inflammation.

Carver BJ, Plosa EJ, Stinnett AM, Blackwell TS, Prince LS. Interactions between NF-kB and SP3 connect inflammatory signaling with reduced FGF-10 expression. J Biol Chem. 2013 May 24;288(21):15318-25.

Expression of Sp3 (green) in fetal lung mesenchymal cells. Interactions between NF-κB and Sp3 inhibit expression of the critical lung growth factor FGF-10.



Neurobiology





Alysson R. Muotri, Ph.D. Assistant Professor of Pediatrics, Division of Genetics Assistant Professor of Cellular & Molecular Medicine

Modeling premature AGING AND CANCER SUSCEPTIBLY WITH IPS CELLS

Cockayne syndrome (CS) is a human premature aging disorder associated with neurological and developmental abnormalities, caused by mutations mainly in the CS group B gene. At the molecular level, CS is characterized by a deficiency in the transcription-couple DNA repair pathway.

To understand the role of this molecular pathway in a pluripotent cell and the impact of CSB mutation during human cellular development, Dr. Muotri, generated induced pluripotent stem cells (iPSCs) from CSB skin fibroblasts (CSB-iPSC). Dr. Muotri showed that the lack of functional CSB does not represent a barrier to genetic reprogramming. However, iPSCs derived from CSB patient's fibroblasts exhibited elevated cell death rate and higher reactive oxygen species (ROS) production.

Moreover, these cellular phenotypes were accompanied by an up-regulation of TXNIP and TP53 transcriptional expression. Our findings suggest that CSB modulates cell viability in pluripotent stem cells, regulating the expression of TP53 and TXNIP and ROS production.

Andrade LN, Nathanson JL, Yeo GW, Menck CF, Muotri AR. Hum Mol Genet. 2012 Sep 1;21(17):3825-34 (2012). Evidence for premature aging due to oxidative stress in iPSCs from Cockayne syndrome.



IPSC-derived teratomas developed under the skin of nude mice. In control cells, the tumor grows due to the ability of the pluripotent cells to different into blood vessels. When the CSB gene is mutated, pluripotent cells failed to differentiate, reducing the formation of blood vessels and, as a consequence, the size of tumors is small compared to controls.

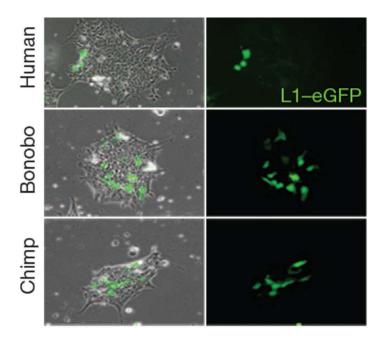
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L1 MOBILIZATION IN NON-HUMAN PRIMATE PLURIPOTENT STEM CELLS

hat make us unique compared to other primates? Humans have more cognitive variation than chimpanzees and bonobos. However, we lack the genetic diversity. In this manuscript, the Muotri labexplored the activity of L1 retroelements (jumping genes) as a source of genetic diversity in primate embryonic stem cells. The Muotri Lab reprogrammed skin cells from humans, chimps and bonobos to test if these cells can accommodate L1 mutations. They showed that not only chimps and bonobos' pluripotent

stem cells lack the expression of genes that control the activity of L1 elements, but also allow them to cause more mutations than human cells. Our findings confirm the idea that modern humans lack genetic diversity because we acquire protection from genetic insults at the embryonic level. That may be related to evolutionary bottlenecks that humans overcome. The work opens new ways of thinking about infectious diseases but most important, it shows how the reprogramming technology could be used for evolutionary studies, helping to test hypotheses.

Marchetto MC, Narvaiza I, Denli AM, Benner C, Lazzarini TA, Nathanson JL, Paquola AC, Desai KN, Herai RH, Weitzman MD, Yeo GW, Muotri AR, Gage FH. Differential L1 regulation in pluripotent stem cells of humans and apes. Nature. 2013 Nov 28;503(7477):525-9 (2013).



Chimpanzee's pluripotent stem cells are able to support L1 activity. Here, we use a L1-eGFP to visualize the activity of jumping genes in these cells under the fluorescent microscope



Albert La Spada, M.D., Ph.D.

Division Chief of Genetics, Department of Pediatrics
Professor of Cellular & Molecular Medicine
Professor of Neurosciences
Professor, Division of Biological Sciences
Associate Director, Institute for Genomic Medicine

FROM PGC-1a to TFEB: MILEPOSTS ON THE ROAD TO THERAPY DEVELOPMENT FOR NEURODEGENERATIVE DISEASE?

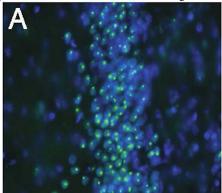
Neurodegenerative disorders comprise one large category of untreatable human disease characterized by age-dependent neuronal demise, initiated by the cell's inability to deal with a specific misfolded protein stress. Huntington's disease (HD) is one such disorder, resulting from the production of huntingtin (htt) protein containing a polyglutamine expansion tract. HD patients display a progressive movement disorder and cognitive decline, for which no suitable therapy is currently available. HD pathogenesis stems from mitochondrial dysfunction and altered nuclear gene transcription, linked

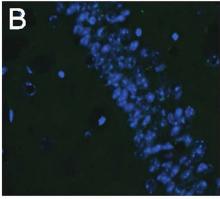
to impaired action of the transcription activator PPARy co-activator 1α (PGC- 1α). In a study published in Science Translational Medicine, Dr. La Spada's lab tested if increased PGC- 1α function could ameliorate HD neurological phenotypes and neurodegeneration by crossing an inducible PGC- 1α over-expression mouse model with HD transgenic mice. It was found that not only does PGC- 1α improve HD neurological function in PGC- 1α over-expressing HD transgenic mice, but PGC- 1α also virtually eradicates htt protein aggregates in the brains of HD mice (see Figure). When the mechanistic basis of this genetic rescue in neuronal cell lines were analyzed, HD striatal-like cells, and in the brains of PGC- 1α -over-expressing HD mice, it was documented

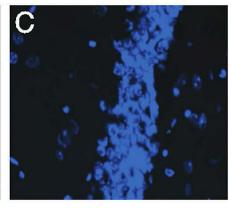
that increased PGC-1a expression promoted mitochondrial function by boosting oxidative phosphorylation activity and reduced oxidative stress by turning on reactiveoxygen species defense genes. However, PGC-1a 's most significant action in eliminating misfolded htt protein was to induce the expression of TFEB, a master regulatory transcription factor that activates genes in the autophagy-lysosome pathway of protein / organelle turnover. PGC-1a regulation of TFEB underscores the importance of maintaining mitochondrial quality control under conditions of accelerated mitochondrial biogenesis and high-level ATP generation. As altered energy production and impaired protein / organelle quality control are key features of neurological disease, these findings establish PGC-1a and TFEB as attractive therapeutic targets for HD and for other neurological disorders characterized by protein misfolding.

Tsunemi T, Soriano KR, Morrison BE, Au J, Vázquez Roque RA, Lazarowski ER, Damian VA, Masliah E & La Spada AR. (2012). PGC-1□ rescues Huntington's disease by preventing oxidative stress and promoting TFEB function. Science Translational Medicine 4: 142ra97

Because of the importance of this work, Discover magazine selected this publication as one of the top 100 science discoveries of 2012 http://discovermagazine.com/2013/jan-feb/72-protein-boost-halts-huntingtons#. Uv7OBvZur2k>.







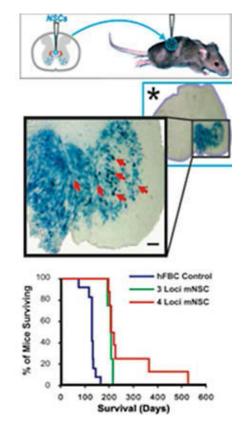
Here we see representative sections from the hippocampus of 18 week-old HD mice (A), non-transgenic littermate controls (B), and HD mice induced to express PGC- 1α (C). Sections were stained with anti-htt antibody EM48 (green) and counterstained with DAPI (blue). Htt aggregates are virtually eliminated in the brain of the HD + PGC- 1α mice (C)



Evan Snyder, M.D., Ph.D.
Professor of Pediatrics
Director, Stem Cell and
Regeneration Program
Sanford-Burnham Institute
for Medical Research

STEM CELLS WORK THROUGH MULTIPLE MECHANISMS

Dr. Evan Snyder's group recently was the first to illustrate the multiple facets of stem cell action in a therapeutic context and how stem cells can be used to better understand disease. Neural stem cells (NSCs) were used as the index stem cell type and Amyotrophic Lateral Sclerosis (ALS) or Lou Gehrig's disease was used as the prototype for a complex disease state. NSCs isolated from the nervous system and grown in a dish can turn into any type of nervous system cell and help to restore function to damaged tissues. NSCs may therefore be useful in repairing injured brain and spinal cord neurons. ALS is a condition in which nerve cells in the brain and spinal cord (particularly motor neurons) degenerate. Evan Snyder lead a team of investigators that analyzed the results of 11 studies they performed in which NSCs were transplanted into the spinal cords of mice with ALS. The authors found that transplanted NSCs (both of mouse and human origin) could slow both the onset and progression of symptoms and prolong survival in diseased mice. In some cases, mice trocytes) produced within the mouse's own nervous system (presumably by changing the program of the mouse's own diseased NSCs) & replace them



Neural Stem Cells (NSCs) transplanted into a mouse model of ALS engraft into the spinal cord (as visualized by blue staining) and prolong survival (green and red survival curves).

with ALS that normally die within 120 days were able to survive for more than one year. Improvement was particularly noticeable in animals where the transplanted stem cells covered a large part of the spinal cord, including regions responsible for breathing and other vital functions. The benefits of NSCs seemed to derive not from replacing degenerating motor neurons but rather from their ability (1) to produce survival-promoting substances called trophic factors that rescued and protected the animal's own diseased motor nerve cells and their connections, (2) to reduce inflammation, and (3) to suppress the number of disease-promoting neural cells (aswith well-behaved astrocytes (derived from the transplanted, fresh, non-diseased NSCs). The results suggest that transplanted NSCs, through multiple modulatory mechanisms, could be a potential new therapy for treating ALS and other untreatable degenerative diseases.

Teng YD, Benn SC, Kalkanis SN, Shefner JM, Onario RC, Cheng B, Lachyankar MB, Marconi M, Li J, Yu D, Han I, Maragakis NJ, Lládo J, Erkmen K, Redmond DE Jr, Sidman RL, Przedborski S, Rothstein JD, Brown RH Jr, Snyder EY. Multimodal actions of neural stem cells in a mouse model of ALS: a meta-analysis. Sci Transl Med. 2012 Dec 19;4(165):165ra164.



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Assistant Adjunct
Professor,
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Mark Nespeca M.D.
Clinical Professor,
Neurosciences & Pediatrics
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Sonya Wang M.D. Assistant Clinical Professor, Neurosciences & Pediatrics Pediatric Neurology

THE ROLE OF CONTINUOUS ELECTROENCEPHALOGRAPHY IN CHILDHOOD ENCEPHALITIS

Demonstrated that continuous recording of the EEG in patients with suspected viral encephalitis identified electrographic seizures, some of which were not clinically apparent, and also demonstrated a high incidence of seizures (46%)

in children with suspected encephalitis.

Children with encephalitis are at high risk for seizures, and continuous EEG monitoring can guide treatment by identifying seizures accurately.

Gold JJ, Crawford JR, Glaser C, Sheriff H, Wang S, Nespeca M. The Role of Continuous Electroencephalography in Childhood Encephalitis. Pediatr Neurol. 2013 Dec 19. Pii: S0887-8994(13)00752-2. Doi: 10.1016/j.pediatrneurol.2013.12.014. [Epub ahead of print]

TABLE 5.Seizures recorded and concerning clinical events shown not to be seizures by EEG

	n	Clinical or Subclinical	Subclinical	Shown Not to be a Seizure	
		Seizure Recorded	Seizure Recorded		
All EEG	143	34 (23.8%)	30 (21.0%)	26 (18.2%)	
rEEG*	129	12 (9.3%)	8 (6.2%)	9 (7.0%)	
cEEG*	54	30 (55.6.0%)	28 (51.9%)	19 (35.2%)	

Abbreviations:

EEG = Electroencephalograph

cEEG = Continuous electroencephalograph

rEEG = Routine electroencephalograph

* Compared with rEEG, cEEG was more likely to record a clinical or subclinical seizure, or demonstrate that a concerning event was not a seizure (all comparisons P < 0.0001 by Fisher exact test).



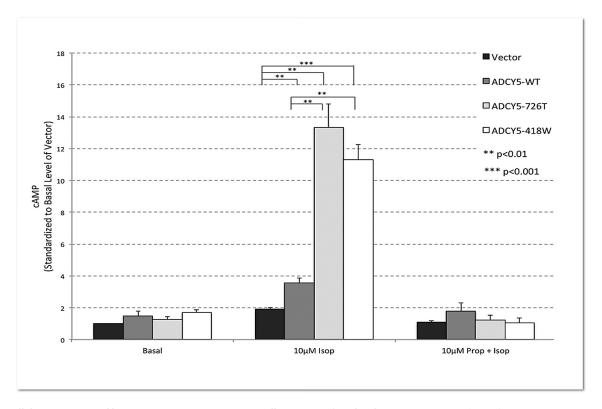
Jennifer Friedman M.D.
Associate Clinical Professor of Neurosciences
Neurology

GENETIC LINK IDENTIFIED IN RARE MOVEMENT DISORDER

Familial dyskinesia with facial myokymia is a newly described, rare movement disorder affecting children and adults. FDFM is caused by gain-of-function mutations in different domains of adenylyl cyclase 5 (ADCY5) – the first definitive link between adenylyl cyclase mutation and human disease. We have illustrated the power of hypothesis-free exome sequencing in establishing diagnoses in rare disorders with complex and variable phenotype. Mutations in ADCY5 should be considered in patients with undiagnosed complex movement disorders even in the absence of a family history.

Identification of the first definitive link between adenylyl cylcase and human disease. Demonstrated the power of exome sequencing in establishing diagnoses in rare disorders. Characterization of phenotypic variability in newly described movement disorder.

Friedman*, Jennifer R.; Chen*, Ying-Zhang; Chen*, Dong-Hui; Chan Guy C. K.; Bloss, Cinnamon S.; Hisama, Fuki M; Topol, Sarah E. Carson, Andrew R.; Pham, Phillip H.; Bonkowski, Emily S.; Scott, Eric R.; Lee, Janel K.; Zhang, Guangfa; Oliveira, Glenn; Xu, Jian; Scott-Van Zeeland, Ashley A.; Chen, Qi; Levy, Samuel; Topol, Eric J.; Storm, Daniel; Swanson, Phillip D.; Bird, Thomas D.; Schork, Nicholas J.; Raskind, Wendy H.; Torkamani, Ali. Gain-of-Function ADCY5 Mutations Cause Familial Dyskinesia with Facial Myokymia. Annals of Neurology (in press)



Intracellular cAMP assessed by enzyme immunoassay. HEK293 cells were transfected with empty EGFP vector (Vector) or expression constructs of EGFP tagged WT-, 418W- or 726T-ADCY5 for 24 hours. Cells were treated under different adenylyl cyclase enzyme activating conditions - carrier alone, isoproterenol (Isop), or propranolol (Prop) plus Isop for 15 minutes - and cAMP level was assessed using an enzyme immunoassay. Treatment with Isop resulted in markedly increased cAMP from the carrier treated basal levels. In comparison to ADCY5-WT, both mutant constructs demonstrated significantly increased levels of intracellular cAMP. Pretreatment with Prop abrogated the stimulatory effect of Isop for all constructs.



Joseph Gleeson M.D.
Professor of Neurosciences and Pediatrics
Neurology

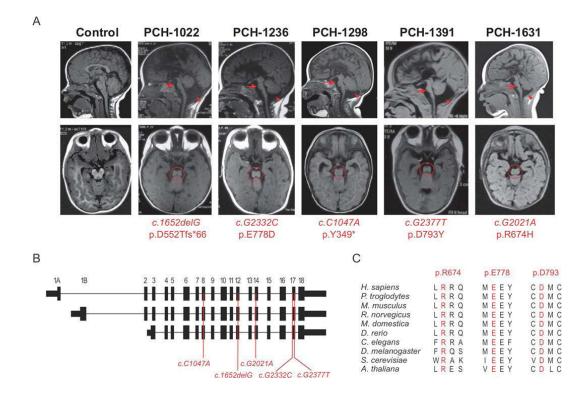
Novel Purine Metabolism Disorder Identified

Despite the discovery over the past decades of

myriad disorders affecting purine nucleotide metabolism, the mechanisms of cellular toxicity are not well understood. Dr. Gleeson identified a novel purine metabolism disorder, manifested as brainstem and cerebellum neurodegeneration, caused by mutations in AMPD2. Neural toxicity underlying AMPD2 deficiency results from guanine nucleotide depletion and consequent protein synthesis inhibition, which can be prevented through bypass of the block in de novo purine synthesis.

Our work suggests AMPD2 as a potentially treatable neurodegenerative disorder.

Akizu N, Cantagrel V, Schroth J, Cai N, Vaux K, McCloskey D, Naviaux RK, Van Vleet J, Fenstermaker AG, Silhavy JL, Scheliga JS, Toyama K, Morisaki H, Sonmez FM, Celep F, Oraby A, Zaki MS, Al-Baradie R, Faqeih EA, Saleh MA, Spencer E, Rosti RO, Scott E, Nickerson E, Gabriel S, Morisaki T, Holmes EW, Gleeson JG. AMPD2 regulates GTP synthesis and is mutated in a potentially treatable neurodegenerative brainstem disorder. Cell. 2013 Aug 1;154(3):505-17.



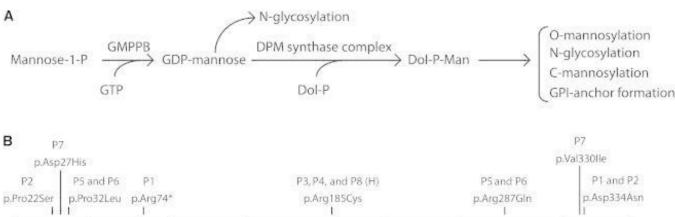
Recessive mutations in AMPD2 cause loss of brainstem and cerebellar structures, typical of PCH. (A) Brain MRI from one of the affected patients for each PCH family. Control child brain MRIs are from pediatric brain atlas (http://www.seattlechildrens.org/healthcare-professionals/education/radiology/pediatric-brainatlas/). Upper: midline sagittal MRI, Lower: axial MRI at optic chiasm level. Images show smaller cerebellum (red arrowhead) and brainstem hypoplasia (red arrow) typical of PCH in all patients compared with the control. All patients show near complete absence of the corpus callosum. All patients show typical "Figure 8" shape of the brainstem (red circle). Corresponding mutation in cDNA (c) and protein (p) is below each scan. Fs: frame shift; *: stop codon.(B) Schematic representation of three AMPD2 isoforms. Mutations indicated relative to their position in cDNA.(C) Mutated amino acids in PCH are fully conserved.

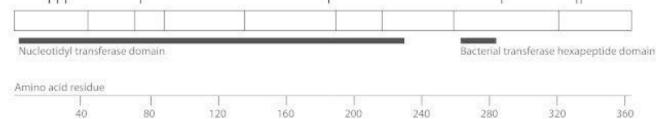


Carla Grosmann M.D.
Associate Clinical Professor of
Neurosciences & Pediatrics
Pediatric Neurology

A new gene responsible for congenital muscular dystrophy (CMD) was discovered in one of our patients due to a mutation in the GDP-Mannose Pyrophosphorylase B. This mutation is added to the list of disease causing mutations in alpha-dystroglycan associated CMDs.

Keren J. Carss, Elizabeth Stevens, A. Reghan Foley, Sebahattin Cirak, Moniek Riemersma, Silvia Torelli, Alexander Hoischen, Tobias Willer, Monique van Scherpenzeel, Steven A. Moore, Sonia Messina, Enrico Bertini, Carsten G. Bo"nnemann, Jose E. Abdenur, Carla M. Grosmann, Akanchha Kesari, Jaya Punetha, Ros Quinlivan, Leigh B. Waddell, Helen K. Young, Elizabeth Wraige, Shu Yau, Lina Brodd, Lucy Feng, Caroline Sewry, Daniel G. MacArthur, Kathryn N. North, Eric Hoffman, Derek L. Stemple, Matthew E. Hurles, Hans van Bokhoven, Kevin P. Campbell, Dirk J. Lefeber, UK10K Consortium, Yung-Yao Lin and Francesco Muntoni. Mutations in GDP-Mannose Pyrophosphorylase B Cause Congenital and Limb-Girdle Muscular Dystrophies Associated with Hypoglycosylation of α-Dystroglycan. American Journal of Human Genetics 93(1) pp. 29 – 41.





GMPPB Function, Structure, and Identified Substitutions. (A) The function of GMPPB in glycosylation pathways. (B) GMPPB has 360 amino acids and two predicted Pfam functional domains: a nucleotidyl transferase domain and a bacterial transferase hexapeptide domain. In this schematic diagram, the blocks represent regions encoded by exons and the substitutions identified in individuals with dystroglycanopathy are shown. The following abbreviation is used:

H, homozygous.









Richard Haas M.D.
Professor of Neurosciences and Pediatric
Neurology

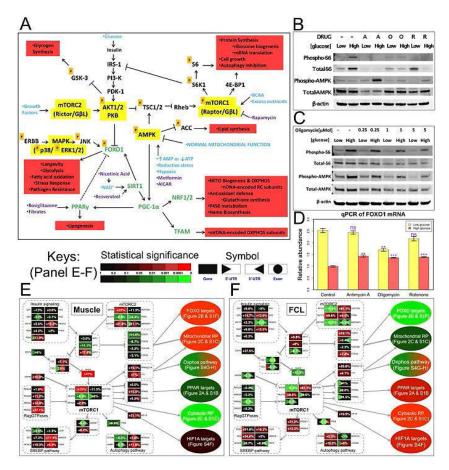
TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL DYSREGULATION IN MITOCHONDRIAL RESPIRATORY DISORDERS

Dr. Haas studied skeletal muscle and fibroblast transcrip-

tomes from patients with a number of different mitochondrial respiratory chain (RC) disorders. Using integrated gene, pathway, and systems biology analyses, significant changes were found in muscle across diverse RC complex and genetic etiologies that were consistent with prior reports in other primary RC disease models and involved dysregulation of genes involved in RNA processing, protein translation, transport, and degradation, and muscle structure. Global transcriptional and post-transcriptional dysregulation was also found to occur in a highly tissue-specific fashion.

The study provides a transcriptional profile of RC disease that may be of potential utility as a diagnostic aid in the clinical setting. Identification of novel adaptive mechanisms at transcriptional and post-transcriptional levels to primary RC disease can also serve as a means to monitor primary RC disease progression or even response to therapy in clinical trials.

Zhang Z, Tsukikawa M, Peng M, Polyak E, Nakamaru-Ogiso E, Ostrovsky J, McCormack S, Place E, Clarke C, Reiner G, McCormick E, Rappaport E, Haas R, Baur JA, Falk MJ. Primary respiratory chain disease causes tissue-specific dysregulation of the global transcriptome and nutrient-sensing signaling network. PLoS One. 2013 Jul 24;8(7):e69282. doi: 10.1371/journal. pone.0069282. Print 2013. PubMed PMID: 23894440; PubMed Central PMCID: PMC3722174



Primary RC dysfunction transcriptionally and post-transcriptionally dysregulates the integrated nutrient-sensing signaling network.

- (A) Integrated overview modeling general interactions between central nutrient-sensing signaling pathways. Arrows and bars convey activating and inhibiting effects, respectively. TFs, physiologic signals, and drugs known to modulate this pathway are indicated in green, blue, and purple font, respectively. "P" indicates pathway components whose activity is modulated by phosphorylation. Red boxes detail physiologic effects.
- (B) Oligomycin-based pharmacologic RC inhibition in human FCLs alters mTORC1 and AMPK pathway activities. To confirm primary mitochondrial RC dysfunction was sufficient to alter mTORC1 signaling, FCLs from a healthy individual were treated in DMEM medium containing 20% fetal bovine serum for 24 hours and either low (1 g/L or 5 mMol) or high (4.5 g/L or 25 mMol) glucose, with either the complex V inhibitor, Oligomycin, ("O", 5 uMol), an AMPK activator, AICAR ("A", 2 mMol), or the mTORC1 inhibitor, rapamycin ("R", 100 nMol). Regardless of glucose concentration, expression of a standard readout of mTORC1 pathway activity, phospho-S6 protein level, was reduced by oligomycin treatment, while increased phospho-AMPK expression was evident in high glucose media.



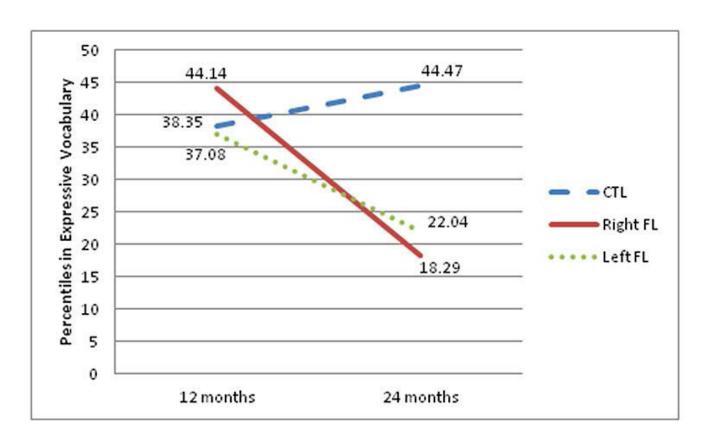
Doris Trauner M.D.
Professor of Neurosciences and Pediatrics
Co-Director, Neurology

EARLY LANGUAGE DEVELOPMENT AFTER PERINATAL STROKE

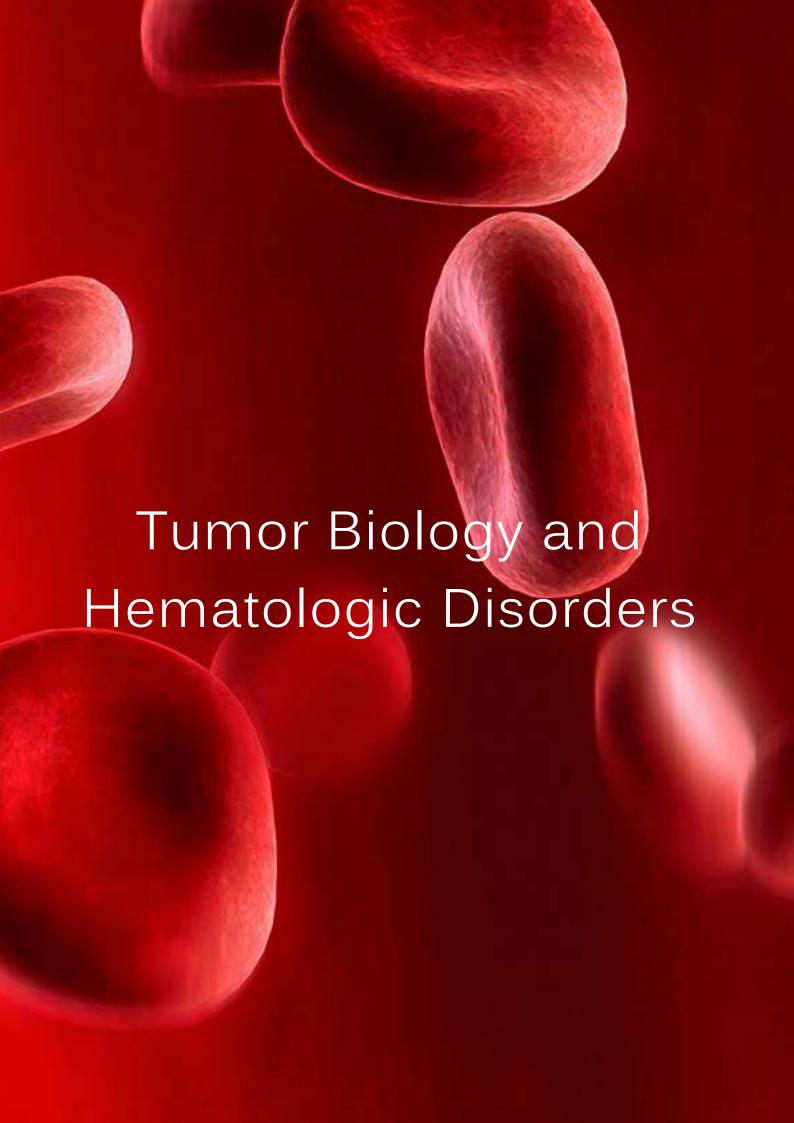
Dr. Trauner has described a different pattern and trajectory of early language development in children who had perinatal unilateral brain injury from stroke compared with non-brain-damaged children with typical language development. The difference in language development may reflect ongoing brain reorganization secondary to plasticity in the developing brain.

In the case of early focal brain injury, early language differences do not necessarily imply permanent impairment, but may indicate that a different means of organizing language is taking place in the face of that injury.

Trauner DA, Eshagh K, Ballantyne AO, Bates E. Early language development after perinatal stroke. Brain and Language 2013 May 24. doi:pii: S0093-934X(13)00090-4. 0.1016/j. bandl.2013.04.006. [Epub ahead of print]



Percentile scores for expressive vocabulary on the McArthur-Bates Communicative Development Inventory for children with right (Right FL) or left hemisphere (Left FL) perinatal stroke and typically developing controls (CTL) at 12 and 24 months. While all groups are equivalent at 12 months, both right and left lesion subjects drop off significantly from controls by 24 months, and there is no difference in expressive vocabulary based on the side of the lesion.





Jing Yang, M.D.
Assistant Professor of Pediatrics
and Pharmacology
Hematology/Oncology

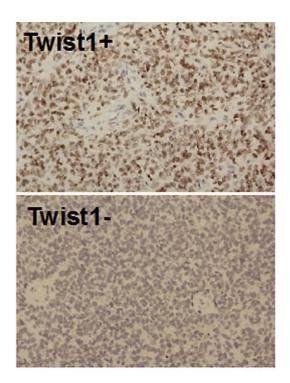
Developing a Novel Therapeutic Approach for Ewing's Sarcoma Metastasis

Dr. Yang's research aims to understand the molecular basis of tumor metastasis and to develop effective therapeutics against metastatic diseases1,2,3. Ewing's Sarcoma is a poorly differentiated malignant cancer of bone and soft tissue that usually occurs between 10-20 years of age. Despite aggressive therapy and marked improvement in survival among patients with local disease during the past 40 years, almost no improvement has been seen in patients with metastatic disease (80% mortality). The failure to stop metastasis is partly due to the lack of understanding about the molecular pathways that regulate its spread. To address this unmet need, she hypothesized that a group of genes that regulate the generation and function of a specialized group of embryonic stem cells (neural crest cells) are reactivated to allow Ewing Sarcoma cells to metastasize. The data from her studies show that expression of one such gene (Twist1) is associated with metastasis and poor survival in Ewing Sarcoma patients diagnosed at Rady Children's Hospital. Currently, her group is using various cell and molecular biology tools,

and mouse tumor models to dissect the role of the neural crest migration gene program in Ewing Sarcoma metastasis and to identify novel small molecule inhibitors against this pathway to block metastasis.

Dr. Yang hopes that the research from her group will reduce morbidity from ineffective conventional therapies and save the lives of countless children affected by metastatic Ewing's sarcoma.

Mark A. Eckert, Thinzar M. Lwin, Andrew T. Chang, Jihoon Kim, Etienne Danis, Lucila Ohno-Machado, and Jing Yang. (2011) Twist1-inudced invadopodia formation promotes tumor metastasis. Cancer Cell. 19(3): 372-86.



Jeff H. Tsai, Joana Liu Donaher, Danielle Murphy, Sandra Chau, and Jing Yang. (2012) Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. Cancer Cell. 22(6): 725-36.

Janine M. Low-Marchelli, Veronica C. Ardi, Edward A. Vizcarra, Nico van Rooijen, James P. Quigley, and Jing Yang. (2013) Twist1 induces CCL2 and recruits macrophages to promote angiogenesis. Cancer Research. 73(2): 662-71.

Expression of Twist1 in Ewing's sarcoma is a prognostic marker for poor survival. A) Examples of Ewing's sarcoma tumor sections that are stained positive or negative for Twist1. B) Kaplan-Meier survival curve based on Twist1 positivity in Ewing's sarcomas.

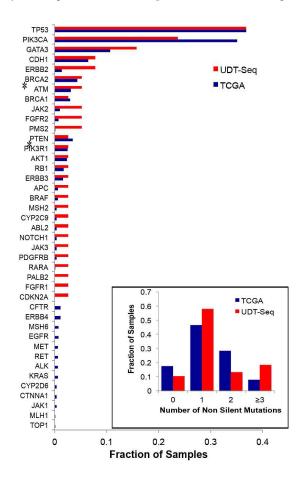


Olivier Harismendy, Ph.D.
Adjunct Assistant
Professor of Pediatrics
Genome Information Sciences

Evaluation of Ultra-Deep Targeted Sequencing for Personalized Breast Cancer Care.

The increasing number of targeted therapies, together with a deeper understanding of cancer genetics and drug response, have prompted major healthcare centers to implement personalized treatment approaches relying on high-throughput tumor DNA sequencing. However, the optimal way to implement this transformative methodology is not yet clear. Current assays may miss important clinical information such as the mutation allelic fraction, the presence of sub-clones or chromosomal rearrangements, or the distinction between inherited variants and somatic mutations. Here, Dr. Harismendy, present the evaluation of ultra-deep targeted sequencing (UDT-Seq) to generate and interpret the molecular profile of 38 breast cancer patients from two academic medical centers. Dr. Harismendy sequenced 47 genes in matched germline and tumor DNA

samples from 38 breast cancer patients. The selected genes, or the pathways they belong to, can be identification of tumor sub-clones in 3 patients, the suggestion of potential adverse drug effects in 3



Comparison to the TCGA cohort. Bar graph representing the fraction of samples with non-silent somatic mutations in the TCGA cohort (N=507, blue) and the studied cohort (N=38, red). (*) indicates a statistically significant difference (Fisher Test p-value<0.05). The inset bar graph indicates the fraction of samples with none, one, two, or three or more non-silent mutations over the entire TCGA cohort (blue) or studied cohort (red).

targeted by drugs or are important in familial cancer risk or drug metabolism. Relying on the added value of sequencing matched tumor and germline DNA and using a dedicated analysis, UDT-Seq has a high sensitivity to identify mutations in tumors with low malignant cell content. Applying UDT-Seq to matched tumor and germline specimens from the 38 patients resulted in a proposal for at least one targeted therapy for 22 patients, the

patients and a recommendation for genetic counseling for 2 patients.

Overall the study highlights the additional benefits of a sequencing strategy, which includes germline DNA and is optimized for heterogeneous tumor tissues. Dr. Harismendy shows that potentially important information is gained by sequencing at high depth, including identification of sub-clonal mutations. Additional information

is also gained from the sequencing of matched germline DNA and from the inference of tumor DNA copy number alterations. Dr. Harismendy therefore demonstrates that in comparison to other high-throughput sequencing methods, UDT-Seq of matched tumor-germline in a clinical setting generates more actionable findings for a greater number of patients.

Olivier Harismendy, Richard B Schwab, Hakan Alakus, Shawn E Yost, Hiroko Matsui, Farnaz Hasteh, Anne M Wallace, Hannah L Park, Lisa Madlensky, Barbara Parker, Philip M Carpenter, Kristen Jepsen, Hoda Anton-Culver and Kelly A Frazer. Evaluation of Ultra-Deep Targeted Sequencing for Personalized Breast Cancer Care. Breast Cancer Research.2013 Dec 10; 15(6):R115, doi:10.1186/bcr3584. Pubmed PMID:



Shawn E. Yost, Phd
Adjunct Assistant Professor of Pediatrics
Genome Information Sciences

MUTASCOPE: SENSITIVE DETECTION OF SOMATIC MUTATIONS FROM DEEP AMPLICON SEQUENCING

Summary: We present Mutascope, a sequencing analysis pipeline specifically developed for the identification of somatic variants present at low-allelic fraction from high-throughput sequencing of amplicons from matched tumor-normal specimen. Using datasets reproducing tumor genetic heterogeneity, we demonstrate that Mutascope has a higher sensitivity and generates fewer false-positive calls than tools designed for shotgun sequencing or diploid genomes.

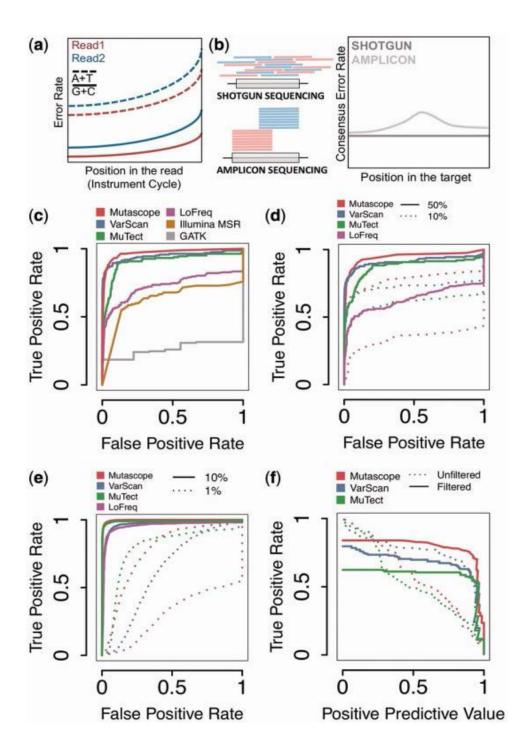
Clinical Relevance: The accurate detection of somatic mutations in tumors is critical for precise diagnostic and selection of targeted therapies, but the low-allelic fraction frequently encountered in heterogeneous or poor cellularity clinical specimens renders this task challenging. Here we describe a tool we developed that specifically optimizes the mutation detection and filtering for deep amplicon sequencing. The

resulting higher accurate detection of somatic mutations at low-allelic fraction increases utility of next generation sequencing in cancer molecular diagnostics.

Shawn E. Yost, Hakan Alakus, Hiroko Matsui, Richard B. Schwab, Kristen Jepsen, Kelly A. Frazer, and Olivier Harismendy. Mutascope: sensitive detection of somatic mutations from deep amplicon sequencing. Bioinformatics. 2013 May 27: 29(15) 1908–1909. doi: 10.1093/bioinformatics/btt305. Epub 2013 May 27. Pubmed PMID:23712659



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Mutascope principle and performance. (a) The sequencing error rate varies based on the read type (blue and red), position in the read (x-axis) or reference base sequenced (lines). (b) Paired reads (red and blue) from shotgun and amplicon sequencing distribute differently over the targeted region (gray box) resulting in different consensus error rates (right panel). (c-e) Comparison of 4-6 tools by ROC analysis showing the classification of mutations at low-allelic fraction (1-10%) in the MIX samples (c), after down-sampling reads to 50 or 10% of maximum coverage (d), or using 1 and 10% allelic fraction variants from TNS pairs. (f) Evolution of the true-positive rate and positive predicted value from the MIX sample low-allele frequency variants (1-10%) before (dotted line) and after (continuous line) application of high-confidence filters



Courtney Thornburg, M.D.
Associate Clinical Professor of
Pediatrics Hematology/Oncology
Medical Director, Hemophilia
and Thrombosis Program
Co-Director, Hemophilia &
Thrombosis Research Program

Innovation in the Field of Sickle Cell Disease

Sickle cell disease is an inherited red blood cell disorder which causes significant morbidity starting in early childhood. Children with sickle cell disease suffer from severe pain, pneumonia, stroke and other life-threatening complications. Hydroxyurea is approved for the prevention complications in adults with sickle cell disease. Dr. Thornburg has conducted research related to hydroxyurea treatment in children.

Dr. Thornburg was a principal investigator in the NHLBI and NICHD sponsored Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG; NCT00006400) which demonstrated safety and efficacy of hydroxyurea in children as young as 1-2 years of age.1 Children who took hydroxyurea during the trial had decreased dactylitis, pain, acute chest syndrome, and hospitalizations compared to children who took placebo.2 Dr. Thornburg is passionate about implementing hydroxyurea treatment starting in childhood. To this end, she has studied barriers to implementation of hydroxyurea,3-5 impact of hydroxyurea on quality of life,6 hydroxyurea in stroke prevention, 7,8 and impact of hydroxyurea on peri-operative management of children with sickle cell disease.9 Dr. Thornburg was an investigator for a NICHD sponsored study evaluating the pharmacokinetics the liquid hydroxyurea in children with sickle cell disease (NCT01506544). Ongoing sickle cell research at Rady Children's hospital includes evaluation of iNKT cells as a biomarker for vasoocclusive pain crisis in collaboration with Dr. Jenny Kim and Dr. Joel Linden, and participation in a phase III study of purified polymer 188 in children in crisis in collaboration with Mast Therapeutics.

Dr. Thornburg is hopeful that her prior and ongoing work will lead to FDA approval of hydroxyurea for children, wider use of hydroxyurea, and, ultimately, decreased burden of disease.

Thornburg CD, Files BA, Luo Z, et al. Impact of hydroxyurea on clinical events in the BABY HUG trial. Blood 2012;120:4304-10; quiz 448.

Thornburg CD, Rogers ZR, Jeng MR, et al. Adherence to study medication and visits: data from the BABY HUG trial. Pediatr Blood Cancer 2010;54:260-4.

Thornburg CD, Calatroni A, Telen M, Kemper AR. Adherence to hydroxyurea therapy in children with sickle cell anemia. J Pediatr 2010;156:415-9.

Hayashi M, Calatroni A, Herzberg B, Ross AK, Rice HE, Thornburg C. Impact of hydroxyurea on perioperative management and outcomes in children with sickle cell anemia. J Pediatr Hematol Oncol 2011;33:487-90.









Donald L. Durden, M.D., Ph.D.
Professor of Pediatrics, Division
of Hematology/Oncology
Vice Chair for Research,
Department of Pediatrics
Associate Director,
Pediatric Oncology

Macrophage Syk-Rac2 Signaling Axis is Required for Metastasis

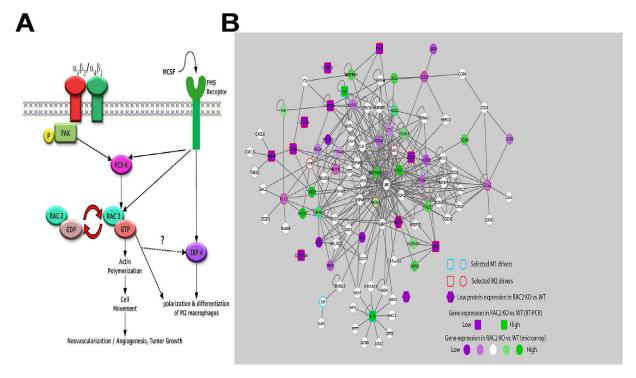
Macrophages (MQs), a major leukocyte population present in tumors, play an essential role in promoting tumor growth by affecting angiogenesis, immune suppression, invasion and metastasis. Despite considerable research efforts, the signal transduction events within macrophages which encode the complex cascade of events required for tumor growth and polarization of macrophages are poorly understood. Our previous findings demonstrate that α4β1 and αvβ3 integrin directed cell migration on specific extracellular matrices (ECM) requires a specific kinase-GTPase pair, Syk-Rac2. This pathway was shown to regulate the ECM dependent postnatal angiogenic response with no defect in vascular development. Herein, we have linked this ECM dependent signaling pathway in macrophages to the regulation of M2 macrophage differentiation and to the regulation of tumor growth, invasion and metastasis. Using mouse genetic models, we provide direct evidence that a macrophage specific, α4β1 integrin dependent Syk-Rac2 signaling axis acts in concert with the p110g isoform (PTEN-PI-3 kinase pathway) to control HIF1α levels, tumor growth and metastasis. We report a novel proteasome/E3 ligase dependent mechanism by which PI-3 kinase regulates HIF1α levels under hypoxic conditions in the stromal compartment. Moreover, treatment with Syk or PI-3 kinase inhibitors demonstrate potent activity in multiple metastatic models. The results define a novel molecular mechanism for the regulation of HIF1a and a macrophage autonomous signaling pathway that is required for alterations in the ECM and the provisional integrin to regulate tumor metastasis and suggest treatment for metastatic disease targeting this pathway in the M2 macrophage compartment.

These results have significant therapeutic implications for the treatment of metastatic disease.

Joshi, S., Singh, A.R., Zulcic, M., Bao, L., Messer, K., Ideker, T., Dutkowski, J. and Durden, D.L. Rac2controls tumor growth, metastasis and M1 to M2 macrophage differentiation in vivo. PLoS One. 2014, In Press.

Joshi, S., Singh, A.R., Zulcic, M. and Durden, D.L. PTEN/PI-3 kinase signaling axis regulated hypoxia induced HIF1a and HIF2a stability in macrophages and controls tumor growth, angiogenesis and metastasis. Mol. Canc Res. (In press, 2014).

Joshi, S., Singh, A.R. and Durden, D.L. MDM2 regulates hypoxic HIF1alpha stability in an E3 ligase. Proteasome and PTEN-PI-3 kinase-AKT dependent manner. J. Biol. Chem. (In press, 2014).



A. Schematic diagram of signaling pathway downstream of MCSF receptor and provisional integrin $\alpha 4\beta 1$ that controls tumor metastasis via an effect exerted from the stromal/M $\mathbb Z$ compartment.

B. Interactome map constructed from multiple omic analysis of target genes and proteins that regulate the M1 to M2 macrophage differention pathway and control metastasis in vivo.

Developmental and Behavioral Science





Kerri Boutelle, Ph.D.
Associate Professor of Clinical
Pediatrics and Psychiatry
Pediatric Gastroenterology, Hepatology &
Nutrition

WILLPOWER AND OBESITY

Childhood obesity affects 1 in every 3 children, and 80% of overweight children will become overweight adults. Being overweight or obese is associated with significant medical and psychological consequences. Although family-based treatments exist for childhood obesity, only one third of children who participate are no longer overweight in adulthood, suggesting that 2/3 do not respond as well. New treatments are needed to assist children in managing their weight over the long term, to prevent adult obesity. Recent functional magnetic imaging data suggests that some people think about food, are more sensitive to food cues in the environment , and eating "feels better" to them than others. These responses have been correlated with being overweight, and weight gain over time. These strong neurocognitive relationships are created between food cues and overeating through Pavlovian conditioning, and over time, cues to eat can trigger changes in the neural reward circuitry. Dr. Boutelle's group has developed a novel treatment for childhood obesity, based on Pavlovian extinction, that attempts to break the relationship between food cues and overeating.

In this treatment, called Cue Exposure-Food, children and their parents are taught to resist overeating in the presence of food (i.e. develop willpower). We have two preliminary studies that suggest that this method could be use-

ful in decreasing overeating in overweight and obese children, to ultimately give them a more durable treatment to lose weight. The Boutelle lab (Center for Healthy Eating and Activity Research; CHEAR) aims to use these and other methods based on basic behavioral sciences to develop and deliver treatments for obesity based on neurocognitive models.

Boutelle, K.N., Zucker, N., Peterson, C.b., Rydell, S., Carlson, J., Harnack, L.J. (2014). An Intervention Based on Schachter's Externality Theory for Overweight Children: The Regulation of Cues Pilot. Journal of Pediatric Psychology. doi: 10.1093/jpepsy/jst142

Boutelle, K.N., Zucker, N., Peterson, C.B., Rydell, S., Cafri, G., Harnack, L. (2011) Two Novel Treatments for Overeating in Overweight Children: A Randomized Controlled Trial. Journal of Clinical and Consulting Psychology, 79(6), 759-771.





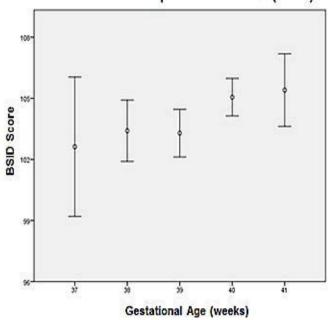
Sheila Gahagan, M.D. Division Chief, Professor of Clinical Pediatrics Academic General Pediatrics, Child Development & Community Health

DEVELOPMENTAL SCORES AT 1 YEAR ASSOCIATED WITH GESTATIONAL AGE FROM 37 – 41 WEEKS Cognitive and motor developmental test scores of preterm and late preterm infants increase with gestational age. Developmental test scores in fullterm infants have not previously been considered to relate to gestational age. In a cohort of healthy, full-term infants, 37 to 41 weeks, 12-month mental and psychomotor scores on the Bayley Scales of Infant Development, increased with gestational age suggesting that neurodevelopment is optimal in infants born at 39 to 41 weeks.

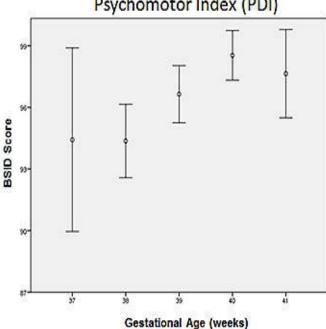
Pediatricians are encouraged to be mindful of the role gestational age related to adaptation to the extrauterine environment realizing that infants born at 37 to 38 weeks will have lower tolerance for labor and other stressors (handling, ambient temperature, and mild hypoxia).

Rose O, Blanco E, Martinez SM, Castillo M, Lozoff B, Vaucher YE, Gahagan S. Developmental Scores at 1 year with Increasing Gestational Age 37-41 Weeks. Pediatrics. 2013 May;131(5):e1475-81.

Mental Developmental Index (MDI)



Psychomotor Index (PDI)



Mental Developmental Index and Psychomotor Index (Bayley Scales of Infant Development II) at 12 months according to week of gestation in a cohort of full term healthy infants*

*Mental Developmental Index and Psychomotor Developmental Index scores are expressed as means and 0.95 confidence intervals



Kay Rhee, M.D.
Assistant Adjunct Professor of Pediatrics
Academic General Pediatrics, Child
Development & Community Health

BODY MASS INDEX DURING A PEDIATRIC WELL-CHILD VISIT

Rates of BMI calculation and plotting remain low. Dr.

Rhee's group examined whether providing a BMI wheel and brief education to pediatric residents and attendings would increase rates of 'BMI recognition' and obesity-related counseling. A delayed-control design was used to evaluate a 20-minute intervention. 1640 records of well-child visits were reviewed to determine the proportion of records in which BMI was calculated and plotted and counseling provided. In clinic A, there was a significant increase in the proportion of records in which BMI was recognized from pre to post intervention (p<0.01). No changes in clinic B occurred until after the delayed intervention. Obesity-related counseling was more likely to occur if BMI was recognized (see Table 1).

Brief education and BMI wheel increased rates of BMI recognition. BMI recognition was associated with increased obesity management. Additional efforts should be incorporated to further increase BMI recognition and assist providers in treating these children.

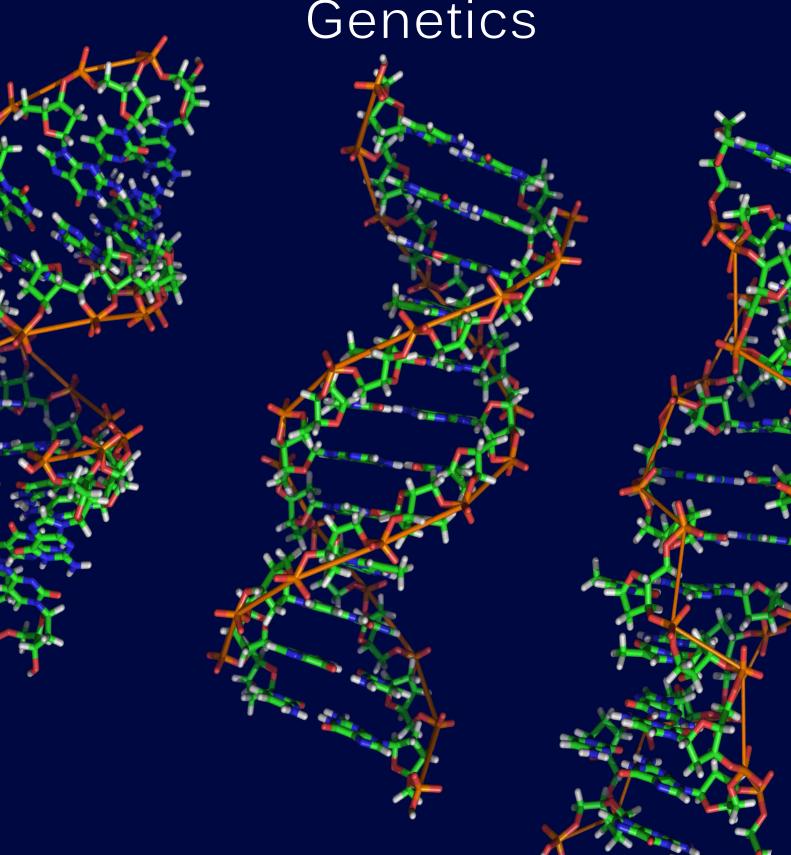
Rhee KE, Phan TL, Barnes RF, Benun J, Wing RR. A Delayed-Control Trial Examining the Impact of Body Mass Index Recognition on Obesity-Related Counseling

	Discussed	Discussed	Discussed	Discussed	Made follow-	Refer to
	dietary	physical	screen time	weight	up	subspecialist
	behaviors	activity		control	appointment	
				behaviors		
BMI recognized	3.22 (1.31-	1.66 (1.18-	1.55 (1.17-	1.33 (0.90-	4.84 (2.14-	1.75 (1.12-
	7.94)**	2.33)**	2.04)**	1.95)	10.95)**	2.74)**
Intervention	0.96 (0.48-	0.94 (0.67-	0.82 (0.63-	1.52 (1.03-	1.10 (0.54-	0.39 (0.25-
period	1.91)	1.30)	1.07)	2.24)*	2.25)	0.60)*
Age	0.89 (0.83-	1.39 (1.34-	0.99 (0.97-	1.53 (1.45-	1.12 (1.04-	1.02 (0.98-
	0.96)**	1.45)**	1.02)	1.62)**	1.21)**	1.07)
Gender	1.17 (0.60-	1.09 (0.80-	0.88 (0.69-	0.74 (0.51-	0.46 (0.24-	1.02 (0.66-
	2.28)	1.49)	1.14)	1.07)	0.91)*	1.56)
BMI %ile	0.99 (0.97-	1.01 (1.00-	1.00 (0.99-	1.01 (1.00-	1.10 (1.05-	1.00 (0.99-
	1.01)	1.01)*	1.01)	1.02)*	1.14)**	1.01)
Insurance	0.58 (0.33-	1.08 (0.78-	1.01 (0.78-	1.29 (0.89-	0.87 (0.39-	1.03 (0.68-
	1.02)	1.49)	1.30)	1.88)	1.92)	1.60)
Race/ethnicity	0.92	1.03	1.02	0.94	1.00 (0.83-	1.05 (0.93-
	(0.75-	(0.94-	(0.95-	(0.84-	1.21)	1.19)
	1.12)	1.13)	1.09)	1.05)		

Table 1. Odds of physician behaviors/discussion topics if BMI was recognized⁺

OR (95% CI) ** p≤0.01 * p≤0.05

Epidemiology and Genetics



Gastroschisis is a congenital abdominal wall defect that is one of only two major birth defects that has been increasing in incidence world-wide for the last several decades.



Kenneth Lyons Jones, M.D.
Division Chief, Distinguished
Professor of Pediatrics
Dysmorphologist/Clinical Teratologist
Dysmorphology/Teratology

ALTERED LIPID METABOLISM IN GASTROSCHISIS: A NOVEL Hypothesis

Gastroschisis is a congenital abdominal wall defect that is one of only two major birth defects that has been increasing in incidence world-wide for the last several decades. Of interest, teen mothers are the group at highest risk of having a child with this defect. However, to date, no genetic or environmental factors have been identified that explain the increasing trend in incidence of this birth defect and/or the particularly high risk among teens.

It was hypothesized that maternal diet and fatty acid levels during pregnancy may play a role in disruption of the abdominal wall through an inflammatory mechanism. It was further hypothesized that differentially poor diet among very young women might help explain their vulnerability to having children with this defect.

In a small case-control study of 13 women carrying a baby prenatally diagnosed with gastroschisis and 9 comparison mothers carrying normal infants, mid-trimester and postnatal fatty acid biomarkers were examined and it was found that mothers of babies with gastroschisis and the babies themselves had consistently different levels of various specific fatty acids relative to controls, several of which are markers of inflammation.

These findings suggest that early maternal inflammation resulting from imbalance of fatty acids could lead to vascular disruption. If this hypothesis is borne out, this may have important implications for prevention of this serious congenital condition.

Chambers CD, Johnson D, Xu R, Luo Y, Louik C, Mitchell AA, Schatz M, Jones KL; OTIS Collaborative Research Group. Risks and safety of pandemic H1N1 influenza vaccine in pregnancy: birth defects, spontaneous abortion, preterm delivery, and small for gestational age infants. Vaccine. 2013;31(44):5026-32. Chambers CD, Yevtushok L, Zymak-Zakutnya N, Korzhynskyy Y, Ostapchuk L, Akhmedzhanova D, Chan PH, Xu R, Wertelecki W. Prevalence and predictors of maternal alcohol consumption in 2 regions of Ukraine. Alcohol Clin Exp Res. 2014;38(4):1012-9.Jones KL, Weiss LA, Hagey LR, Gonzalez V, Benirschke K, Chambers CD. Altered lipid abolism in gastroschisis: a novel hypothesis. Am J Me





Christina Chambers, Ph.D.
Professor of Pediatrics
Director of Clinical Research,
Rady Children's Hospital
Associate Director, Clinical &
Translational Research Institute
Perinatal Epidemiologist

RISKS AND SAFETY OF PANDEMIC H1N1 INFLUENZA VACCINE IN PREGNANCY

Pregnant women are at higher risk of complications of influenza infection that can be life threatening for the mother and her fetus. Despite recommendations that all pregnant women be vaccinated for influenza, uptake is generally poor, in part due to maternal perception of safety concerns. This situation was exacerbated during the 2009/10 influenza season with the H1N1 influenza pandemic and the addition of a monovalent vaccine containing the new strain. In collaboration with the U.S. Department of Health and Human Services, Biomedical Advanced Research and Development Authority, Dr. Chambers led a national cohort study of pregnancy outcomes in 1,032 women who were or were not vaccinated for influenza.

They were able to establish that there was no evidence of an increased risk for major congenital anomalies, spontaneous abortion, or small for gestational age infants in mothers who were vaccinated compared to mothers who were not. A modest increased risk for preterm delivery was found in mothers who were vaccinated, but the shortened length of gestation was approximately 3 days. These findings



help support the safety of influenza vaccination during pregnancy with regard to multiple infant outcomes.

PREVALENCE AND PREDICTORS OF MATERNAL ALCOHOL CONSUMPTION IN 2 REGIONS OF UKRAINE

Fetal Alcohol Spectrum Disorders are thought to be the most common known cause of developmental disabilities worldwide. However, in many countries throughout the world there are no data on alcohol consumption patterns in pregnant women that can help inform public health practice and interventions.

This group established for the first time in an Eastern European country a standard alcohol screening protocol in Ministry of Health-supported prenatal care settings in two regions in Ukraine. Between 2007 and 2012, clinical staff successfully screened 11,909 pregnant women.

Over 90% reported being ever drinkers, more than half consumed alcohol in the first month of pregnancy and 46% continued to drink into midpregnancy or beyond. Several predictors of heavier or "binge" drinking were identified. However, a single question about tolerance for alcohol when not pregnant was the best predictor of alcohol consumption in pregnancy.

The screening program has now been established in these clinical care settings, and findings have helped inform public health education and intervention strategies for high-risk pregnancies.

Chambers CD, Johnson D, Xu R, Luo Y, Louik C, Mitchell AA, Schatz M, Jones KL; OTIS Collaborative Research Group. Risks and safety of pandemic H1N1 influenza vaccine in pregnancy: birth defects, spontaneous abortion, preterm delivery, and small for gestational age infants. *Vaccine*. 2013;31(44):5026-32.

Chambers CD, Yevtushok L, Zymak-Zakutnya N, Korzhynskyy Y, Ostapchuk L, Akhmedzhanova D, Chan PH, Xu R, Wertelecki W. Prevalence and predictors of maternal alcohol consumption in 2 regions of Ukraine. *Alcohol Clin Exp Res.* 2014;38(4):1012-9.



Erin Smith, Ph.D. Assistant Project Scientist Genome Information Sciences

GENETIC ANCESTRY OF PARTICIPANTS IN THE NATIONAL CHILDREN'S STUDY

The National Children's Study (NCS) is a prospective epidemiological study in the USA tasked with identifying a nationally representative sample of 100,000 children, and following them from their gestation until they are 21 years of age. The objective of the study is to measure environmental and genetic influences on growth, development, and health. Determination of the ancestry of these NCS participants is important for assessing the diversity of study participants and for examining the effect of ancestry on various health outcomes.

We estimated the genetic ancestry of a convenience sample of 641 parents enrolled at the 7 original NCS Vanguard sites, by analyzing 30,000 markers on exome arrays, using the 1000 Genomes Project superpopulations as reference populations, and compared this with the measures of self-reported ethnicity and race. For 99% of the individuals, self-reported ethnicity and race agreed with the predicted superpopulation. NCS individuals self-reporting as Asian had

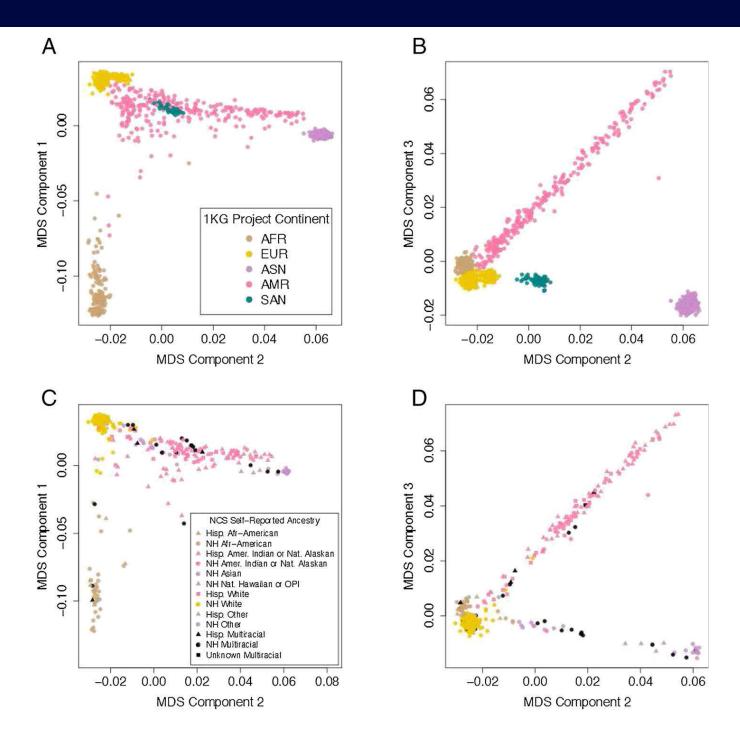
genetic ancestry of either South Asian or East Asian groups, while those reporting as either Hispanic White or Hispanic Other had similar genetic ancestry. Of the 33 individuals who self-reported as Multiracial or Non-Hispanic Other, 33% matched the South Asian or East Asian groups, while these groups represented only 4.4% of the other reported categories. Our data suggest that self-reported ethnicity and race have some limitations in accurately capturing Hispanic and South Asian populations. Overall, however, our data indicate that despite the complexity of the US population, individuals know their ancestral origins, and that self-reported ethnicity and race is a reliable indicator of genetic ancestry.

Race and ethnicity are associated with environmental risk factors for disease, such as tobacco smoke, air quality, and food environments. While the relationship between race and genetics has been contentious, it is clear that genetic factors associated with disease can vary with racial background, resulting in common disease loci differing between ancestral groups. These environmental and genetic differences could result in ethnicity and race being associated with various health outcomes, such as cancer treatment and toxicology, and are therefore important to consider in large epidemiological studies of environmental influences on development, such as the NCS.

We show that self-reported ethnicity and race have some limitations in accurately capturing Hispanic and Asian populations, highlighting the relevance of using genetically determined ancestry rather than self-reported ancestry alone.

Genome Biol. 2014 Feb 3;15(2):R22. [Epub ahead of print] Genetic ancestry of participants in the National Children's Study.

Smith EN, Jepsen K, Arias AD, Shepard PJ, Chambers CD, Frazer KA



Genetic clustering between participants in the 1000 Genomes Project (1KG) and National Children's Study (NCS). In total, 1445 unrelated individuals from the 1KG and 641 from the NCS were clustered on genotypic profiles using multidimensional scaling. (A, B) The 1KG individuals are color-coded by superpopulation and plotted according to their scores on (A) the first two dimensions and (B) the second and third dimensions. (C,D) NCS participants are color-coded by their expected superpopulation group and plotted according to their scores on (C) the first two dimensions and (D) the second and third dimensions. Abbreviations: Hisp, Hispanic; NH, Non-Hispanic; Afr, African; Nat. Native; Amer., American; OPI, Other Pacific Islander





Jane Kim, M.D.
Assistant Adjunct Professor of Pediatrics
Endocrinology

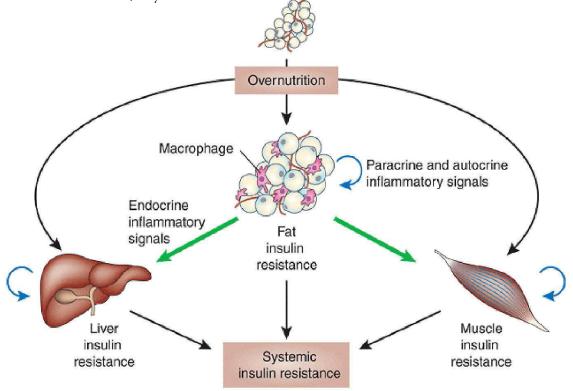
RESISTING INSULIN RESISTANCE

G protein-coupled receptors mediate a variety of physiological functions and are the targets of approximately half of all modern medicinal drugs. GPR105, a G protein-coupled receptor for UDP-glucose, is highly expressed in several human tissues and can regulate leukocyte chemotaxis. Since insulin resistance is associated with chronic inflammation in insulin target tissues, Dr. Jane Kim and her colleagues hypothesized that GPR105 might play a role in insulin resistance, a key factor in the development of type 2 diabetes. Using GPR105-deficient mice, they showed that GPR105

regulates the recruitment of pro-inflammatory macrophages to the liver in obesity, significantly increasing liver inflammation, liver fat, and systemic insulin resistance. This study reveals a role for GPR105 in the development of insulin resistance in diet-induced obesity and encourages investigation of this receptor as a promising therapeutic target for type 2 diabetes.

Part of the cause of type 2 diabetes is the patient's resistance to insulin. Studies like Dr. Kim's discovery help to understand the biological mechanism of insulin resistance and potential targets for new drugs to treat type diabetes

Xu J1, Morinaga H, Oh D, Li P, Chen A, Talukdar S, Mamane Y, Mancini JA, Nawrocki AR, Lazarowski E, Olefsky JM, Kim JJ. GPR105 ablation prevents inflammation and improves insulin sensitivity in mice with diet-induced obesity. J Immunol. 2012 Aug 15;189(4):1992-9.



Xu J1, Morinaga H, Oh D, Li P, Chen A, Talukdar S, Mamane Y, Mancini JA, Nawrocki AR, Lazarowski E, Olefsky JM, Kim JJ. GPR105 ablation prevents inflammation and improves insulin sensitivity in mice with diet-induced obesity. J Immunol. 2012 Aug Scientific Discoveries - 2014 15;189(4):1992-9.

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Ulupi Jhala, Ph.D. Associate Research Scientist Endocrinology

Destruction of Beta Cells

Dr. Ulupi Jhala has focused on identifying pharmacologic targets for delaying the progression of beta cell death. She recently identified a family of mixed lineage kinases (MLKs), that are specifically activated by cytokines early in proinflammatory destruction of beta cells. These kinases are responsible for damaging mitochondria early in the death process, and increasing their susceptibility to inflammation. Current results strongly indicate that the cross talk between phosphorylation and ubiquitination is a common theme in inflammation and may constitute a code that can enable or dampen the inflammatory process. Autoimmune destruction of insulin

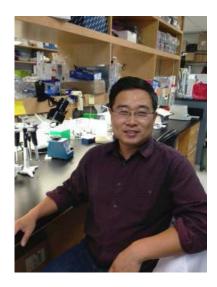
secreting beta cells marks the turning point in the incidence of type 1 diabetes. Widespread loss of beta cell and the near absolute dependence on exogenous insulin are accompanied by unpredictable swings in blood glucose and risk of sudden death. Therefore preservation of even a small insulin secretory capacity, could translate into better glycemic control and could mark a major improvement in the quality of life for T1D patients.

Type 1 diabetes mellitus is the result of the immune system attacking and destroying the beta cells in the pancreas which produce insulin. Therapies to date have tried unsuccessfully to prevent the immune system from attacking. Dr. Jhala's discovery could lead to novel approaches helping the beta cell to survive the immune attack.

Humphrey, R. K. et al. Lysine 63-linked ubiquitination modulates mixed lineage kinase-3 interaction with JIP1 scaffold protein



Humphrey, R. K. et al. Lysine 63-linked ubiquitination modulates mixed lineage kinase-3 interaction with JIP1 scaffold protein in cytokine-induced pancreatic beta cell death. The Journal of biological chemistry 288, 2428-2440, doi:10.1074 (2013).



Jianhua Shao, M.D., Ph.D.
Associate Proffesor of Pediatrics
Endocrinology

ADIPONECTIN REGULATES FETAL LIPID METABOLISM

Maternal obesity increases offspring birth weight and susceptibility to obesity. Adiponectin is an adipocytesecreted hormone with a prominent function in maintaining energy homeostasis. In contrast to adults, neonatal blood adiponectin levels are positively correlated with anthropometric parameters of adiposity. Dr, Jianhua Shao investigated the role of adiponectin in maternal obesity- enhanced fetal fat deposition. By using high-fat diet-induced obese mouse models, he demonstrated that maternal obesity increased fetal fat tissue mass, with a significant elevation in fetal blood adiponectin. However, adiponectin gene knockout (Adipoq-/-) attenuated maternal obesity-induced high fetal fat tissue mass. He further studied the effects of fetal adiponectin on fetal fat deposition by using a cross breeding approach to create Adipoq-/+ and Adipoq-/- offspring, whereas maternal adiponectin was null. Adipoq-/+ offspring had more fat tissue mass at both birth and adulthood. Significantly high levels of lipogenic genes, such as sterol regulatory element-binding protein 1c and fatty acid synthase, were detected in the livers of Adipoq-/+ fetuses. In addition, expression of genes for placental fatty acid transport was significantly increased in Adipoq-/+ fetuses. Together, our study indicates that adiponectin enhances fetal fat deposition and plays an important role

in maternal obesity-induced high birth weight.

Pregnant mothers who are obese are likely to have fatter babies who are at greater risk of becoming obese later in life. Dr. Shao has discovered the important role a hormone from the fetal fat causes the fetus to gain excess weight when the mother is overweight.

ADIPONECTIN SUPPRESSES THERMOGENESIS AND ENERGY EXPENDITURE

Adiponectin is an adipocyte-derived hormone that plays an important role in maintaining energy homeostasis. Dr. Jianhua Shao investigated whether adiponectin regulates brown adipose tissue (BAT) activation and thermogenesis. Using mouse models with genetically altered blood adiponectin levels, Dr. Shao's lab demonstrate that adiponectin inhibits thermogenesis

by suppressing BAT activation and subcutaneous fat browning. He also discovered that the anti-thermogenic effect of adiponectin is not mediated by its receptor AdipoR1 and AdipoR2. Interestingly, in contrast to the anti-thermogenic effects of adiponectin, AdipoR2 and, to a less extent, AdipoR1 are required for BAT activation. He found that adiponectin inhibits β3 adrenergic receptor gene expression in adipocytes through increasing PPARy activity. Therefore, he proposed that the suppression of BAT activation and thermogenesis is a major mechanism whereby adiponectin reduces energy expenditure. This study reveals a novel characteristic of adiponectin as an adipocyte-secreted starvation hormone that favors energy conservation and could be a target for

anti-obesity therapy.

Unlike our regular fat stores, brown adipose tissue can burn up calories. Dr. Shao discovered that adiponectin, a hormone produced by our fat stores inhibits the ability of brown fat to burn calories. Potentially developing a drug to inhibit adiponectin could be a treatment for obesity

L. Qiao, H.S. Yoo, A. Madon, B. Kinney, W.W. Hay, J. Shao, Adiponectin enhances mouse fetal fat deposition. Diabetes 2012,61,3199–3207.

L. Qiao, H.S. Yoo, C. Bosco, B. Lee, G.-S. Feng, J. Schaack, et al., Adiponectin reduces thermogenesis by inhibiting brown adipose tissue activation in mice. Diabetologia 2014,57,1027–1036.

Scientific Discoveries - 2014

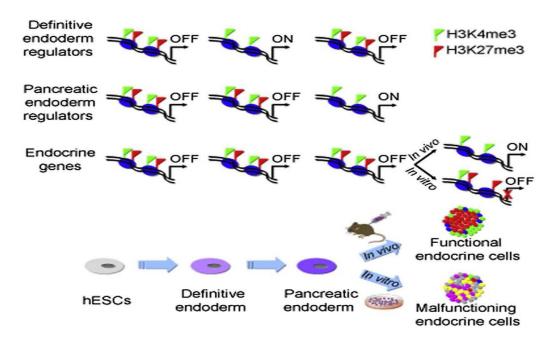


Maike Sander, M.D.
Professor of Pediatrics
Endocrinology
Director, Pediatric Diabetes Reserch Center

PANCREATIC ENDOCRINE DIFFERENTIATION AND CHROMATIN MODIFICATIONS

Using genome-wide analysis of RNA and histone modifications, Dr. Maike Sander reported the first transcriptional and epigenetic map of the developing pancreatic beta cell. By defining the changes in gene transcription and chromatin architecture associated with normal and aberrant endocrine cell differentiation, insight was provided into chromatin remodeling mechanisms underlying the production of beta cells. Moreover, she demonstrated that endocrine cells produced from pluripotent stem cells in vitro do not fully eliminate repressive chromatin marks on endocrine-specific genes, likely contributing to their malfunction. Dr. Sander's work suggests novel strategies for cell manipulations to achieve production of mature beta cells in vitro. This will be important not only for cell therapies, but also for identifying disease mechanisms that underlie the pathogenesis of diabetes.

The ability to convert stem cells to pancreatic cells and ultimately into beta-cells which produce insulin as a cure for diabetes requires a thorough understanding of the multiple steps in beta cell development. Dr. Sander's study identified one of the reasons why it has not yet been possible to create beta cells from pancreatic cells.

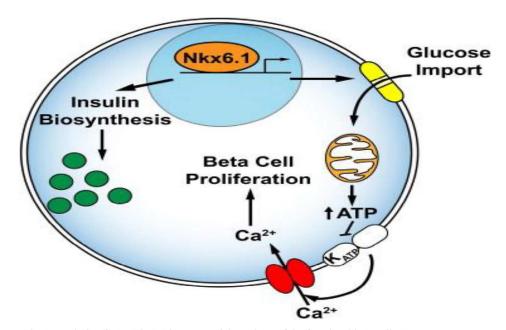


Insufficient induction of endocrine genes in vitro is associated with aberrant histone modification patterns during terminal differentiation into endocrine cells.

NKX6.1 IS NECESSARY FOR ESTABLISHING BETA CELL IDENTITY

Dr. Maike Sander identified the transcription factor Nkx6.1 as an essential regulator of the functional beta cell state by showing that beta cell-specific deletion of Nkx6.1 in mice results in diabetes due to loss of beta cell features, including insulin production, glucose sensing and the capacity to proliferate. By identifying genes that are bound and regulated by Nkx6.1 in beta cells, she

provided molecular insight into beta cell-specific gene regulation. Given recent observations that Nkx6.1 levels are reduced in type 1 and type 2 diabetic beta cells, these findings provide a possible mechanism for pancreatic beta cell failure in diabetes and suggests that restoring Nkx6.1 levels could be a therapeutic strategy in Type 2 diabetes. In both type 1 and type 2 diabetes pancreatic beta cells fail to continue to produce insulin. Dr. Sander has discovered a regulator factor in the nucleus of beta cells which is essential for the beta cell to make insulin. When the beta-cells of mice lacks this factor the mice develop diabetes.



The transcription factor Nkx6.1 is an essential regulator of the functional beta cell state.







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SOX9-DEPENDENT ACINAR-TO-DUCTAL REPROGRAMMING

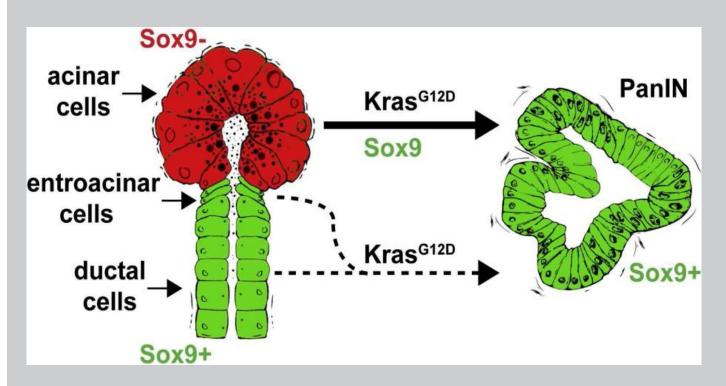
Dr. Maike Sander's lab has discovered that preneoplastic lesions for pancreatic ductal adenocarcinoma predominantly originate from acinar cells but rarely from ductal and stem-like centroacinar cells. They show that cell-specific activation of oncogenic Kras in mice readily induces duct-like premalignant lesions from acinar cells whereas ductal cells are surprisingly refractory to Kras-mediated transformation. Furthermore, using loss- and gain-of-function approaches, we identify the ductal fate determinant Sox9 as a critical mediator for the induction of premalignant lesions from acinar cells. Our results imply that therapeutic targeting of signaling pathways involved in ductal reprogramming of acinar cells could prevent initiation of pancreatic ductal adenocarcinoma.

Dr. Sander discovered a unique genetic regulator factor, Sox9, which is responsible to converting precancerous cells in the pancreas into pancreatic cancer. These observations could lead to the development of drugs which could block Sox9 function

Xie, R., Everett, L.J., Lim, H.W., Patel, N.A., Schug, J., Kroon, E., Kelly, O.G., Wang, A., D'Amour, K.A., Robins, A.J., Won, K.J., Kaestner, K.H, Sander, M. (2013) Dynamic chromatin remodeling mediated by Polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells, Cell Stem Cell, 12:224-37.

Taylor, B.L., Liu, F.F., Sander, M. (2013) Nkx6.1 is essential for maintaining the functional state of pancreatic beta cells, Cell Reports, 4: 1262-1275.

Kopp, J.L., von Figura, G., Mayes, E., Liu, F.F., Dubois, C.L., Morris, J.P., Pan, F.C., Akiyama, H., Wright, C.V.E., Jensen, K., Hebrok, M., Sander, M. (2012) Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma, Cancer Cell, 22, 737-50.



The ductal fate determinant Sox9 is a critical mediator of Kras-induced premalignant acinar cell reprogramming.

HIGH FAT DIET AND AMINO-ACID METABOLISM:

EXPERIMENTS IN FLIES



Gabriel Haddad, M.D.
Chair, Department of Pediatrics
Distinguished Professor,
Department of Pediatrics
and Neurosciences
Chief Scientific Officer &
Physician-in-Chief
Rady Children's Hospital

The Haddad laboratory has been interested in a variety of questions related to hypoxia sensing, and mechanisms of injury and survival when cells and tissues are exposed to low O2. Human conditions with low oxygenation usually arise in lung, heart or neurologic diseases as well at high altitude. Often, in mammals, these situations, if severe and prolonged enough, lead to cell injury and organ and system failure and consequently cell and tissue death. Herein below, are some of the discoveries from the Haddad laboratory, from

this past year.

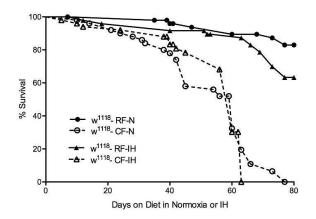
Obesity is associated with many diseases, one of the most common being obstructive sleep apnea (OSA), which in turn leads to blood gas disturbances, including intermittent hypoxia (IH). Obesity, OSA and IH are associated with metabolic changes (such as insulin resistance, lipid disturbances), and while much mammalian work has been done, mechanisms underlying the response to IH, the role of obesity, and the interaction of obesity and hypoxia remain unknown. As a model organism, Drosophila offers tremendous power to study a specific phenotype and, at a subsequent stage, to uncover fundamental mechanisms, given the conservation of molecular pathways. Herein, we characterize the phenotype of Drosophila on a high-fat diet in normoxia, IH and constant hypoxia (CH). We found that flies on a high-fat diet show increased triglyceride levels and a shortened lifespan in normoxia, IH and CH. Furthermore, flies on a high-fat diet in normoxia and CH show diminished tolerance to stress, with decreased survival after exposure to stress such as extreme cold or very low oxygen levels. Of interest, IH seems to rescue this decreased cold tolerance, as flies on a high-fat diet almost completely recovered from cold stress following IH. In addition, metabolomics revealed altered fatty acid, amino acid, and carbohydrate metabolism with high

fat. Microarray analysis uncovered transcriptional changes in nitrogen metabolism, including a homolog of human argininosuccinate lyase (ASL). Knockdown of this homolog in flies phenocopied traits observed with high fat, namely increased triglyceride levels and decreased cold tolerance and its restoration ameliorated some traits. We conclude that the cross talk between hypoxia and a high-fat diet can be either deleterious or compensatory, depending on the nature of the hypoxic treatment.

These results draw an important link between regulation of amino acid metabolism and the response to dietinduced obesity.

Heinrichsen, E.T., Zhang, H., Robinson, J.E., Ngo, J., Diop, S., Bodmer, R., Joiner, W.J., Metallo, C.M. †, Haddad, G.G. † Metabolic and transcriptional response to a high-fat diet in Drosophila melanogaster. Molecular Metabolism 2014; 3(1): 42-54.

Nitin Udpa, Roy Ronen, Dan Zhou, Junbin Liang, Tsering Stobdan, Otto Appenzeller, Yuanping Du, Lixia Guo, Rui Cao, Yu Wang, Xin Jin, Chen Huang, Wenlong Jia, Dandan Cao, Guangwu Guo, Claydon Victoria E, Roger Hainsworth, Jorge L. Gamboa, Mehila Zibenigus_Wuhib, Guta Zenebe, Jin Xue, Siqi Liu, Kelly Frazer, Yingrui Li, Vineet Bafna, Gabriel G. Haddad-Whole genome sequencing of Ethiopian highlanders reveals evolutionarily conserved genes that regulate hypoxia tolerance. In Press, Genome Biology





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Altered lifespan due to high-fat diet and hypoxia. Adult w1118 flies (3-5d old) were placed on regular (RF) and high-fat (CF) diets in normoxia (N) or intermittent hypoxia (IH). There was a significant difference between the regular and high-fat diet curves in each comparison, as well as between normoxia and IH when flies were on a regular diet. Flies had a shorter life span if on high fat diet and slightly shorter when hypoxia was intermittent.

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