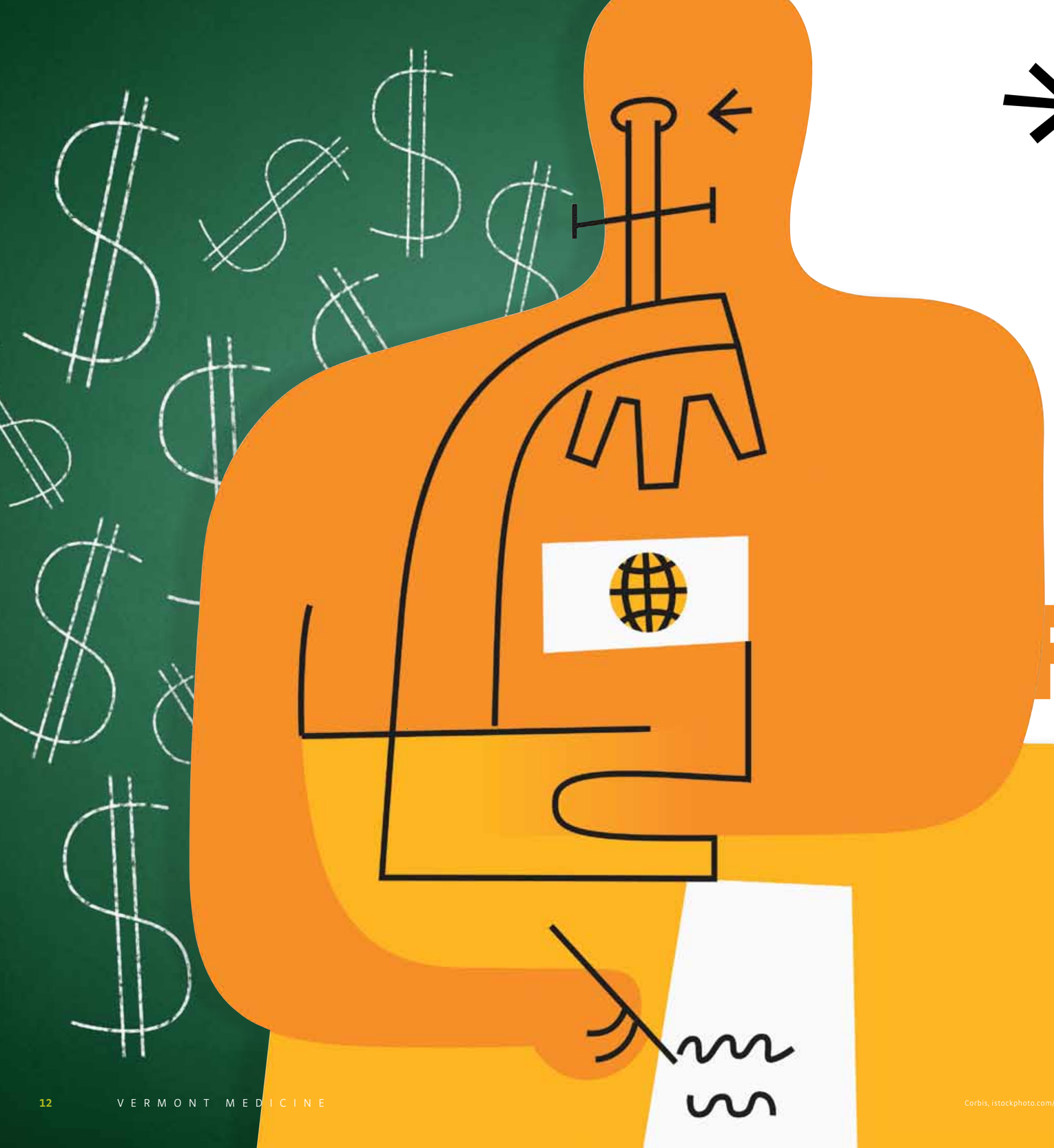

With the economy in the grip of the worst recession in 70 years, the passage of the American Recovery and Reinvestment Act (ARRA) in 2009 provided federal funding to a long list of public work projects across the nation. While allocations for highway and bridge repairs received extensive media coverage, less well known was the outgrowth of $5 billion in new medical research grants offered through the National Institutes of Health (NIH).

The NIH itself identified a new initiative called the "Challenge Grants in Health and Science Research," through which approximately 200 grants were allocated for innovative projects that "focus on specific knowledge gaps… that would benefit from an influx of funds to quickly advance the area in significant ways."

Patience, and a high tolerance for repetition, is mandatory in the world of biomedical investigation, where proving a hypothesis can easily consume a decade, and years pass in the blink of an eye. Three $500,000 NIH Challenge Grants were secured by investigators at the College of Medicine. These researchers only had two years to obtain results, but they rose to the challenge to deliver solid and sometimes surprising findings.

FILLING THE GAP

by **Jennifer Nachbur** | photographs by **Mario Morgado**

Three intensive research projects at the College of Medicine yield solid results and prepare new paths for further investigations.

Corbis, istockphoto.com/tumpikuja

Professor of Anatomy and Neurobiology Rae Nishi, Ph.D., gathered experts from the university and across the region to examine the effects of nicotine and prototoxin genes on adolescent behavior in her NIH Challenge Grant project.

STARTING FROM SCRATCH

A large multidisciplinary study with both animal research and human study arms, the Challenge Grant led by Rae Nishi, Ph.D., professor of anatomy and neurobiology, faced the steepest hurdle. She'd gathered experts from the university to help examine the effects of nicotine and prototoxin genes on adolescent behavior, and in particular, whether teens who smoke experience any long-term changes in their brains. With the Challenge Grant in hand, Nishi and her colleagues, at a remarkably amped-up pace, ticked through their long to-do list. For the animal arm of the study, they bred bioengineered mice — breeding colonies without the prototoxin gene and cross-breeding them with mice whose neurons carried a fluorescent protein, so that the structure of the neurons could be studied in "wild type" or non-bioengineered mice versus the genetically-manipulated "knockout" mice. In another arm of the study, they recruited 200 human participants, conducted behavioral testing, and collected and genotyped hundreds of DNA samples to determine the genetic makeup of the subjects who were tested.

"We proposed something no one had ever thought about before — we really 'dreamt big,'" says Nishi.

> "These molecules are more tied to anxiety and depression, which are both big factors in the need for a cigarette."
>
> —Rae Nishi, Ph.D.
> Professor of Anatomy and Neurobiology

Nicotine mimics a normal brain chemical, acetylcholine, a neurotransmitter in the brain. This activates the brain's reward center through receptors called nicotinic acetylcholine receptors. Nishi and her team wondered if a teen's smoking addiction could be tied to a difference in the sensitivity of the nicotinic receptors to acetylcholine. Specifically, the group examined the novel LY6-neurotoxin-like gene — or LYNX1 — suspected to be the likeliest gene to be involved in this process. Prototoxins — molecules that associate with nicotinic receptors in the brain — act like little brakes in the reception process. These molecules can prevent the nicotinic receptor from fully opening, thus slowing down its function. Nishi and her colleagues wondered if addiction to smoking was tied to an inefficient LYNX1 gene.

The study's subjects — adolescent mice and adolescent humans — underwent behavioral testing with and without the influence of nicotine. The team compared mice without the gene to normal mice, administered nicotine at the dose required to achieve the most pleasing response and observed how it influenced behavior. They then compared the genotyping data to determine any correlations to the mouse's susceptibility to nicotine. In the human subjects, Alexandra Potter, Ph.D., assistant professor of psychiatry, and colleagues employed a computer test, "Go/No Go," that measures stop signal reaction time, along with surveys and a variety of psychiatric behavior measures. While the human behavior failed to exactly match the animal behavior results, both arms clearly illustrated how nicotine benefitted subjects.

Nishi and her team continue to work on a no-cost extension of the grant, and are in the process of analyzing the data. They have identified some very interesting trends from both arms of the study that follow a different path than the original hypothesis — a genetic link to anxiety versus susceptibility to addiction. "We found that it's a more complicated issue than just the activities of the reward center," says Nishi. "It looks like these prototoxin molecules are more tied to anxiety and depression, which are both big factors in the need for a cigarette. The hypothesis is more complicated than we originally thought: people get hooked because the initial hit activates the reward center. But then, when you stop, you feel more anxious and depressed and that drives you to smoke again. Our work showed what genetically may tie to that response." Members of the research team presented an abstract on their preliminary findings at the 2011 Society for Neuroscience meeting and will be submitting several papers and applying for grants once the analyses are complete.

This intense study notably generated results, but also concrete economic gains. "We hired three full-time research assistants, paid partial salaries for four investigators, purchased major equipment from two local Vermont vendors, MBF Bioscience and Med Associates. We also paid two consultants to assist in analyzing the human genetic data and several part-time staffers to collect data and run the human studies," says Nishi, as she details the sort of expenditures that these kinds of intense research studies generate. "It was a hugely ambitious project to pursue in two years, but we got quite a bit out of it."

A COMBUSTION CONUNDRUM

Recognizing a significant correlation between air pollution and the development of disease, Professor of Medicine Naomi Fukagawa, M.D., Ph.D., and colleagues used Challenge Grant funding to analyze the biological effects

The Given Building laboratory of Professor of Medicine Naomi Fukagawa, M.D., Ph.D., was the setting for a Challenge Grant study of petrodiesel and biodiesel emission particles.

of emission particles — both petrodiesel and biodiesel — from a food systems perspective.

"Very little is known about whether biodiesel is better, worse or the same for the environment and for people," says Fukagawa, who adds that the world's push to break free from fossil fuel dependence has spurred on research in this realm.

She points out that biodiesel, often produced from food sources, can have a significant impact on food availability and pricing — raising corn prices, and shifting soybean use into biodiesel production. With about 925 million people categorized as hungry in the world, this creates a conflict: Are agricultural crops for humans, animals, or fuel?

Over 25 years of basic and applied research have led to the acceptance that petrodiesel fuel emissions have an impact on such health conditions as asthma, chronic bronchitis, chronic obstructive airway disease, cardiopulmonary diseases, and cancer. But, the mechanisms for the effects of the airborne particulate matter produced by these emissions remain unknown.

"Combustion of biodiesel fuels is associated with lower emission of particulate matter," explains Fukagawa. "However, the health consequences of exposure to exhaust from combustion of pure soy biodiesel or a 20 percent soy biodiesel blend are unclear."

As a first step, Fukagawa and her team previously published data on the mechanisms and outcomes associated with asbestos exposure, extending this to studies of diesel versus biodiesel emissions and fine particulate matter concentrations. In one experiment, biodiesel exhaust particles appeared to induce more lung inflammation and oxidative stress than petrodiesel particles after three days. The researchers also examined the impact of these particles in mice without the gene for apolipoprotein E (ApoE), a major component of very low-density lipoproteins, which is involved in the uptake and distribution of blood lipids. Mice deficient in ApoE are characterized by the development of atherosclerosis. After six weeks of inhalation of pure soy biodiesel, the ApoE-deficient mice appeared to have more atherosclerotic lesions than those exposed to filtered air.

> "Very little is known about whether biodiesel is better, worse or the same for the environment and for people..."
>
> **—Naomi Fukagawa, M.D., Ph.D.,**
> Professor of Medicine

With several journal manuscripts in progress, the group is already anticipating its next challenge, to determine the relative contribution of pure soy biodiesel versus the presence of petrodiesel in a 20 percent soy biodiesel blend and pure petrodiesel in producing the differential responses, which would affect exhaust particle size and composition.

INVESTIGATING THE CLOT MYSTERY

Assistant Professor of Medicine and hematologist Neil Zakai, M.D., harnessed the ongoing NIH-supported REGARDS (Reasons for Geographic and Racial Differences in Stroke) study to investigate potential causes of a higher incidence in African-Americans of venous thromboembolism (VTE). VTE consists of deep vein thrombosis (DVT) — a blood clot that develops in the deep veins of the leg — and pulmonary embolism (PE), which occurs when a piece of the clot breaks off and travels to the lung.

The U.S. Centers for Disease Control estimates that 300,000 to 600,000 Americans suffer VTE each year and between 60,000 to 100,000 people die each year, as a result. However, the reasons underlying the 30 to 60 percent higher incidence in African-Americans of VTE were unknown.

For five years, REGARDS has been regularly surveying the health of more than 30,000 people, about half of whom are African-American, throughout the country. Between 2003 and 2007, the project focused on the collection of baseline information on medical conditions and blood samples from these volunteers, which are currently stored at UVM's Colchester Research Facility.

First up for Zakai and colleagues was a review of the data on hospitalizations for the African-American participants, who were then administered a phone questionnaire to determine whether or not they had suffered a VTE. If the answer was "yes," the team retrieved their hospital records regarding the event. In total, the group identified more than 1,000 potential events to review. Zakai and colleagues Mary Cushman, M.D., professor of medicine and a REGARDS co-investigator, and Aaron Folsom, M.D., from the University of Minnesota, reviewed the clinical characteristics surrounding 470 potential events following a protocol that required two reviewers per event and a discussion among the three clinicians to resolve any disagreements.

"We want to understand if and why African-Americans have higher rates of VTE events than whites," says Zakai, whose recent article in the *Journal of Thrombosis and Haemostasis* on this topic provided a review of VTE trends in diverse racial groups, as well as a review of genetic and

> "By understanding why there are differences and who is at most risk for VTE, we can begin to develop better and more targeted preventive measures to help reduce VTE in everyone..."
>
> **—Neil Zakai, M.D.**
> Assistant Professor of Medicine

Assistant Professor of Medicine Neil Zakai, M.D., used the enormous resource of millions of blood samples stored at UVM to study the reasons for higher incidence of deep vein thrombosis in African-Americans.

environmental risk factors for VTE and information about how these factors add to differences in VTE according to race.

"By understanding why there are differences and who is at most risk for VTE, we can begin to develop better and more targeted preventive measures to help reduce VTE in everyone," say Zakai and his co-author Leslie McClure, Ph.D., of the University of Alabama at Birmingham.

Zakai, who with his colleagues is currently constructing the database to house all of the collected information that will allow an analysis of the clinical characteristics of the VTE events, is hopeful about the results yielded from the project.

The team's next goal is to combine the new data with information gleaned from the national Longitudinal Investigation of Thromboembolism Etiology (LITE) study, which has followed 20,000 people, mostly white, for roughly 18 years.

"While differences in VTE by race due to genetic predisposition will probably always be present," say Zakai and McClure in the *Journal of Thrombosis and Haemostasis* article, "understanding the reasons for racial differences in VTE will help providers develop strategies to minimize VTE in all populations." VM