ISSUES

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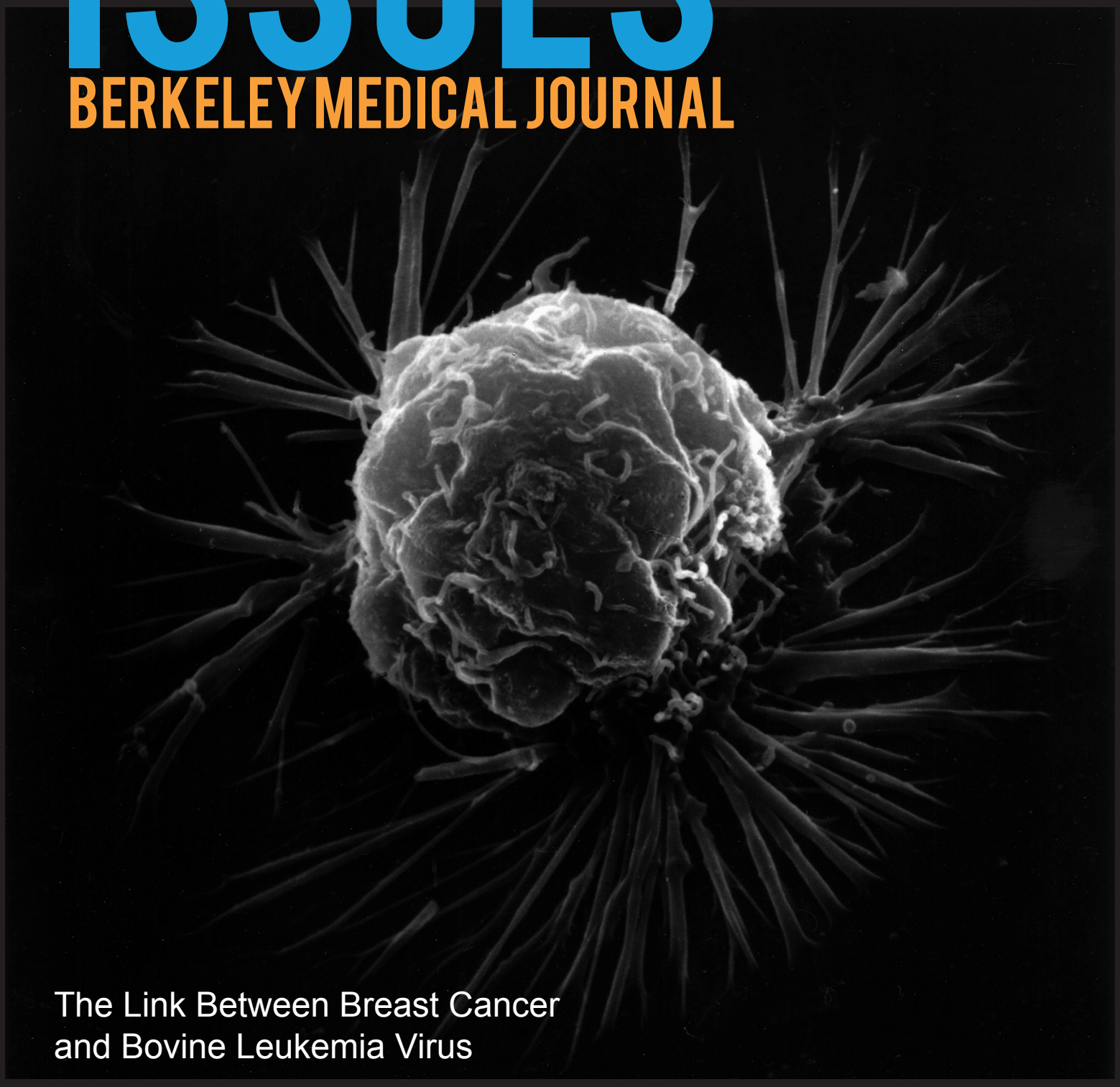

The Link Between Breast Cancer and Bovine Leukemia Virus

ISSUES

ISSUES Berkeley Medical Journal launched in the fall of 1993 with the aim to entertain and foster dissucssion about medical developments.

As the University of California, Berkeley's premiere medical and health sciences undergraduate publication, we are sponsored by the Associated Students of the University of California (ASUC) and hope to serve students interested in healthcare. Our student-writers focus each semester on chronicling the recent developments in biomedical research, current issues in public health, and quandaries surfacing in contemporary medical practice.

Cover Image: Scanning electron-microscope photograph of breast cancer cell courtesy of National Cancer Institute
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Questions or comments? Contact us at: berkeleymedicaljournal@gmail.com

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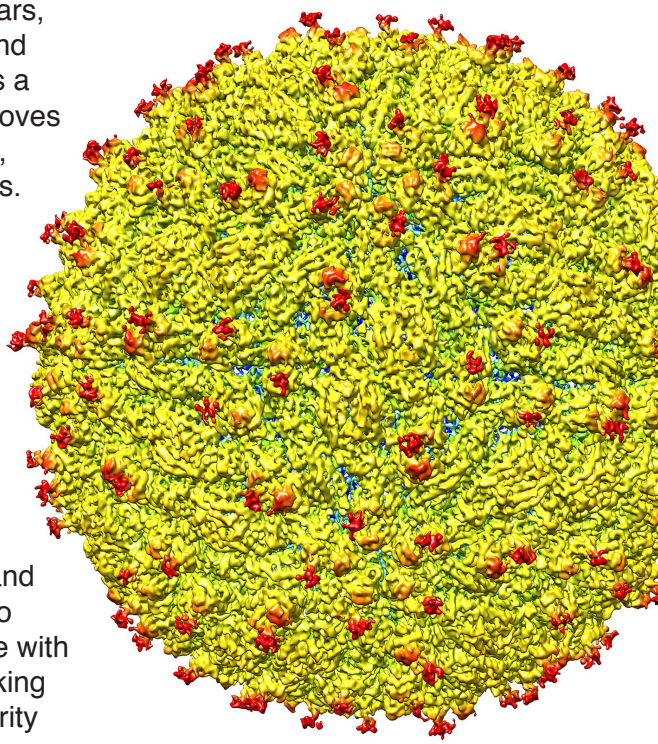

Image: Representation of Zika Virus Courtesy of Kuhn and Rossmann research groups

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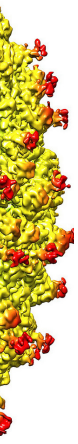


SHORT REPORTS

Bovine Leukemia Virus:
A Link Between a Prevalent Virus and Breast Cancer

BY CHELSEA MUENNICHOW

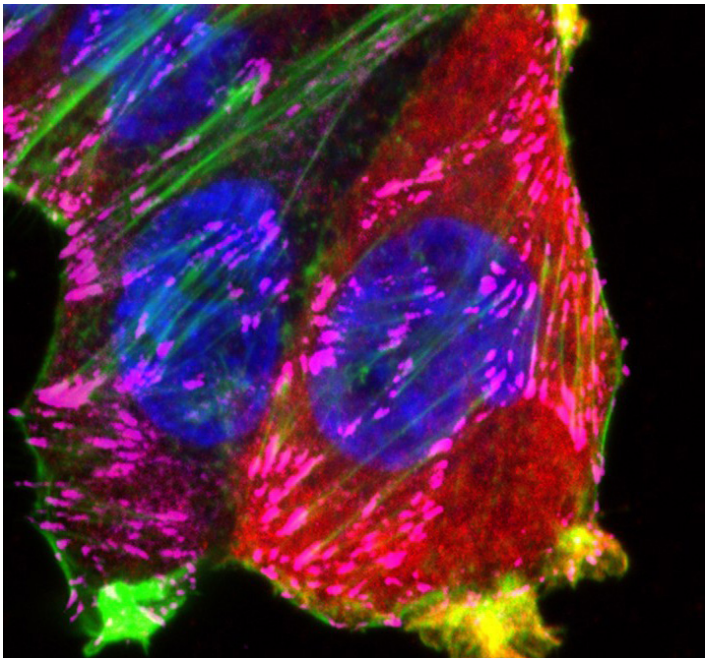


PHOTO COURTESY BUHRINGER FRIEDRICH, (LEFT) PHOTO OF BREAST CANCER CELL COURTESY NATIONAL CANCER INSTITUTE (RIGHT)

Could drinking milk increase risk for breast cancer?

Breast cancer is the second most common cancer in women, and was projected to kill 40,290 women in the US in 2015. There are many factors which contribute to and cause breast cancer, and Dr. Gertrude Case Buehring, a professor of virology in the Infectious Diseases and Vaccinology Division of the School of Public Health, is interested in investigating the link between breast cancer and Bovine Leukemia Virus. In her recent study, 239 samples from women with and without a history of breast cancer were analyzed for the presence of Bovine Leukemia Virus (BLV). The results were astounding, because they supported the correlation between bovine leukemia virus and breast cancer development in patients. The next step, which is currently transpiring at Berkeley, is to discover if the virus infected the samples before or after the cancer developed.

Bovine Leukemia is also known as Bovine leukosis, a retrovirus first discovered in Lithuania in 1871, causing B-cell leukemia or lymphosarcoma in cattle. B-cell leukemia is the most common type of cancer of white blood cells. It specifically affects B-Cell lymphocytes, which originate in bone marrow and combat infection in healthy individuals. At the time of its discovery, bovine leukemia virus was thought to be an infectious disease because of how quickly it was able to spread through the cattle herds in Lithuania. The virus was isolated in 1969, and has since been extensively studied. Bovine leukemia virus is not just a retrovirus, but a deltaretrovirus. Deltaretroviruses are very closely related to the human T-cell leukemia virus, and it has genomic region in their DNA that thought to be oncogenic, which means to cause the growth or development of tumors. The genomic region is called Tax, or trans activating region of x-gene. Tax is oncogenic due to its ability to cooperate with other oncogenes, disrupt growth control mechanisms, and disrupt DNA repair. These functions of Tax allow the development of tumors to be widespread and rampant.

However, cancer is not always caused by viruses or retroviruses. Cancer is usually caused by defects in regulatory circuits that govern cell proliferation and homeostasis. Why then, has this random virus from Lithuania virus sparked so much interest? In the most recent study on bovine leukemia virus in dairy cattle

completed in 2007, over 82% of the entire US dairy herd from the nation's 17 major dairy producing farms were sampled. Nearly 84% of the dairy operations sampled tested positive for bovine leukemia virus. Products from animals that develop malignant lymphona and lympho sarcoma cannot be sold on the market, but less than 5% of those animals with BLV develop these conditions. Therefore, products from many of the animals containing the virus are sold to the public. Even more astonishing was that only 7.5% of those sampled had independently reported the presence of BLV in their dairy cows. Moreover, approximately 38% of beef herds and 100% of all large-scale dairy operation herds are infected with the virus. Naturally, concerns were raised about the transmission rates of Bovine leukemia to humans, and consequently 10 studies were completed in the 1970s. These experiments used immunologic methods to test serum samples from 1,761 humans, but none of them tested positive for antibodies for BLV. Therefore, it was concluded that BLV could not be transmitted from animals to humans through consumption. However, since the 1970's, much more sensitive immunoblot techniques have been made which allowed Berkeley researchers to detect antibodies for BLV in 39% of 257 human volunteers. It was unclear, however, if these antibodies were indeed present in blood serum because of infection with bovine leukemia virus, or only present because of exposure to bovine leukemia virus that had been inactivated with heat in dairy products that were consumed.

To investigate this, researchers injected sheep with pasteurized milk and others with raw milk from Bovine leukemia infected cows. The former did not develop infection and subsequently antibodies, whereas the latter did. Researchers then began to investigate human tissues for evidence of infection with bovine leukemia virus, focusing specifically on breast tissue. Although it is not known exactly how the virus infects breast tissue, it is thought to be through human-to-human transmission, unpasteurized milk, or uncooked meat.

This case-control study from UC Berkeley used "archival formalin fixed paraffin embedded breast tissues" from 239 donor women from the Cooperative Human Tissue Network. In the study, participants were classified as having breast cancer or no history of the disease through a variety of means including medical records and anatomic pathological examination of tissues. Using in situ polymerase chain reaction, which is a DNA diagnostic test to amplify DNA for examination, the exposure of the breast tissue to BLV was determined to be localized within the epithelium of the mammary tissue. It was found that the mammary epithelium for women with breast cancer contained markedly higher concentrations of BLV than did the controls: 59% of the samples with breast cancer had exposure to BLV, whereas only 29% of the tissue samples with no history of the disease showed exposure to BLV.

The most shocking thing about this study, however, is the fact that the odds ratio for the development of breast cancer after exposure to bovine leukemia virus is proportional to that of other recognized risk factors, including obesity, alcohol consumption, hormones, lifestyle, reproductive history. The only risk factors with an odds ratio that exceeded BLV was genetics, familial breast cancer history, high dose ionizing radiation, and age.

Although these findings do not prove that high levels of BLV exposure will guarantee the development of breast cancer, they are very important first steps. If further research shows that BLV is causally linked to breast cancer, then preventative approaches to breast cancer treatment can start to be created. Currently, Dr. Buehring's laboratory is researching other questions involving this topic. These questions include: does the infection of normal human breast cancer cells with BLV in a culture cause them to acquire the characteristics of a malignant cell? And how exactly do humans become infected with BLV? Can women infected with BLV pass the virus to their children through breast milk or through the placenta? And are other human tissue and organs besides mammary tissue infected by BLV? Once these questions are answered, our understanding of BLV and its link to cancer in humans will be much more defined. If BLV is found to have a causal contribution to cancer, it would have profound implications to public health education and policy. Information about the consumption of contaminated meat and its associated risk with breast cancer should be provided to the public, and it would be imperative that preventative health measures be implemented to increase standards and sanitation of the dairy and meat industry.

"the odds ratio for the development of breast cancer after exposure to bovine leukemia virus is proportional to that of other recognized risk factors, including obesity, alcohol consumption, hormones, lifestyle, reproductive history."

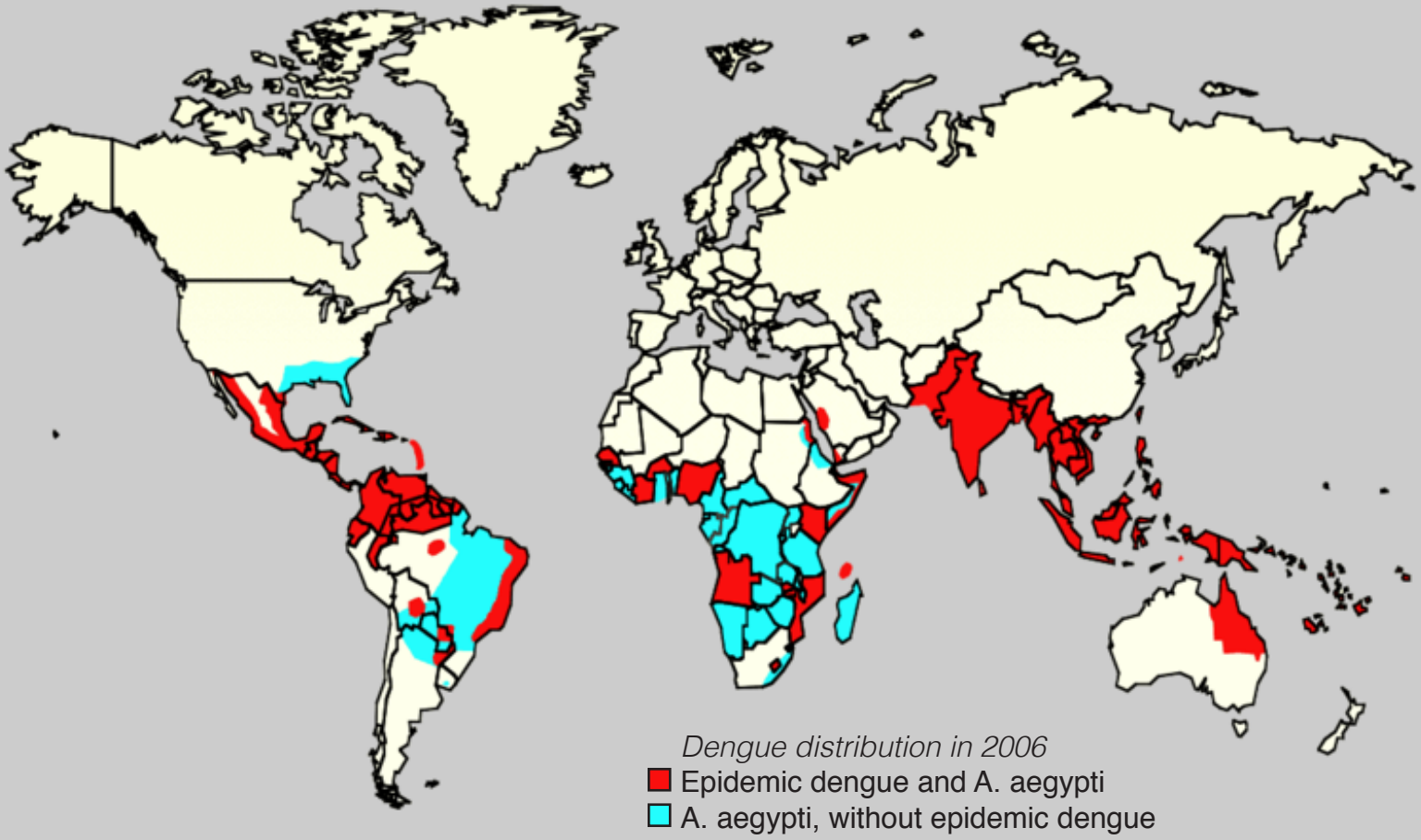


IMAGE COURTESY GARY CLARK

PUBLIC HEALTH SPOTLIGHT: DENGUE

Dengue Vaccine Research Reaches New Heights

BY AHAANA SINGH

Even before modern medicine, the prevalence of mosquito-borne illnesses has been rampant. Today, 128 countries worldwide are at risk of suffering from one of the most rapidly growing mosquito-related viruses. Causing severe joint pain, nausea, high fevers, and countless other symptoms, this virus infects approximately 390 million people every year according to the World Health Organization's (WHO) studies. The number of cases is not only locally increasing with the spread of the disease to new areas, but there are also explosive outbreaks occurring worldwide. In 2013, the Americas alone reported 2.35 million cases, and 37,687 of those cases were severe.

Up until now, no treatments have been discovered for this growing epidemic; however, the combined efforts of a variety of research institutes have resulted in the development of six possible vaccine candidates to tackle the Dengue Fever. Of the six vaccine candidates in clinical trials, one has made it to the most advanced clinical development stage.

While it was initially concentrated in tropical areas, the Dengue Fever is now spreading far beyond its tropical origins. The virus is closely related to the viruses that cause West Nile and Yellow Fever. Typically, cases within the US have been attributed to international travel and contraction of the virus from more, but recent developments show that there is an increased risk along the Texas-Mexico border, as well as in Florida. The Aedes mosquito, found on every continent today, is the transmitter of the four-strain virus, posing risk to anyone exposed to it.

"[Dengue] is an increasing public health issue because the spread of mosquito vectors," says Dr. Robert Beatty, a lecturer of immunology at the Univer-

sity of California, Berkeley, whose research focuses on developmental recombinant proteins for dengue vaccines. "Urbanization is also a huge factor--this is urban disease as opposed to rural disease."

Typical symptoms of Dengue mirror those of the flu--sudden high fever, severe headaches, fatigue, nausea, vomiting, and skin rashes. Although most cases are fairly mild and can be taken care of over the typical ten-day symptomatic period, there are still many cases that have become significantly more severe and sometimes fatal. Extreme cases result in the development of hemorrhagic fever, damage to lymph and blood, enlargement of the liver, and circulatory system failure. In some cases these symptoms may progress to what is known as dengue shock syndrome (DSS)--causing abdominal pain, hemorrhage, and circulatory collapse. It also may lead to the dengue hemorrhagic fever (DHF), which begins abruptly with high continuous fever and headache in addition to respiratory and intestinal symptoms such as a sore throat, cough, nausea, vomiting, and severe abdominal pain.

These unsettling symptoms paired with the increased spread of the virus has lead to a major push for Dengue prevention and treatment worldwide. However, the development of the live recombinant tetravalent vaccine--a four-strain vaccination that targets all four of the Dengue viruses--has progressed without the benefit of a full understanding of the pathogenesis of dengue. Triggering an immune response to each of the four Dengue viruses is predicted to minimize the overall risk of disease enhancement. At the same time, it must be kept in mind that antibody-dependent enhancement of the virus may still occur with the antibodies that are meant to counter the virus. Accordingly, a vaccine must attack the virus but not remain in the patient's immune system for too long. Several other concerns exist regarding live attenuated virus vaccines, such as the tetravalent strain undergoing testing; these include cell-culture-derived adventitious agents, community spread of the vaccine virus by resident vector mosquitoes, vaccine virus causing disease in the nervous system, and the effects of vaccine administration to immunocompromised hosts. These, however, are very unlikely although theoretically possible and must be accounted for.

"It's hard to gauge effectiveness [of potential vaccines] because people can get repeatedly infected with dengue," according to Beatty, "Looking at complete prevention and looking at reduced disease, reduced disease is a more important focus."

The first major efforts to create a vaccine have been unsuccessful for several different reasons, including problems of unbalanced immune responses and inconsistent reactions to the vaccine. The US National Institutes of Health, however, has introduced new Dengue vaccine research with direct mutation-altering technology. The National Institutes of Health has licensed the resulting vaccine candidates to several institutions for further testing.

Of the several vaccines--including a chimeric virus, inactivated virus, subunit vaccines, DNA vaccines, and vectored vaccines--the tetravalent chimeric dengue virus vaccine has progressed to phase III testing. The Center for Disease Control and Prevention (CDC) created the vaccine by inserting DENV-1, -3 and -4 prM and E genes into cDNA derived from the attenuated DEN-2 component that was developed in the Sanofi Pasteur live attenuated dengue virus vaccine at the Mahidol University in Bangkok, Thailand. The chimeric tetravalent vaccine candidate was then formulated and licensed to Inviragen, Inc. and Takeda, respectively, and has undergone clinical testing with those pharmaceutical companies. After additional testing and manipulation, the candidate vaccine has now progressed to efficacy studies--required to prove efficacy against dengue in the field and to build a robust safety database, according to research conducted by Wallace, D.

The live chimeric dengue vaccines, when inoculated into dengue-immune children or adults, have not resulted in enhanced disease caused by vaccine

IMAGE COURTESY JAMES STEWART

virus, which suggests that the live virus in the vaccine is controlled and unlikely to cause vector transmission of the dengue virus. Numerous dengue vaccine developers have also performed risk assessments to gauge this. According to Sardelis et al, "The results of published studies indicate a very low likelihood that a vaccine could transmit vaccine-derived dengue viruses to a mosquito." Still, however, studies must continue in order to observe the interference that may be caused by the mixture of four dengue viruses in a single strain vaccine.

Despite the advances in research and development, Professor Beatty still has concerns about the ultimate utilization of the vaccine, "How do you decide whether or not it's worth public health dollars? If you can commit big nations to buy it then public health money will provide for other [needy] nations." If this is not a focus during development, vaccine production is essentially useless because it will not reach the countless nations that need it. Currently, a licensed dengue vaccine is not available but the studies conducted and that continue to progress, are providing increased knowledge of the dengue virus and vaccine development. Although only one vaccine has progressed to phase III testing, several other possible candidates are undergoing preclinical and clinical trials and appear to be promising. "It's going be very difficult to settle on one vaccine that is going to be very successful," says Professor Beatty, "There's a lot of challenges to deciding on one [of the several] vaccines to work." Continued research, however, along with economic pushes and technological advancements, shows promise of a dengue vaccine reaching the markets shortly.

With each academic, technological, and economical investment contributed towards this growing Dengue vaccine research, there are hopes of eliminating one of the largest, growing mosquito-transmitted viruses in the world.

Environmental [In]Justice:
Population Demographics and Environmental Health Hazards

BY KELSEY ABKIN

IMAGE COURTESY ARISTOCRATS-HAT ON FLICKR

When discussing environmental health hazards, our inclination is to think of it in strictly scientific terms. What process is causing the degradation of our atmosphere? Is it possible that the release of harmful chemicals are warming our planet? While these questions are of utmost importance, there is another crucial component to environmental concerns, a social component. Think communities burdened unequally by hazardous waste, toxic incinerators, and health-threatening chemical contamination. Think race playing a prominent role in who receives the weight of this issue. In a new study, published in the American Journal of Public Health by a UC Berkeley graduate student team lead by Lara Cushing, light was shed on the previously indeter-

minate relationship between race and environmental health hazards. Until now, it was known that neighborhoods with higher percentages of Hispanic and African American residents had poorer air quality, closer proximity to hazardous waste sites, and fewer parks--but what makes this study groundbreaking is its simultaneous focus on multiple hazards and its consideration of specific factors that make populations more vulnerable to the effects of pollution. In doing this, they were able to see that the risk of exposure to environmental health hazards, such as air pollution and toxic waste, is related to race, and in no insignificant way. In California, Hispanics were 6.2 times higher, blacks were 5.8 times higher and Asians and Native Americans were two times higher, when compared with Whites, to be exposed to such health hazards. With such staggering comparisons found, it is important to ask what are the implications and what can be done?

To address the concern that communities of color unequally carry the burden of environmental hazards, the University of California, Berkeley and the California Environmental Protection Agency (CalEPA) used California Communities Environmental Health Screening Tool (CalEnviroScreen 1.1) developed by the state EPA's Office of Environmental Health Hazard Assessment. This tool was used to evaluate how environmental indicators affected populations differently. Briefly, the CalEnviroScreen 1.1 took 11 indicators of pollution burden and 6 of population vulnerability. Of these 11 indicators, 6 were indicators of exposure such as pollutant sources, releases, and environmental concentrations, while 5 were environmental effects indicators measured as threats to the environment such as hazardous waste sites. These latter 5 were counted as half the weight of the indicators of exposure. This number, titled the pollution burden score, was then multiplied by the vulnerable pollution score. This score of vulnerability included biological traits (e.g., age and disease status) and factors related to socioeconomic status (e.g., poverty and education level) that can increase susceptibility to adverse health impacts of pollutants. The indicators are all aggregated into a cumulative impact score, and they are matched to communities defined by ZIP codes from 2010.

Using information from 2010 Census data, relationships between race and ethnicity and cumulative impact scores were addressed. To assess which aspects of pollution burden were most unevenly distributed, concentration curves were plotted and a concentration index calculated for each indicator with respect to zip code—level racial/ethnic makeup and the percentage of the population living in poverty. The research compared, from lowest to highest, the percentage of the population that is either non-Hispanic, White, or living above twice the federal poverty line to the cumulative share of the environmental hazard. In doing so, they were able to note that curves above the equality line indicated an excessive burden on colored communities while curves below the equality line showed a burden on the White and wealthy. For instance, the concentration curve most dramatically emphasized disparity when it came to pesticide use. The graph illustrated that the 60% of zip codes with the highest proportion of residents of color host more than 95% of California's agricultural pesticide use.

So what exactly did this study tell us? It was found that the median cumulative impact score was found to be 75 percent higher for Hispanics and 67 percent higher for African Americans when compared with non-Hispanic White populations. In other words, these two racial groups had a higher rate of exposure to and were more likely to be vulnerable to pollution hazards. Native Americans had the third highest median population vulnerability score but a lower median pollution burden score than did other groups. Asian/Pacific Islanders had the third highest median pollution burden score but lower median population vulnerability scores than did Hispanics, African Americans, and Native Americans. Further emphasizing the disparity, it was found that the odds of living in one of the 10% most affected communities were higher for all racial groups other than Whites. Specifically, odds were 6.2 times higher for Hispanics and 5.8 times higher for African Americans. Going against what would be expected, it was revealed that disparities in pollution burden were generally greater with respect to race/ethnicity than they were with respect to poverty. To answer the question of what exactly this study told us, it showed that race is not a factor we can ignore when discussing the burdens of environmental hazards but rather is the greatest indicator of environmental inequality.

Given the enormity of this issue, a solution must be discussed. While these findings cannot directly indicate the probability that people of particular races will develop health problems such as asthma or cancer, they do indicate which communities are most likely to be exposed to environmental stressors that impact overall health. Making this information public allows policymakers to address the communities that warrant the most attention. Potential legislation could enforce stricter pollution laws and better regulate current, pressing environmental issues in these particular areas. Unequally burdened communities of color require priority in the environmental reform movement. Environmental inequality is not, at its core, an environmental issue--it is a social issue with its' roots deeply set in prevalent inequalities and thus should be treated as such.

2015 Nobel Prize in Medicine: Artemisinin

Traditional Medicine in Pharmacology

BY SHANNON PAI

TU YOUYOU (RIGHT) WITH MENTOR IN 1951

As a result of institutionalized bias against herbal remedies in the 20th century, alternative medicines are not as widely accepted as western medicines in the United States. In fact, after the passage of the Dietary Supplement Health and Education Act in 1994, herbal medicines became officially labeled by the FDA as "herbal supplements". However, after the 2015 Nobel Prize in Medicine was awarded to Tu Youyou for the development of artemisinin, a key antimalarial drug, herbal medicines have gained more recognition in the United States.

One reason that western medicines are often more trusted and widely used in the United States is that they undergo extensive research studies and clinical trials before they can be sold to the public. Herbal medicines have also gone through extensive studies, but in the form of trial and error. Traditional herbal medicine has been developed through hundreds of years of experimentation and passed down orally each generation. Healthcare providers in non-western societies often know the medicinal uses of different plants, their side effects or toxicity, dosage, and their interactions with other medicines. Some even store seeds in granaries to preserve certain genetic strains that are more effective treatments.

The power to dictate which drugs are legitimate and safe for public use is held by Western institutions such as the FDA, which results in marginalization of traditional treatments. Interestingly, many important and widely used pharmaceutical drugs such as Aspirin (Salix spp.), a drug for pain, headaches, and colds, and Taxol (Taxus brevifolia), an anti-cancer drug, are derived from molecules isolated from plants. These plants were introduced to Western researchers and physicians through collaboration with traditional healers and interpreters. Dr. Thomas J. Carlson, a medical doctor, ethnobotanist, and professor at UC Berkeley, believes that the western approach to medicine can be greatly complemented by working with local communities to better understand ethnomedicine.

One example that supports the efficacy of traditional plant-based medicine and a future for research into non-western medicines is the discovery of the anti-marlarial drug Artemisinin by Tou Youyuo. To those living in the developed world, malaria is no longer a health concern. However, the burden of malaria is still high in developing countries. According to the World Health Organization, an estimated 3.2 billion people are at risk for being infected with malaria. In 2013, there were 198 million cases globally that resulted in 584,000 deaths.

In the 1960s, the main treatments for malaria were chloroquine and quinine. But these treatments had many adverse side effect some of which were so severe that they decreased compliance to drug therapy, meaning that patients were more likely to stop us-

ing the drug. Over time, the development of resistant strains of malaria made these drugs became increasingly ineffective.

Due to the urgent need for anti-malarial drugs, China set up Project 523 to spur research in drugs that fight against malaria. Tu Youyou understood the medicinal properties of Chinese herbs, and screened over 2,000 Chinese herbs for an antimalarial drug. Eventually she and her colleagues discovered Artemisia annua, the plant artemisinin is derived from.

This plant was already known to have medicinal properties, and is documented in The Handbook of Prescriptions for Emergency Treatments written by Ge Hong in 340 A.D. In previous studies, extracts from Artemisia annua (Qinghao), a type of wormwood native to Asia, were shown to inhibit parasite growth by up to 68%. Follow-up studies, however, only achieved 12% to 40% inhibition. Tu reasoned that the low inhibition could be due to a low concentration of the active ingredient in the preparation and began to improve the methods of extraction.

Because conventional methods to isolate chemicals utilize high temperatures that could damage the active ingredient, Tu decided to switch from ethanol to ether extraction, which occurs at a lower temperature. The result was an extract with undamaged active molecules and hence improved antimalarial activity. The extract was then separated into an acidic toxic portion with no antimalarial activity and a neutral nontoxic extract with antimalarial activity. In October of 1971, the neutral extract (number 191) was tested on a strain of rodent malaria, Plasmodium berghei, and achieved 100% inhibition of the malarial parasite. While Tu's team struggled to obtain high-quality crystals from the plant, two teams (Zeyuan Luo, Yunnan Institute of Drug Research and the late Zhangxing Wei, Shandong Institute of Chinese Traditional Medicine) of Project 523 used Tu's information and methods to obtain pure artemisinin crystals that were highly active against rodent malaria parasites. These artemisinin crystals were then tested in human clinical trials and were very effective in treating and reducing the symptoms of malaria.

MONOCHROME PAINTING OF SWEET WORMWOOD BY LAN MAO IN 14TH–15TH CENTURY
IMAGE COURTESY WELLCOME LIBRARY, LONDON

Even though the discovery of artemisinin took place in the late 1960s and the 1970s, Tu Youyou only recently gained widespread recognition after being awarded the Nobel prize. This drug has saved the lives of millions of people around the world and is currently being investigated for its potential use in cancer therapy.

Unfortunately, malaria has not been a major target of drug companies, which have historically focused their efforts on more profitable treatments for diseases prevalent in affluent countries. Thus, the discovery of artemisinin has been significant for populations more susceptible to malaria and with less access to healthcare, particularly because artemisinin has less severe side effects than synthetic pharmaceutical drugs. Often, people who have difficulty with the general side effects of pharmaceuticals can benefit from using herbal medicines that have been proven to be safe and effective through animal studies and clinical trials.

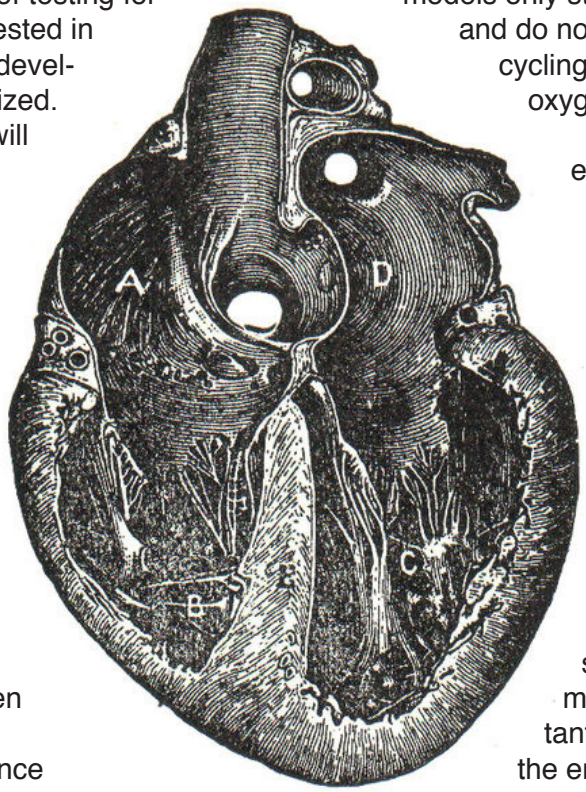
Artemisinin is currently available in a pill form and is most effective when used in combination with other anti-malarial drugs to reduce the likelihood of drug resistance. Its drawbacks include low bioavailability (the amount of each administered dose that reaches the circulation system) and high cost. People in developing countries could benefit from renewed interest in anti-malaria research in addition to research of possible chemical molecule derivatives of Artemisia annua that have higher bioavailability. Malaria is still an ailment that afflicts much of the Earth's population, and those living in developed countries who have more access to modern research and technology should not neglect the health challenges facing less privileged populations.

Biotech: Wearing your Heart on your Chip

The Development of a Living Heart on a Microchip

BY ISABEL MARCHAND

From the creation of the self-driven car to the rapid, continuing progression of the Internet, scientists and science-fiction aficionados alike have dreamed of a new era of technological innovations. Fortunately, for both researchers and sci-fi fans alike, dreams of technological advancements in the context of biological systems has now become a reality. Researchers at the University of California, Berkeley recently developed a silicone-based device that accurately and successfully models the human heart. Led by Professor Kevin Healy, this bioengineering research team developed the organ-on-a-chip model in order to advance the efficiency of the drug development industry. By creating an easier mode of testing for toxicity, the time and money invested in this drug-testing aspect of drug development will be drastically minimized. Additionally, these organ chips will reduce, and possibly eliminate, the need for testing these drugs on animals and in vitro human tissue and organ samples.



Compared to purely technological industries, the drug development process is both expensive and inefficient, taking on average a decade and billions of dollars to produce just one federally approved drug. Additionally, current testing methods using animals are enveloped in scientific issues. The various physiological differences between humans and other species often complicate results, and the reliance on these simpler animal models can yield unintentionally inaccurate results that could put lives at risk and waste a decade of research and billions of dollars. Animal testing is also surrounded in bioethical issues.

This experiment was not the first to recreate human physiological functions. The Grosberg Lab, a 2011 research group at Harvard University, used mouse embryonic stem cell-derived cardiomyocytes (CMs) to recreate the structure of cardiac ventricles in 2D form. While they were successful in modeling the cardiac ventricles, this 2D form could not be recreated as a 3D model. Furthering the Grosberg Lab experiment, the Boudou group from the University of Pennsylvania was successful in creating a freestanding 3D tissue model; however, both experiments were limited by their use of animal cells, as opposed to human-derived tissue. In recent years, successful 2D models have been created that used human stem cell-derived CMs; however, there is still a lack of models that successfully recapitulate the three-dimensional physiology of in vivo human structures. Additionally, these models only statically model human physiology, and do not take into consideration nutrient cycling, tissue exposure to drugs, and oxygen transport.

Taking all of these previous experiments into consideration, the Healy Lab at UC Berkeley developed a two-part hypothesis to test if combining genetically accurate human cells with "tissue-like" drug gradients would yield a CM model accurate enough to successfully predict the pharmacological toxicity of various drugs. Researchers in the Healy Lab did so by developing a microphysiological system (MPS) that uses 3D confinement to arrange human stem cell-derived CMs into a 3D microtissue, a system that importantly mimics the flow protection of the endothelial barrier.

To accurately mimic the structure of the human heart, this chip was developed using a three-pronged concept: cardiac fibers, microcirculation, and diffusive transport to the tissue were all prioritized. The system is comprised of a central cell chamber with two adjacent media channels, and various connecting microchannels. The media channels allow for precise delivery of nutrients. In order to create a reproducible cardiac microtissue in the cell

chamber, the researchers relied on a previously used method developed by the Lian Group from the University of Wisconsin. Cells were injected into the cell to promote cardiac tissue formation without extracellular or synthetic matrices.

Within twenty four hours, the human induced pluripotent stem cell cardiomyocytes (hiPSC-CMs) formed a 3D cardiac tissue that could beat spontaneously without any external stimulation. After seven days, the beating of all cells temporally aligned, and the model was comprised of multiple cell layers. Structure alignment and consistent beating are crucial for a cardiac MPS–without either of these two, the structure would not properly model the human heart, and would therefore be nonfunctional.

Upon developing this system, the researchers assayed the cardiac response with four drugs of known toxicity, ultimately showing that this human stem cell-derived system significantly improved the accuracy of toxicity testing over more involved trials and previous 2D models. The researchers assayed the toxicity of Isoproterenol, E-4031, Verapamil, and Metoprolol. These pharmacological agents were selected because together they represent four important pharmacological classes that have characteristic clinical responses and known toxicity, so the measured toxicity could be compared to known values. Measuring heart beat, the researchers obtained spontaneous and consistent beat rates between 55 and 80 beats per minute (bpm) in the MPS model across all four drug trials. The data obtained from the Isoproterenol administration showed increased beat rate and more accurate beat values compared to the data obtained from tests administered on the questionable 2D models. Additionally, the MPS data was comparable to data obtained from tests administered on slices of human heart tissue. This proves that the MPS model was more consistent with actual human tissue compared to the synthetic models.

The tests conducted using Verapamil were also significant. Verapamil is a calcium antagonist that blocks the calcium ion channels in cardiac tissue. The blocking effects differ based on drug concentration, making testing on animal-cell derived models difficult due to the high reliance on human cell physiology. Testing Verapamil with the animal systems yielded a decrease in beat rate and increase in irregular heartbeat, known as arrhythmia, as well as a complete lack of beating. Clinical observations of Verapamil, however, report very few incidences of arrhythmias. Testing Verapamil on the MPS yielded data that mirrored the clinical observations and larger scale animal testing. These findings show that the MPS system may be more efficient in modeling tests, which can lead to the MPS replacing animal testing and eradicating the unethical methods that may be undertaken with the current methods of animal testing.

Overall, the MPS creates a 3D microtissue that responds to drug testing similar to more mature hiPSC-CMs, a known accurate clinical model of the human heart. Additionally, this MPS is more viable because it uses human cells, which alleviates any inaccuracies that can arise from the discrepancies between animal and human models. These findings are ground-breaking in the realm of drug testing and medicine, with potential to greatly decrease both time and money spent on testing pharmaceuticals. Due to the quicker response of the MPS, the time spent modeling the effects of certain pharmacological agents can be shortened, and time spent on toxicity research can be greatly reduced. The MPS could also decrease money spent on these tests because it would reduce the amount of total material needed to perform these tests from various animals and lab materials to just the MPS chips. Additionally, this change in drug testing can completely eradicate the need for animal testing.

Future directions include modification of the MPS for more personalized drug testing, as a patient could use their own cells to test their own reaction to certain pharmacological agents. MPS also has the potential for development outside of the drug screening and testing world and could be used to assay other biological factors. The possibility of advancing MPS for other organs outside of the heart to test reactions with pharmaceuticals, and perhaps even environmental factors, marks it as a potentially revolutionary system for in vitro testing of biomolecular agents on the human body.

Addyi: The Female Libido Pill

BY SHANNON PAI

IMAGE COURTESY JAMIE ON FLICKR

Hypoactive sexual desire disorder (HSDD) is the most common female sexual dysfunction, affecting approximately 10% of females. It is defined as a persistent or recurring lack of interest in sex to the point of negatively impacting the ability to partake in romantic relationships, and consequently emotional and mental health. HSDD is commonly misunderstood as a psychological or personal problem rather than a biological disorder. As such, it is often left untreated because the lack of research on its biological basis by the scientific community. The few interventions that have been used to treat the disorder were developed through evidence-based psychological methods.

Until recently, there was no pharmacological drug approved to treat HSDD. Research was done primarily on the physiological, psychological, and sociocultural factors underpinning the development of HSDD. Medical therapeutic strategies used prior to Addyi concentrated on the modulation of hormone levels, such as testosterone administration. Lack of testosterone has been shown to cause not only low libido, but also decreased sexual receptivity and pleasure. Therefore, administering doses of testosterone has a positive effect on the treating symptoms of HSDD. Psychological therapeutic strategies concentrate on cognitive behavioral therapy (CBT). CBT assumes that changing harmful thinking, like unrealistic perceptions about normal sexuality, can lead to changes in emotions and behavior. Because of the importance of relationship health in the development of HSDD, the therapist helps both partners improve their communication, identify the meanings that they associate with sex, and work on non-sexual intimacy.

Addyi is a new FDA-approved drug being used for HSDD. It is being incorrectly referred to as the female "Viagra" pill despite its mechanism of action not being related to vasodilation, the primary mechanism of Viagra. Instead, Addyi functions as both a serotonin agonist and antagonist regulating the levels of dopamine, norepinephrine, and serotonin in accordance with specific regions of the brain. Different levels of these neurotransmitters can excite or inhibit the sexual response. Dopamine and norepinephrine have been identified as excitatory factors, while serotonin has inhibitory effects.

The effectiveness of Addyi was evaluated in three 24-week randomized, double-blind, placebo-controlled trials in about 2,400 premenopausal women with acquired, generalized HSDD. They concluded that Addyi causes statistically significant increases in the number of satisfying sexual events and in sexual desire scores (measured using the Female Sexual Function Index [FSFI]). FSFI is a brief 19-item questionnaire used as a multidimensional self-report instrument for assessing the sexual function of a woman. In addition, Addyi was found to reduce the female sexual distress of premenopausal women diagnosed with HSDD.

There are some improvements that remain to be made to Addyi. Risks associated with the drug include potential interaction with alcohol and the risk of severely low blood pressure and loss of consciousness. To combat this, Addyi is taken at bedtime to help decrease the risks associated with central nervous system depression, such as hypotension and sedation. Addyi has also revived criticisms of HSDD being classified as a disorder. Critics claim that the distinction made between what is "normal" and "abnormal" sexuality is inherently flawed because of the stigma it places on asexuality.

While Addyi may have some flaws, its development remains significant to women suffering from HSDD who previously had only hormone administration and psychological therapy available to them. It has the ability to improve the number of satisfying sexual events and decrease distress related to sexual desire. This has the potential to improve satisfaction in romantic relationships and overall mental and emotional well-being. On a broader scale, its development may also prompt more interest into the research of sexual desire.

The Giggle Gene: Are Our Genes a Laughing Matter?

BY ISABEL MARCHAND

IMAGE COURTESY RUSSIA INTERNATIONAL NEWS AGENCY

Long has the question been asked if there is a link between genes and personalities. Due to the joint efforts of researchers at the University of California, Berkeley and Northwestern University, including Robert Levenson and Dacher Keltner of UC Berkeley and Claudia M. Haase of Northwestern, the existence of a "laughing gene" in the human genome was discovered in 2015. Individuals in the study possessing the short version of a specific gene, known as 5-HTTLPR, were more likely to smile or laugh than those with the longer version of the gene. This discovery may offer insight into the interaction between our genes and our responses to the environment.

The relationship between genes and personality has perplexed scientists for quite some time. Until now, very little was known about the relationship between genetics and personality. However, the discovery of this link between 5-HTTLPR and emotional responses may change that. The findings associating a short 5-HTTLPR allele with positive emotions refutes prior research that asserted this same allele caused more somber emotions in certain individuals. Prior to this study, the neurotransmitter 5-HTTLPR was thought to create feelings of depression and anxiety, in addition to some degree of Post-traumatic Stress Disorder. Occasionally, it has even been referred to as the "depression gene."

In this experiment, three different groups of individuals watched different scenarios that would evoke both positive and negative emotional responses. Over three hundred individuals participated in this study. The first group of young adults watched cartoons, the second group watched a more subtly comical movie, while the third group watched a martial conflict unfold. The researchers filmed the test subjects and then tested the recordings with a decoding system that observed and recorded the emotional microexpressions of participants. These microexpressions, which we make subconsciously in response to emotional situations, reveals what emotions we are feeling. The researchers wanted individuals to express genuine emotion. The most important clue for this is engagement of the eye muscles responsible for creating "crow's feet." If an individual's smile or laugh was accompanied with crow's feet, the researchers knew it was a genuine, positive reaction. In addition to recording these microexpressions, the researchers took saliva samples to test for the allele itself.

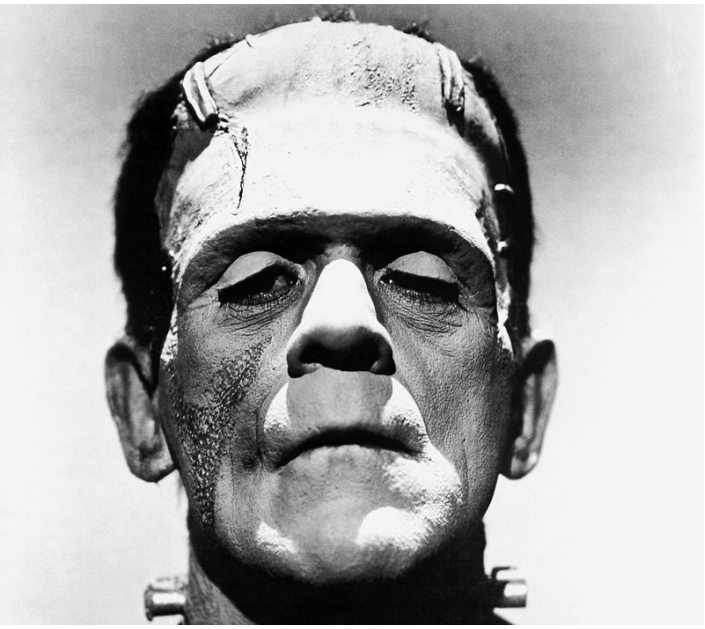
The findings are ground-breaking in the discipline of psychology. Not only did individuals possessing the short 5-HTTLPR allele show more genuine positive emotion, but these results held true even after taking into account gender, race, and ethnicity—strengthening the argument that this allele and its effects are present in the entire human genome. This new research argues that individuals with this short 5-HTTLPR allele are not simply more predisposed to negative emotional reactivity, like originally thought, but are rather more likely to feel the emotional highs and lows of life as a whole. In other words, individuals with the short allele are more likely to experience the extremes of the emotional spectrum, while those with the long-allele are less likely to react in such drastic ways.

It is important to note that this research may indicate a link between genes and personality, but it does not mean that there is necessarily a causal relationship between the two. Further research would need to be conducted in order to test if the alleles themselves are solely responsible for evoking emotional responses to stimuli. Different upbringings and other personality contributors could also be responsible for our varied emotional responses. Hopefully, though, this study

can lead researchers to eventually determine whether this cause and effect relationship does exist .
The possibilities for the applications of this research ranges from issues involving mental health and personality psychology to the relationship between humans and our environment. Although much more research would be needed in both gene therapy and personality mapping, finding a link between the human genome and our personalities may mean that gene therapy could become a possible treatment method for some mental illnesses.

Cryogenics: The Real Frankenstein

BY ALBERT WANG



Dr. Victor Frankenstein created a monster: a monster that proceeded to attack and ravage before finally coming head-to-head with its creator in perhaps the most emotional and tense of encounters. Despite creating a monster in the process, Dr. Frankenstein achieved something humans have longed to do; he acquired the skills necessary to revive the dead, the potential for humans to never die. Now, for the first time, humans may actually possess such knowledge. Before considering whether eternal life is something we want, we must sit back and revel at the possibilities and technology that may allow us to do that. Specifically, we have knowledge of how to freeze bodies, in the hope that in the future there will be cures for insufferable diseases.

About 200 individuals have signed up to give their bodies to cryonics research or research in the technology of freezing bodies, in hopes that they will be rewarded with eternal life in the future. The biggest stumbling block for scientists, however, is not the freezing process, but rather the unfreezing process, because of problems with maintaining the body's functionality. As scientists spend hours in labs all over the world looking for a way to decode this complicated process, Nature, the very power we are trying to work our way around, has already gifted the answer to a species. Alaskan Wood Frogs earned their fame through the incredible temperature changes they withstand. Able to withstand temperatures below those viable for human life, these frogs seem frozen in time during the winter months, then melt and return back to life in the spring. These frogs provide a significant linkage between the challenge to eternal life cryonics aims to solve and what this species naturally seems to possess.

A key factor to the wood frogs' success has been their ability to produce cryoprotectants. Cryoprotectants increase the chance of survival after freezing by preventing intracellular ice formation, stabilizing membranes and macromolecules, and serving as antioxidants, metabolic substrates, and metabolic regulators. As described by D.J. Larson, Ph.D in the Journal of Experimental Biology in 2004, these cryoprotectants allow Alaskan wood frogs to survive in temperatures below freezing for up to six months with a minimum of near -20 degrees Celsius. With this information, researchers have begun considering the possibilities of cryoprotectants in the study of cryonics, since the biggest problem associated with cryonics has been the process of survival upon unfreezing.

It is already known that bodies can be unfrozen and function for a short period of time, but survival never lasts longer than a few hours. The freezing and subsequent unfreezing processes have simply caused too much damage to the human body for it to function as well as it previously did. Scientists have already learned why cryoprotectants work for wood frogs. Currently, the issue is not that cryoprotectants cannot be used in humans, but rather, it is not known how they would function. In fact, cryoprotectants are already naturally produced in the human body; however they are an artifact of ancient human history, and carry out a slightly different function than they do in wood frogs.

While researchers continue to investigate the possibilities of cryoprotectants in the human body, two major biotech companies, Cryonics Institute and Alcor, have already begun accepting clients who wish to be frozen in hopes of being revived in the future. Since these companies have already developed a working

process of freezing, the future of the field lies in the unfreezing process. Scientists continue to experiment and test different species of wood frogs, their cryoprotectants, and their respective freezing and unfreezing capabilities. These teams hope that a more conclusive analysis can be reached in the next few years on the workings of cryoprotectants, as well as how they could potentially be applied to humans.

Cryonics and cryoprotectants hold important information in the quest for eternal life, holding potentially major implications for the future of medicine. Other areas, such as organ transplantation, are in dire need of improvement as demand increases, and cryonics could very well offer it. The thought of bringing a body back to life has long been thought as science fiction as there is a reason why the story of Frankenstein has persisted. Now, we are closer than ever to reviving a real frozen body–and this time, it might not be pure science fiction.

Research in Reversing Age

BY YASH SHAH

In the sixteenth century, Ponce de Leon dedicated the rest of his life to search America for the Fountain of Youth. Although he never found it, researchers studying aging are finding that certain techniques have been found to minimize the aging effect. Harnessing and using this research could have extensive applications in the real world, who doesn't want to stay young?

It is strange to think about how and why aging occurs, despite being such a common part of our lives. The Villeda Lab at UC San Francisco is tackling these questions. In one of its most recent publications the lab shows that certain proteins circulating in an organism's blood slow down aging. These researchers explain that systemic manipulations such as exercise, caloric restriction, and changing blood composition can stop and potentially reverse many effects of aging, especially age-related loss of plasticity in the brain. Although genetic influences do largely affect how one will grow, studies show a surprising level of malleability in an organism's lifespan.

The research group explored how much of a role genetics plays in aging. Through numerous studies, it has been found that an organism's lifespan can be affected at a molecular level; this is through complex interactions and relationships between many different genes. They are usually involved in cellular processes such as homeostasis; also, many pro-longevity signaling pathways have also been shown to play important roles in higher-level brain function. Therefore, there is evidence for a connection between the variability in aging and the plasticity of the Central Nervous System (CNS) after time. In fact, it shows that because the CNS is intimately involved in every other aspect of the body, it is in the unique position of potentially being influenced by and influencing the plasticity of the rest of the body. Gene therapy does not seem to be a viable option because there is not a specific genetic target.

However, an alternative, more systemic approach to could be just as successful, according to researchers at UCSF. Broad changes in the systemic environment (such as blood composition), rather than point manipulations in the CNS, may provide the means for rejuvenation. This can be done through exercise, CR, and changes in blood composition by heterochronic parabiosis or young plasma administration.

One feature common among aging tissues is loss of regenerative capacity, and the maintenance of adult stem/progenitor cells which have the ability to self renew and produce new cells in adult tissue is critical in preserving this capacity.

Exercise seems to be a method through which aging can be slowed or reversed. Physical exercise increases blood delivery to most tissues, which leads to change in the systemic environment. It also affects molecular and cellular processes that play major roles in stem cell function both in vitro and in vivo. This is because exercise induces autophagy, the clearance of cellular debris, which protects hematopoietic stem cells from metabolic stress. It has also been shown to enhance neural progenitor proliferation and neurogenesis to a higher level. In the blood, circulating levels of IGF-1 decrease with age but systemic manipulations seem to restore these levels to previous standards. To-

gether, these factors show the ability research has in rejuvenating adult stem cell function across tissues.

In addition, caloric restriction also provides a method for systemic manipulation that successfully can reverse the effects of aging, through a reduction of 20-40% of caloric intake without malnutrition. This effect can be attributed to increasing glucose metabolism, reducing oxidative stress, and the increasing ability of cells to counteract DNA damage as well as influencing aspects of the aging immune and neuroendocrine system. Caloric restriction has also been shown to rejuvenate tissue regeneration through mechanisms similar to what happens through exercise. This occurs by the release of steroids by the body in response to caloric restriction or exercise.

Finally, heterochronic parabiosis, which is a process by which the blood streams of two organisms are connected, can also have the same effect as exercise and caloric restriction. The effects that the blood of younger animals can have on the physiology and behavior of older animals when transfused to them have been found to be significant. Through this model it has been shown that exposure to young blood can enhance the regenerative capacity of peripheral tissues and CNS in aged animals. “Pro-youthful” factors in the younger blood are credited for the physiological and behavioral changes. It has been shown that these factors change the systemic environments for the tissues of the body, reversing the effects of aging upon them. This is done by restoring growth factors such as GDF11 to more youthful states which has been shown to reverse age-related muscular and cardiovascular impairments in mice. It has also been found that daily administration of recombinant GDF11 increases tissue function.

Though seemingly impossible, the reversal of aging is becoming more realistic. However, these findings do not contain the root cause or causes of aging, and as such there is no existing “cure” to aging. This research does promise extension of life through personal health and heterochronic parabiosis.

Photonic Polymerase Chain Reaction (PCR)

BY CHELSEA MUENNICHOW

Imagine the endless possibilities that could result from faster DNA diagnostics, especially in a world where DNA sequencing is becoming increasingly relevant in all aspects of science and medicine; for example, in molecular biology, genetic recombination and transgenic animals have become increasingly important tools. An important aspect of these and many other diagnostic tests is the polymerase chain reaction (PCR), a tool used by molecular biologists to amplify DNA and generate millions of copies of a specific DNA sequence. Researchers at UC Berkeley in the lab of Dr. Luke Lee, the co-director of the Berkeley Sensor and Actuator Center and a professor of bioengineering, have engineered a novel approach to high-speed DNA concentration recording. This technique allows for direct optical DNA sequence detection.

In traditional PCR, approximately thirty cycles of repeated heating and cooling separate double-stranded DNA, allowing the strands to be bound to a matching primer, and allow for DNA replication by a high temperature-stable DNA polymerase enzyme. The PCR amplifies one copy of DNA to produce millions of copies. It is absolutely essential in various aspects of genomics, including forensic analyses, cloning research, and even paternity tests. Nonetheless, it can take over an hour to complete these thirty thermal cycles in order to successfully amplify the DNA. According to Dr. Lee, “PCR is powerful…[but] it takes a lot of power and is expensive.” Therefore, traditional PCR assays are simply impractical for rapid point-of-care diagnostics, especially when one considers the high cost, impracticality, and power requirements for the heaters and coolers of these thermal cyclers.

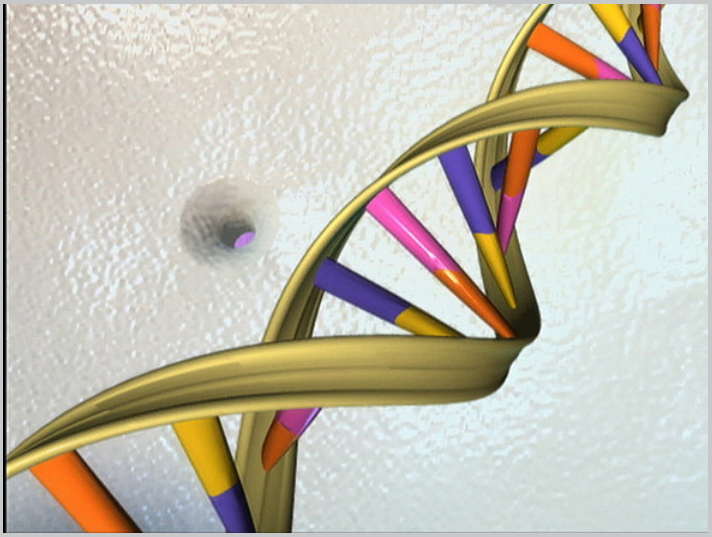
UC Berkeley bioengineers have recently developed an advanced cycling system which is expected to vastly expand all aspects of polymerase chain reaction tests, from clinical performance to research applications. This exciting new technology uses photonics, an extremely cheap, fast, and accurate system that can yield results in as little as five minutes. The photonic system involves alternating cycles of heating and cooling that are powered by energy from photons of light from light-emitting diodes, or LEDs. Researchers foresee the implementation of this technology in a wide range of settings beyond research in molecular biol-

ogy. For example, point-of care-diagnostics is medical diagnostic testing performed outside of a clinical laboratory, but still in close proximity to the patient's place of care. The cheap, fast system of photonics for polymerase chain reactions will allow for such testing to be used for critical, on-the-spot decision making in these and other fields. This method promises to transform point-of-care diagnostics in everything from medicine to evolutionary biology, food security, ancient DNA sample analysis, diagnosis of infectious and hereditary diseases, and many more academic disciplines and healthcare fields.

The photonic PCR system is grounded in plasmonics. Plasmonics involves the interaction of light with free electrons on a metallic surface, usually to produce heat. The researchers' design involves thin gold films that are approximately 120 nm thick, which they deposited onto a plastic chip with several microfluidic wells filled with DNA in solution. Gold is usually used in photonic heating because of its high efficiency in absorbing light, as well as its lack of activity in biological systems, making it perfect for this interaction with DNA. Then, using a 3.5 Watt LED array with a wavelength of 450 nm emitting light from beneath the film, the electrons in the gold strip are excited. The resulting heat generated from this processes is used to warm the DNA in solution, accelerating the thermal denaturation step that is required to separate the double-stranded DNA strand into usable single-stranded DNA templates. To complete the cooling cycle, the LED array shuts off, cutting off heat flow to the electrons, causing rapid cooling of the system.

Not only was the accuracy of this method identical to traditional polymerase chain reaction methods, but the researchers at Berkeley found that thirty thermal cycles could be completed in less than five minutes, over ten times faster than traditional PCR. In fact, the researches clocked the solution's heating speed at approximately 55 degrees Fahrenheit per second, with the cooling rate coming in at about 43.9 degrees Fahrenheit.

The possibilities and ramifications of this discovery are seemingly endless. One of the professors working on the project at UC Berkeley says the photonic system would be perfectly suitable for "ultrafast point-of-care, on-chip genomic diagnostics" that could be used everywhere from emergency rooms to rural areas. This is a truly revolutionary technology that will allow for the direct optical detection of DNA-protein interactions, protein-protein interactions, RNA-protein interactions, and many other biological systems.



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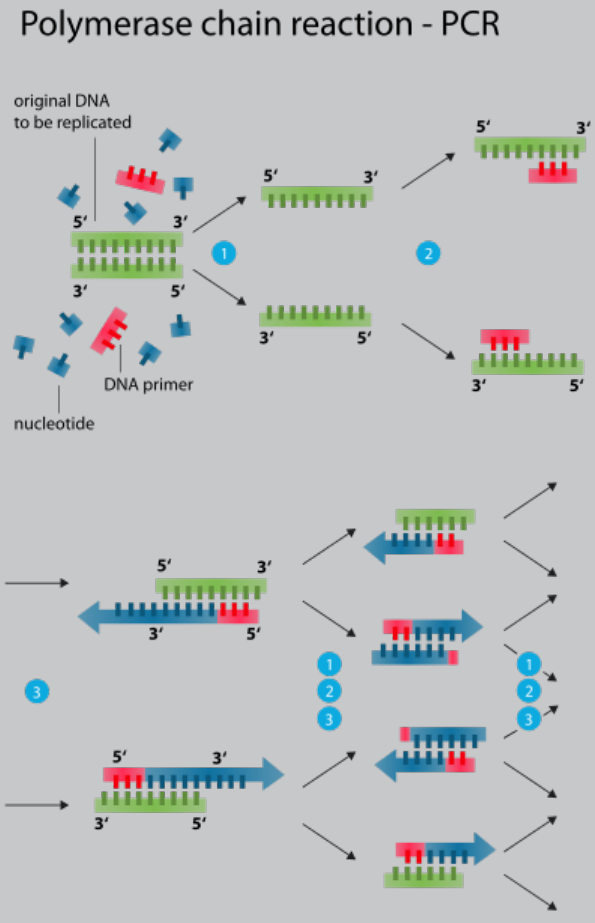

DIAGRAM OF PCR
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