

Hüttemann lab develops tech that can stop cellular damage

The lab of Dr. Maik Hüttemann has developed a new infrared light technology capable of halting cellular damage before it can take root.



Dr. Maik Hüttemann

“This is a platform technology that can be applied to several disease conditions in which mitochondria are dysfunctional,” says Dr. Hüttemann, Professor of Molecular Medicine and Genetics, and of Biochemistry, Microbiology & Immunology.

The work was performed mostly on the brain, and findings were reported in the February 2018 issue of *Scientific Reports*, “Inhibitory modulation of cyto-

chrome c oxidase activity with specific near-infrared light wavelengths attenuates brain ischemia/reperfusion injury,” authored by Sanderson et al.

“The literature is full with articles that propose that infrared light activates mitochondria, which we have confirmed for some wavelengths,” Dr. Hüttemann explains. “However, what we did was unique in that we scanned the near-infrared light range between 700 and 1000 nm and discovered for the first time novel wavelengths that slowed down mitochondrial function.”

The infrared light is absorbed by copper atoms of the cytochrome c oxidase (COX) enzyme. The COX enzyme catalyzes the terminal step of a process known as the electron transport chain which provides over 90% of the cell’s energy. COX is also known to be the rate-limiting enzyme in this process. This means that slowing down COX will slow down

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Granneman lab expands with fellowship, supplement, and new investigator awards

James Granneman, PhD is Professor of Molecular Medicine and Genetics and of Internal Medicine. His work towards the treatment of obesity-related disorders has been prolific, and continually supported by grants and industry contracts. His laboratory is expansive and home to numerous post-doctoral fellows, research scientists, students, and research assistants. In addition to Dr. Granneman’s research related accomplishments he has also found success in training the next generation of scientists. This fact is best demonstrated by the project awards recently received by his trainees. Some of those projects and scientists include:

Rayanne Burl is in the Molecular Genetics and Genomics PhD program and has been awarded a three-year NIH F31 fellowship from the National Institute of Diabetes and Digestive and Kidney Diseases.

Burl’s fellowship is a collaboration between Gran-

neman’s lab and the labs of Roger Pique-Regi, PhD and Francesca Luca, PhD, both Assistant Professors of Molecular Medicine and Genetics and of Obstetrics and Gynecology. Dr. Pique-Regi oversees Burl’s computational analysis for single-cell RNA-sequencing datasets.



Dr. James Granneman

Burl’s research with Dr. Granneman focuses on neogenesis of adipose tissue; specifically, how cold temperature triggers the proliferation of multiple cell types in classic brown adipose tissue (BAT), addressed in her fellowship proposal, “Deconstructing the brown neogenic zone in classic brown adipose tissue.”

With the incidence of Type 2 diabetes reaching epidemic numbers globally, expanding catabolic

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Samavati lab develops screening tool for cystic fibrosis and other illnesses

Lobelia Samavati, MD, is overseeing a team that is working with a technology capable of successfully screening for various illnesses, initially cystic fibrosis (CF).



Dr. Samavati is Associate Professor of Medicine and of Molecular Medicine and Genetics in WSU's School of Medicine.

She began her research on this project nearly 12 years ago, and her lab has now developed a T7

Dr. Lobelia Samavati

phage display library derived from sarcoidosis tissue. They are using it to differentiate among diseases; in this case, they could differentiate CF-specific antigens from healthy controls and lung cancer patients. Phage display is a technique using bacteriophages to connect proteins with the gene that encodes them.

Their findings were published in the *Nature* journal *Scientific Reports*. The article, "Detection of Cystic Fibrosis Serological Markers Using a T7 Phase Display Library," showcases the team's method for the creation of the T7 Phage Library (<https://www.nature.com/articles/s41598-017-18041-2>). The work may aid in the development of molecular therapy as well as in diagnostics and therapies.

Dr. Granneman, from Pg. 1

adipocyte phenotypes such as brown adipocytes offers a way to improve energy balance and insulin sensitivity. In order to exploit this remodeling for therapeutic benefit, researchers need to understand the mechanisms by which populations of catabolic adipocytes are expanded *in vivo*. BAT is a thermogenic organ, mobilizing lipid stores to create heat. Expanding BAT in rodent models improves metabolic health. Adult humans also have BAT, and BAT activity is correlated with metabolic health. However, the amount of BAT in humans is variable and its activity is low. Therefore, to expand the thermogenic activity of BAT in humans, researchers need to understand the source of new brown adi-

The lab used a high-throughput method to develop a novel platform based on a T7 phage library – a cDNA library derived from mRNA isolated from bronchoalveolar lavage (BAL) and leucocytes of sarcoidosis patients. The library underwent a biopanning selection technique and 1,070 potential antigens were collected for the CF study. They constructed a microarray platform and immunoscreened with healthy controls, lung cancer (LC) and CF subjects. Applying statistical models, they selected the top 20 frequently significant clones that discriminated CF antigens from healthy controls and LC patients. The performances of the models were validated on an independent validation set. They also identified CF-specific clones that correlate highly with clinical values such as a common CF diagnosis sweat chloride test, Body Mass Index and Tiffeneau-Pinelli Index predicted values.

"For the first time, we show that CF-specific serological biomarkers can be identified through immunoscreenings of a T7 phage display library with high accuracy, which may have utility in development of molecular therapy," according to the *Scientific Reports* paper.

According to the researchers, there is a great need for serum-based biomarkers for detecting various diseases including cancer, inflammatory diseases, infections, and genetic disorders. Non-invasive serological biomarkers can help measure disease progression or gauge therapeutic response. Markers previously identified have not been helpful in

phenotypes.

To this end, Burl's work seeks to better understand how BAT neogenesis occurs during cold exposure. Her proposal uses single-cell RNA-sequencing of mouse BAT over a period of cold-induced BAT neogenesis to identify cell types present in the BAT neogenic zone. The proposal also uses single-molecule fluorescence *in situ* hybridization to locate cell types in the neogenic zone and transgenic mouse models to provide mechanistic analysis of adrenergic receptor signaling during cold-induced neogenesis. Ultimately, this work will advance the understanding of the cellular plasticity of BAT and guide therapeutic efforts for establishing beneficial cellular

a clinical setting. Using high-throughput technology to create phage-protein microarrays, in which peptides from a unique sarcoidosis cDNA library were expressed as a phage fusion protein, helped bridge that gap.

Samavati's team examined other conditions, such as cancer, but the lung cancer tissue samples did not work so effectively in creating a library. Most tissues extracted from cancer will quickly decompose after being removed from the patient, and the cells also don't carry the antigen, so progress was more difficult to track. Optimal results were derived from the sarcoidosis tissue, including all lung cells obtained during the bronchoscopy from lungs of various patients, as well as whole peripheral blood cells; using live cells set in a solution to preserve antigenicity of the sample. After the success of the immunoscreening library for CF, Samavati and her team are turning their focus toward using the technology on tuberculosis, and in the future they would like to tackle other diseases such as rheumatoid arthritis and Crohn's.

For her tuberculosis biomarker project, Samavati's group confirmed that the T7 Phage display library can identify classifiers for active tuberculosis, which was published in a special issue of *Viruses* on phage derived protein, July 19, 2018: <http://www.mdpi.com/1999-4915/10/7/375/htm>. The development of a cDNA library was funded by the National Heart, Lung and Blood Institute of the National Institutes of Health (HL104481).

phenotypes.

Vanessa Ramseyer Payant, PhD, was recently awarded a grant supplement for her work with a new protein, VPS-13C.

Dr. Granneman used proteomics analysis to discover the presence of VPS-13C in lipid droplet fractions in brown adipose tissue, Dr. Ramseyer Payant says.

"When I joined the lab, I became interested in this protein because VPS-13C had not been reported on lipid droplets before and because variations in the gene that encodes this protein are associated with changes in glucose levels and diabetes. Using a custom-made

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The genetic counseling profession grows, but still faces provider status and licensing challenges

In May, Wayne State University graduated its 17th class of genetic counseling graduate students. In the intervening years there have been many changes to the program, influenced by increase in demand for genetic counseling services and the rapid expansion of genetic and genomic tests.



Angela Trepanier, MS, CGC

Genetic counselors are health professionals who specialize in identifying individuals and families at risk of genetic conditions and helping them understand and adapt to the medical and psychosocial consequences. They receive the MS in Genetic Counseling degree. Currently, there are 4,626 certified genetic counselors in the U.S. and Canada (abgc.net). The profession has doubled in the last 10 years and is expected to double again in next 10 years – a much faster growth rate than average. Factors contributing to this growth include the rapidly increasing number of clinical genomic tests available and an increasing number of non-patient facing genetic counseling job opportunities such as with genetic testing laboratories, insurance companies, and others.

Despite this growth, a recent workforce analysis showed that there is a shortage of genetic counselors in the U.S. The analysis estimated that demand for genetic counseling services may not be fully met until 2023 or later. In light of this shortage, five national genetics organizations have collaborated to develop and implement a workforce strategic plan. The overarching objective of the plan is train a sufficient number of genetic counselors to meet the demand in the U.S.

One way to increase the number of practicing genetic counselors is to increase the number

of students in training. This is being accomplished in two ways – by increasing the number of students trained per program and by increasing the number of accredited programs. The main limitation to increasing the number of students trained per program is the availability of clinical training sites. Despite this limitation, programs, including the Wayne State program, have increased their size, expanding from a median of ~6 new students per year to ~8 new students per year. The number of programs has expanded as well. Currently there are 43 accredited programs in the U.S. and Canada. Of these, 8 are new programs accredited in the last three years. As a result of these changes, the number of students admitted each year to a genetic counseling program has increased from 237 in 2011 to 403 in 2018, a 70% increase.

Although the number of genetic counselors is growing, the profession continues to face challenges in the areas of approved Medicaid billers and of state licensing. Currently, genetic counselors lack recognition as a provider by the Centers for Medicare and Medicaid Services (CMS). In order to bill Medicare for services, a profession must be recognized as an authorized provider, which requires an act of Congress. However, before legislation will even be considered, the profession has to show that it will not add cost to the Medicare system. To address this issue, the National Society of Genetic Counselors commissioned a study that showed genetic counselors would actually save Medicare several billion dollars in genetic counseling and testing costs. NSGC is working with Representative Erik Paulsen (R-MN) to introduce federal recognition legislation soon.

In addition to lack of recognition by CMS, genetic counselors are not licensed in every state. The purpose of licensure is to help the public recognize which providers are qualified to provide a service (title protection) and to protect the public from unqualified providers. This is important because there are

many documented cases of harm in Michigan and nationally in which failure to accurately interpret family history and/or genetic testing information led to a missed diagnosis or the wrong diagnosis. Unfortunately, licensure is also tightly tied to the ability to bill for services. In states without genetic counseling licensure, genetic counselors are at a disadvantage compared to health professionals who can bill for the services they provide. To date, 25 states have passed genetic counseling licensure legislation, including our neighbors – Minnesota, Illinois, Indiana, Ohio, and Pennsylvania. Michigan has been working on passing licensure legislation since 2003. A bill (SB 331) is currently under consideration in the Michigan Senate and scheduled for a committee vote in May. Wayne State Genetic Counseling Program faculty members Angela Trepanier and Erin Carmany are actively involved in this effort as members of the Michigan Association of Genetic Counselors (MAGC) Licensure Subcommittee. Professor Carmany also serves as the president of MAGC and Professor Trepanier serves as the Chair of the National Society of Genetic Counselors Licensure Subcommittee.

Even with the challenges imposed by the lack of federal recognition and state licensure, the outlook for genetic counseling billing is improving. For instance, some insurance providers, like Priority Health and Cigna, now require genetic counseling by a certified genetic counselor or geneticist before a genetic test is ordered for certain indications. These policies were put in place because of strong evidence that the more appropriate testing that takes place when a certified genetics professional is involved reduces the cost of genetic testing.

For more information about genetic counseling or how to support licensure or recognition efforts, please contact the genetic counseling program at geneticcounseling@med.wayne.edu or visit www.genetics.wayne.edu/education

Dr. Granneman - Continued from Pg. 2

antibody and different methodologies including biochemical methods, immunofluorescence, and electron microscopy, I confirmed the presence of this protein on lipid droplets.”

With the data, she secured a two-year post-doctoral fellowship from the American Heart Association. “Under that fellowship, I found that VPS-13C is enriched in brown adipose tissue on lipid droplets and that it presents a unique subspherical distribution on those lipid droplets. This is interesting because, unlike VP-S13C, most lipid droplet proteins distribute homogeneously on the lipid droplet surface and it suggests that VPS-13C forms a complex that mediates the interaction between lipid droplets and other organelles. Although it is known that lipid droplets interact with other organelles, the members and function of such complexes have never been shown. We also found that brown adipocytes that lack VPS-13C through genetic manipulations have reduced lipid storage and increased lipolysis. Using sub-cellular fractionation (biochemical methods) we found that in the absence of VPS-13C, ATGL, the main enzyme that hydrolyzes triglycerides (lipids), accumulates on lipid droplets suggesting that VPS-13C suppresses lipolysis by preventing ATGL translocation to lipid droplets.” Those findings were published in January, “Vacuolar protein sorting 13C is a novel lipid droplet protein that inhibits lipolysis in brown adipocytes,” in *Molecular Metabolism*.

Ramsayer Payant used the data gathered during her fellowship, and co-wrote a proposal for a two-year NIH Research Supplement, where the investigators sought to study the role of VPS-13C using transcriptomic, phospho-proteomic, and lipidomic analyses. “We also proposed to characterize the lipid droplet subdomain where VPS-13C concentrates and to identify the proteins that form this structure. Finally, we proposed to study the *in vivo* metabolic profile of mice lacking VPS-13C.”

She is in the final year of the grant, and the team hopes to use the findings to secure a transitional grant in an effort to get a deeper understanding of the role of VPS-13C in lipid metabolism. “Understanding the role of VPS13C would help us identify novel targets for the regulation of lipid storage and mobilization, ultimately providing new insights in the regulation of energy and metabolism.”

“Working in Dr. Granneman’s lab allowed me to learn many techniques and exposed me to many creative and state-of-the-art techniques while learning to do research

and write grant proposals more independently,” Dr. Ramseyer Payant says.

Emilio Mottillo, PhD is a K99 grant recipient. “With the grant we hope to understand how fatty acids signal,” Dr. Mottillo says. “We know that obesity causes various metabolic disorders and that excess lipids are the problem, but exactly how fatty acids cause various diseases is not completely understood. We hope to improve our understanding of fatty acid metabolism, which has important implications for metabolic diseases such as diabetes and fatty liver disease.”

Dr. Mottillo’s approach involves using fluorescence microscopy to image lipid metabolism and protein interactions in real time, providing new insights to lipid mobilization.

“It was an exciting moment to receive the grant, especially as a young scientific investigator as it will allow me to expand my training, support my research, and provide a road map to become an independent investigator,” Dr. Mottillo says, “with a goal of solving problems that will hopefully ultimately improve the health of our society.”

Alexander Yang, WSU School of Medicine MD student and Molecular Genetics and Genomics PhD student was awarded an NIH F30 fellowship, covering three years of his PhD studies and the two years of remaining medical school to follow his PhD. He finished two years of medical school before starting his graduate studies in Dr. Granneman’s laboratory. He chose to work in Dr. Granneman’s laboratory after a summer rotation and developed rapport with Dr. Granneman and other laboratory members.

Yang is studying lipid storage, particularly the mechanism of how the I148M variant of PNPLA3 causes fatty liver disease. The variant was identified in 2008 by Dr. Helen Hobbs and Dr. Jonathan Cohen at the University of Texas and is present in 60% of Hispanics. The goal of the project is to elucidate the function of PNPLA3 and how the I148M variant affects PNPLA3.

“Understanding the mechanism will hopefully lead to new drug targets for fatty liver disease, as there are currently no drugs to treat fatty liver disease,” he says. Among the collaborators is Dr. Christopher Kelly in the Department of Physics, who is helping the team measure the affinity of pure proteins. “It’s an exciting cross-discipline collaboration, and I’m humbled that the NIH also agrees.” Yang said.

James Granneman, PhD is a Professor of Molecular Medicine and Genetics and of

Internal Medicine. He is also the Director of Wayne State’s Center for Integrative Metabolic and Endocrine Research, a joint research effort of the Departments of Pathology and of Internal Medicine (Endocrinology) that was established to address the worldwide epidemic of obesity and diabetes. Dr. Granneman’s research focuses on understanding how excessive fat accumulation contributes to disease and how to therapeutically target adipose tissue for treatment of obesity-related disorders, like diabetes and cardiovascular disease.

His current work uses team science approaches to address two interrelated areas. The first seeks to identify mechanisms that promote ‘catabolic remodeling’ of adipose tissue; that is, how to turn typical white fat from a tissue that stores excess energy to one that burns it. This work uses single cell transcriptomics and genetic lineage tracing to identify the cells that are destined to become fat cells in adipose tissues and to devise methods for guiding these cells into beneficial phenotypes. This project now in its 13th year of NIH funding and is a collaboration with Dr. Roger Pique-Regi of CMMG and Dr. Yun-Hee Lee of Yonsei University in South Korea. The project is also supported by a Visiting Scientist Fellowship from the Danish Diabetes Academy and by the NIH F31 award to CMMG graduate student Rayanne Burl discussed above.

The second area of research addresses how muscle and fat cells mobilize lipid energy for local and systemic use. Inappropriate mobilization of fatty acids by fat and muscle can lead to insulin resistance and diabetes. The long-term goal of this work is to develop and validate new preclinical drug candidates for development as obesity and diabetes therapeutics. The research uses several innovative techniques, including molecular imaging, medicinal chemistry, and structural biology, and involves collaborations with numerous WSU colleagues including Drs. Jeffrey Tseng (CMMG), Emilio Mottillo (CMMG), and Chris Kelly (Physics), as well as researchers at the Scripps Research Institute in Florida and Southern Denmark University in Odense, Denmark. This project is also supported by an NIH F30 award to CMMG graduate student Alexander Yang.

Dr. Granneman is currently PI/PD of 4 NIH research or training grants, has a contract with Eli Lilly. He is co-PI of the multidisciplinary Diabetes Obesity Team Science (DOTS) initiative through the WSU Division of Research. His office and laboratory are located in the IBio building at 6135 Woodward Ave.

Dr. Maik Hüttemann- Continued from Pg. 1

overall mitochondrial activity.

“The importance of our studies is the discovery of specific wavelengths that have the exact opposite effect of what was previously known and inhibit mitochondria. Our wavelengths can therefore be applied when mitochondria are hyperactive, which happens during conditions of cell stress. Important examples for such a condition is ischemia-reperfusion injury as seen in stroke and heart attacks. Those are conditions in which hyperactive mitochondria generate bursts of so-called free radicals, which cause massive damage and trigger cell death.”

The COX-inhibitory wavelengths, 750 and 950 nm according to the published findings, reduced mitochondrial respiration, essentially eliminated free radical production, and robustly attenuated neuronal death, whereas near infrared (NIR) light that activates COX provided no benefit.

The researchers looked at the COX-inhibitory NIR as a possible therapy for cerebral reperfusion injury via a rat model of global brain ischemia. Hüttemann credits Dr. Thomas

Sanderson, a former Associate Professor of Emergency Medicine at Wayne State University (now at the University of Michigan) for his contributions toward the success of this highly collaborative study. In addition, Dr. Karin Przyklenk, Director of the Cardiovascular Research Institute, and Dr. Lawrence Grossman, Director of CMMG, provided research support and a research environment that was instrumental to the success.

In the study, animals that did not receive the treatment showed 86-percent neuron loss in the CA1 hippocampus post-reperfusion while the inhibitory NIR groups showed much lower neuron loss, ranging from only 11 percent to 35 percent. Arguably the most striking finding was that neurologic function was preserved to control levels when rats were treated with the combination of the two COX-inhibitory wavelengths. These findings suggest that COX-inhibitory NIR may work as a non-pharmacologic and non-invasive treatment therapy for cerebral reperfusion injury, according to the researchers.

“Our infrared light technology is ideal in that it is non-invasive and does not rely on blood flow,” Dr. Hüttemann says. “Another major advantage is that it can be initiated exactly

when needed, which means when blood flow is restored. This sets us apart from all pharmacological approaches, which have failed, that we think can at least in part be explained by the fact that the early reflow phase is missed because drugs have to reach the tissue through the restored blood flow and build up before they can have an effect. In conclusion, our technology prevents the damage from happening.”

As the ultimate goal, Drs. Hüttemann and Sanderson and their colleagues are now working towards translating their findings into the clinic. Doing so involves moving forward on two fronts. One is showing efficacy in larger animals than the rodents used in the original study. The other is fabricating a medical device that could be used in hospitals and clinics as compared to the home made laboratory version. Work on both fronts is progressing, bringing a potentially major life-saving invention closer to reality and also bringing an exciting new direction into the lives of the inventors.

This work is currently supported by the U.S. Department of Defense Technology Development Award PR151051, and the National Institutes of Health grant R01 NS091242.

Faculty and trainee accomplishments

Francesca Luca, PhD; Roger Pique-Regi, PhD; and Ren Zhang, PhD were each promoted to Associate Professor with tenure effective August 2018.

Michelle Cichon, MS, CGC was promoted to Academic Services Officer III in August 2018.

Leonard Lipovich, PhD has been again invited to participate in Bio-IT World (2018) in Boston as both a Speaker and a Session Chair. This is his fourth invited presentation and session chairmanship at this prestigious joint academic and industry annual conference in the last six years.

Maik Hüttemann, PhD and Kezhong Zhang, PhD were each awarded the WSU School of Medicine Distinguished Faculty Award in December 2017.

Stephanie Gladycyk (Graduate Research Assistant in Dr. Grossman’s lab) received 1st place honors and **Hasini Kalpage** (GRA in Dr. Hüttemann’s lab) received 3rd place honors at the Graduate and Postdoctoral Research Symposium held on March 6, 2018, which was spon-

sored by the Graduate School.

Bhanu Jena, PhD was named in January 2018 an advisory board member for the Victor Babes National Institute, Bucharest, Romania.

Jaime Stafford, PhD (lab of Dr. Michael Tainsky) successfully defended her PhD thesis on March 1, 2018. She has accepted a postdoctoral position at the Mayo Clinic.

Samiran Ghosh, PhD was awarded the Young Researcher Award from the International Indian Statistical Association on December 29, 2017

Jordan Zhou, GRA in Dr. Kang Chen’s lab, was the American Association of Immunologists (AAI) Young Investigator Awardee for placing 1st in oral presentations at the 2018 Translational Research Cancer Centers Consortium meeting.

Cynthia Kalita, GRA in the Luca/Pique-Regi group, was selected for a podium presentation of her research project at the Cold Spring Harbor Meeting on Systems Biology: Global Regulation of Gene Expression.

Leonard Lipovich, PhD was awarded the

Charles H. Gershenson Distinguished Faculty Fellowship by the WSU Board of Governors.

Angela Trepanier, MS, CGC was awarded the inaugural University of Minnesota College of Biological Sciences Alumni Achievement Award in April 2018.

Bhanu Jena, PhD and colleagues launched in May 2018 a new biobank company named Quantum Pathologies in collaboration with Harvard Medical School.

Douglas Depoorter and **Marissa Petitpas**, MGG master’s students, each received awards from the 2018-2019 WSU Graduate School Master’s Scholarship Competition.

Siddika Venkatachalam, Andrea Filthaut, Erica MacDonald, Taylor Hayes, and Nicole Lester, Genetic Counseling master’s students, received 2018-2019 scholarships from the WSU Graduate School.

Markku Kurkinen, PhD was made a scientific advisory board member for NeuroActiva in June 2018.

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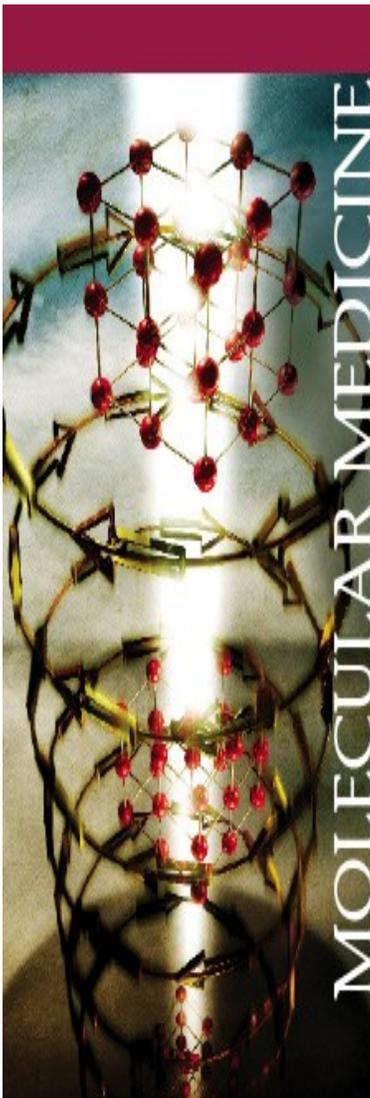
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