

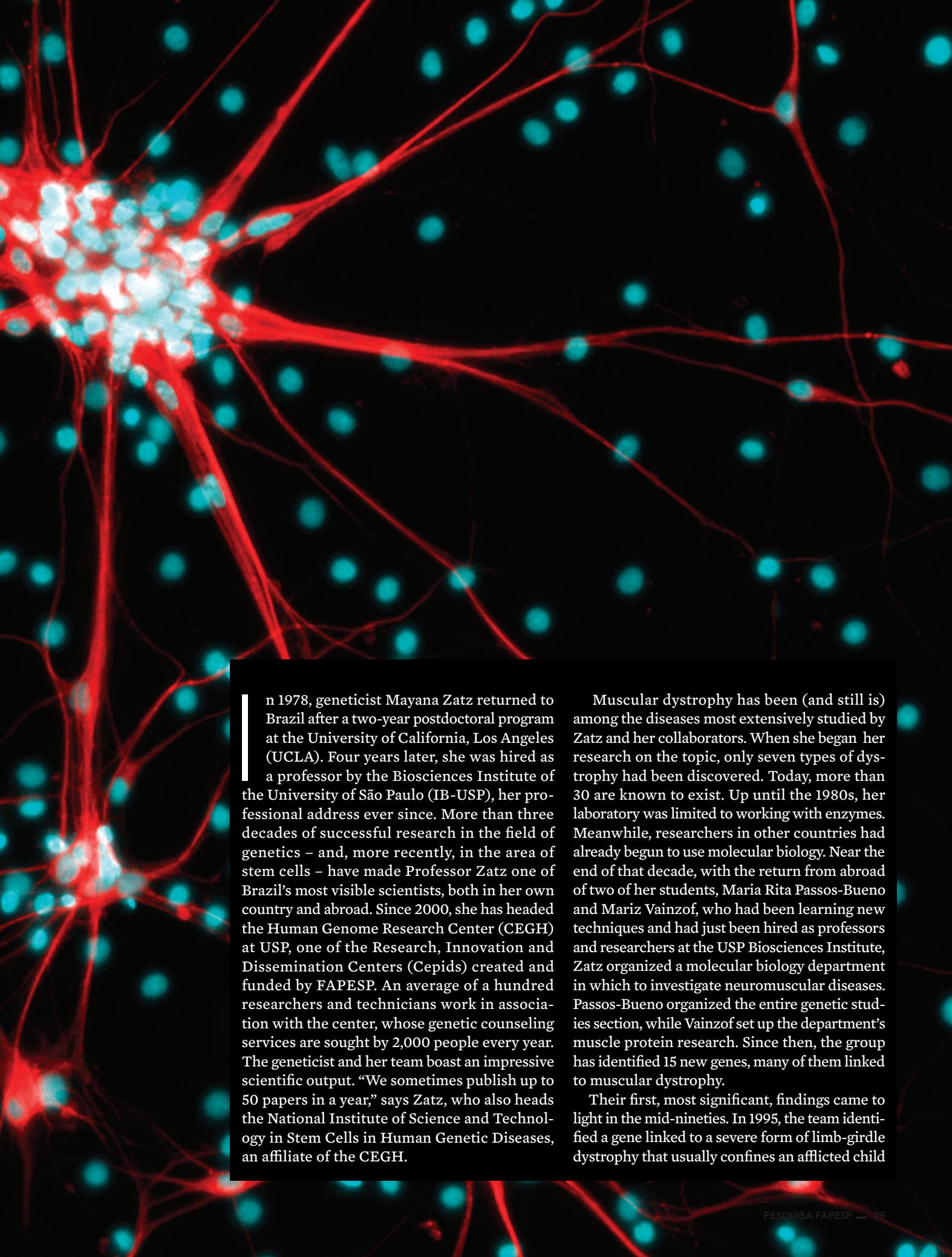
HUMAN GENETICS

# The dystrophy center

Research group in São Paulo identifies new genes and new forms of neuromuscular disease in Brazil

Marcos Pivetta

Fluorescence microscopy image of embryonic neural stem cells forming neural networks. Red represents the protein tubulin and blue depicts the cell nucleus



In 1978, geneticist Mayana Zatz returned to Brazil after a two-year postdoctoral program at the University of California, Los Angeles (UCLA). Four years later, she was hired as a professor by the Biosciences Institute of the University of São Paulo (IB-USP), her professional address ever since. More than three decades of successful research in the field of genetics – and, more recently, in the area of stem cells – have made Professor Zatz one of Brazil's most visible scientists, both in her own country and abroad. Since 2000, she has headed the Human Genome Research Center (CEGH) at USP, one of the Research, Innovation and Dissemination Centers (Cepids) created and funded by FAPESP. An average of a hundred researchers and technicians work in association with the center, whose genetic counseling services are sought by 2,000 people every year. The geneticist and her team boast an impressive scientific output. "We sometimes publish up to 50 papers in a year," says Zatz, who also heads the National Institute of Science and Technology in Stem Cells in Human Genetic Diseases, an affiliate of the CEGH.

Muscular dystrophy has been (and still is) among the diseases most extensively studied by Zatz and her collaborators. When she began her research on the topic, only seven types of dystrophy had been discovered. Today, more than 30 are known to exist. Up until the 1980s, her laboratory was limited to working with enzymes. Meanwhile, researchers in other countries had already begun to use molecular biology. Near the end of that decade, with the return from abroad of two of her students, Maria Rita Passos-Bueno and Mariz Vainzof, who had been learning new techniques and had just been hired as professors and researchers at the USP Biosciences Institute, Zatz organized a molecular biology department in which to investigate neuromuscular diseases. Passos-Bueno organized the entire genetic studies section, while Vainzof set up the department's muscle protein research. Since then, the group has identified 15 new genes, many of them linked to muscular dystrophy.

Their first, most significant, findings came to light in the mid-nineties. In 1995, the team identified a gene linked to a severe form of limb-girdle dystrophy that usually confines an afflicted child

to a wheelchair by the age of 10. That same year, the researchers identified another gene that, when mutated, is responsible for the rare type of progressive blindness known as Knobloch syndrome. To date, every known mutation that causes the syndrome has been allocated to the two copies of the COL18A1 gene, which was identified by Passos-Bueno's team at IB-USP. The story of the discovery of the association between Knobloch disease and COL18A1 – a gene in chromosome 21 that codes a protein called collagen XVIII – was a tale of hard work, patience, and a bit of luck, the essential ingredients for scientific progress.

When a family afflicted with limb-girdle dystrophy was discovered in the city of Euclides da Cunha in the Brazilian state of Bahia, the researchers observed that there were also numerous cases of blindness in that group of people, which included a number of consanguineous couples. To find out what was going on with those individuals, Passos-Bueno traveled to Euclides da Cunha, talked with the family members, and collected material for her research. "Some members of the family have dystrophy and others are blind," Passos-Bueno explained in 1997, in a story for *Notícias FAPESP*, the precursor of *Pesquisa FAPESP*. As usual in such cases, every family involved in the research projects conducted by Zatz and her team received genetic counseling in order to learn how to deal with their disease and become aware of the risks of transmitting mutations to their future offspring.

In their search for genes associated with neurodegenerative diseases, the USP team turned up some surprising results. Their work on VAP-B, a gene in chromosome 20, is a good example. In a paper published in November 2004 in the *American Journal of Human Genetics*, researchers from the São Paulo center showed that a mutation in VAP-B could cause three distinct types of degenerative motor neuron diseases: adult-onset progressive spinal

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muscular atrophy; the classic form of amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease); and a new, atypical variant of amyotrophic lateral sclerosis, dubbed ALS8. A dysfunction in this gene was detected in 34 individuals from seven families: 16 people had spinal muscular atrophy, 15 presented ALS8, and three had the classic form of ALS. Hundreds of carriers of the mutation were later identified in Brazil and other countries.

The three illnesses are similar and, in certain aspects, hard to tell apart. The similarity might be attributed to the discovery that the VAP-B gene mutation may be the cause of the anomalies. In general terms, all three are categorized under the broad umbrella of motor neuron diseases. The diseases produce lesions that compromise the specialized brain and/or spinal cord cells that convey electrical impulses to the muscles. These muscles contract or relax based on the commands transmitted by the upper (brain) and lower (spinal cord) motor neurons. People with ALS8 usually show the first symptoms while in their forties, with life expectancy ranging from five to 25 years after diagnosis. Agnes Nishimura, the researcher chiefly responsible for the discovery and description of the new type of ALS, formerly a researcher at the USP human genome research center and now at King's College in London, won the Paulo Gontijo Award for Medicine in 2007 for her work on the disease.

Ringo and Sulair:  
both dogs have a  
mutation that causes  
muscular dystrophy,  
but surprisingly don't  
manifest the disease.  
USP researchers are  
attempting to find  
genes that prevent  
dystrophy from  
manifesting in zebrafish



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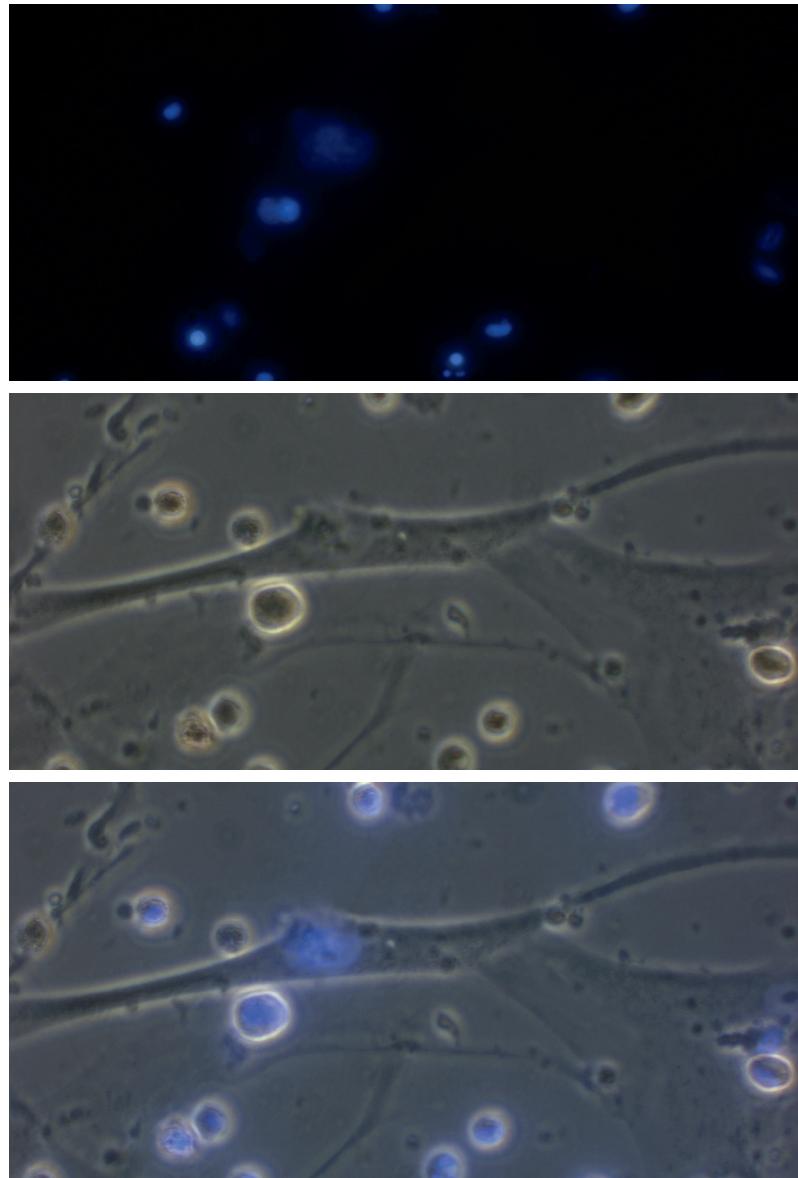
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**F**or Professor Zatz and her team, the research on VAP-B and the new form of ALS is ongoing – and interesting results continue to emerge. Last year, in collaboration with Brazilian and international colleagues from research centers in the U.S., the CEGH researchers at USP uncovered a clue to the mechanism that seems to be involved in the destruction of motor neurons in people afflicted with the disease. The scientists successfully generated motor neurons from ALS8 patients and were able to observe that VAP-B protein levels were lower in those cells. “It was the first time that this had been done for this hereditary form of amyotrophic lateral sclerosis,” says Zatz, who published the experiment’s results in *Human Molecular Genetics* in June last year. The motor neurons were grown *in vitro* from induced pluripotent stem cells (iPSC), which in turn had been generated from fibroblasts, a type of skin cell, taken from patients who have the disease. The neurons were then compared to those of non-afflicted individuals from the same family.

#### SPOAN AND GOLDEN RETRIEVERS

In 2005, in an increasingly difficult feat when it comes to genetics research, a group of researchers from CEGH and the USP Hospital das Clínicas discovered a previously unknown neurodegenerative disease called Spoan (short for spastic paraparesis, optic atrophy and neuropathy) in Serrinha dos Pintos, a small town in the state of Rio Grande do Norte where consanguineous marriages are common. As soon as they were certain that no report of the clinical condition yet existed in the scientific literature, the USP researchers initiated an effort to provide the 4,300 inhabitants of the little town with some notion of basic genetics, as well as information about the disease – which manifests in the offspring of marriages between cousins who carry the genetic mutation. The pathology, then unknown to science, was described in the American journal *Annals of Neurology*. “We examined patients of various ages who had the syndrome, from age 10 to 63,” biologist Silvana Santos, the researcher chiefly responsible for discovering the unknown disease, said at the time during an interview for

Umbilical cord stem cells (in blue) merge with cells of a muscular dystrophy patient (in gray). The merge (last image) is necessary in order for muscle cells to be formed



*Pesquisa FAPESP*. “We were able to observe the evolution of the disease. Over time, these people close in on themselves like a flower.”

The disease is incurable, but not fatal, and has no effect on patients’ cognitive abilities. It does not cause mental retardation, pain, or deafness. But its effects on the quality of life of patients, who become physically disabled, are devastating – especially in rural, healthcare-deficient populations such as those in Serrinha dos Pintos. Before the researchers came along, the local inhabitants believed that the disease was caused by a hereditary syphilis, said to have spread through the family’s bloodline by a womanizing ancestor. The feet turn outward, the head droops, and the patient ends up in a wheelchair.

The gene where the disease-causing mutation occurs has not yet been identified. But scientists have analyzed DNA samples from dozens of families who live in the city, both sick and healthy, and

the results suggest that the Spoan gene might be somewhere in chromosome 11. The problem is that, seven years after the new disease was discovered, the research efforts have yet to determine the exact location of the gene responsible for the disease.

For news of a more encouraging nature, let's look at a recent study involving a pair of golden retrievers: nine year old Ringo and his son Suflair, age 6. The doggy duo appears to carry protective genes or mechanisms that totally or partially neutralize the detrimental effects of the mutation that causes muscular dystrophy. Both animals carry a genetic mutation associated with the disease, which prevents them from producing dystrophin, a protein essential to muscle integrity. Surprisingly, however, neither of them presents the classic signs of dystrophy, such as difficulty walking and swallowing; if they had developed the disease,



In the wheelchair, a Spoan syndrome patient: new neurodegenerative disease was discovered in Serrinha dos Pintos by researchers at CEGH-USP

they would likely already be dead. Ringo was recently diagnosed with prostate cancer, unrelated to his lack of dystrophin and quite usual in a dog his age. Suflair is also not dystrophic. He does drag his hind paws a little, but that's it. His brothers were not as lucky: they all died a few days after birth or developed severe muscular dystrophy.

In an experiment carried out in collaboration with the laboratory of Sergio Verjovski-Almeida, from the USP Chemistry Institute, the researchers saw that asymptomatic dogs expressed (activated) some genes less intensely than did sick dogs. "Our hypothesis is that the reduced expression of these genes may be offering these dogs some kind of protection. This might be important in finding a way of fighting the disease," says Mayana Zatz. "We are breaking a paradigm and showing that the protein's absence does not always lead to dystrophy. The thing now is to discover what the protective genes are doing." The zebrafish, commonly used as a biological model, is also helping in that task.

In the 2000s, alongside her more traditional genetic studies, Zatz assigned a part of the CEGH's efforts to stem cell research. "We are a Cepid, so we had the administrative and financial flexibility to enter that field very quickly," reveals the geneticist, who has also been taking active part in the recent battles for the regulation of stem cell research in Brazil. It didn't take long for the first results to emerge. In a paper published in 2009, researchers from the CEGH and from Sergio Verjovski-Almeida's team at IQ-USP uncovered evidence that the stem cells in the blood of the umbilical cord and the ones in its walls – tissues that can be stored away for future therapeutic use if needed – have different genetic profiles. In 2008, the team had already shown that cord wall tissue is much richer than cord blood in mesenchymal stem cells, a special type of cell that has the potential to differentiate into a wide range of tissues. The particular properties of each type of stem cell can affect their medical use, should it be proven that the genetic differences entail reduced versatility.

In April last year, biologists from the CEGH, in a project coordinated by Oswaldo Keith Okamoto jointly with neuroscientists at the Federal University of São Paulo (Unifesp), published a paper in *Stem Cell Reviews and Reports* showing that the presence of human fibroblasts in rats with induced Parkinson's disease cancels out the potential positive effects of implanted mesenchymal stem cells obtained from the umbilical cords of newborns.

## PROJECT 80+

"When we administered the stem cells exclusively, the rats showed improvement in the symptoms of the disease," says Zatz. "But when we also injected the fibroblasts, the beneficial effects disappeared and the symptoms actually became worse. It's possible that many of the poor results observed in scientific work involving cell therapy are due to this type of contamination." Fibroblasts are a type of skin cell that have strong similarities to some types of stem cell, but with completely different properties. They are frequently used as sources of induced pluripotent stem cells (iPSC), whose properties resemble those of embryonic stem cells.

In addition to representing an advance in basic knowledge about the potential benefits of cell therapy in such a complex and delicate organ as the brain, the results of the work served as a warning for relatives of Parkinson's patients. No country in the world offers officially approved stem cell treatments to fight this or any other neurodegenerative disease. "You have to carefully examine the stem cell research and not make false promises of a cure," stated a co-author of the paper, neuroscientist Esper Cavalheiro from Unifesp, who heads the research at the National Institute for Translational Neuroscience, a project conducted jointly by FAPESP and the Ministry of Science, Technology and Innovation (MCTI). "Before we propose therapies, we need to fully understand the mechanism of stem cell differentiation into the various tissues in the body, and understand how the brain 'talks' to and directs the activities of these cells." To date, the only diseases with approved stem cell treatments are blood diseases, particularly cancers (leukemias). In the battle against these diseases, physicians have been resorting for decades to bone marrow transplants, since marrow is rich in hematopoietic stem cells, i.e., the precursors of blood cells.

Parkinson's is actually one of the diseases whose study might benefit from the most recent CEGH project. The initiative is called Project 80+, and was launched last year with the goal of sequencing the complete genomes of 1,000 healthy individuals ages 80 and older, in a quest to discover genes or other factors that enable people to enjoy a good quality of life as they age.

The CEGH is building a database of octogenarian genomes. DNA samples have already been collected from 400 healthy individuals ages 80 and up and will be compared with the genetic material of another thousand people, both healthy and with medical conditions, but starting with people over age 60. With this approach, Professor Zatz is hoping to identify gene mutations that might help doctors predict the future physical condition of their patients.

Should it be proven that many or all of the healthy octogenarians have a certain genetic mutation whose long term effects are proven to be innocuous or negligible, there is no reason for a sexagenarian or younger healthy individual who also presents the same mutation to be alarmed. It will probably be no more harmful to that individual than it was to the 80 year olds. "Genome sequencing technology is getting progressively cheaper," says Zatz, whose partners in the initiative are Maria Lucia Lebrão and Yeda Duarte from the School of Public Health at USP, both of them specialists in the aging process. "With costs declining, we will be able to analyze a large number of samples." Project 80+ will also study the brain activity of individuals ages 60+ through magnetic resonance imaging exams, in a partnership with the Albert Einstein Israelite Institute for Education and Research. ■

## The CEGH is mapping octogenarian genomes in a quest for genes that favor healthy aging

## PROJECT

Human Genome Research Center - No. 1998/14254-2 (2000-2012)

### GRANT MECHANISM

Research, Innovation and Dissemination Center Program (Cepid)

### COORDINATOR

Mayana Zatz – IB-USP

### INVESTMENT

R\$ 34,412,866.53

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## FROM OUR ARCHIVES

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