Dancing genes

Incor teams reduce from 2,000 to 80 the number of DNA fragments capable of explaining the origin of hypertension

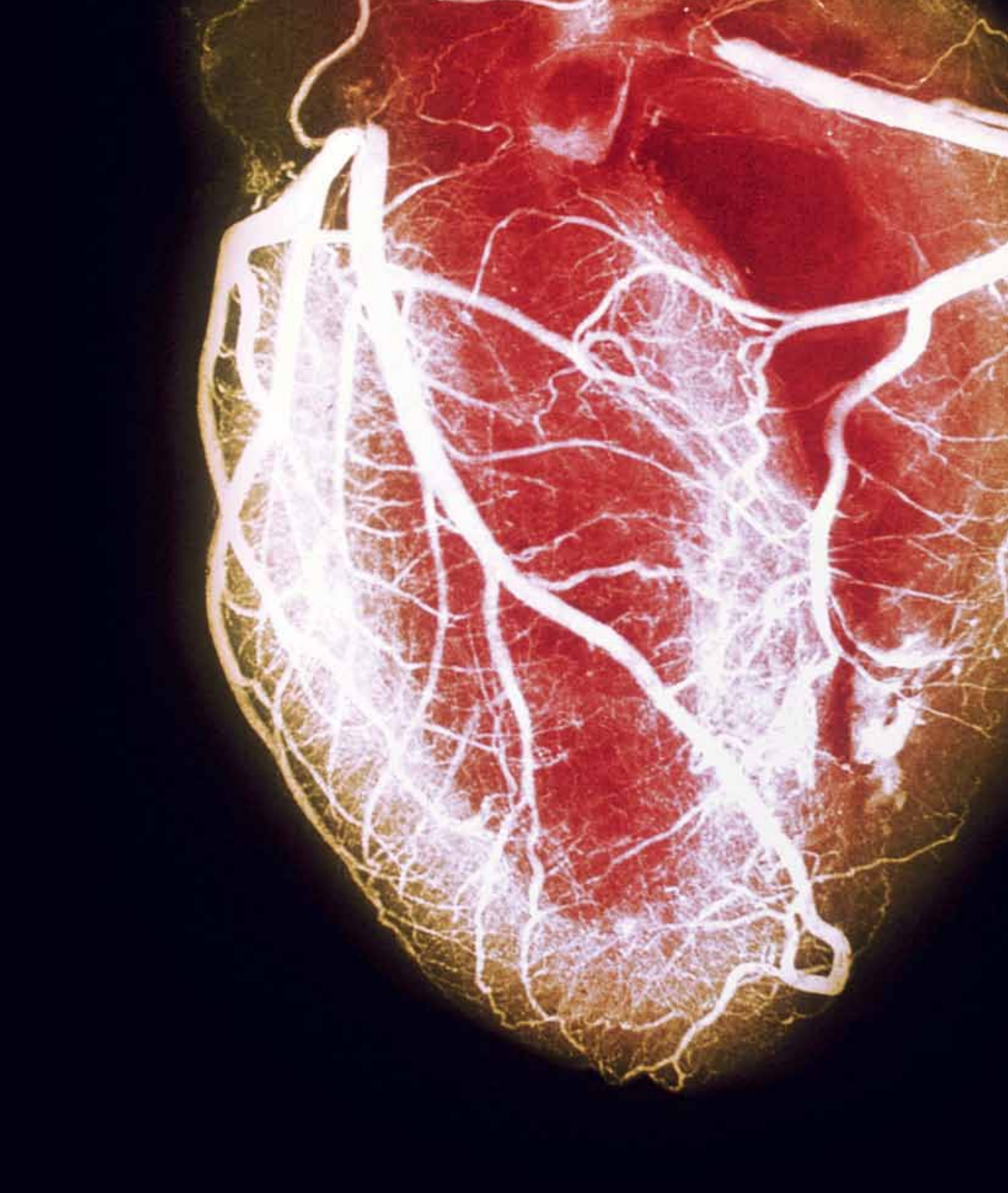
Mariluce Moura and Ricardo Zorzetto

In a back-and-forth interview granted in May for the June 2012 edition of *Pesquisa FAPESP*, Prof. Eduardo Moacyr Krieger, who in 1985 established the Hypertension Unit of the Heart Institute (Incor) of the University of São Paulo School of Medicine (FM-USP), home of one of the most important research groups on hypertension in Brazil, with a respected international following, reminded us that the blood pressure regulatory system is closely linked to our genes. "We receive our blood pressure control mechanisms as a genetic blueprint; they synthesize the pressor and depressor mechanisms. This blueprint can facilitate the production of pressor substances or form fewer hypotensor substances. This is where it begins: from the start, we either are predisposed to having hypertension or not," he said.

However, this predisposition alone is not sufficient. For the problem to appear, there must be a decisive participation by the environment, "which is constantly leading to regulation of blood pressure." And this environment, Krieger explained, "is salt consumption, inactivity, obesity, stress--all those things that one way or another affect the regulatory system." Someone with a very good regulation system could be subjected to all the environmental pressures and continue to have normal pressure. "But someone else, with a very poor system, could go without eating any salt at all, lie in a hammock, and still be hypertensive." This is the "current state-of-the-art" about essential or primary hypertension. This "combination of the lay of the land and the environment" is known, and we do not know how to "change the lay of the land" preventively, but it's what we would like to do. If the genes involved in hypertension are identified, if it becomes possible to know everyone's genetic blueprint, and based on this, to work with genetic counseling, then perhaps the essential hypertension that affects 50% of the adults over the age of 60 could become an epidemic of the past.

Many years earlier, in 1999, another Krieger, Prof. José Eduardo Krieger, director of the Genetics and Molecular Cardiology Laboratory of Incor, team member and son of Prof. Eduardo Moacyr Krieger, told *Pesquisa FAPESP* (issue 47, October): "We are not under the illusion that we will be able to explain hypertension as a single genetic defect." Prof. José Eduardo Krieger is the coordinator of the entire genetic part of the thematic project *Physiological bases of hypertension: an integrated study*, which has been supported by FAPESP since 1995. At that time, he noted that in no complex biological problem, or to be more precise, in none of the complex diseases that normally correspond to the major lines of public health programs, such as diabetes, cancer, asthma, atherosclerosis, epilepsy and schizophrenia, is there a single gene that is responsible for the disease. "What explains these diseases are defects in several genes, which, under the influence of

SCIENCE PHOTO LIBRARY



Angiography shows in fine detail the arteries that irrigate the heart

Physiology and genetics of hypertension

NATURAL VASOACTIVE SUBSTANCES Maurício Rocha e Silva, Eduardo Braun-Menéndez and Irvine Page identified, respectively: bradykinin, angiotensin and renin

PHYSIOLOGY OF HYPERTENSION

1940/
1950

MOLECULAR BIOLOGY

DOUBLE HELIX Francis Crick and James Watson defined the three dimensional structure of DNA

different environmental factors, determine the manifestations of the disease

There have been almost 13 years of systematic studies and more than 200 scientific articles have been published in the most important journals in the field, describing advances in theoretical understanding of the physiology and genetics of cardiovascular diseases, presenting new data for a cardiovascular epidemiological profile of Brazilians or exploring the results of producing more and more refined lineages of hypertensive rats. Nevertheless, neither the Incor group, nor any other group in the world, has been able to answer the central and implacable question that has remained a challenge in the field of cardiovascular disease: what are the genetic determinants of primary hypertension, this disease that affects 22% of the adult Brazilian population and a total of 970 million people around the world?"

"We have already reduced the number of chromosome regions in which we experimentally sought to find the genes that could play a clear role in the emergence of hypertension from five to four, after the development of 12 generations of hypertensive rats; in other words, of animal models for investigation, in our laboratory," says José Eduardo Krieger, full professor of molecular medicine at USP. And using all the sophisticated arsenal of tools that molecular biology offers today to search for these genes, crossing them with cardiac and vascular phenotype studies that direct data from Incor patients and other epidemiological studies in different population groups provide them, Krieger's team continues to search for "the address," as he calls it; that is, the address of the cause of hypertension.

Today, it is supposed that the emergence of primary hypertension is the result of several minor defects in multiple genes linked to the organism's means of homeostatic regulation (which maintains a balance of temperature, blood pressure, blood glucose level, etc.). At the same time, there is a consensus that in 90% of the cases, this contemporary epidemic results from a close interaction between environmental and genetic factors. However, in the remaining 10% of the cases, where the most resistant forms of hypertension, as well as those produced by rare diseases are found, there are cases that are not in any way related to environmental factors, and in which either multiple genes or a single defect on a single gene can determine the pathological condition of arterial pressure.

According to Krieger, it is surprising and somewhat frustrating that the ceaseless search since 1990s for the genetic components of hypertension has led to more definitive findings precisely in these situations of rare diseases rather than in the more general and common manifestations of the problem. "There are studies in these cases of a single defect described in all its detail." For example, over the past fifteen years, Richard Lifton of the Genetics Department at Yale University and his colleagues described a series of genetic alterations that lead to hypertension that is dependent on unwanted retention of salt and water in the organism.

TROPICAL ANTI-HYPERTENSIVE
While studying inflammation, Sergio Henrique Ferreira identifies the bradykinin potentiating factor (BPF) in jararaca (pit viper) venom

CONTROL PROGRESSES
Squibb develops and begins testing of the first pharmaceuticals designed to inhibit the angiotensin converting enzyme (ACE)

1960

1970

GENETIC SCISSORS AND GLUE
Identification and isolation of restriction enzymes and ligases that make it possible to cut and paste DNA, opening the way to genetic engineering

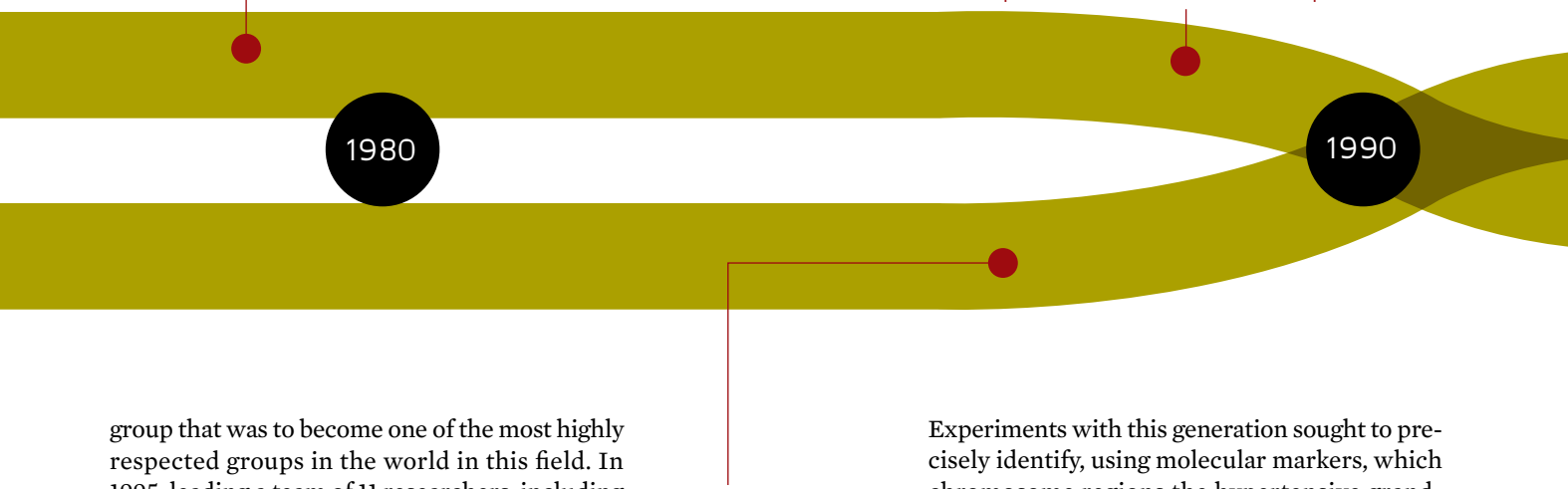
Among those cases unrelated to primary hypertension are those with well-identified renal causes. "When the cause of hypertension is renal artery stenosis, for example, it is curable. The defect is easily fixed using a stent, similar to the one used in cases of narrowing of the coronary artery." But according to Krieger, a *sine qua non* condition for the hypertension to be controllable is for the kidney to be preserved. A follower in theoretical terms of the famous American physiologist Arthur Guyton (1919-2003), Krieger argues that the nervous system and stress, for example, will play a permanent role in continuation of hypertension only if the renal system has been altered. "A preserved kidney permits infinite gains in the battle to control blood pressure," he says. This viewpoint was not always settled and accepted among the members of the Incor group, and the head of the Genetics and Molecular Cardiology Laboratory says pragmatically that "conflicting theoretical viewpoints are actually very healthy, because they increase the possibilities for moving forward."

Even though the genes from remote cellular regions that supposedly help to push the diastolic reading above 90 and the systolic reading above 140 millimeters have not been served up on a silver platter, so to say, the trove of scientific work done by the Kriegers' group on them has shown continual and consistent advances since it began at Incor. This includes the many theoretical and experimental studies surrounding the mechanisms of blood pressure control, and later also in the area of hypertension genetics, but also includes an investigation into the remodeling that veins undergo when they are used to replace arteries blocked by fatty plaques, as is the case of a coronary bypass, and the line of research with stem cells for cardiac muscle recovery.

Using veins, which are vessels specialized in transporting blood under low flow and pressure conditions, instead of arteries, which transport blood under pressures up to 20 times higher, limits the durability of some saphenous grafts to about a decade. A blockage, if only partial, occurs in the implanted segments of this vein removed from the leg to re-establish the heart's blood supply. In a series of experiments with rats and human blood vessels, Krieger's group noted that the blood pressure along the vessel walls alters the programming of the cells of veins subjected to arterial functioning conditions. As a result, the vein walls become excessively thick a few years after the heart revascularization surgery. This investigation has already resulted in the identification of several proteins involved in the thickening of the implants, two of which have been described in full by the Incor team: the protein produced by gene p21, which inhibits cellular reproduction and is generally less active in these conditions, and the protein produced by gene CRP3, which is usually active only in the arteries, but is also produced by veins used in arterial functions, as Krieger reported to *Pesquisa FAPESP* in June 2009 (issue 160).

Prof. Eduardo Moacyr took over as head of the Incor Hypertension Unit after 28 years of a respected career as professor and researcher of the USP School of Medicine at Ribeirão Preto. There, his principal line of research had been to study blood pressure regulation mechanisms, principally neurogenic mechanisms, in experimental hypertension models (pioneering the use of rats). Therefore, when he arrived at Incor, he brought with him considerable expertise, and was able to begin forming a multidisciplinary research

INFOGRÁFICO TIAGO CIRILLO



group that was to become one of the most highly respected groups in the world in this field. In 1995, leading a team of 11 researchers, including molecular biologists, physiologists and general practitioners, he submitted to FAPESP the thematic study *Physiological bases of hypertension,* which was linked to an international effort to establish the genetic bases of blood pressure. In some specific studies, researchers from the Medical College of Wisconsin and the Universities of Harvard and North Carolina have collaborated with the Brazilian research.

In 1999, a report published in *Pesquisa FAPESP* (issue 47, October) stated that "some very concrete results" had been achieved. The first, published in 1995 in an article in *Genome Research*, was "the identification of five chromosome regions in lab animals (rats) that explained a large part of the increase in blood pressure in these test animals." There seemed to be genes directly involved with hypertension in these regions, and multiple experiments would be used to try to identify them. In the rats, two of the regions were located on chromosome 2 and the others were located on chromosomes 4, 8 (which later studies refuted) and 16. Then there was a strong inclination to look for the corresponding human chromosome regions.

The five, and later, four chromosome regions were found during continuous crossings and phenotypic characterizations of laboratory animals, as well as efforts to describe the molecular markers used to search for the genes. According to the 1999 report, "the purpose of crossing hypertensive and normotensive (with normal blood pressure) animals was to obtain grandchildren; that is, a second generation of animals whose genetic blueprint would feature a random distribution of the genes of each of the two types." In all, the group obtained 12 generations of rats and multiple sub-lineages of congenic animals. Experiments with this generation sought to precisely identify, using molecular markers, which chromosome regions the hypertensive grandchildren had inherited from their hypertensive grandparents.

The search for genes linked to hypertension is not a rather deterministic adventure, as it may seem. Finding them increases the chances of a precise description of the different blood pressure control systems, such as the renin-angiotensin system, described back in 1949, and increases the possibility of understanding the basis for the differences in behavior of the control systems in equally hypertensive people. Finally, it would make it possible to establish prevention methods and therapies that are more appropriate for each patient.

In spite of the fact that the work by the Incor group is closing in on the genes that could explain hypertension, the universe of genes to be eliminated is still quite large, since each of the four regions identified contains some 500 genes. Since he arrived at these regions, Krieger has managed to restrict the number of genes of interest from almost 2,000 to around 80. And work is still underway with a variety of exploratory tools (DNA chips, transcriptome, micro RNA and others) to select candidate rat genes, alongside genotype and phenotype studies made possible by observation and examination of Incor patients and material from major epidemiological studies such as the cross-sectional study of the population of Baipendi, in Minas Gerais, conducted on 1,700 people (14% of the adult population) from 100 families. "If we find one or two candidate genes in each of the four animal models, and working with comparative genomic techniques, we test the human genome to see whether there is an alteration in the same genes, or at least in the biochemical means by which these genes are implicated," Krieger says, "we will have taken a big step forward." ■

INFOGRAPHICS TIAGO CIRILLO **SOURCE** TIMELINE AND INFOGRAPHICS, JOSÉ EDUARDO KRIEGER / INCOR-USP

NEW STATUS FOR BIOLOGY
The start of human genome sequencing brings biology into the field of big science

THE HUMAN GENOME
The first drafts appear of the sequencing of the human genome and genomes of other species

THE HYPERTENSION GENES
From a possible pool of 2,000 genes, 80 are identified as possibly being involved in control of blood pressure

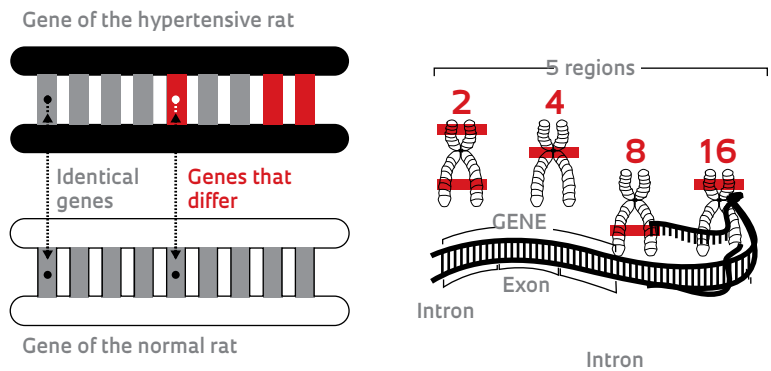
2000

2010

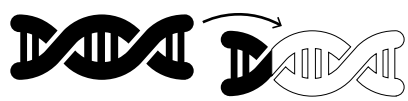
MAPPING OF SYNDROMES
Several chromosome regions associated with the genesis of complex diseases are identified in lab animals

Genetic prospecting

Researchers combine genetics and molecular biology in search of genes that may be involved in high blood pressure



IN SEARCH OF AN IDEAL LINEAGE Breeding rats with normal blood pressure with others that have high blood pressure for successive generations made it possible to identify five chromosome regions linked to high blood pressure



400 to 500 candidate genes in each chromosome region

MIXTURE OF GENOMES Animals from carrier lineages in each region were again crossed with hypertensive rats, which made it possible to reduce the number of suspect genes

20 candidate genes for each animal

CLOSING IN using molecular techniques, researchers seek to identify evidence in renal tissue of gene participation in arterial hypertension

PROJECTS

Integrated study of arterial hypertension: molecular and functional characterization of the cardiovascular system – No. 2001/00009-0 (2001-2006)
From the bench to the clinic: development of biomarkers as predictors of the response to therapy and injury of target organs in systemic arterial hypertension – No. 2007/58942-0 (2009-2012)
Genetic mapping of cardiovascular risk factors in the Brazilian population – Hearts of Baipendi project – No. 2007/58150-7 (2008-2012)

GRANT MECHANISMS
1. and 2. Thematic Project
3. Regular research assistance line

COORDINATORS
1. and 2. Eduardo Moacyr Krieger – Incor/FMUSP
3. José Eduardo Krieger – Incor/FMUSP

INVESTMENT
R$6,110,874.19
R$3,306,336.56
R$1,832,181.66

SCIENTIFIC ARTICLES

Storck, N. J. *et al.* A biometrical genome search in rats reveals the multigenic basis of blood pressure variation. **Genome Research**. 1995.

CAMPOS, L.C. *et al.* Induction of CRP3/MLP expression during vein arterialization is dependent on stretch rather than shear stress. **Cardiovascular Research**. 2009.

FROM OUR ARCHIVES

Closing in on the silent killer Issue No. 47 – October 1999
Molecular Alarm Issue No. 69 – October 2001
Reconnected heart Issue No. 160 – June 2009
Unexpected Effect Issue No. 171 – May 2010