TOPIC

Novel Genetic Approaches to the Resolution of Complex Diseases

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SUMMARY

Genetics has been, up to very recent times, the science of monogenic disorders based on Mendelian Laws. Completion of the human genome sequencing as well as that of other species, including mainly the mouse and rat, are now providing the opportunity to resolve complex diseases, i.e., those based on several genes and significantly modified by the environment. This complex paradigm, however, requires novel strategies. We propose an integrated use of experimental genetic animal models, with that of relatively isolated human populations with large families and accessible genealogical data. Forseeably, genetics will be part of this novel medicine in which the prediction of diseases will be based on the individual genomic make-up.
Key words: genetics of cardiovascular disease, complex diseases, experimental genetics, human genetics.

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After the establishment of "Mendel's law" in 1866 in a monastery in Brno, now the Czech Republic, genetic research has progressed from Archibald Garrod's *Inborn Factors in Disease* introduced in 1909 (1) via the "one-gene one-disease" understanding of monogenetic disorders in the 60s and 70s to more complex approaches in the resolution of common human diseases in this century. These accomplishments are brilliantly summarised in *"DNA, the Secret of Life*" by James D. Watson (2), published on the occasion of the 50th anniversary of the discovery of the double helix of DNA and dedicated to Francis Crick in 2003.

Common diseases are usually of polygenic, heterogeneous character, i.e. caused by different types of disease-susceptibility genes and impacted by interactions with the environment (3). Major progress was made in February 2001 when a public consortium, the Human Genome Organisation (HUGO) and a private company, Celera Genomics, published the first draft of the sequence of the human genome and its 30,000 genes. The lessons in humility learned from this initial description published both in *Science* and *Nature* is that 45% of the human genome is composed of transposons, repetitive elements that are propagated by creating their own copies anywhere in the genome and the roles of which are only slowly being discovered. Two hundred genes of bacterial origin are telling us that we should be extremely cautious in dreaming about xenotransplantation, which may have the potential to awaken the dormant foreign genes of donor species put in the human environment. We have 300 genes more than the mouse and only 1.5-fold more than the worm (*nematode*) or only twice as much as the fly (*drosophila*). We are indeed part of the animal kingdom.

Fortunately, there are some human particularities, especially in our immune system, in neurotransmitters, transcription elements and genes responsible for cell apoptosis. Even at the time of writing these seminal papers, reports were already making news of so-called paralog genes, genes with similar structures identified in *silico*, i.e. on screen, just from their sequences. Very quickly, these novel genes, including 5-HT3B, CYST2 and BACE2, were explored further and have started to be novel therapeutic targets.

GENES AND ENVIRONMENT

Genes and environment interactions are a major unresolved pathophysiological component as most diseases affecting a large proportion of today's populations, including hypertension, dyslipidemia, cancer and atherosclerosis, are not only based on our genomic make-up but are also largely influenced by the environment. Sometimes, it is seen only as additive components, as the noise of one or another, depending on the point of view, either epidemiological or genomic, of the researcher. Some researchers such as David Moore propose that both components always co-exist. As described in his book, "*The Dependent Gene*", he calls the debate "the fallacy of nature versus nurture". Others, e.g. Matt Ridley (4), clearly understand the differential roles of genes and environment when he states that "genes are designed to take their cues from nurture".

We propose the simplified scheme presented in Figure 1 where genes and environment act in a complementary fashion and to different degrees. For instance, in situations like testicular cancer in uranium miners, or when diabetes is due to pancreatic damage or even in renovascular hypertension, the genetic component is minimal while the environment predominates. At the other extreme, in Huntington's disease or cystic fibrosis, the genetic impact is close to 100%. Such is also the case in medullary thyroid cancer and when mutations of insulin or its receptor result in diabetes. In hypertension, several cases of monogenetic diseases, such as Liddle's syndrome or glucocorticoid-remediable hypertension, greatly contributed to the resolution of phenotype/genotype correlation. However, in most common diseases, the role of genes and environment is almost 50–50, yet how these half and half proportions influence each other is only slowly being unfolded.

There are several emerging areas in the genetics of hypertension and dyslipidemia where total genome scans have been performed and increasing numbers of quantitative trait loci (QTL), compatible with the polygenic nature of disease, have been identified (5, 6). The repetitive character of some of these QTL suggests the presence of underlying haplotypes. The

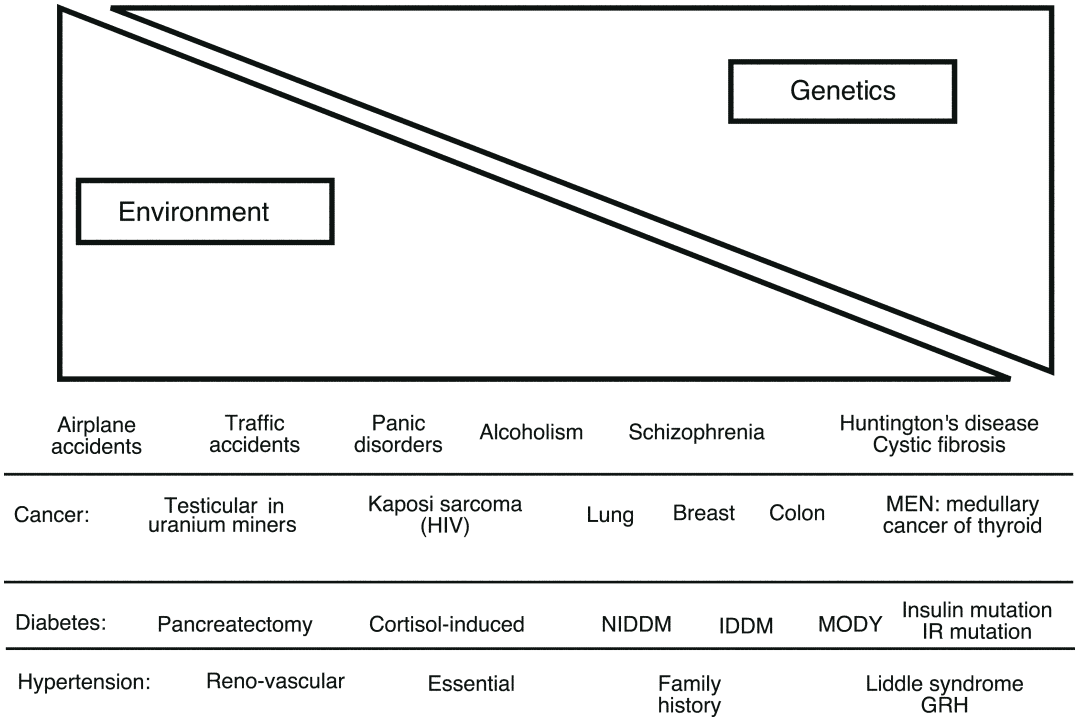

Fig. 1. Gene and environment

existence of inter-species (syntenic) regions, where genes are in the same order on chromosomes in mice, rats and humans, demonstrates the relevance of comparative genomics as a new emerging field of research. Novel trans-disciplinary approaches are clearly needed to characterize gene and environment interactions in complex traits, with the participation of epidemiologists, nutritionists, behaviourists, molecular biologists and geneticists.

Our group in Montreal considers as a main research objective the investigation of the separate and combined effects of genetic and environmental components in the development of cardiovascular disorders. Gene and environment interactions in our paradigm occur when the outcome of a given environmental exposure differs according to the individual's genotype. Figure 2 summarises our research strategy aimed at better understanding gene and environment interactions in complex traits. Naturally, all these interactions happen over time, involving different sets of genes and the influence of different environments, playing different roles in utero, in early development, in adulthood and in senescence.

We are studying various types of environmental exposures, including nutrition, drugs, toxins, etc. which can be examined in either genetically-defined populations or genetically-designed rodent models with known loci of susceptibility to diseases and their complications. We are investigating physiological processes through which the interactions of target genes and different exposures are expressed with the final goal of identifying clinically-relevant outcomes of these interactions.

EXPERIMENTAL GENETICS

In this search we are greatly aided by the availability of rodent models of disease, allowing genetic crosses generated from unaffected and affected inbred strains. Traditionally, the F2 generation is studied, and usually 200 to 300 animals are required. Each of them has to be phenotyped and genotyped; then, genomic markers are positioned throughout the genome, and basically genotype-phenotype correlations establish linkage of a percentage of phenotypic variance of a complex trait (such as blood pressure, BP) with markers on chromosomes at a particular locus. This allows the localisation of candidate genes that are then called "positional candidates". After this step, we display comparative genetic maps for rats, mice and humans to better define corresponding chromosomal loci between these species.

Recently, we started using transcriptomics, i.e. the localisation of the differential expression of the 30,000 genes on chromosomes with specific narrowing of QTL. We have tested, for instance, a novel rat model of metabolic syndrome X that exhibits the hypertensive, dyslipidemic and insulin-resistance triad (7). This experimental genetic research permitted us to establish synteny between the dyslipidemia of this strain and human combined hyperlipidemia.

A more advanced model is represented by recombinant inbred strains (RIS). This model has a particular history in Prague where it was developed at the Biology and Genetic Institute of the Charles University Faculty of Medicine and the Physiological Institute of the Czech Academy of Sciences. Collaboration between Professors V. Kren, M. Pravenec and J. Kunes has resulted in a unique set of RIS, bred from the widely-used spontaneously hypertensive rat (SHR) and a normotensive Brown Norway strain of a particular subtype. After more than 25 generations, homozygous replicas of the F2 generation have been produced. These animals have been mapped with genetic markers on all chromosomes and have been widely shared by several international collaborators of Charles University and the Czech Academy of Sciences, resulting in landmark studies.

We were fortunate at the University of Montreal to participate in this international endeavour. It allowed us to map the stress response in these strains along with the genetic determinants of kidney and heart weight in neonatal and adult rats, which were feasible only in this model (8). We were also able, for the first time, to map QTL of the differential expression of stress

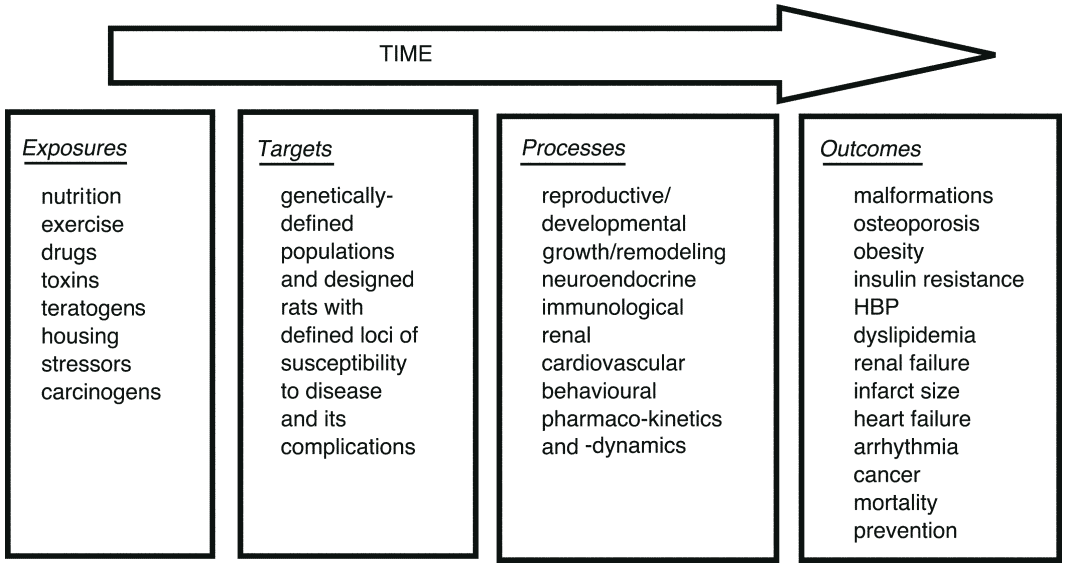

Fig. 2. Gene and environment interactions

genes after immobilisation stress (9). This was the first attempt to map messenger RNA quantitatively. Table 1 summarizes the expression of 5 stress genes in the adrenals, heart and kidneys mapped to rat chromosomes. Noticeably a common locus was found for all heat stress proteins (HSPs) in the 3 organs studied, at a specific QTL on rat chromosome 7 (at the D7Cebrp187s3 marker). We then searched for positional candidate genes at the locus. By comparative genomics, we identified a polymorphism between the hypertensive and normotensive strains in the 3' untranslated region of heat stress transcription factor (Hstf1) that affected its expression and was responsible for the differential expression of HSPs after stress. Hstf1 is thus a candidate gene for the differential expression of HSPs in hypertension and is proposed to be the cellular thermometer of stress (8, 9).

It is, therefore, possible that in diseases, such as hypertension, not just the gene sequence but rather the regulatory components responsible for the degree of gene expression or the speed of its metabolism may exert pleiotropic effects. Furthermore, we were able to identify the ecogenetic components of electrolyte excretion in these strains (10), a clinically relevant story. We have clearly demonstrated that in different RIS bearing half of the genome from each of the 2 progenitor strains, phenotypes such as urinary sodium excretion are variable but only when the environment is perturbed. This can be seen in the strain distribution pattern in Figure 3. The left side of the figure shows little difference between the strains when they are fed a normal diet and are in a steady state. However, in the first 24 hours of fasting, i.e. when there is a change from the steady state to the adaptive state, a genetic gradient appears between the strains. This allowed quantitation of the relative influence of genes and environment, clearly demonstrating an excess of environmental impact on the steady state while the genetic contribution prevailed during the stress of fasting.

HUMAN STUDIES

Naturally, all these experimental studies are aimed at better understanding the genetics of human hypertension. We have the unique opportunity to explore a population of French origin living in the Saguenay-Lac-St-Jean region in the Quebec Province of Canada (11–13). The population originated from a small number of settlers who initially migrated from France in the late 17th century and then moved from the southern part of Quebec to this region. An homogeneous population with large families up to the 40s, it is endowed with computerised genealogical records dating back to the original settlers (14, 15).

Such relatively isolated populations, present in other parts of the world, such as in Iceland and Finland, have the additional

Tab. 1. Expression of stress genes in RIS (summary of results)

Gene	adrenal	heart	kidney
hsp27	D7Cebrp187s3	D7Cebrp187s3 Myh3	D7Cebrp187s3
hsp70	D7Cebrp187s3	D7Cebrp187s3 Y Chrom	D7Cebrp187s3 Y Chrom Myh3
hsp84	D7Cebrp187s3 D9Cebr204s1	D4Mit19	D7Cebrp187s3
hsp86	D7Cebrp187s3	D7Cebrp187s3 Myh3	D7Cebrp187s3 Y Chrom Myh3
hsp105	D7Cebrp187s3	D7Debrp187s3 Myh3	D7Cebrp187s3 Y Chrom D12Cebrp97s9

(Reproduced with permission from ref. 9)

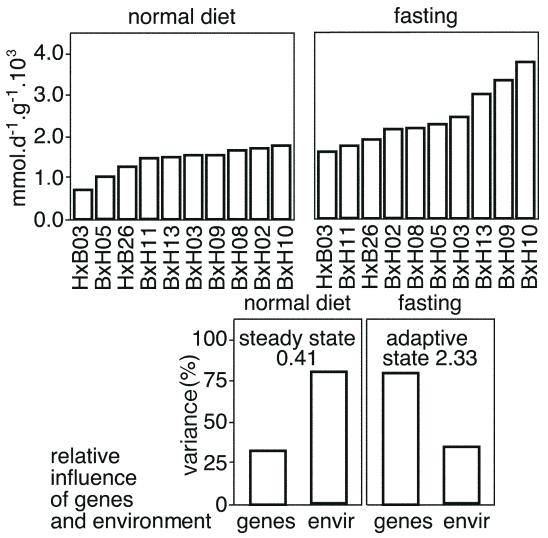

Fig. 3. Strain distribution pattern of Na excretion in RIS
(Reproduced with permission from ref. 10)

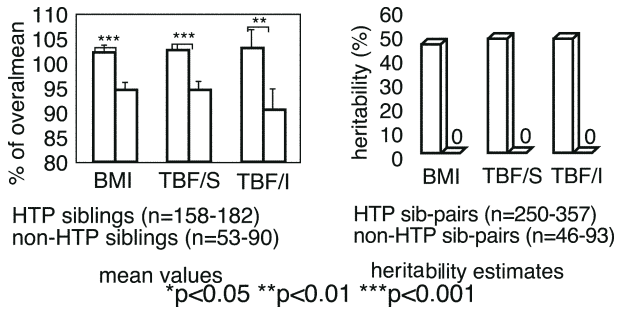

Fig. 4. Global obesity measures
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power of uncovering the genetic determinants of hypertension. In our set of 120 multiplex families, we have proceeded with an extensive collection of more than 250 phenotypes recorded under standard operating procedures for anthropometric (body mass index, impedance, skinfolds, circumferences), cardiovascular (SORBA, echography, 24-h BP-monitoring, vessel wall thickness, posterior wall thickness, elasticity), renal (urinary collection of electrolytes), and metabolic (neuroendocrine) parameters in unstimulated and stimulated (emotional stress, postural changes) states (16).

Recently, we added environmental components and epigenetic markers (C-reactive protein /CRP/, interleukins, end-glycosylated products, DNA methylation, free radicals) to our investigations. The first step usually evaluates heritability (h2) estimates, and sibling/sibling correlations were determined in families for each trait, which permitted us to establish that some traits (Figure 4) such as global measures of obesity, are higher in siblings with hypertension than in those without hypertension, suggesting that hypertension, at least in some situations, is clearly genetically linked with obesity. The degree of heritability actually is about 30% for cardiovascular functions during mathematical stress, and increases to nearly 100% for some stable phenotypes apparently little influenced by the environment, such as ApoA or ApoB.

Initial genome scans, first presented at the Congress of the International Society of Hypertension in Prague in 2002, demonstrated that numerous QTLs throughout the genome usually agglomerate in clusters, such as on chromosomes 1, 3 and others (17). What does the clustering of these QTLs mean? As the question is still unresolved, one has to make analogies to other diseases, such as irritable bowel syndrome, where a group at MIT uncovered haplotype clusters (18). Thus, in Crohn's disease and ulcerative colitis, the causes are not confined to a single gene but to a set of genes, where the pathological forms are associated with haplotypes and result in these pathological states. This brings a new understanding of complex diseases that deserves to be explored further. Progress has been made with the availability of very detailed and accurate genealogical records of our population in search of their coefficients of kinship. We have already reported that hypertensive subjects with and without obesity have a high coefficient of kinship, indicating different ancestors for those having hypertension with and without obesity (12).

We can summarise our thoughts in Figure 5. 150,000 years ago, our human ancestors originated from Africa and this common origin is now being witnessed by studying genes such as ApoE, demonstrating the position of additional mutations accumulating throughout human history (19). These ancestral genes were submitted to environmental selection which operated particularly before the end of the reproductive age. We were all submitted to the stress of infection, starvation, pests and

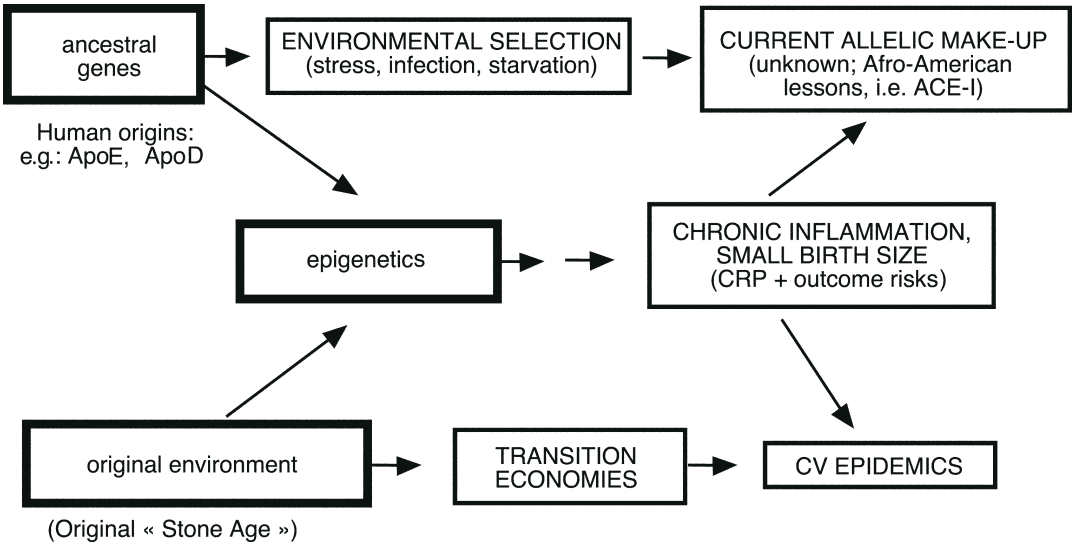

Fig. 5. Gene-by-environment of HBP

cholera from the Middle Ages and much before, but we know that we already had genes such as those of susceptibility to obesity. One has to just look at the statues of Venus of Vestonice, Venus of Lespugue, Venus of Willendorf and others, which clearly record the existence of obesity some 20,000 to 30,000 years ago in human subjects perhaps due to the beginning of sedentarism accompanying periods of caloric plentiful. The original environment, such as the Stone Age, has certainly changed very much in countries of economic transition. Epigenetic influences, including those of chronic inflammation or birth size, are now detectable by biomarkers such as plasma CRP, and glycosylated end products, etc. Together with the current allelic make-up, epigenetic factors and changes from our original to the current environment have resulted in today's cardiovascular epidemics.

Let us conclude with Philip R. Reilly in his preface of *Guiding Icarus: Merging Bioethics with Corporate Interests,* published in 2002: *"During the 21st century, we will sharply reduce our risk of disease, greatly improve our therapies, recalibrate our longevity, modify the plant and animal species that we most depend upon, and begin to enhance our capacity for intelligent action ... It is important to ponder how these manifold applications will be guided. Who will act as the gatekeepers? Who will lead the way? Who will decide which of the new products that this vast scientific army could develop is most likely to benefit the world?*" (20).

We all hope to benefit from knowledge of the genetic determinants of drug efficacy and side-effects. We also want to understand the genomic determinants of therapeutic and preventive efficacy in the environmental context, such as the impact of low-salt diets: should be administered to everybody or only to those likely to benefit from them? We need to understand the epigenetic modulation of inflammatory components in the development of cardiovascular diseases, but at the same time we have to realise the impact of this new knowledge on our patients, their relatives and descendents to reduce the danger of genetic discrimination that may arise from the unthoughtful application of genetic information as we have summarised in our reflection on the insurability of subjects during this period of the birth of predictive medicine based on individual genetic make-up (21).

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Abbreviations

BP	–	blood pressure
CRP	–	C-reactive protein
HBP	–	high blood pressure
h2	–	heritability
HSPs	–	heat stress proteins
Hstf1	–	heat stress transcription factor
HUGO	–	Human Genome Organisation
QTL	–	quantitative trait loci
RIS	–	recombinant inbred strains
SHR	–	spontaneously hypertensive rat
SORBA	–	noninvasive hemodynamic monitoring

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COMMENTARY

Comment on Pavel Hamet's "Novel Genetic Approaches to the Resolution of Complex Diseases"

Over the last couple of years molecular genetics has made significant progress in the understanding of basic life processes in general, and of the essence of human health and disease in particular. This has been aided in particular by the sequencing of the human genome and of those of a number of other organisms, including the mouse and the rat, when a plethora of experimental models of pathological states in man were developed. There is no need to emphasize that these achievements spring from the amazing technological development of methods in molecular biology and genetics, hand-in-hand with IT data-processing methods, without which rapid development of sequencing analysis and comparative genomics would be unimaginable.

It would be appropriate to point out – to focus on medical issues – that fewer than 1 per cent of human diseases are of a monogenic nature, and that the majority of human health disorders result from numerous gene–gene as well as gene–environment interactions. The authors of the reviewed article, Pavel Hamet and Johanne Tremblay, and their team in Montreal have focused on the analysis of genetic and environmental components affecting the development of cardiovascular disorders. To analyze the genetics of essential hypertension in man, the Montreal team has exploited the unique opportunity of investigating Quebec's population of French origin, comprising the numerous descendants of the small number of colonists who came to Quebec from France in the end of the 17th century. The significant advantage of this population group is the size of the individual families and the existence of detailed genealogical records. The analysis of this population has yielded a number of findings on, for example, the relation between hypertension and obesity, or the discovery that QTLs agglomerate in certain chromosomes of the human genome. The results achieved by the linkage analysis of the human population thus not only provide new factual findings but also pose new questions about the meaning, or possibly the importance, of the new discoveries and molecular genetic mechanisms of these complex processes in both normal and pathological states.

Advances in comparative genomics allow the gradual processing of the findings and the targeted formulation of questions addressed to experimental models of human pathological states set up in laboratory rats or mice, whose genomes have been sequenced recently (the rat in 2003). This brings us to the experimental genetics of hypertension and the metabolic syndrome, which has been a permanent aspect of our co-operation with the Montreal group since the early 1990s. The formal embodiment of this co-operation is the existing official agreement between the University of Montreal, Charles University and the Academy of Sciences of the Czech Republic. The core of the joint project is the study of cardiovascular and metabolic phenotypes in segregating populations of model inbred rat strains and, especially, in unique models of recombinant inbred and congenic rat strains developed within the frame of the co-operation between the Institute of Biology and Medical Genetics of the First Faculty of Medicine of Charles University and General Teaching Hospital, and the Institute of Physiology of the Academy of Sciences of CR over more than twenty years. Reciprocal recombinant inbred strains derive from the spontaneously hypertensive SHR strain and the normotensive Brown Norway strain - the significant advantage of the latter is the fact that it was this particular strain which had been selected for genome sequencing.

The co-operation between our two centers involves regular exchange study visits dedicated to work on joint projects, as documented by the citations from common titles in the commented article. It is encouraging that the long-lasting joint effort of teams from the medical faculty and the Institute of Physiology of the Academy of Sciences of CR devoted to the development of RI strains and other experimental models of metabolic disorders has attracted the renowned Canadian center; last but not least, the fact that the head of the Montreal center and co-author of the article in question is a graduate of the First Faculty of Medicine reflects considerable credit on Charles University.

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