Genetic Determinants of the Stress Response in Cardiovascular Disease

Pavel Hamet and Johanne Tremblay

Originating from Hans Selye's general concept, the biological notion of the stress response implicates neuroendocrine and endocrine pathways as well as their cellular mediators as components of the general adaptation syndrome and its consequences. A highly variable degree of response to psychological and physical stresses has been noted in experimental animals and in human populations. Some stressors, such as the "cold pressor test," have been developed as tools for disease prediction. They apply to variable conditions, from hypertension to insulin resistance, but mostly in relation to cardiovascular outcomes. Other environmental factors, including the degree of salt intake, potentiate the stress response in animals and humans. Our group has undertaken to explore the genetic determinants of the stress response in inbred strains, recombinant congenic and congenic strains, as well as founder human populations. We have succeeded, initially, in linking increased body temperature, a major phenotypic response to stress, to the quantitative trait loci (QTL), one at myh3 on chromosome (Chr) 10, one at *hsp27* on Chr 12, and one on Chr Y in the rat. The expression of several stress proteins is under the dominant influence of *hstf1* at the rat Chr 7 locus (Mit3). The high prevalence of cardiovascular diseases with traits of enhanced stress responsiveness is discussed here in the context of a paradigm, such as hypertension, in the general population.

STRESS AND DISEASE

HISTORY NOTES that Hans Selye[1] was drawn to human adaptation after overhearing a conversation between assistant professors of anatomy and pathology at Charles University in Prague towards the end of the First World War. They had noticed, during autopsies, that "those boys who did not make it in the trenches had unusually large adrenals."

Experimental investigation from the 1930s, and discussions in student pubs in Prague at that time, focused on experiments in rats exposed to physical and mental challenges (eg, long-lasting starvation), where it was reported that their organs were undergoing atrophy with the notable exception of the adrenals.[2,3] These rats responded with strong adrenocorticotropic hormone (ACTH)-corticoid stimulation.

After his studies in Prague, Paris, and Rome, Professor Selye introduced the concept of stress in Montreal in the mid-1950s.[1] He described the "general adaptation syndrome" with its three successive phases: first, the alarm reaction; second, the resistance phase; and, third, the phase of exhaustion. He mainly focused on the importance of stimulation of the hypothalamo-pituitary-adrenal (HPA) axis, represented by the release of cortisol.

After 40 years of research on stress, Selye realized the principal remaining problems and misconceptions surrounding the clinical application and theoretical evaluation of the "stress concept," which he summarized in 10 points: (1) the correct definition of stress, stressors, and the general adaptation syndrome; (2) the concept of nonspecificity in biology and medicine; (3) conditioning of the stress responses by diverse endogenous (mainly genetically determined) and exogenous (environmental) factors; (4) the relationship between the general and local adaptation syndromes; (5) the difference between direct and indirect pathogens; (6) the definition of morbid lesions in whose pathogenesis stress plays a particularly prominent role—the so-called diseases of adaptation; (7) the role of genetics versus that of factors under voluntary self-control in mastering biologic stress; (8) the mode of action of hormones, drugs, and behavioural attitudes; (9) the so-called first mediator of the stress response, which carries the message that a state of stress exists from the directly affected area to the neurohormonal regulatory centers; and (10) the prophylaxis and treatment of stress-induced damage by pharmacologic and behavioral techniques.[4] Altogether, Selye published 5,968 pages of medical research findings on these topics.[2]

Such historical concepts nevertheless have to be weighed, in the context of Selye's "general adaptation syndrome," against novel evidence supporting the specificity of the stress response by the demonstration of "neurochemical signatures" of *c-fos* expression mapping selective brain regions upon exposure to different stressors, eg, immobilization, haemorrhage, cold, pain, and hypoglycemia, as recently reviewed by Pacak and Palkovits.[5]

While keeping in mind all these 10 points, which remain true even 25 years later, the present article will focus on points 3 and 7, which are related to interactions between genetic and environmental factors in a common stress-related disease, hypertension, using it as an experimental paradigm.

For a long time, a wealth of anecdotal accounts have associated stress with the development of many diseases. The psychosomatic causes of cancer, asthma, ulcers, allergies, etc are well recognized. The links between the stress of modern life and cardiovascular diseases were explored deeply in the early seventies. NHANES I (first National Health and Nutrition Examination Survey) and NHES (National Health Examination Survey) suggested an association between professional stress

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and the prevalence of infarct. An investigation of 2,409 men in 1960 to 1961 and of 2,424 men between 1971 and 1975[6] showed that lack of control at work, perceived as a psychological stress, was associated with past myocardial infarction (MI). Low decision latitude was linked to an increased prevalence of MI in men in both NHES and NHANES I.

STRESS AND HYPERTENSION

Essential hypertension is one of the most common cardiovascular "disorders of civilization." Increased blood pressure (BP) has been reported in response to psychoemotional factors, immobilization, pain, cold, heat, light, noise, and vibration (for review, see Folkow[7]). The stress response has long been considered a predictor of hypertension. Thus, in 1934, Wood et al from the Mayo Clinic tested 142 children between the ages of 7 and 17 years whom they tracked for 45 years.[8] The BP response to the cold pressor test (considered positive when systolic BP rises by 25 mm Hg and diastolic BP by 20 mm Hg) was initially used to categorize normo-reactors and hyper-reactors. At the end of follow-up, hypertension was observed in 34% of hyper-reactors in contrast to only 18% in the normo-reactor group. When subjects with a positive family history were analyzed separately, 71% of hyper-reactors became hypertensive compared with only 19% of normo-reactors. This was a pioneering study because it suggested for the first time that the stress response was a predictor of hypertension and postulated its familial aggregation. A similar predictability of hypertension by the response to the cold pressor test was also noted in a long-term investigation of medical students by Menkes et al.[9] Jan Brod and his colleagues in Prague used arithmetic tests to detect precursors of so-called labile hypertension, essentially with a family history of the disease.[10,11]

Since then, it has frequently been discerned that hypertensive or prehypertensive subjects with a positive history are characterized by increases in BP when subjected to mental or physical stress compared to subjects with matching BP but of normotensive parents.[12] Heritability (h_2) estimates have been directly determined in monozygotic and dizygotic twins. Heritability of the BP response to stress appears similar to that of basal BP, as reported by Hunt et al,[13] with h_2 estimates between 44% and 49% for systolic and diastolic BP upon challenge by mathematical tests. Furthermore, the correlations of 0.40 for systolic BP and 0.51 for diastolic BP recorded in monozygotic twins decrease to 0.18 and 0.29 in dizygotic twins.[13] Other studies have demonstrated that the response to mental arithmetic may not only be a predictor of BP levels, but also of left ventricular hypertrophy.[14] All work now points to a genetic contribution to variance of the stress response.

Hypertension, which is a multifactorial trait but where psychosocial stress seems to be of crucial importance, is characterized by a polygenic mode of inheritance.[15] But genes alone are seldom enough to cause hypertension if certain environmental factors linked to modern life are not also present, with the exception of monogenetic cases of hypertension.[16] These two components, genes of susceptibility (component 1) and environmental factors (component 2), are often accompanied by a third component of increasing importance, which is time-dependent and which has been defined by Folkow[7] as the structural cardiovascular adaptation that occurs in the heart and vessels when exposed to a prolonged, chronic stress load and contributes to the maintenance of sustained, elevated BP. Thus, perpetual mental stress has been shown to cause a significant rise in BP in "free-living" women in Italy who were followed for 30 years compared to a group of nuns living in a secluded order, with similar salt intake and food choices.[17] Thus, around menopause, BP in the control group rose while the curve remained flat in the nun's group, and the incidence of death from cardiovascular disorders was more than twice higher in the control group. The cardiovascular response to stress appears to be the product of three components: genetic susceptibility $\times$ stress load and/or perception $\times$ time.

Experimental studies in animals have shown that spontaneously hypertensive rats (SHR) and mice (SHM) are more reactive to stress than their normotensive counterparts.[18-21] Exposure to several psychogenic stressors, such as cage switch, placement in an open field, or immobilization, leads to greater changes in heart rate, BP, and body temperature in hypertensive rodents.[20,22] Selye was the first researcher to use immobilization stress, which led to the manifestation of his stress syndrome in rats.[3] Thus, immobilization stress induces activation of various stress effector systems, including changes in body temperature. The increase in body temperature with stress is part of an intermediate phenotype of the stress response in this species. Environmental stress susceptibility includes thermosensitivity,[19] which is found in various strains of hypertensive rats and mice as well as in hypertensive humans.[23] Thermosensitivity can be assessed by various means in rodents: the rate of body temperature increase can be measured noninvasively when the rodents are submitted to 44°C heat stress, or following immobilization in restriction cages for 30 minutes, or in response to intraperitoneal injection of endotoxin.[24]

GENETIC DISSECTION OF THE STRESS RESPONSE IN HYPERTENSION

In our first genetic studies, we submitted the rate of body temperature increase in response to body heating to segregation analysis in the F_2 population of crosses between normotensive and hypertensive mice. The faster rate of body temperature increase behaved as a recessive trait determined by a single major gene and constituted the thermosensitivity (*tms*) locus. We also found that in the F_2 population, this locus, which corresponded to a phenotype exceeding a 1.4°C increase per minute, was associated with an 11-mm Hg elevation of BP in homozygotes with the hypertension allele, compared to thermoresistance in heterozygotes or homozygotes with the normotension allele. BP was thus correlated with the rate of body temperature increase: $r = 0.323$, $P = .012$. These initial data suggested that the response to environmental stress in the form of heat may constitute a significant determinant of BP variance.[19] An exaggerated rise in body temperature has also been observed in hypertensive rats in response to immobilization stress.[25] The increase is evident in several genetically hypertensive rat lines, compared to various normotensive strains, including Wistar-Kyoto (WKY), Brown Norway (BN), and Sprague-Dawley (SD) rats.[26]

Only a few studies have investigated the genetic contribution

to the stress response using quantitative trait locus (QTL) analysis.[27-32] To identify the genetic determinants of the body response to immobilization stress, we applied full genome scan in a set of recombinant inbred strains (RIS) originating from reciprocal crosses of SHR and normotensive BN strains. In this permanent replica of the F_2 generation, we discerned QTLs of the stress response variance with increased body temperature after immobilization stress as a phenotype.[31] Two suggestive QTLs were revealed: one on chromosome (Chr) 10 (logarithm of the odds score [LOD] 2.2) and the other on Chr 12 (LOD 1.3). The effect of these QTLs was enhanced to a significant level by a high-sodium diet: LODs 4.0 and 3.3 for Chr 10 and 12, respectively, which point to a salt-sensitive component of the phenotype (Fig 1). We then confirmed the existence of the Chr 10 QTL by the use of congenic strains for this chromosome. Both QTL and the salt effect were demonstrated in the congenic strains. Interactions between the loci of Chr 10 and 12 were observed with rat strains bearing the SHR alleles at both loci having the highest thermal response to immobilization stress.[31] Furthermore, our studies also showed that the Y Chr of SHR origin enhanced the response to immobilization stress, as demonstrated in the RIS panel and in consomic strains for Chr Y.

These findings provided the first evidence of the genetic determination of reactivity to stress in the context of hypertension, with interaction between autosomal loci and between Y and autosomal Chr explaining nearly 50% of variance of the stress response in such strains.[31]

CELLULAR STRESS RESPONSE

In neurons, the rapid expression, soon after stress, of immediate early genes such as *c-fos*, *c-jun*, and *jun D,* was used to map central stressor-specific neuroendocrine pathways in the brain.[5] The cellular response to stress is also characterized by the expression of heat stress genes (*hsps*) under the transcriptional control of heat shock transcription factors (HSTFs). The heat stress protein (HSP) family represents a set of highly conserved proteins that are rapidly induced by various stressors such as heat, metal ions, nutrient deprivation, or other cell insults. Four major families of stress proteins can be distinguished according to the molecular mass of their members: 110, 90, 70, and 27 kd. Stress proteins are believed to be involved in the adaptative response of the cell to stress, and to protect essential cellular functions from denaturation, by acting as molecular chaperones.[33] Upon stimulation by stressors, specific HSTFs (1 and 2) bind to heat stress element (HSE), a *cis*-acting sequence in the promoter of stress-responsive genes, to turn on their expression.[34] HSTFs are considered to be sensors of the stress response.[35-37] Tolerance to a lethal temperature is acquired via stress proteins. The cells also become resistant to other stressors, a phenomenon referred to as "cross-tolerance." We have reported an increased expression of *hsp27* and *hsp70* in hypertensive mice, rats, and humans in response to several stressors.[38-40] Similar observations were made by other groups.[41] The mRNA levels of these *hsps* rose more rapidly in hypertensive strains, but were followed by a more rapid decline to baseline for both mRNA and proteins.[38,39,42] This is apparently due to the enhanced activation of HSTFs in

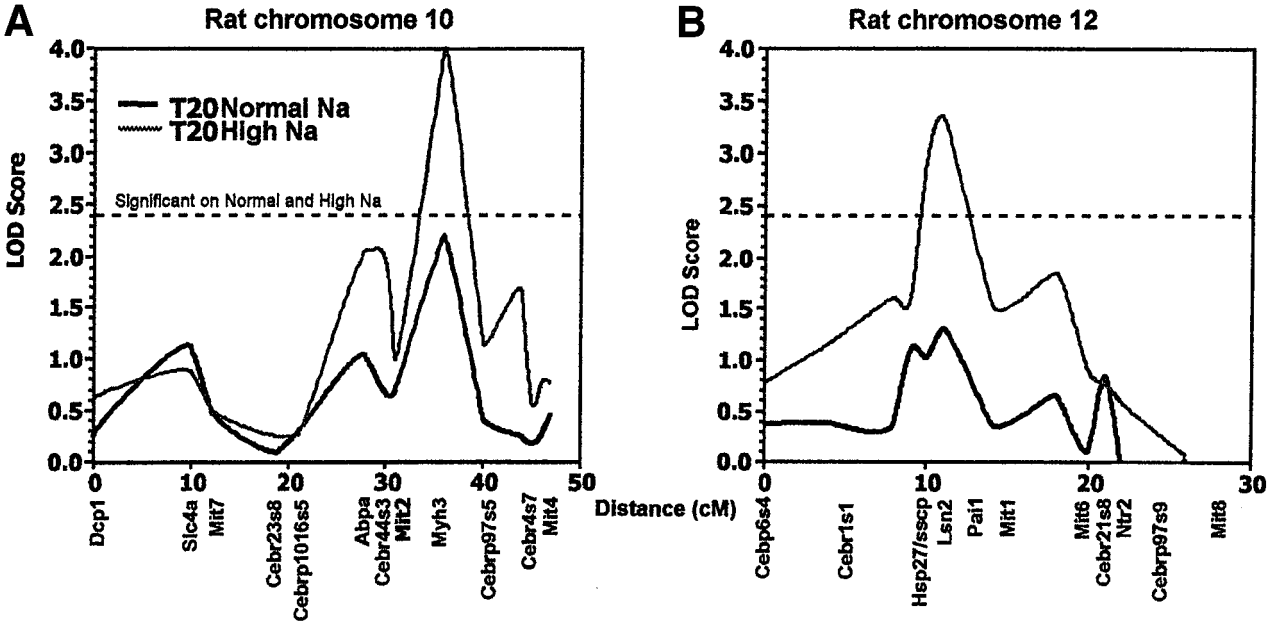

Fig 1. QTL plot for T20 measured in 21 RIS under normal and high Na intake. Dotted line indicates the significance threshold according to the permutation test. 95% confidence intervals (CI) were calculated according to the method of Darvasi and Soller.[99] Data (strains) from both conditions were combined. (A) Chr 10. Partial linkage map from Dcp1 to Mit4 from Pravenec et al.[100] Chr designation (D10) is omitted. CI = 26 cM. (B), Chr 12. Partial linkage map from Cebr6s4 to Mit8 from Pravenec et al.[101] Chr designation (D12) is omitted. 95% CI = 33 cM. (Reprinted with permission.[31] © 2000, Lippincott Williams & Wilkins.)

SHR.[39] Two loci containing *hsps* have been found to cosegregate with BP and left ventricular mass: the first, within the major rat histocompatibility complex (RT1) on rat Chr 20, contains *hsp70*, and the second, on rat Chr 12, bears *hsp27*.[43,44]

Recently, we realized that despite identical stress being applied to all RIS, *hsp* expression shows up to a 12-fold gradient within the RIS panel, with little intrastrain variability, indicative of a strong genetic contribution to the trait. When we examined the strain distribution patterns of different stress gene expressions, we identified a common locus on Chr 7 at the D7Cebrp187s3 marker, which was consistently associated with all *hsp* expression in most of the organs studied, with a LOD score of 3.0 for *hsp27* expression. Interestingly, we mapped rat *HSTF1* at the same locus, suggesting that 42% of the observed interstrain variability of *hsp* levels in response to stress could be due to *HSTF1*, which was found as a positional candidate at this locus.[32] A single base mutation was identified at the 3' untranslated region (UTR) of *hstf1*, which could be responsible for differences in the metabolic fate of its mRNA.[32]

EFFECT OF HIGH SALT

The effect of high-salt intake on BP has been studied by several groups, and an abundance of reports have been published on the subject. The actual consensus is that the hypertensive effect of nutritional sodium is difficult to demonstrate. Epidemiological studies and interventional trials suggest only a marginal effect of nutritional sodium on arterial pressure in the general population. However, it is generally accepted that there is a subpopulation of individuals who are more sensitive to salt and who will see their BP increase in response to salt loading. In our studies, we have observed, in a salt-sensitive substrain of SHR, that high sodium intake heightens the BP response to stress, particularly when accompanied by low calcium intake[25] (Fig 2). The body temperature rise in response to immobilization was also dependent on the Na/Ca ratio in the diet, being maximal on high-sodium and minimal on high-calcium diets. This was also confirmed in congenic animals, suggesting that a QTL on Chr 10 could mediate the salt effect on the stress response.[31] Thus, the impact of sodium on BP could be a result of modulation of the stress response. Hyperactivity of the sympathetic nervous system appears to be a prerequisite for the hypertensive effect of nutritional sodium.[45-47] Increased sodium in the diet augments BP and catecholamine levels in certain brain regions of stressed SHR, compared to unstressed SHR.[48] Anderson et al[49] showed that stress induces hypertension and salt retention. Their dogs received saline infusion for a period of 12 days and were submitted daily to 30 minutes of stress. Hypertension slowly established in parallel with positive sodium balance. When stress was omitted from the study, BP returned to normal, even on continuous saline infusion, and the effect of sodium was reversible in the absence of stress. Similar results were obtained in the rat.[50,51] Interactions between occupational stressors and nutritional sodium[52] were observed in humans. In an epidemiological study, 384 men with a high stress index (according to their response to a questionnaire), and who were at the 90th percentile of sodium excretion, showed higher systolic and diastolic BP increases of 6.3 and 5.9 mm Hg, respectively, compared to control subjects. In-

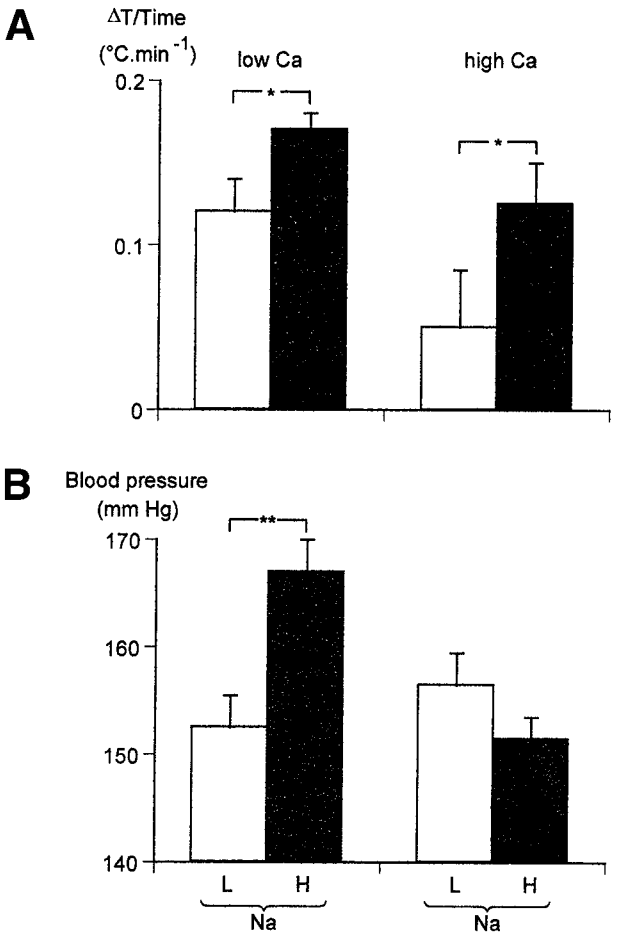

Fig 2. Effect of dietary sodium and calcium on (A) body temperature increase and (B) blood pressure increase in a salt-sensitive substrain of SHR. ANOVA *$P < .05$, **$P < .01$, n = 7 in each group. (Adapted with permission.[25] © 1994, Lippincott Williams & Wilkins.)

versely, the low-sodium diet of subjects, who also presented a high stress index, was associated with lower diastolic and systolic BP. An interventional study with standardized diets[53] performed on 27 normotensive and 21 hypertensive men submitted to a psychogenic stress (competitive video games), showed that 69% of subjects increased while 31% reduced their sodium excretion during stress, and the BP rise associated with stress was less in the sodium excretors. It does seem that modulators and nonmodulators of sodium excretion, in response to activation of the renin-angiotensin system, can be regulated by stress. Noticeably, nonmodulators have a significantly higher prevalence of hypertension than modulators.[54] It is also interesting that all aspects of sodium balance are modulated by stress. Thus, rats that have the choice between water and a 1% saline solution will increase their salt consumption under psychogenic stress.[55] Furthermore, SHR will also raise their salt consumption, compared to normotensive WKY controls, in the presence of stressors, strongly supporting a genetic difference between the strains.[55]

Table 1. Genetic Input Into the Pathophysiological Understanding of Stress

1. Distinction of secondary from primary events
2. Delineation between genetics versus environmental factors
3. Demonstration of ancestral selection pressure
4. Determination of relevance of physiological pathways
5. Discovery of novel relationships
6. Role of hormones and cytokines with respect to time and stimuli
7. Definition of fine "sub-syndromes"
8. Development of novel targets for therapy and prevention

GENETICS AS A TOOL IN BROADENING OUR COMPREHENSION OF THE IMPACT OF STRESS IN CARDIOVASCULAR DISEASES

How will the novel era of genetics and genomics assist in our understanding of the pathophysiological mechanisms of the stress response and its impact on cardiovascular diseases? Table 1 summarizes the potential contribution of genetic studies to our understanding of the pathophysiology of several diseases. Clearly, many of these features can apply to the stress response:

Distinction of Secondary From Primary Events

The establishment of allelic dependency on environmental modulation of a phenotype points to its primordiality. An example can be the above-discussed single base mutation in the 3'UTR of the *hstf1* gene in SHR, which leads to the heightened expression of several *hsps*.[32] In this review, we have concentrated our attention on polygenic models, such as RIS and congenic strains, as the stress response is fundamentally of a polygenic character, particularly in diseased states. This does not conflict with the importance of research aimed at single gene subtraction or addition (transgenesis, knock-out, knock-in and antisense technology), which teaches us about the contribution of a single gene to a specific pathway or trait. Successful targeting was achieved with alterations in components of the HPA axis, including corticotropin-releasing hormone and its receptors, arginine-vasopressin, glucocorticoid receptors, neuropeptide Y, neurokinin, 5-hydroxytryptamine transporter, α_2-adrenergic receptor, and interleukin-6 genes among others, as reviewed recently by Steckler.[56] While these studies have led to an improved understanding of the impact on HPA axis activity in the basal state or after a stress load, they are isolated "snapshots" from a "movie of the stress response." Gene expression arrays (using single nucleotide polymorphisms [SNPs]) and proteomics have the potential to incorporate a more global approach to identifying the "primary" genetic components, as initiated by QTL detection. With the draft sequences of the human genome available, and those of the rat and mouse on the way, the patterns of QTL clustering will help to define physiological profiles that can be used for gene decoding and by comparative genomics, translate the data obtained in the rat and mouse to the human.[57,58]

Delineation Between Genetics Versus Environmental Factors

The estimation of h_2 is a useful approach to evaluating the genetic contribution to variance of a phenotype. It does, however, bear some heavy assumptions concerning shared environment, life-long habits, etc. Keeping in mind these limitations, our studies in French-Canadian families indicate a sizeable genetic contribution to the stress response, particularly in pulse rate after mathematical test.[59] Thus, while there was no apparent h_2 in the resting state, sibling pairs responded in a concordant manner, with $h = 0.68$, indicating rather substantial allele-sharing of this response. Animal models are particularly well suited for delineation between "genes and the environment," as they are inbred, homozygous at all loci, and the environment can be strictly controlled. In our above-mentioned study, the response to immobilization stress was localized on autosomes 10 and 12 and on Chr Y of the rat.[31] We have discussed the heightening of this response (and increasing the LOD scores of these QTLs) by a high-sodium diet. Together, in interaction, these loci explain 46% of variance of the response to immobilization stress. Yet another stressor, the fasting state, leads to sodium retention. Na excretion has been localized on Chr 10 and that of potassium on Chr 14,[60] explaining 47% to 55% of these traits. While the remainder of variance may be contributed by some minor gene(s), it can be assumed that the major component of the "remaining half" is indeed environmentally sustained.

Nevertheless, the impact of genetic components is higher in the adaptive phase (unsteady state) of fasting, while the environmental component predominates in a steady state.[60] From our experience, it is particularly with the RIS panel that our capacity to delineate between genes and environmental components is strongest, owing to the fact that this set of strains shares only 2 genomes (BN*lx* and SHR). A polygenic trait, such as the stress response, will result in a range of responses that can then be ranked into a strain distribution pattern (SDP). The sum of variance within each strain, genetically identical, is of course the reflection of the environment. The gradient between strains is solely supported by genes. The ratio between the two provides us with a comprehension of their relative importance (Fig 3).

Demonstration of Ancestral Selection Pressure

With a few of the world's genealogically documented founder populations, for example, Finland and the Saguenay Lac St-Jean region in Québec, Canada, it is conceivable to evaluate ancestral selection pressures. Selection pressure, until only a few generations ago, was exerted for survival from infection, caloric sparing, and famine resistance, which were all potentially beneficial in times of famine, the plague, and cholera. We have devised a new method[61] of layered founder analysis, which permits demonstration of the founder effect, separating families with hypertension and obesity from those with hypertension without obesity as far back as 15 generations. Obviously, selection pressure applies only until the end of the reproductive period of human life. With doubling of our species' survival expectancy, these beneficial traits of "defense" may turn into "risk factors" at age 60 to 80 years. Does this "thrifty gene" paradigm apply also to stress susceptibility and to the capacity of "general adaptation"? What, if any, is the putative evolutionary advantage of an increased stress response in hypertensives, suspected to be present when considering its

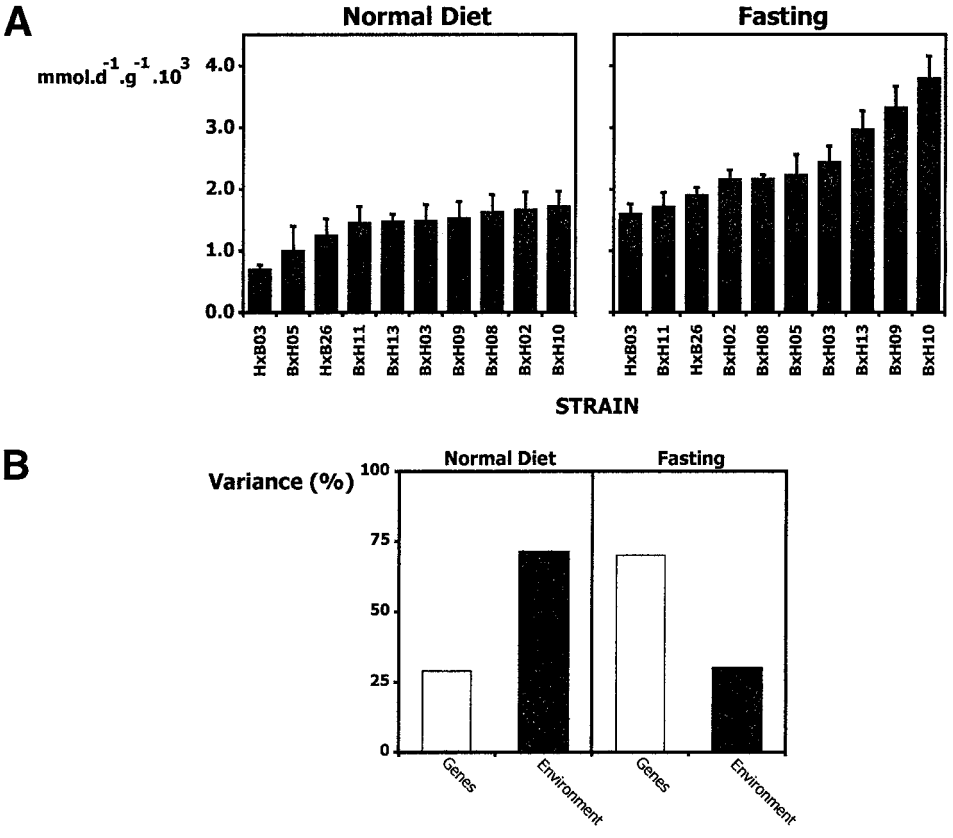

Fig 3. (A) Strain distribution pattern of Na excretion in a RIS subset of 10 strains under normal diet (left) and fasting (right) conditions. Four to 6 rats per strain were tested. Strains sorted according to increasing Na excretion. On the normal diet, Na excretion denotes Na intake, whereas under fasting, Na excretion represents Na wasting. Na wasting exhibits a strong gradient among the RIS tested, indicative of a genetic difference. (B) Variance of Na excretion in a subset of 10 RIS tested. Interstrain variance denotes genetic variance, whereas intrastrain variance reflects environmental variance. On the normal diet (steady state), most of the variance is due to the environment (Na intake). Under fasting (adaptative state), 70% of the variance in Na excretion is due to the difference in excretion between strains, which is indicative of a strong genetic component. Shown for both cases is the ratio of genetic/environmental variances under our experimental conditions. (Reprinted with permission.[60] © 2002, Lippincott Williams & Wilkins.)

prevalence in populations. Susceptibility to salt retention and caloric sparing may have obvious historical survival advantages, but what about the increase of BP in response to physical and mental challenges? While many of these responses are still to be gathered, we now have tools to resolve them: first, to identify QTLs of stress susceptibilities and then to demonstrate the putative "founder effect," which may turn into an "ancestral protective phenotype."

Determination of Relevance of Physiological Pathways

A further task is to position the genes, which respond to stress (HSPs, immediate early genes, stress kinases, cytokines such as tumor necrosis factor [TNF], etc) into relevant physiological pathways. The current task is to establish the gene expression profiles of thousands of genes and to assign them to specific cellular pathways. The technology is now available for such a task, with the recent development of expression chips, including gene and expressed sequence tag (EST) arrays.[62] Large numbers of EST repertoires have been generated for cardiovascular diseases[62] and we also have a greater appreciation of variance within pathways in the basal state.[63,64] Thus, the Seattle "Project Normal" teaches us that stress-induced and hormone-regulated genes are among the few highly variable pathways of gene expression. These include, for instance, HSP*40* in the testes, MKP-1, Gadd 45, and cytochrome cyp4a12 in the liver and kidneys. Several of these highly variable genes are under the control of growth hormone and can be modulated by caloric intake.[64]

In the near future, these stress response pathways have to be investigated for their allelic dependency and phenotype amplification, both in rodent models and in humans.

Discovery of Novel Relationships

Understanding of the gene profiling of stress-related pathways holds the potential for uncovering novel relationships. Gene × gene interactions are now being investigated. As an example, we may cite the interaction of angiotensin-converting enzyme (ACE) and guanylyl cyclase-A (GC-A) alleles on the development of heart hypertrophy in rat models of hypertension[65,66] or our description of interactions between Chr 10 and 12 on the stress response.[31] Consomic, congenic, and multiple congenic strains, as well as RIS and recombinant congenic strains, are all tools now available to test such gene interactions in rodent models and then to assess their pertinence in human cohorts.

Role of Hormones and Cytokines With Respect to Time and Stimuli

The genetics of the stress response should be rewritten with a global understanding of the role of hormones and cytokines using gene array pathways (as above), and then submitted to searches for the roles of their genetic variation in diseases, including cardiovascular disorders.[67] Unrelated population and multiplex family studies are foreseeable, using both candidate gene association and genome-wide linkage analysis. SNPs will

expectedly be favored tools in this work. SNPs are defined by their presence in more than 1% of at least one population with stable inheritance and leading to haplotype building blocks.[68] SNPs occur at every 1,000 base pairs of DNA throughout the genome and are bi-allelic in nature, suitable for high-throughput analysis. Some of them code for amino acid changes (conservative or not), others are relevant for their presence in the 5'UTR or 3'UTR of noncoding regulatory regions of genes.

We have uncovered at least two 3'UTR SNPs in SHR—one in *hsp70* and the second in *hstf*. While 5'UTR mutations can modulate the degree of gene expression, those in 3'UTR are potentially relevant for the metabolic fate of mRNA, being responsible for their increased lability. We have recently described a "dynamic linkage" of the 3'UTR allele of angiotensin II type 1 receptor (*AT1R*) gene in our set of French-Canadian families: the response of pulse rate and heart contractility was shown to be in significant linkage with this 3'UTR allele, but only in specific physiological situations (change in posture).[69] The rapidity with which a significant linkage appears and disappears points to the involvement of a rapidly-intervening system, such as the sympathetic nervous system. Thus, the putative lability of *AT1R* mRNA may, as a consequence, be reflected as a variability of sympathetic nervous system regulation in a given individual.

Naturally, when present, mutations in coding regions of environmentally or hormonally responsive genes have potential pathophysiological consequences. Thus, 2 common SNPs within the β_2 adrenergic receptor at codons 16 and 27 lead to complete receptor desensitization in homozygotes with Arg at codon 16, while homozygotes at Gln 27 have maximal venodilation in response to isoproteronol. These common polymorphisms thus have the potential for distinct responses to exogenous as well as endogenous β-adrenergic stimulation and are potentially important determinants of the vascular response to stress.[70]

Table 2 lists some of the genetic variants potentially relevant to the stress response in humans and rodents.

Definition of Fine "Sub-syndromes"

Over the past several decades, researchers have developed strains with particular sensitivities to the environment and/or outcomes, including Dahl's salt-sensitive and SHR stroke-prone strains. These background strains are now characterized extensively by total genome scans allowing for targeted development of congenic strains bearing QTLs of environmental sensitivity. The only available genetic strain of "stress-induced hypertension," developed by Professor Markel's group in Novosibirsk, deserves full genomic characterization.[71]

Further research in human subjects will have to be directed in an analogous way to establish clinical sub-syndromes and their molecular diagnostics. The genetic components of salt retention (modulators and nonmodulators) and cortisol regulation have been described but need validation in other populations. Eventual sub-syndromes involving environmental susceptibilities should also be evaluated for their relationhips with metabolic components of high BP, ie, insulin resistance, obesity, and dyslipidemia.

Development of Novel Targets for Therapy and Prevention

Gene therapy is probably only a far-off futuristic expectation in stress modulation, but its successes in animal models often teach us the relevance of the pathways under study. Thus, the viral delivery of *Drd2*, encoding the dopamine receptor, permitted Thanos et al to dramatically decrease alcohol preference and intake levels.[72]

Understanding the exact genomic and proteomic pathways leading to pathological levels of the stress response has the potential for identifying susceptible individuals by genotyping relevant alleles as well as suitable individualized targeting of preventive measures. Clearly, as depicted in Table 2, individuals with defective alleles of *RYR* should avoid anaesthesia with succinylcholine and halothane, and subjects with P12A

Table 2. Examples of Genetic Variants in Stress Response Pathways

Gene	Polymorphism (variant)	Phenotype	Species
Susceptibility and outcomes			
Hsp70/TNFα	RFLP	BP increase	Rat[43]
TNFα	Microsatellite	Lower body obesity	Human[73]
Leptin		Central body obesity	Human[74]
HSTF	3′UTR	Temperature increase with immobilization	Rat[32]
Hsp27	3′UTR	LVH	Rat[44]
PPARG	P12A	NIDDM	Human[75]
ACE	I/D	BP increase LVH, renal damage	Human[76-86]
Pharmacogenomics			
RYR	Many described	Malignant hyperthermia	Human
ADRB2	G16R	β desensitization and vasodilatation	Human[70]
FMO	E158K, V257M, E308G	Trimethylaminuria, defect in FMO3, a hepatic phase 1 drug-metabolizing enzyme	Human[87]
CYP2C9	Many described	Metabolic defect of tolbutamide, warfarin NSAIDs	Human[88-90]
CYP2D6	Many described	Metabolism of β-blockers, antidepressants	Human[91-98]

Abbreviations: TNF, tumor necrosis factor; HSP, heat stress protein; PPARG, peroxisome proliferator-activated receptor-γ; ACE, angiotensin-converting enzyme; RYR, ryanodine receptor; ADRB2, adrenergic receptor B2; FMO, flavin-containing monooxygenase; CYP, drug-metabolising enzyme cytochrome P450; RFLP, restriction fragment length polymorphism; UTR, untranslated region; LVH, left ventricular hypertrophy; NIDDM, non–insulin-dependent diabetes mellitus.

mutation in the *PPARG* gene should be surveyed for susceptibility to diabetes.

The day is within reach when a specific diet, type and intensity of exercise, as well as dose adjustment of drugs and protection/expression to stressors will be recommended on the basis of the individual's genetic makeup.

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APPENDIX

Glossary of Terms

Allele: Alternative forms of a single gene.

Comparative genomics: Analysis of the conservation of gene order on chromosomal regions between species.

Congenic strains: Inbred strain generated from a control strain and another strain bearing a trait of interest (eg, hypertension) by successive backcrosses and which differs from the control strain only by a specific locus from the other strain.

Consomic strains: Inbred strain generated from a control strain and a strain bearing a trait of interest by successive backcrosses and which differs from the control strain only by one entire chromosome from the other strain.

Founder effect: A form of genetic drift that occurs when an isolated population is formed by a small number of founders. The population often exhibits genetic diseases at a higher frequency than expected. The French-Canadian population of the Saguenay-Lac St-Jean area has been widely studied for its characteristics perpetuating a subset of alleles of the original population.

Genetic marker: A variant allele, often polymorphic, that is used to construct a genetic (genomic) map.

Genome scan: Analysis of the entire genome with genetic markers.

Genomic map: A map showing distances between genetic markers on a chromosome. Distances are measured in centimorgans, cM, and are calculated by analysis of recombination of alleles at meiosis.

Haplotype building blocks: A series of known sequences linked on a chromosome.

LOD score: Logarithm of the odds score. Logarithm of the ratio of the likelihood of the observed linkage under the proposed hypothesis to the likelihood under the null hypothesis. A LOD score greater than 3 is a 1,000:1 ratio and is often taken as a threshold that the loci are linked.

Quantitative trait locus: A locus linked to genes that may contribute to a quantitative trait.

QTL clustering: Clusters of QTL for different traits at the same chromosomal locus.

Recessive trait: Caused by recessive genes that direct the expression of the trait only in the absence of a dominant gene.

Recombinant inbred strains: Inbred strains derived from two existing inbred strains (founders) that are produced by inbreeding F2 animals derived from founders.

Segregation analysis: Analysis of the separation of pairs of alleles into different gametes during meiosis. It is used to determine the genetic mode of inheritance of a trait and combined with a genome scan can be used to localize a trait in a chromosomal locus.

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