Genetics of Essential Hypertension: From Families to Genes

*Division of Nephrology, Dyalisis and Hypertension, University "Vita e Salute" San Raffaele Hospital, Milan, Italy; and [†]Department of Science and Biomedical Technologies State University of Milan, Milan, Italy.

Abstract. Family studies demonstrated the contribution of genetic factors to the development of primary hypertension. However, the transition from this phenomenologic-biometric approach to the molecular-genetic one is more difficult. This last approach is mainly based on the Mendel paradigm; that is, the dissection of the poligenic complexity of hypertension is brought about on the assumption that the individual genetic variants underlying the development of hypertension must be more frequent in hypertension in families. The validity of these assumptions was clearly demonstrated in the so-called monogenic form of hypertension. However, because of the network of the feedback mechanisms

In almost any textbook dealing with this issue it is stated that hypertension arises in families (1). Studies on natural-adopted children (2-4), parent-offspring relationships (5-9), and concordant and discordant twins (10-13) support this definition. In these studies, the phenotype used to detect the genetic component was the level of BP, but most of the subjects studied were below the age of 40 yr and had normal BP levels. The relevance of these results to the understanding of the genetics of hypertension depends on what we believe is the mechanism of hypertension. If we believe that hypertension is just the tail of a normal distribution of BP considered a quantitative physiologic trait, as suggested by Oldham et al. (14), we may use these results to gain information about the genetics of hypertension, also considering the tracking phenomenon (15-19). Clearly, we must assume that the genetic network underlying hypertension is not qualitatively different from that underlying the regulation of normal BP. An opposite view may propose that hypertension arises at a certain age from the interaction of some peculiar genetic network, which is present in the hypertensive patients only, interacting with specific environmental factors. This last condition is observed in secondary forms of hypertension in which the molecular-pathophysiologic mechanisms involved do not seem to operate for normal BP control in the normotensive population. For instance, it is likely that the etiologic factors leading to the

1046-6673/1300-0155

Journal of the American Society of Nephrology Copyright © 2002 by the American Society of Nephrology

DOI: 10.1097/01.ASN.0000032524.13069.88

regulating BP, it is possible that that the same gene variant may have an opposite effect on BP according to the genetic and environmental backgrounds. Independent groups of observations (acute BP response to saline infusion, incidence of hypertension in a population follow-up of 9 yr, age-related changes on BP) discussed in this review suggest a positive answer to this question. Therefore the impact of a given genetic variant on BP level must be evaluated within the context of the appropriate genetic epistatic interactions. A negative finding or a minor genetic effect in a general population may become a major gene effect in a subset of people with the appropriate genetic and environmental backgrounds.

fibrodysplastic alterations in the renal arterial wall are present only in the subset of subjects who develop renovascular hypertension. Likewise, in primary hypertension, BP could be controlled by the interaction of those genetic mechanisms at work only in the subset of subjects who will become hypertensive later in their life.

The lack of a bimodal distribution of BP (20) between this subgroup and the normotensive population is not a valid argument against this hypothesis for the following reasons:

- 1. The "bimodal distribution" has been found when searched with the appropriate methods (21).
- 2. In highly penetrating monogenic diseases, there is an enormous quantitative variation in the phenotypic expression of the trait of interest among carriers of the culprit allele (22).
- 3. When a trait is under the influence of many genetic and environmental factors, it has been demonstrated that the normal distribution of the trait is not a good argument against the involvement of well-defined individual genetic mechanisms (23).

When the genetic and environmental components of total BP variation are evaluated, we should also take into account that the effect of the environment on BP is age-dependent. For instance, it has been demonstrated in both humans and rats that dietary salt intake in early life is extremely important on the subsequent BP level. Normal newborn babies maintained on a 6-mo reduction in salt intake had a significantly lower BP compared with babies on a normal sodium intake, both in the short term and in the long term, though both groups had been reverted to the same amount of sodium (24,25). Even though these results have been questioned, similar observations have been made in rats (26–28). The BP effects of short-term changes in sodium intake act age-dependently. In fact, the

Correspondence to Dr. Giuseppe Bianchi, Division of Nephrology, Dialysis and Hypertension, University Vita e Salute, San Raffaele Hospital, Milan, Italy. Phone: 39-02-2643-3006; Fax: 39-02-2643-2384; E-mail: Bianchi.Giuseppe@hsr.it

magnitude of BP changes is greater in older people than in younger ones (29-32). The discrepancy in the relationship between dietary sodium and BP between interpopulation studies, where there is a strike relationship between dietary sodium and BP, and intervention studies in adult population, where sodium effect is less evident, may be due, at least in part, to the fact that in the former dietary sodium is established since birth. Translating these age-dependent effects on BP to genetics, we have to consider the possibility that also the pressure effect of genes involved in renal sodium handling could be age-dependent.

The topic of this paper requires additional considerations on the relationships between the phenomenologic-biometric approach (the Galton paradigm) that has been used to assess the genetic and the environmental components of BP in families and the molecular genetic approach (the Mendelian paradigm) used to study genes in individuals. The first approach describes the relationship between genetic (nature) and environmental factors (nurture) in causing hypertension by measuring BP in related and unrelated individuals under conditions of random mating. This approach tries to define the portion of total BP variance due to genetic factors (V_G) and that due to environmental ones (V_E). Values of V_G ranging from 20% to 55% are reported in the literature (1,33). However, there are many problems when trying to link these biometric values to more precise individual genetic molecular mechanisms. The addition rule of variance $(V_T = V_G + V_E)$ applies only when genotypic and environmental factors are independent of each other (34). However we know that this is not true for hypertension, because there are environmental factors (salt, for instance) that produce their hypertensive effect only in the presence of a particular genotype and vice versa. In both rats and humans, there are many data supporting this notion (35–39). In humans, a low-salt diet produces a range of individual responses ranging from +17 mmHg to -15 mmHg, the average fall being -1.8 mmHg (40). Similar findings are obtained when diuretics are given (35,41,42). A very significant portion of this variability, including the pressor effect, is due to genetic factors, and this portion is going to increase as our understanding of the genetics of this phenomenon progresses and the number of gene polymorphisms included in the analysis rises. Therefore the precise interaction between the genetic and the environmental component may be calculated only after having reached an exhaustive understanding of these phenomena and having performed the appropriate measurements. The addition of V_{G} to V_{E} is also affected by epistatic (43,44) or additive interactions and by dominance and recessiveness, defined by the phenotype-genotype relationship. Again, no sophisticated analysis or assumption may replace the direct measurements of the influence of these phenomena on the precise partition of V_{T} into V_G and V_E .

With this proviso, we may use the ratio VG/VT = h^2 as a population statistical parameter of hereditability. These considerations have practical implications. In fact, assuming that the biallelic system of interest accounts for a 4% of total BP variation in a population and that the genetic component represents 40% of the total BP variation, then the biallelic system

accounts for 10% of the total genetic variation; our apparent negligible value of 4% becomes a more relevant 10% of the total genetic variation. However, we must be aware of the fact that the exact relationship between the h^2 value and the genetic-molecular mechanism at work in that population can be established only after having performed the appropriate measurements.

The results so far obtained in well-studied monogenic diseases like thalassemia (45) or phenylketonuria (46) demonstrate that a single mutation is associated with an enormous phenotypic diversity (47). The same picture also emerges from studies on monogenic forms of hypertension (48) even though the data so far accumulated are not so clear. These data demonstrate that the phenotypic diversity is determined by layer upon layer of complexity. At each level of biologic organization, there are modifier genes that modulate the effect of the gene variant of interest. Therefore all the experts of monogenic diseases stress the importance of a multidisciplinary approach that combines organ and cellular pathology from the molecular to the physiologic-biochemical level to properly understand the underlying genetic mechanism. However, the strategies commonly used to dissect the genetic complexity of hypertension do not always take these considerations into account. Consequently, in spite of considerable efforts of the researchers in this field (49-58), the results of the studies are so far rather scanty in terms of (1) consistency among the genetic findings in different populations and (2) agreement about the most appropriate criteria to disclose the involvement of a given genetic mechanism.

What is really most surprising is the intrinsic inconsistency of some proposals. For instance, though it is widely recognized that the genetic and environmental factors involved in hypertension are heterogeneous, the most usual proposal to overcome this substantial problem is to increase the sample size to acquire the appropriate power to detect small differences. However, increasing the sample size, we also add more factors, either genetic or environmental. Therefore we are embarking on an endless job, and there is no convincing analysis in the literature trying to overcome this limitation.

The common paradigm underlying the strategies of statistical genetics predicts that the gene variant of interest must always be more frequent in cases than in controls or must cosegregate with a higher level of BP in families (the Mendelian paradigm) (59,60). This paradigm was proved to be fruitful in understanding the genetics of monogenic diseases or diseases where a clear-cut abnormality of a chemical, morphologic, or clinical phenotype exists. However, in view of the results so far obtained, we should wonder if the same paradigm could be equally fruitful for a quantitative phenotype so variable as BP (61,62).

Back to Pathophysiology

The most relevant data we got from more than 50 yr of studies on the pathophysiology of arterial hypertension are:

1. The pivotal role of the kidney in the long-term control of BP (63-65). This does not necessarily mean that the primary fault leading to hypertension must be located within the kidney

but simply that when the primary cause is outside the kidney, some modifications in the pressure-natriuresis relationship must occur to shift the set point of BP regulation.

2. The nervous system (66) and alterations of adrenal glands (67–69) may also lead to hypertension (always through some modification of renal function). However, it is important to note that in most cases there are peculiar functional features that distinguish these forms of hypertension from the primary essential one.

3. No convincing evidence has so far been presented for a primary hypertensive mechanism arising within the heart or the muscular portion of the vessels. It is not clear whether the findings showing that alteration in endothelial function may lead to modifications of BP could be considered suggestive of a primary involvement of the endothelial function in causing primary hypertension (70).

4. When the relationship between a renal cause of hypertension and the level of BP is studied, two observations emerge:

a. When groups of patients (or animals) are compared, the degree of renal alterations (renal artery stenosis, renal insufficiency) is related to BP increase.

b. For the same degree of renal alteration, there is a wide individual variation in BP levels, even if this variation is not generally properly underlined.

Therefore, any definite cause of hypertension may produce different levels of BP according to the individual genetic or environmental background.

Both secondary renal forms of hypertension and monogenic forms of hypertension are generally associated to morphologic, functional, or biochemical features that distinguish them from primary hypertension. Therefore, the genetic mechanism(s) leading to primary hypertension must be more subtle than those leading to a monogenic form and very well interlinked with all the homeostatic mechanisms to produce a selective change in BP without overt alterations in electrolyte metabolism, hormone levels, or renal or nervous system function. Two possible hypotheses could reconcile these observations with the fact that primary hypertension occurs in the absence of overt alterations:

- 1. Genetic alterations at multiple sites that lead to BP increase without the intervention of counter-regulatory mechanisms.
- 2. A genetic alteration of some basic mechanism involved in the regulation of body fluids, electrolyte metabolism, and kidney function. In such a condition, all these functions could be reset at a higher BP level without triggering any compensatory mechanism. Both hypotheses are likley. Only genetic models that can accommodate epistatic or additive interactions are appropriate to test the first hypothesis.

The feedback mechanisms that regulate BP are reciprocally influenced; therefore, it is obvious that the best way to disentangle this complexity is to combine the existing knowledge on the pathophysiology of hypertension with the genetic approach. According to this view, genetics should be used as an additional tool that must be integrated with pathophysiology, clinical medicine, and pharmacology. This type of integration is needed to hypothesize the possible network of epistatic or additive interactions among gene loci of which the variants may differently modulate the physiologic mechanisms involved in BP regulation. The results obtained may then be analyzed with the models of epistatic or additive interaction within the framework of physiologic genetics.

There is a long list of candidate genes that are likely to be involved in the relationship between sodium (Na) intake and BP regulation (52–57). These genes can either directly regulate renal Na excretion or can modify the BP response to body sodium changes. Therefore, their genetic interactions are complex and may differ in the individual patient according to the available alleles at the loci involved in a specific mechanism. The complexity of this approach may discourage its practical application, because the wide number of genes involved requires very large samples to reach a sufficient statistical power. However, we do not see any valid alternative approach.

The elucidation of the physiologic mechanisms that link BP and renal Na excretion demonstrated that, within the discouragingly long list of putative mechanisms, pressure-natriuresis (63) emerged as the keystone mechanism that regulates the long-term set point of BP level. Likewise, among the various genetic mechanisms that are theoretically involved in this process, it could be possible to outline a hierarchical sequence. From this approach, we can collect appropriate data to test the second type of hypothesis.

The arguments involved in the debate on the relationship between alterations in individual genetic molecular mechanisms and changes in a very complex whole body phenotype, like BP, are similar to the long-lasting debates between supporters of either the reductionistic or the olistic approaches for dissecting the complexity of the living organisms. The central issue of these debates is the following: Is the whole organism just the sum of individual genetic molecular mechanisms, or, because a network of homeostatic mechanisms exists, is the whole organism a new entity that cannot simply be dissected into its individual molecular mechanism?

If the Mendelian paradigm cannot be applied to the genetics of hypertension, all the most sophisticated models of statistical genetics based on it are simply not appropriate to generate valid scientific information. It is surprising that warnings about the limitations of the Mendelian paradigm arose from the in depth knowledge of classical monogenic diseases like phenylketonuria (46) or thalassemia (45), despite of the fact that these diseases gained a lot of important scientific breakthroughs with its application. This type of bell-shaped curve regarding the importance of a given discipline for the understanding of complex phenomena is not new in the history of science. As our knowledge in a given field progresses, we also learn the limitations of the tools we are using to understand it.

Limitations of the Mendelian Paradigm to the Understanding of the Genetics of Hypertension

Is it possible that the same gene variant or allele may have opposite effects on BP depending on the genetic or environmental backgrounds? If the answer is yes, at least under particular circumstances, then the Mendelian paradigm needs a serious revision in this context.

To answer this question, we shall consider the relationship

between body sodium regulation (through changes in renal tubular Na reabsorption) and the activity of the renin-angiotensin system (RAS) in determining BP levels. Indeed, human carriers of gene variants that favor faster tubular Na reabsorption tend to have lower plasma renin (35-71), evoking the very well established pathophysiologic notion that BP is regulated by a correct balance between body Na and RAS activity (72). Whatever the primary genetic or environmental mechanism involved, the pressure-natriuresis phenomenon is central in BP regulation and renal Na excretion (63). Within a few minutes from changes in renal perfusion pressure, there are modifications of both proximal tubular Na reabsorption and cell surface expression of tubular cells Na transporters (73,74). This implies the existence of a biochemical-pressure transducer sensing the changes in hydrostatic pressure and triggering a sequence of biochemical cellular events, which lead to a variation in tubular Na transport capacity. The bulk of knowledge so far accumulated on α -adducin (ADD1) polymorphism is consistent with the notion that these genetic variants affect this type of transducer (75–78).

We have recently tested the interaction between ADD1 and aldosterone synthase genes (CYP11B2), which should favor hypertension affecting tubular Na reabsorption capacity (76,79), and angiotensin-converting enzyme (ACE) gene as modulator of RAS activity. These studies were carried out either in an acute setting (BP response to an intravenous infusion of Na) (75) or in a chronic setting (development of hypertension in a follow-up population study) (80). The acute setting was carried out in never-treated patients on normal Na diet under very carefully controlled environmental conditions to limit confounding factors. The results are consistent with the hypothesis that the functional difference associated to DD and II ACE genotypes is disclosed or magnified in the presence of the gene variant causing renal Na retention both in the acute and chronic setting.

In fact DD, ID, and II ACE genotypes did not have *per se* any influence on acute BP response. However, when patients with the three ACE genotypes were further subdivided according to Gly460Trp polymorphism of ADD1 (Gly460Trp genotype that increases Na retention and Gly460Gly), a linear increase in BP with the D allele was observed in patients carrying also the 460Trp allele; in Gly460Gly patients, a tendency to a BP decrease with the increase in the number of D alleles was found (Figure 1). This means that only the study of epistatic interactions with a gene variant affecting renal Na retention disclosed a pressure effect of ACE polymorphism.

In a longitudinal study on the development of hypertension in a Belgian population (the chronic setting) (80), carriers of ACE DD genotype displayed a slightly higher incidence of hypertension (+31%) compared with ID and II patients. This small difference was greatly magnified (+250%) when ADD1 and CYP11B2 polymorphisms were also taken into account. These two last gene polymorphisms did not *per se* have any effect on the development of hypertension in this study; however, their effect is again disclosed through the interaction with the ACE I/D polymorphism (Figure 2).

Clearly the negative findings with ADD1 and CYP11B2



Figure 1. Mean BP (MBP) changes with intravenous acute sodium load as a function of angiotensin-converting enzyme (ACE) and α -adducin genotype. Reproduced with permission from reference 75.

were due to the fact that they potentiate the effect of DD genotype but conversely depress the effect of the II and I/D genotypes. In fact, when the relative risk (RR) of developing hypertension was calculated according also to the ADD1 and Cyp11B2 polymorphisms, it increased from 1.2 to 2.04 in DD carriers compared with the general population, whereas it decreased from 0.9 to 0.4 in II and ID carriers (Figure 2). Therefore, the net effect being zero, these genes would have never been detected by any sophisticated statistical analysis that does not take into account their interaction.

The interactions among ADD1, ACE, and CYP11B2 were not confirmed by studying their association to hypertension in 128 hypertensive patients and 128 normotensive controls (81). However, the analysis has been carried out comparing very small subgroups of subjects for each combination of genotypes (around 10 to 15 subjects in each subgroup). Power calculations were not given in that article, however everybody can judge the scientific validity of a statistical analysis based on allelic frequencies assessed in such small subgroups (82).

A paradoxical BP response to saline infusion was also observed in one clip two kidneys hypertension in rats, where Na loss by the controlateral kidney may stimulate an excessive secretion of renin that *per se* overrides the hypotensive effect of body Na depletion. Under these circumstances, saline infusion lowers BP (83).

In subjects with a combination of genotypes that reduce the constitutive renal ability to retain Na, there may be an enhanced level of Na retaining hormones, which may *per se* predispose to BP increase if a peculiar genetic background favors their hypertensive effect. In other words, the hypotensive effect of genotypes favoring Na excretion may be overridden by genotypes favoring the vascular and renal effects of



Incidence of Hypertension by ACE Genotype Rate per 1000 Subjects - Year

Figure 2. Incidence of hypertension according to (from left to right) ACE genotype in all subjects, in carriers of the ADD1 460Trp allele, in CC homozygotes of CYP11B2 gene, and carriers of the ADD1 460Trp allele. RR, relative risk to develop hypertension in the different subgroups compared to the whole population. Modified with permission from reference 80.

"pressor" hormones triggered by the former combination of genotypes.

In fact, as discussed above, the whole organism is not simply the sum of its parts (see genes), but it is a new entity in which the interactions among the various feedback loops generate the complexity that stands in its own and contributes to the individual reactivity. Recently the age-dependency of α -adducin polymorphism modulation on BP has been analyzed in a general population. The results obtained suggest that the 460Trp allele is associated to hypertension only in older subjects (>55 yr), whereas at the homozygous status this allele seems to have opposite effects on BP at younger ages (<44 yr) (84). Unpublished data from our group on the relationship between α -adducin polymorphism and the hormonal profile (aldosterone, renin, endogenous ouabain) suggest that at younger ages carriers of Na retaining gene variants have lower levels of hormones with the same effect on renal Na handling as carriers of Na-losing gene variants. Such a homeostatic response at a younger age may affect the overall pressor effect of these gene variants, mainly when age-dependency is not taken into account.

By comparing the different phases of renovascular hypertension (from acute to chronic phase), we may observe sequential changes in the renin-angiotensin-aldosterone system, plasma cathecolamines ouabain-like factor, and structure of the peripheral vessels. All these changes may be modulated by different combinations of gene variants. This interrelationship may be even stronger when the initial triggering mechanism is not external, like experimental constriction of the renal artery, but is genetic, that is at work since fetal life. In this respect, the genetics of developmental phenomena may come into play. For instance, the number of glomeruli in the adult kidney ranges from 300,000 to 1,750,000 (85,86). The genetic mechanism underling this variability is still poorly understood. Certainly this variation *per se* may affect the BP response to any given acquired (or genetic) kidney abnormality in adult life. Knockout of the RAS system genes in mice (angiotensinogen, ACE, AT1R) produces remarkable morphologic kidney changes (87). This suggests that these genes regulate both the day-today physiologic function and are also involved in setting the final morphology of the kidney.

At present it is not known whether the developmental effects of these genes influence BP during the adult life along the same direction as the physiologic ones. Certainly the available data seem in contrast with this hypothesis. In mice, the knockout of angiotensinogen and ACE genes (87) produces renal morphologic changes (glomerulosclerosis, tubular atrophy, interstitial fibrosis) that *per se* should tend to increase BP. Conversely, the consequent enormous reduction in plasma or tissue level of these proteins causes a net decrease of BP in adult life. It is not known whether the more subtle functional changes produced by spontaneous mutations within these genes may then have opposite effects on BP according to their developmental or functional effects.

Variations in salt intake are associated to different activation of RAS. The relationship is strictly related to the districts were RAS is studied. Na depletion activates the circulating RAS and depresses the vascular one. Opposite changes occur with Na load (88). Given this differential regulation with opposite effects on BP, various gene variants can potentiate or depress one type of angiotensin II release over the other. The contrasting data (89,90) so far obtained on the release of angiotensin II after intravenous infusion of angiotensin I according to the different ACE genotypes may also be due to this phenomenon.

It has been shown recently that ACE genotypes influence angiotensin II production after angiotensin I infusion on highsalt diet but not on low-salt diet (91). This interrelationship between ACE genotypes and body sodium levels may influence the pressor effect of the ACE DD genotype that is associated to increased levels of plasma ACE. In other words, a putative difference in the pressor effect between DD and II ACE genotypes may only be disclosed above a certain critical level of body sodium.

Differences in the evolutionary history of the population may also greatly affect the assessment of the role of a gene with statistical genetic techniques. An increased genetic ability to retain Na could have favored fitness during the reproductive age, across the Paleolithic period, when hunters heated a lowsalt diet. However, because the number of genes affecting renal Na retention is large, this selective advantage could have been achieved by varying the frequency of different alleles. Namely, increasing the frequency of alleles that favor Na reabsorption or decreasing the frequency of those inhibiting it. In other words, evolution or selection are interested in functions and not in genes (gene polymorphism is only a tool available for the evolutionary processes, or evolution by bricolage) (92).

To study this aspect we carried out a case-control study in two European populations, divided by a very large genetic distance (around 10,000 yr), one from Northern Italy (around Milan) and the other from Northern Sardinia (around Sassari). The 460Trp allele of ADD1 gene, which is known to stimulate Na reabsorption, was significantly associated to hypertension in the Milan sample (35) but not in the Sassari one (42).

However when BP response after diuretic treatment was studied in hypertensives from Milan and Sassari, the 460Trp allele was significantly associated with a great BP fall in both populations (35,42), together with a lower PRA and faster ion transport of Na across the erythrocyte membrane (93). This result clearly indicates that physiologic genetics has provided similar data in both populations consistent with previous data obtained in humans, supporting the sodium-retaining activity of the 460Trp ADD1 allele, whereas statistical genetics furnished contrasting data. As indicated above, the inconsistency between the two genetic methodologies may be explained as follows: to ensure the required amount of body Na during the paleolithic period, the constitutive renal Na reabsorption was increased. However in more recent periods, because a normal or high salt diet is available, this increased tubular reabsorption may favor the development of hypertension (thrifty-genotype hypothesis) (94,95). This could have happened in the hypertensives of the two populations throughout a different equilibrium of the alleles operating in the genetic network devoted to the regulation of renal Na excretion. The shift of this particular gene network toward sodium retention may be achieved by decreasing the frequency of gene variants favoring Na loss or by increasing the frequency of those variants promoting Na reabsorption. Therefore Sassari hypertensive patients could have a genetic network leading to hypertension different from Milan hypertensive patients. However, in different networks, the 460Trp ADD1 allele does always exert its Na-retaining activity. The relevant message of these results is that each gene variant discloses its own function only when measured in the appropriate conditions. In particular, the predictivity of the pharmacologic response to a drug that somehow interferes with the disease-favoring allele is independent from the demonstration that the culprit allele is significantly associated with the disease.

This is the reason why pharmacogenomics may provide a paramount contribution (96-99). In the last decades, the selective blockade of a given receptor or biochemical activity with an appropriate drug has greatly contributed to our understanding of the complexity of cellular biochemistry and body pathophysiology. Even though the drug selectivity was often not especially high, thus weakening the scientific validity of the information, the use of different drugs with different selectivity was of great help. Along this line, the availability of ACE inhibitors or angiotensin II receptor blockers provided a substantial contribution to the comprehension of the pathophysiology of this system. Why has this contribution been much greater than that resulting from 10 yr of statistical genetics studies on the polymorphisms of RAS genes? Even if there were problems of drug selectivity, it is not possible to ignore this big discrepancy!

As mentioned above, the blockade of a given molecular biochemical function with a drug is one of the most powerful tools for understanding the function of a gene and its contribution to the overall activity of a complex system. Just like a gene knockout! However, we could have problems with both approaches. The selective antihypertensive activity of a drug in patients carrying a given genotype or a combination of them may indicate a common pathway between the drug and the genotype and highlights a given genetic hypothesis over others. Of course, before reaching any conclusion, it is necessary to take into account possible confounding factors such as: (1) interference with previous treatment; (2) stage of the disease; (3) pharmacokinetics and metabolism of the drug; (4) interference with possible counter-regulatory mechanisms limiting the fall of BP produced by the drug.

For all these reasons, the most sophisticated methods of statistical genetics based on the Mendelian paradigm seem unsuitable for approaching the complexity of the genetics of hypertension if solid pathophysiologic, pharmacogenomics, biochemical, and molecular data are not simultaneously taken into account. According to Schork (61) and Terwillinger and Goering (62), the available tools of statistical genetics are not simply capable of decoding the genotype-phenotype relationship in this type of disease. Even if such an explicit admission has not been made by other experts, their endless debates on the suitability of the various methods certainly indicates a lack of agreement on a gold standard. Therefore, the genetics of

hypertension is facing a very crucial dilemma: on one hand, it has to rely on statistical genetics methodology to define the role of a given gene; on the other hand, there are no gold standard methods to apply.

Conclusions

The data so far accumulated on the pathophysiology and genetics of hypertension discussed above led us to conclude that it would be highly unlikely that a major gene will emerge from these studies. In the future, we shall have a definite number of gene polymorphisms, each contributing for 2 to 10% of genetic variation depending on the genetic and the environmental background of the population considered. Gene interactions will emerge as the most productive approach to explain why a very sophisticated network of feedback loops may result in a BP increase without any clear-cut abnormality of those hormonal, humoral, or structural parameters reflecting body fluids status, kidney and nervous system functions, and all the other systems involved in BP regulation.

The biometric value of inheritability of hypertension may have its molecular basis on these networks of genetic interactions with some type of hierarchical organization. The crucial question regards the most appropriate tools for understanding these interactions. Each physiologic function may have a discrete number of alternative genetic variants, which may replace each other according to the evolution by bricolage illustrated above. For these reasons, the relevance of physiologic and pathophysiologic genetics and of pharmacogenomics is much greater than that of any sophisticated statistical analysis based on allelic frequency or cosegregation. Indeed, we are facing this dilemma: the geneticists are entitled to define the role of a given gene with tools that are, however, unsuitable to cope with the pathophysiologic complexity of hypertension. Pathophysiologists are aware of this complexity, but they are not generally entitled to define the role of a given gene. Only the integration of these disciplines with all their know-how may overcome these problems. This dilemma is also accentuated by less scientific and more sociologic aspects. Science is now deeply embedded in the industrial world of our society. This has certainly accelerated its development, but the competition for funding coming from different sources is leading to a strenuous defense of each one's know-how as the most appropriate to solve a given problem.

Acknowledgments

This work has been supported in part by a grant of the Ministry of Health of Italy (ICS 030.6/RF00-49), Cofin MM06A9241_001, and Eurnetgen QLG1-2000-01137 to Giuseppe Bianchi, and FIRST 2000 to Chiara Lanzani.

References

- Ward R: Familial aggregation and genetic epidemiology of blood pressure. In: *Hypertension: Pathophysiology, Diagnosis and Management*, Vol.1, edited by Laragh JH, Brenner BM, New York, Raven Press, 1990, pp 81–100
- Biron P, Mongeau JG: Familial aggregation of blood pressure and its components. *Pediat Clin* 25: 29–33, 1978

- Biron P, Mongeau JG, Bertrand D: Familial aggregation of blood pressure in 588 adopted children. *Can Med Assoc J* 115: 773– 774, 1976
- Mongeau JG, Biron P, Sing CF: The influence of genetics and household environment upon the variability of normal blood pressure: The Montreal adoption survey. *Clin Exper Hypertens* A8: 653–666, 1986
- Miall WE, Henage P, Khosal T, Lovell HG, Moore P: Factors influencing the degree of resemblance in arterial hypertension in close relatives. *Clin Sci* 33: 271–283, 1967
- Longini IM, Higgins MW, Minton PC, Moll PP, Keller JB: Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. *Am J Epidemiol* 120: 131–144, 1984
- Havlik RJ, Garrison RJ, Feinleib M, Kannel WB, Castelli WP, McNamara PM: Blood pressure aggregation in families. *Am J Epidemiol* 110: 304–312, 1979
- Wolanski N: An approach to the problem of inheritance of systolic and diastolic blood pressure. *Genet Pol* 10: 263–268, 1969
- Hayes CG, Tyroler HA, Cassel CJ: Family aggregation of blood pressure in Evans county, Georgia. *Arch Intern Med* 128: 965– 975, 1971
- Stocks P: A biometric investigation of twins and their brothers and sisters. Ann Eugenet, 1930
- McInhai ML, Shaffer JW, Hines EA: The heritability of blood pressure. An investigation of 200 twin pairs using the cold pressure test. J Hopkins Med J 136: 57–74, 1975
- Havlik RJ, Garrison RJ, Katz SH, Ellison LC, Fienlieb M, Myrianthopulos NC: Detection of genetic variance in blood pressure in seven-years-old twins. *Am J Epidemiol* 109: 512– 516, 1979
- Feinlieb M, Garrison RJ, Fabsitz R, Chriastian JC, Hrubec Z, Borhani NO, Kannel WB, Rosenman R, Schwartz JT, Wagner JO: The NHLBI twin study of cardiovascular disease risk factors: Methodology and summary of results. *Am J Epidemiol* 106: 284–295, 1977
- Oldham PD, Pickering G, Fraser R, Sowry GSC: The nature of essential hypertension. *Lancet* 1: 1085–1093, 1960
- Wilsgaard T, Jacobsen BK, Schirmer H, Thune I, Lochen ML, Njolstad I, Arnesen E: Tracking of cardiovascular risk factors: The Tromso study: 1979–1995. *Am J Epidemiol* 154: 418–426, 2001
- Burke V, Beilin LJ, Dunbar D: Tracking of blood pressure in Australian children. J Hypertens 7: 1185–1192, 2001
- O'Sullivan JJ, Derrick G, Foxall RJ: Tracking of 24-hours and casual blood pressure: 1-year follow-up study in adolescents. *J Hypertens* 9: 1193–1196, 2000
- Okasha M, McCarron P, McEwen J, Davey Smith O: Determinants of adolescent blood pressure: Findings from the Glasgow University student cohort. *J Hum Hypertens* 14: 117–124, 2000
- Suh I, Nam CM, Kim SI, Lee KH, Kim HC, Kim CS: Twelveyear tracking of blood pressure in korean school children: The Kangwha Study. *Yonsei Med J* 40: 383–387, 1999
- 20. McManus IC: Bimodality of blood pressure levels. *Statist Med* 2: 253–258, 1983
- Day NE: Estimating the components of a mixture of normal distributions. *Biometricka* 56: 463–474, 1969
- Ledley FD, Levy HL, Woo SLC: Molecular analysis of the inheritance of phenylketonuria and mild phenylalaninemia in families with both disorders. *N Engl J Med* 314: 134–141, 1986

- 23. Harris H: Enzyme polymorphism in man. *Proc Roy Soc B* 164: 298–310, 1966
- Hofman A, Hazebroek A, Valkenburg HAA: A randomised trial of sodium intake and blood pressure in newborn infants. *J Am Med Ass* 250: 370–373, 1983
- Geleijnse JN, Hofman A, Witteman JC: A long-term effects of neonatal sodium restriction on blood pressure. *Hypertension* 29: 913–917, 1997
- Grollman A, Grollman EF: Teratogenic induction of hypertension. J Clin Invest 41: 710–714, 1962
- Dahl LK: Salt intake and hypertension. In: *Hypertension: Physiopathology and Treatment*. Part Two: Physiopathology of Human Experimental Hypertension. Genest J, New York McGraw-Hill, 1983 pp 134–147
- Krecek J, Dlouha H, Zicha J: Salt supply in early life and possible consequences for hypertension in adult life. *Biblthea Nutr Dieta* 31: 121–130, 1982
- Luft FC, Miller JZ, Grim CE: Salt sensitivity and resistance of blood pressure: Age and race as factors in physiological responses. *Hypertension* 17[suppl I]: I102–I108, 1991
- Myers JB, Morgan TO: Effect of alteration in sodium chloride intake on blood pressure in normotensive subjects. *J Cardiovasc Pharmacol* 6[suppl I]: S204–S209, 1984
- Zeme I MB, Sowers JR: Salt sensitivity and systemic hypertension in the elderly. *Am J Cardiol* 61: 7H–12H, 1988
- 32. Mc Grecor GA, de Wardener HE: *Salt, Diet and Health*, Cambridge, Cambridge University Press, 1998, pp. 82–99
- 33. Levy D, De Stefano A, Larson MG, O'Donnell R, Lifton P, Gavras H, Cupples LA, Myers RH: Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the Framingham heart study. *Hypertension* 36: 477–483, 2000
- Vogel F, Motulsky AG: *Human Genetics: Problems and Approaches*, 3rd edition, Berlin, Springer-Verlag, 1997
- Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, Glorioso N, Lanzani C, Manunta P, Righetti M, Rivera R, Stella P, Troffa C, Zagato L, Bianchi G: Polymorphism of alphaadducin and salt sensitivity in patients with essential hypertension. *Lancet* 349: 1353–1357, 1997
- Dahl LK, Heine M, Tassinari L: Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. *Nature* 194: 480–482, 1982
- Rapp JP: Dahl salt susceptible and salt-resistant rats. *Hypertension* 4: 753–763, 1982
- Poch E, Gonzales D, Giner V, Bragulat E, Coca A, de la Sierra A: Molecular basis of salt sensitivity in human hypertension. Evaluation of renin-angiotensin-aldosterone system gene polymorphisms. *Hypertension* 38: 1204–1209, 2001
- Hunt S Cook N, Oberman A, Cutler JA, Hennekens CH, Allender AS, Walker GW, Whelton PK, Williams RR: Angiotensinogen genotype, sodium restriction, weight loss and prevention of hypertension. Trials of hypertension prevention, phase II. *Hypertension* 32: 393–401, 1998
- Miller JZ, Weiberger MH, Daugherty SA, Fineberg NS, Chtistian JC, Grim CE: Heterogeneity of blood pressure response to dietary sodium restriction in normotensive adults. *J Chron Dis* 40: 245–250, 1987
- Turner ST, Schwartz GL, Chapman AB, Boerwinkle E: C825T polymorphism of the G protein beta(3) subunit and antihypertensive response to a thiazide diuretic. *Hypertension* 37: 739– 743, 2001

- 42. Glorioso N, Manunta P, Filigheddu F, Troffa C, Stella P, Barlassina C, Lombardi C, Soro A, Dettori F, Pinna Parpaglia P, Sciarrone Alibrandi MT, Cusi D, Bianchi G: The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be excluded by a negative association study. *Hypertension* 34: 649–654, 1999
- 43. Cheverud JM, Routman E: Epistasis and its contribution to genetic variance components. *Genetics* 139: 1455–1461, 1995
- Frankel W, Schork N: Who's afraid of epistasis? *Nature Genet* 14: 371–373, 1996
- Weatherall DJ: Phenotype-genotype relationships in monogenic disease: Lessons from the thalassaemia. *Nature Rev* 2: 245–255, 2001
- 46. Scriver C, Waters P: Monogenic traits are not simple. *TIG* 15: 267–272, 1999
- 47. Wolf U: Identical mutations and phenotypic variation. *Hum Genet* 100: 305–321, 1997
- Jeck N, Konrad M, Peters M, Weber S, Bonzel K, Seyberth H: Mutations in the chloride channel gene, CLCNKB, leading to a mixed Barter-Gitelman phenotype. *Ped Res* 48: 754–758, 2000
- 49. Lifton RP: Molecular genetics of human blood pressure variation. *Science* 272: 676–680, 1996
- Morris B: Genes for essential hypertension: the first decade of research. In: *Progress in Hypertension*, Part 4, edited by Frossard PM, Utrecht, VSP, 1999, pp 1–44
- Hamet P, Pausova Z, Adarichev V, Adaricheva K, Tremblay J: Hypertension: Genes and environment. J Hypertens 16: 397– 418, 1998
- Jeunemaitre X, Soubrier F, Kotelevstev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM, Corvol P: Molecular basis of human hypertension: role of angiotensinogen. *Cell* 71: 169–180, 1992
- Casari G, Barlassina C, Cusi D, Zagato L, Muirhead R, Righetti M, Nembri P, Amar K, Gatti M, Macciardi F, Binelli G, Bianchi G: Association of the alpha-adducin locus with essential hypertension. *Hypertension* 25: 320–326, 1995
- 54. O'Donnell CJ, Lindpainter K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Myers RH, Levy D: Evidence for association and genetic linkage of the angiotensin-convertin enzyme locus with hypertension and blood pressure in men but not in women in the Framingham Heart Study. *Circulation* 97: 1766–1772, 1998
- 55. Kruskhal J, Xiong M, Ferrell R, Sing CF, Turner ST, Boerwinkle E: Linkage and association of adrenergic and dopamine receptor genes in the distal portion of the long arm of chromosome 5 with systolic blood pressure variation. *Hum Mol Genet* 7: 1379–1383, 1998
- Svetkey LP, Chen YT, McKeown SP, Preis L, Wilson AF: Preliminary evidence of linkage of salt sensitivity in black Americans at the b2-adrenergic receptor locus. *Hypertension* 29: 918– 922, 1997
- Kainulainmen K, Perola M, Terwilliger J, Kaprio J, Koskenvuo M, Syvanen AC, Vartiainen E, Peltonen N, Kontula K: Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. *Hypertension* 33: 844–849, 1999
- Lifton RP, Gharavi AG, Geller DS: Molecular mechanisms of human hypertension. *Cell* 104: 545–556, 2001
- Lander ES, Schork N: Genetic dissection of complex traits. Science 265: 2037–2047, 1994
- 60. Weeks DE, Lathrop M: Polygenic disease: Methods for mapping complex disease traits. *TIG* 11: 513–519, 1995
- Schork NJ: Molecular genetics of hypertension, In *Genetics of Hypertension*, edited by AD Dominiczack, Connell, Sonbrier, Oxford, Bios S.P. Ltd, 1999, 9–15

- Terwilliger JD, Goering HHH: Gene mapping in the 20th century and 21st centuries: statistical methods, data analysis and experimental design. *Hum Biol* 72: 63–132, 2000
- 63. Guyton A, Hall J, Coleman T: The dominant role of the kidneys in the long-term regulation of arterial pressure in normal and hypertensive states. In: *Hypertension, Pathophysiology, Diagnosis and Management*, Vol. 1, edited by Laragh JH, Brenner BM, New York, Raven Press, 1990, pp 1029–1052
- Bianchi G, Fox U, Di Francesco G, Giovannetti AM, Pagetti D: Blood pressure changes produced by kidney-cross transplantation between spontaneously hypertensive and normotensive rats. *Clin Sci Mol Med* 47: 435–448, 1974
- Dahl L, Heine M, Thompson K: Genetic influence of the kidney on blood pressure: Evidence from chronic renal homografts in rats with opposite predisposition to hypertension. *Circ Res* 34: 94–101, 1974
- 66. Wiss MJ, Oparil S, Chen YF: The role of central nervous system in hypertension. In: *Hypertension, Pathophysiology, Diagnosis* and Management. Vol.1, edited by Laragh JH, Brenner BM, New York, Raven Press, 1990, pp 679–701
- Biglieri EG, Irony I, Kater CE: Adrenocortical forms of hypertension. In: *Hypertension, Pathophysiology, Diagnosis and Management*. Vol.1, edited by Laragh JH, Brenner BM, New York, Raven Press, 1990, pp 1609–1624
- Manger WM, Gifford RW: Pheocromocytoma. In: Hypertension, Pathophysiology, Diagnosis and Management. Vol.2, edited by Laragh JH, Brenner BM, New York, Raven Press, 1990, pp 1639–1660
- Wilson FH, Dise-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Dessiter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP: Human hypertension caused by mutations in WNK kinase. *Science* 293: 1107–1112, 2001
- Klahr S: The role of nitric oxide in hypertension and renal disease progression. *Nephrol Dial Trnsplant* 16[Suppl 19]: 60– 62, 2001
- Grant F, Romero JR, Jeunemaitre X, Hunt SC, Hopkins PN, Hollenberg NH, Williams G: Low-renin hypertension, altered sodium homeostasis and alpha-adducin polymorphism. *Hypertension* 39: 191–196, 2002
- 72. Hall JE, Guyton A: Control of sodium excretion and arterial pressure by intrarenal mechanisms and the renin-angiotensin system. In: *Hypertension, Pathophysiology, Diagnosis and Management*. Vol.1, edited by Laragh JH, Brenner BM, New York, Raven Press, 1990, pp 1105–1129
- Zhang Y, Mircheff AK, Hensley CB, Magyar CE, Warnock DG, Chambrey R, Yip KP, Marsh DJ, Holstein-Rathlou NH, McDonough A: Rapid redistribution and inhibition of renal sodium transporters during acute pressure natriuresis. *Am J Ph Physiol* 39: F1004–F1014, 1996
- Magyar CE, Zhabg Y, Holstein-Rathlou NH, McDonough AA: Downstream shift in sodium pum activity along the nephron during acute hypertension. *J Am Soc Nephrol* 12: 2231–2240, 2001
- Barlassina C, Schork N, Manunta P, Citterio L, Sciarrone MT, Lanella G, Bianchi G, Cusi D: Synergistic effect of alphaadducin and ACE genes in causing blood pressure changes with body sodium and volume expansion. *Kidney Int* 57: 1083–1090, 2000
- Manunta P, Burnier M, D'Amico M, Buzzi L, Maillard M, Barlassina C, Lanella G, Cusi D, Bianchi G: Adducin polymor-

phism affects renal proximal tubule reabsorption in hypertension. *Hypertension* 33: 694–697, 1999

- 77. Ferrandi M, Salardi S, Tripodi G, Barassi P, Rivera R, Manunta P, Goldshleger R, Ferrari P, Bianchi G, Karlisch SJ: Evidence for an interaction between adducin and Na(+)-K(+)-ATPase: Relation to genetic hypertension. *Am J Physiol* 277: H1338–H1349, 1999
- Tripodi F, Valtorta F, Torielli L Chieregatti E, Salardi S, Trusolino L, Bianchi G: Hypertension-associated point mutations in the adducin alpha and beta subunits affect actin cytoskeleton and ion transport. *J Clin Invest* 97: 2815–2822, 1996
- Paillard F, Chansel D, Brand E, Benetos A, Thomas F, Czekalski S, Ardaillou R, Soubrier F: Genotype-phenotype relationships for the renin-angiotensin-aldosterone system in a normal population. *Hypertension* 34: 423–429, 1999
- Staessen J, Wang GJ, Brand E, Barlassina C, Birkenhager W, Herrmann SM, Fagard R, Tizzoni L, Bianchi G: Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population. *J Hypertension* 19: 1349–1358, 2001
- Clark CJ, Anderson NH, Farmer R, Friel EC, Fraser R, Connell JM: Alpha-adducin and angiotensin-converting enzyme polymorphisms in essential hypertension. *Hypertension* 36: 990–994, 2000
- Lalouel JM, Rohrwasser A: Power and replication in case-control studies. *Am J Hypertens* 15: 201–205, 2002
- Mohring J, Mohring B, Naumann HJ, Philippi A, Homsy E, Orth H, Dauda G, Kazda S, Gross F: Salt and water balance and renin activity in renal hypertension of rats. *Am J Physiol* 228: 1847– 1855, 1975
- 84. Castellano M, Barlassina C, Rossi F, Perani C, Giacche' F, Rivadossi F, Muiesan ML, Beschi M, Tizzoni L, Cusi D, Bianchi G, Agabiti-Rosei E: Age-dependency of alpha-adducin polymorphism modulation of blood pressure [Abstract]. Presented at IX Scientific Meeting of the International Society of Hypertension and XII European Meeting on Hypertension. Prague, June 23–27, 2002
- Manalich R, Reyes L, Herrera M, Melendi C, Fundora I: Relationship between wight at birth and the number and size of renal glomeruli in humans: A histomorphometric study. *Kidney Int* 58: 770–773, 2000
- Quais Al-Awqati: Molecular and cellular events during kidney development. In: *Textbook of Nephrology*, 4th edition, edited by Massry SG, Glassock RJ, Philadelphia, Lippincott, Williams and Wilkins, 2001, pp 9–13
- Guron G, Friberg P: An intact renin-angiotensin system is a prerequisite for normal renal development. *J Hypertens* 18: 123– 137, 2000
- Neri Serneri GG, Boddi M, Coppo M, Chechi T, Zarone N, Martelli M, Poggesi L, Margheri M, Simonetti I: Evidence for the existence of a functional cardiac renin-angiotensin system in humans. *Circulation* 94: 1886–1893, 1996
- Chadwick IG, O'Toole L, Morice AH, Yeo WW, Jackson PR, Ramsay LE: Pressor and hormonal responses to angiotensin I infusion in healthy subjects of different angiotensin-converting enzyme genotypes. J Cardiovasc Pharmacol 29: 485–489, 1997
- 90. Van Dijk MA, Kroon I, Kamper AM, Boomsma F, Danser AH, Chang PC: The angiotensin-converting enzyme gene polymorphism and responses to angiotensin and bradykinin in the human forearm. *J Cardiovasc Pharmacol* 35: 484–490, 2000
- Van der Kleij FGH, de Jong P, Henning R, de Zeeuw D, Navis
 G: Enhanced response of blood pressure, renal function and

aldosterone to angiotensin I in DD genotype are blunted by low sodium intake. *J Am Soc Nephrol* 2002, in press

- 92. Duboule D, Wilkins AS: The evolution of "bricolage". *Trends Genet* 14: 54–59, 1998
- Glorioso N Filigheddu F, Cusi D, Troffa C, Conti M, Natalizio M, Argiolas G, Barlassina C, Bianchi G: Alpha-adducin 460Trp allele is associated with erythrocyte Na transport rate in North Sardinian primary hypertensives. *Hypertension* 39: 357–362, 2002
- Sharma AM: The thrifty-genotype hypothesis and its implications for the study of complex genetic disorders in man. J Mol Med 76: 568–571, 1998
- 95. Neel JV: Diabetes mellitus: A thrifty genotype rendered detrimental by progress? *Am J Hum Genet* 14: 353–362, 1962
- 96. Ferrari P, Bianchi G: Genetic mapping and tailored antihypertensive therapy. *Cardiovasc Drugs Ther* 14: 387–395, 2000
- 97. Evans W, Relling M: Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science* 286: 487–491, 1999
- Roses A: Pharmacoghenetics and the practice of medicine. Nature 405: 857–865, 2000
- McCarthy J, Hilfiker R: The use of singlenucleotide polymorphism maps in pharmacogenomics. *Nat Biotechnol* 18: 505–508, 2000