Biochimica et Biophysica Acta 1802 (2010) 1285–1298

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Genetics of primary hypertension: The clinical impact of adducin polymorphisms

Lorena Citterio, Chiara Lanzani, Paolo Manunta, Giuseppe Bianchi *

Nephrology, Dialysis and Hypertension, San Raffaele Scientific Institute, and Chair of Nephrology, Università "Vita-Salute" San Raffaele, Milan, Italy

ARTICLE INFO

Article history:
Received 15 January 2010
Received in revised form 26 March 2010
Accepted 30 March 2010
Available online 8 April 2010

Keywords:
Complex disease
Hypertension phase
Polymorphism
Network

ABSTRACT

The usefulness of the results so far published on genetics of primary hypertension for establishing the clinical impact of candidate gene polymorphisms is weakened by the scanty information regarding: a) the functional effect of the gene variants of interest in humans; b) the regulatory genetic network (RGN) where the gene is operating with all the interacting environmental–biological factors and the respective hierarchical organization; c) the consistency between the natural history of the established pathophysiological mechanisms underlying hypertension and the new molecular mechanism detected with genetics; d) the limitations regarding the translation of animal data to human due to the differences among species of the genetic molecular mechanisms underlying similar organ function changes in the different species. Of course, not all these information are available for adducin polymorphisms. In this review, being aware of their importance, the evaluation of the clinical impact of adducin has been focused on data obtained together with the interacting genetic-environmental or biological factors. Adducin polymorphisms and endogenous ouabain (EO) were detected by a top-down approach in rodents after having demonstrated, at cellular and kidney level, that an increase in tubular Na reabsorption could underlies the transition from normotension to hypertension both in rodents and humans. Therefore, we hypothesized that adducin polymorphisms and EO may operate within the triggering RGN that initiates the increase in blood pressure in both species. The distinction between triggering RGN and the secondary RGN is important both to limit the level of genetic complexity arising from secondary changes, and to detect the molecular target to develop tailored therapeutic approach. The pharmacogenomic approach, both in rodents or humans, with newly discovered and never treated hypertension, may be useful to strengthen the "causation" of genetic mechanism. Mutant adducin increases tubular reabsorption: diuretics, because of their effect on overall tubular reabsorption, or rostafuroxin, because of its selective inhibition of the adducin and ouabain effects, may be used for this purpose. Indeed the pharmacogenomic approach with both drugs have provided data consistent with the role of adducin and EO. Taken together, all these findings indicate a clear impact of adducin polymorphism and EO in a subset of patients when the appropriate environmental, biological or genetic context is taken into account. The size of this impact is variable and affected by the context.

1. Introduction

In spite of the considerable technological progress in studying the relationship between DNA variations and cellular or whole organism phenotypes in experimental settings, the estimation of the clinical impact of specific molecular mechanisms in complex human diseases remains the major challenge [1,2]. After 20 years of research on candidate genes in primary arterial hypertension a consensus on their role has not been reached yet [3,4]. On the other hand, the whole genome scanning is producing convincing and replicable data supporting the involvement of some SNPs, but the effect size of these SNPs (or the portion of the hereditability that they are able to explain) is very modest (0.5–3.3 mmHg) [5–9]. Most of the association studies data so far published on adducin polymorphisms have the same limitations [10].

Genetic heterogeneity (the same phenotype may be produced by different genetic mechanisms) and epistasis (modulation by other genes of the effect of a particular gene) taken together with other

Abbrevations: EO, endogenous ouabain; RGN, regulatory genetic network; CV, cardiovascular; BP, blood pressure; RAAS, renin–angiotensin–aldosterone system; MNS, Milan Normotensive Rats; MHS, Milan Hypertensive Rats; ADD1, adducin 1; ADD2, adducin 2; ADD3, adducin 3; WNK1, WNK lysine deficient protein kinase 1; NEDD4L, neural precursor cell expressed, developmentally down-regulated 4-like; QTDT, quantitative transmission disequilibrium test; ACE, angiotensin-converting enzyme; SEM, structural equation modelling; NPPA, natriuretic peptide precursor A; GNB3, beta polypeptide 3; G protein, guanine nucleotide binding protein; SCNN1A, sodium channel, nonvoltage-gated 1 alpha; AGT, angiotensinogen; IgA, immunoglobulin class A; EPOGH, European project on genes in hypertension; CYP11B2, aldosterone synthase; AGTR1, angiotensin II receptor, type 1; PRA, plasma renin activity; CHD, coronary heart disease; IHD, ischemic heart disease; MI, myocardial infarction; CIMT, carotid intima-media thickness; HCTZ, hydrochlorothiazide; NSAID, nonsteroidal anti-inflammatory drugs

* Corresponding author. San Raffaele Scientific Institute, Division of Nephrology and Dialysis, Università "Vita-Salute" San Raffaele, Chair of Nephrology, Via Olgettina 60, 20132 Milan, Italy. Tel.: +39 0226435330; fax: +39 0226432384.
E-mail address: bianchi.giuseppe@hsr.it (G. Bianchi).

0925-4439/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2010.03.014

confounders weaken the significance of association studies. This complexity is also involved in human monogenic disease [11,12]. The same gene variant within the same family tree may be associated to a mild clinical symptoms at adult age or to a devastating disease at young age [11,12]. Certainly, the effect of the gene modifiers is magnified in polygenic multifactorial disease like hypertension. The tendency to increase the size of the sample to reach an adequate statistical power cannot replace the requirements arising from the facts mentioned above. The replications of findings across different populations are certainly important; however, the lack of knowledge on the context difference among these populations limits the validity of this important criterion. Even in a typical monogenic disease like phenylketonuria the clinical symptoms are related to the amount of phenylanine in the diet.

The contemporary genetics is developing the concept of genetic–biological–environmental network to properly handle this genetic complexity [2,11–17]. Many convincing data have been so for obtained in experimental settings [18,19]. However, the translation of the animal data to human clinical symptoms requires the assessment of possible similarities or differences among the regulatory genetic networks (RGN) across species [20]. On the other hand the animal model of complex diseases is indispensable to detect the regulatory RGN by the top-down approach with the appropriate genetic manipulation, protein–protein or protein–tissue interactions. Four groups of problems hamper the capacity of the current genetics strategies to provide appropriate data to improve the present clinical approaches to primary hypertension (in terms of etiopathogenesis, diagnosis therapy and cardiovascular (CV) risk assessment).

1.1. Regulatory genetic networks (RGN) across species and within species: The comparison with the developmental biology

Recent data on single or double gene inactivation effects revealed important RGN differences across species [21,22]. Single gene inactivation seems to produce more consistent effects across species implying similar gene function [21]. Conversely, double inactivation unmasks much larger differences across species [21]. The most likely explanation for these data is that the network rearrangement after inactivation of one gene occurs throughout molecular mechanism pathways that differ among species. However, some of these double inactivation effects are well conserved across species that separated by 1 billion years of evolution [22,23]. Therefore, there are well conserved networks (core networks) as well as species-specific RGNs. The development of a given level of blood pressure (BP) may be assimilated to the development of a given organ function. In fact in both settings the cooperation among a variety of cell lineages, hormones, nervous signals, load and environment is needed to achieve a specific level of organ structure and function or BP. Consequently, the bulk of knowledge on the RGN underlying organ development may help to understand the RGN in hypertension.

The concept of organ homology across species [24], that is, similarities of organ function and structures across species, may also be applied to the approaches aimed at establishing similarities or differences among RGNs regulating BP across species. Developmental studies have clearly established that the same organ, structure and function existing between two species may be achieved by species-specific molecular mechanisms [25,26]. Because of the variety of pleiotropic functional effect of each gene product (or node within the RGN) the gene function used by a particular organ RGN may differ across species. It is the peculiar molecular context of the tissue at the given time of its development that determines the connection among the single nodes within the RGN [26]. This implies that both structure and function of each gene product or node must be assessed in each species to draw conclusions about molecular comparison between two species. Within developmental RGNs there are two types of nodes (or gene products) [26]. A first type can be inactivated without functional alterations, because they are nodes with low level of connectivity with other nodes. Conversely, the inactivation of the second types of nodes, that have a much higher level of connectivity with other nodes, produces much greater functional consequences. In other words, the functions of the first type of nodes may be buffered by the RGN, while the functions of the second type are not easily buffered by the RGN. When perturbed by mutations, these latter nodes, also called "hub proteins," may produce a shift or change in the function of RGN. In the literature the genetic molecular findings in rodents are frequently translated to humans without considering the problems illustrated above. The combination between genetic structure and function is emerging as the most appropriate approach to unravel the key protein function (or hubs) that determines the overall RGN function.

1.2. Pathophysiological data

1.2.1. Plasma renin and blood pressure level

In the past, the controversy on the role of plasma renin in the regulation of BP was fostered by contrasting levels of BP associated with high plasma renin levels. In fact, high renin levels may be associated with hypotension, normotension and hypertension. Therefore, the conclusion was: renin could not be involved in the regulation of BP[27]. The progress of our knowledge on the variety of factors involved in the BP regulation, and, especially, the availability of the selective inhibitors of the renin–angiotensin–aldosterone system (RAAS) led us to have a more balanced and correct view on the role of the RAAS [28,29]. This role must be assessed within the framework or factors involved in the regulation of body sodium (Na intake and renal Na excretion), body fluids and other CV functions (cardiac output, peripheral resistance) or tissue production of the various RAAS components [30,31]. The same factors affecting the plasma renin–blood pressure relationship must also be involved in the relationship between RAAS gene variants and BP level. The evaluation of the clinical impact of any candidate gene must be carried out within the appropriate context of the factors, detected with 50 years of pathophysiological studies.

1.2.2. Phases of hypertension

The hypertension triggered by the reduction of renal blood flow (renal artery constriction) is sustained by a sequence of mechanisms that start with renal secretion of renin, followed by renal Na retention and ending with peripheral vascular autoregulation or vascular remodelling. At this phase structural vascular or organ changes may take over the initial functional changes [32–40]. Regardless of this variety of mechanisms, hypertension may be reverted by removing the renal artery stenosis [41,42]. However, if this manoeuvre is carried out when the vascular structural changes or renal damage reach a critical level that confers to them the ability to maintain hypertension, its effect on BP is negligible [43]. The time (or phase)-dependent changes of the various mechanisms supporting hypertension occur in spite of the fact that a "triggering" mechanism, the renal artery clip maintains the same degree of artery constriction. The same phase-dependent changes of the various hypertensive mechanisms occur in rodents with genetic hypertension [44]. Also in humans, with primary hypertension, renovascular hypertension or monogenic forms of hypertension there are data supporting the phase-dependent variations of the mechanisms underlying hypertension or organ damages [45–47]. Therefore, in order to correctly evaluate the genotype-intermediate phenotype relationship, the phase-dependent changes illustrated above must be take into consideration the natural history of hypertension (both the initial triggering mechanism and its secondary ones) [32,45–47].

1.3. Integration between genetic and pathophysiological clinical data

In monogenic forms of hypertension we may have different types of RGNs. All these forms involve the ability of the kidney to handle Na and water [48] either as a renal constitutive change (renal RGN) [49] or secondary kidney change triggered by an increased production of adrenal endogenous substances, aldosterone or other mineral

corticoids hormones (adrenal RGN), affecting this renal function [50]. In these hypertensive forms, the characteristic of being "triggering" RGN is conferred by a highly penetrant genetic mutation affecting either Na channel endocytosis [49] (Liddle syndrome) or the steroid synthetic pathway [48]. This latter alteration, with or without the neoplastic growth, is responsible for a variety of adrenal forms of hypertension. In the past, through pathophysiological and genetic tools, the triggering RGNs (or gene variants or hormones) were identified and provided the rationale for a "causal antihypertensive therapy" (spironolactone or eplerenone) for the hyperaldosteronism (within the mineral corticoids forms of hypertension) or amiloride for the Liddle syndrome. It is important to realize that also the triggering RGN approach implies that the same gene variant may produce hypertension across a variety of combinations with other gene variants according to the peculiar individual RGN in which the gene of interest must interact within the different tissues or species. A gene variant favouring Na transport in the renal ascending limb may be a primary cause of hypertension if it is associated to some of the following different abnormalities regarding both:

a) a compensatory mechanism such as a decrease of Na reabsorption in the more distal part of the nephron or inhibition of the tubulo-glomerular feed back mechanism [51] that, in turn, may have a variety of molecular mechanisms [52]: 1) Na and Ca transport systems across the luminal membrane of the macula densa; 2) renin release [53]; 3) afferent arteriolar tone etc;
b) the mechanisms regulating the relationship between extracellular volume and BP. For instance, a genetic mechanism that activates the Na–K pump at the basolateral membrane of tubular cell may favour Na retention and expansion of body Na. But this alteration should be coupled with a mechanism that prevent vascular dilatation at the kidneys or at the whole organism levels or even increase vascular tone. The combination of these two types of mechanisms may represent a triggering RGN [54]. Mutated adducin and endogenous ouabain (EO) may well trigger hypertension throughout a combination of renal and vascular effect (see below).

As suggested by the genetics of organ developmental studies a specific genetic variant may also influence more than one of these individual molecular mechanisms [26] (pleiotropic effect) in such a way to modulate their actions towards an increase of BP. Therefore, from the pathophysiological perspective, the RGN may be composed by a complex and variable set of gene variants that may change from a subset of patients to another one. Most of the RGN approaches have been developed with single pathological tissues in human or in others species. Pathophysiological data discussed above demonstrate the cooperation among different tissues (kidney, adrenal, vessels, etc.) during the initial phase. Therefore, the concept of triggering RGN beside the protein–protein interaction within a defined tissue may also include the interactions among relevant molecular mechanisms from different tissues. Again, similarities with the RGN underlying organ development may be found: the statement "complex traits are spatio-temporal collectives of multiple interacting gene products" [55] summarizing the contemporary RGN approach to organ development, may also be applied to arterial hypertension. For RGNs involved in hypertension we should only change spatio-temporal with organ-temporal.

1.4. Translation of animal data to human

As it has been suggested for the organ development, also for the genetics of phase-dependent hypertension illustrated above, we may hypothesize that the RGNs are hierarchical [24,54]. There may be a portion of "core" or "triggering" RGNs controlling the initial stages of hypertension that is at the top of the hierarchy. These RGNs may be considered also as the core triggering RGNs present in all species (like the renal artery constriction that triggers hypertension across different species including human). Then we may have a portion of "secondary" RGNs controlling intermediate phenotypes (renin, body sodium, Na+ intake, etc.) staying in the middle. Finally, we have a portion of RGNs controlling the detailed functions of cells in different organs that are at the periphery or at the bottom of the hierarchy. These RGNs may be involved in vascular remodelling and organ damage. Moving from the top (core or triggering RGNs) to the bottom RGNs, the differences across species may increase. Therefore, translational studies should be more efficient and productive if developed at the level of a core triggering RGN that can only be detected by studying the initial development of hypertension within a normotensive population or background.

This distinction may have therapeutic implications, as discussed above for renovascular hypertension. The definition of the core or triggering networks is also relevant to detect the molecular targets to develop the most appropriate "causal" therapies. The BP responsiveness to drugs in experimental renovascular hypertension may vary across the different phases [56–60]. RAAS inhibitors are particularly active in the I phase, diuretics or reduction of Na intake is needed in the II phase, while Ca^{2+} antagonists may be needed to lower BP when an important vascular remodelling is present. A drug active on the triggering mechanisms (in this case an hypothetical drug that acts as the removal of artery constriction) may have a more stable and a long-lasting effect provided if it is used before the development of severe CV or renal secondary change. Moreover, with this hypothetical drug, the compensatory mechanisms trying to bring the BP to the original high level are less likely to occur [61]. It is noteworthy that J.O. Davis [40] in his review on renovascular hypertension defines this condition "a multifactorial disease" because of the many mechanisms triggered by the clip on the renal artery. However, the removal of the clip is followed by "normalization" of BP and all the other factors, if this manoeuvre is carried out before the appearance of overt organ damage. Therefore, this effect of unclipping may support a definition of a "monofactorial disease" cured by a single manoeuvre. Similarly, the inhibition of initial triggering network in the appropriate subset of new discovered hypertensive patients may block the effects of many secondary genetic changes, resulting in long-lasting normalization of BP. Also, in two other forms of hypertension where the primary cause is known such as primary hyperaldosteronism or Liddle syndrome, the clinical practice has demonstrated that the specific drug spironolactone or amiloride, are able to normalize BP only when administrated before the development of secondary organ changes. On the contrary, these drugs would be less effective if administered in patients with long-lasting hypertension (O. Melander, personal communication).

The Mendelian randomization has been proposed [62–64] to strengthen the detection of a causal relationship between gene variations, intermediate phenotypes and the associated organ damages, including the effects of drugs. This approach is certainly appropriate for those intermediate phenotypes, such as plasma levels of cholesterol or lipoproteins that are relatively stable across the different stages of the disease and are more directly related to the genetic abnormalities or mutations [63]. However, the phase-dependent changes in hypertension weaken the scientific validity of any negative finding obtained with Mendelian randomization if the time-course of the disease is not taken into account. Very advanced technological approaches to measure the overall gene variation or statistical sophistication, taken alone, are unable to capture this complexity. The challenge of the genetics of primary hypertension is to detect this core or triggering RGN with its hubs or major effect gene products both in the lab animal model (rodents) and in humans. This may lead to the subdivision of this heterogeneous human condition into defined subsets of patients relatively more homogeneous for the respective core RGN, clinical characteristics (type of organ damage) and long-lasting response to a selective drug hitting the hub nodes within the core triggering RGN. In our view this triggering RGN may provide the compass or "marker" (similar to a

highly penetrant mutation underlying a monogenic disease) helping as to define new nosological clinical entities in this era of molecular medicine.

2. Adducin

2.1. Detection of adducin as a candidate gene

To what extent do the adducin and EO data so far available in rodents and humans may support the hypothesis that they could function as "hub node(s)" within the above described triggering core RGN at work in both species? Clearly, our studies aimed at detecting molecular mechanisms of primary hypertension triggering a transition from normotension to hypertension started many years ago before the genetic era. The various steps of these studies leading to the identification of a genetic mechanism described in detail elsewhere [10] may be summarized as follow:

1) development of a rat model of primary hypertension (the Milan normotensive rats (MNS) and the Milan hypertensive rats (MHS) strains) and assessment of possible similarities or differences between rodents and humans. This assessment was carried out at different levels of biological organisation: whole organisms, kidney function, plasma levels of hormones and cellular function. Only prehypertensive and early hypertensive stages were considered on the "obvious" assumption that these stages are the most appropriate to detect initial pathophysiological triggering mechanisms;
2) these studies demonstrated that the pathophysiological abnormalities responsible for triggering hypertension in rat could also be at work in a subset of patients;
3) the bulk of data were consistent with the hypothesis that a primary abnormality, within the kidney could be the triggering mechanisms. This hypothesis was strengthened by the observation that hypertension may "travel" with the transplanted kidney in both species;
4) moving from the rat whole kidney function down to nephron segmental function, the data obtained were consistent with the hypothesis that a primary increase in tubular reabsorption (particularly at the proximal level) could be considered as the primary mechanisms;
5) proximal tubular cells of young prehypertensive rats were smaller and had lower Na content and faster membrane Na transport. Similar changes were present in erythrocytes; therefore, a generalized genetic abnormality could be envisaged;
6) through bone marrow transplantation between MHS and MNS rats we demonstrated that these changes of erythrocytes were genetically determined within the stem cells. Therefore, erythrocyte ion transport could be used to assess similarities or differences between humans and rats;
7) the differences in rat erythrocytes ion transport were still present in resealed ghost (still having the membrane actin cytoskeleton) but absent inside vesicles (devoid of the actin of cytoskeleton);
8) actin cytoskeleton from MHS was injected into MNS and vice versa in order to detect immunological differences between the cytoskeleton proteins at the two strains;
9) an antibody towards a cytoskeleton protein was detected and used to screen a DNA library. Adducin was detected in this way.

Therefore, adducin was detected after a long series of studies focused on the triggering pathophysiological mechanisms at work in rats and humans.

Certainly, this strategy differs from that used to develop the contemporary concept of RGN. However, the nine groups of data mentioned above provide the theoretical and experimental support to the notion that adducin polymorphism is involved in a core triggering RGN. The mutations in rodent and human adducin occurred at different position in the two species [10] (see Fig. 1). It has been previously shown that the different lactase gene alleles in African population of different ancestries have similar function [65]. Similarly, mutated adducin of both species produce similar modifications of the protein functions, that is, both stimulate renal Na transport by activating the Na–K pump on the basolateral membrane [10] and produce a greater activation of the Na–K pump-dependent signal transduction (M. Ferrandi and P. Ferrari, personal communication). Simultaneously, studies on EO (see also the chapter of P. Manunta) revealed very interesting interrelationships with the adducin polymorphism. Ouabain modulates the Na–K pump activity in a concentration-dependent manner [66,67]: activating the renal Na–K pump at subnanomolar concentrations, leading to increased renal Na reabsorption, or inhibiting it at slightly higher concentrations, thus favouring renal Na excretion. Moreover, this low concentration EO is also able to increase the vascular tone [68].

Higher levels of plasma EO potentiate the difference in the pressor response to saline infusion between carriers of mutated Trp allele in Adducin 1 (ADD1) gene and in carriers of the wild type allele (rs4961) [69]. This action occurs through a vascular effect because no modification of renal Na excretion was detected. The molecular basis of this vascular effect is not entirely known even though the Na–K pump activity modification is the likely mechanism. From the pathophysiological viewpoint, the relationship between EO and adducin resembles the one between aldosterone and Na channel on the luminal membrane. Both the hormones may adjust the function of the constitutive capacity of tubular cells to transport Na according to the body need. The renal target of aldosterone is the Na channel, while the renal target of EO is the Na–K pump that, in turn, is modulated by adducin polymorphism. Either mutated adducin or EO may be important components of a core triggering RGN at work in the subset of patients carrying the appropriate mutations of the genes coding for adducin or for the enzyme involved in EO synthesis transport or excretion affecting the renal Na transport and vascular tone. This hypothesis is markedly reinforced by the demonstration that the selective inhibitors of these two mechanisms in rodents also normalize BP in the subset of patients carrying the mutations mentioned above (M. Ferrandi, P. Ferrari and G. Valentini, manuscript in preparation).

The results so far available on the relationship between adducin polymorphisms and the various human phenotypes, somehow connected to hypertension and its CV complications, have been collected as follow:

a) association studies between ADD1 or adducin 2 (ADD2) genes alone and presence or absence of hypertension or BP levels subdivided according to the most studied populations: Caucasian, Chinese and Japanese;
b) studies considering interactions between ADD1 and ADD2 or adducin 3 (ADD3) genes or between ADD1 and RAAS genes or other genes coding for protein regulating body Na or increasing vascular tone, such EO and WNK lysine deficient protein kinase 1 (WNK1), neural precursor cell expressed, developmentally down-regulated 4-like (NEDD4L) genes [70]. The interactions among ADD1, ADD2 and ADD3 have to be studied because the genes coding for these proteins map on different chromosomes, but the proteins are working together within the cells as dimer or tetramer (ADD1/ADD2 or ADD1/ADD3). Because of these biochemical characteristics, a genetic interaction among the adducin loci provides an additional argument to support the link between the adducin polymorphisms and the phenotype of interest. Adducin polymorphism affects the constitutive capacity of renal tubular cell to reabsorb Na, thus it influences BP throughout variations on total body sodium [10,71–74]. Therefore, beside the Na intake, any evaluation of the effect of adducin polymorphism on BP must also consider the polymorphism of genes coding for the proteins involved in the activity of the RAAS, EO or other proteins involved in the regulation of renal Na handling or vascular

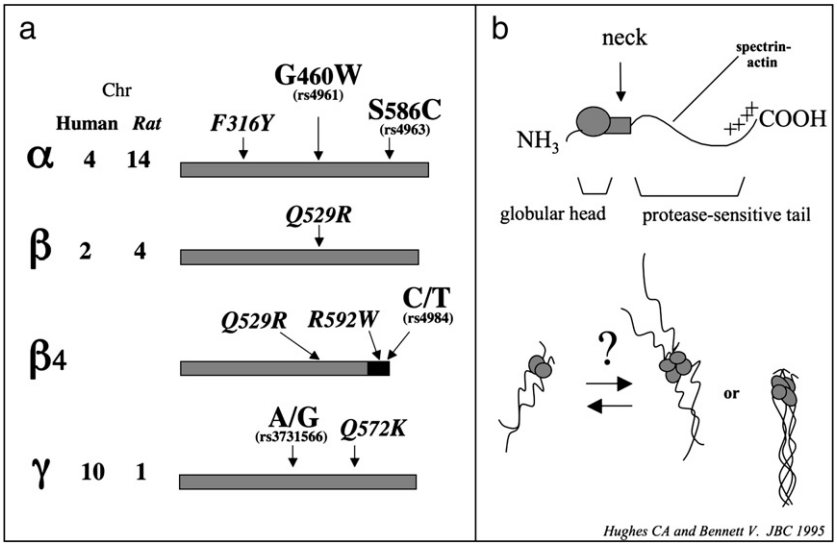

Fig. 1. ADD1, ADD2, ADD3 gene structure and relative adducin protein structure. a) Adducin genes are indicated by boxes; black filled box indicates alternative spliced exons in ADD2-β4 isoform. The position of SNPs is shown by the arrows with the SNP coordinates based on the aminoacid change for only missense mutations. The human SNPs are typed in regular and also report dbSNP ID in brackets while the rat ones are in italics. The human and rat chromosome location is reported on the left. b) Single subunit and dimer/tetramer structure of adducin protein. In the cell adducin works as a dimer (ADD1/ADD2 or ADD1/ADD3) or as a tetramer. Therefore, this provides the biochemical base for the genetic interaction among the various adducin loci.

tone. In fact, more than 30 years of pathophysiological studies have demonstrated the importance of the interaction between body Na and RAAS in the regulation of BP and CV complications.

c) the combination of the genotyping data with the BP lowering effect of diuretics or of another drug that selectively affects mutated adducin or EO effects has also be studied. These pharmacogenomic approaches not only may provide useful information for administering the right drug to the right patients but may also provide a strong additional argument to support the clinical impact of these genetic mechanisms.

In the article published in 2005 [10], the various aspects of the adducin polymorphisms spanning from basic science to clinical application were reviewed. The present review discusses the results from clinical studies published during the last 4 years.

2.2. Association studies in Asian populations

According to a PubMed search covering the period from our review article published in 2005 [10] to the end of 2009, 59 additional articles have been published on the role of adducins in various clinical setting. However, considering the studies measuring only the ADD1 Trp allele frequency in normotensives and hypertensives (association study) the proportion of positive and negative findings were not different from that reported in 2005. Therefore, it may be concluded that mixed results are obtained when the frequency ADD1 Trp allele is considered alone. The many flaws of these studies (in terms of definition of normotension, collection of data, admixture of confounding factors), taken together with the lack of a proper definition of the context, weaken the scientific validity of these data. Already in 1999 [75] we demonstrated that the frequency of the ADD1 Trp allele in hypertensives could be either higher or similar to that of normotensives in two Italian populations. Also the arguments, developed in the previous section, clearly suggest that only the findings at b) and c) are relevant to evaluate the clinical impact of adducin polymorphisms. However, some comments on the association studies carried out in Asian populations may be of interest because the frequency of the ADD1 Trp allele is almost three times higher compared to Caucasians (48% vs. 17%) (NCBI dbSNP site http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs4961). So far 14 studies have been carried out in the Chinese populations. Three studies [76–78] reported genotyping of DNA markers mapping around the ADD1 locus but separated by many haplotype blocks [10]. Therefore, these negative findings are not relevant to evaluate the role of adducin. Four association studies genotyping the ADD1 Trp allele [79–82] are positive (in one of these studies [81] also the familial quantitative transmission disequilibrium test (QTDT) was positive), two studies [83,84] provided negative data. Other positive studies included one study [85] showing an interaction of the Trp allele with the I/D polymorphism of angiotensin-converting enzyme gene (ACE) on systolic BP and in the family-based QTDT analysis. Other two studies showed an association of the Trp allele with intracerebral hemorrhage [86] or with the magnitude of the BP fall with diuretic [87]. A further study [88] examined different SNPs on the ADD1 gene and detected a very significant SNP (rs17833172) with a rather low minor allele frequency (4%) but not the Trp allele SNP (rs4961). Finally, a study applying a structural equation modelling (SEM) to capture the independent and combined effect of several genotypes (including ADD1) on renal function of 1188 type 2 diabetic patients was recently published [89]. ADD1, natriuretic peptide precursor A (NPPA), guanine nucleotide binding protein (G protein), beta polypeptide 3 (GNB3) and sodium channel, non-voltage-gated 1 alpha (SCNN1A) genes were found to have an effect on BP that influenced renal function.

Ten studies were carried out in Japanese and Korean populations, two [90,91] with negative results and eight with positive data. The latter subset of studies included four studies [92–95] considering only the single ADD1 Trp allele. One study [95] showed an increase risk to develop hypertension in carriers to ADD1 Trp allele. Other studies considered Na excretion [96], low plasma renin levels [97] or interaction with angiotensinogen (AGT) [98] or with ACE I/D on the decay of renal function in immunoglobulin class A (IgA) nephropathy [99]. A recent study [100] examined the possible association with hypertension of a larger number of SNPs. A SNP (rs3755351) on ADD2 reached high level significance that failed to persist after Bonferroni correction. ADD1 polymorphism is not mentioned in this study, likely because it did not reach the threshold level of significance. Unfortunately, the interaction between ADD1 and ADD2 has not been analyzed. It is noteworthy that the two SNPs on ADD2 and ADD3 found in Caucasian population (rs4984 and rs3731566) have not been detected or detected at a very low frequency (ADD2 0.03–0.006) in two Chinese populations [101]. Whether Asian populations have different ADD2 or ADD3 alleles, with

effects similar to those of Caucasian population remains to be established but it is very likely. For instance the different African populations from different ancestry have different lactose alleles with similar function [65]. A recent metaanalysis carried out by Niu et al. [83] in 15 association studies, selected from a total of 91 studies found in PubMed, concluded that there was no difference of the ADD1 Trp allele between hypertensives and normotensives. The criteria of selection of these 15 studies (seven in Caucasians and eight in Asian populations) are not entirely clear. In particular, it is not clear why with so selective criteria, two studies [93,102] have been included among the negative ones, while they were considered by the authors to be the positive. The study of Wang [102] was carried out in a Flemish Caucasian general population. A similar frequency of Trp allele in hypertensives and normotensives was found, but the age of the population ranged from 10 to 84 years. Therefore, most of the normotensives were below the age of 50 years that is usually considered the lowest level of age that allows to exclude the development of hypertension. In the past, we and others used much older levels of age to classify as normotensives the controls used in the association studies to avoid the inclusion of subjects that will develop hypertension later in their life. In this paper the most significant finding was the interaction between ADD1 Trp allele and ADD2 T allele (rs4984) in determining the level of BP and the risk to develop hypertension in women. In the other study [93] the small and not significant difference in the Trp allele between hypertensives and normotensives was accompanied by both a positive association of the ADD1 Trp/Trp genotype with hypertension that remains significant after adjusting for various confounders and in nuclear families a significant transmission of the Trp allele to the affected subjects. As mentioned above, in 1999 [75] we showed that the association study based on the difference in the Trp allele frequencies between hypertensives and normotensives may provide positive or negative results in two different Italian populations. However, gene structure–function interactions measured as the BP response to diuretics in the hypertensives of both populations provided more constant results. Carriers of ADD1 Trp allele, compared to carriers of the ADD1 Gly/Gly wild genotype, have a larger BP response to diuretics in both populations. Since then genetics of complex diseases has made some progress and the limitations of association study based on allele frequency have been widely recognized. Apparently, our [75] and other [103–106] efforts along this direction during the last 10 years were unable to convince some investigators of genetics of hypertension. In summary, according to present knowledge the studies of Asian populations on adducin are all positive when some type of interactions was considered. Studies measuring the difference in frequency of the Trp allele between hypertensives and normotensives yielded a positive result only in 8 out of 12. In evaluating the positive results in these two populations we must also consider the publications bias that it is always difficult to assess. However, these data are strong enough to justify appropriate efforts along the direction we are suggesting in this review to evaluate the contribution of adducin polymorphisms and EO to the RGN triggering hypertension in these populations.

2.3. Genetic studies considering the context (risk to develop hypertension)

In order to limit the confounding effect of the population context and of the various experimental designs, we shall discuss together the results obtained by the Staessen's group in Belgium and by other groups in Netherlands.

The Staessen's group, in collaboration with us, has so far published 16 papers dealing with ADD1 alone or in interaction with the other adducin or RAAS genes or with dietary salt. These studies have been carried out on two populations:

a) the Flemish study consists of a random sample of the households living in a definite area of Northern Belgium followed for several years;
b) the European Project on Genes in Hypertension (EPOGH) study, including an European family-based epidemiological survey involving several European countries.

The very high precision of phenotyping and the attempt to define either the genetic or the environmental contexts are the main characteristic of these studies. Some of these results have been already discussed in the previous review; however, they are recalled here to provide a more complete picture of specific aspects.

2.3.1. Interaction of ADD1 with RAAS genes

In the cross-sectional analysis of the Flemish population [107], the ADD1 Trp allele, per se, or in association with the ACE I/D genotypes has no influence on BP. Conversely, the effects of ADD1 Trp allele effects on BP and on the risk to develop hypertension (follow up study) vary according to the presence of the Aldosterone synthase (CYP11B2) CT + TT or CC genotypes. In the presence of the CT + TT genotypes, the ADD1 Trp allele is associated with a decrease in systolic–diastolic BP and with the smallest relative risk to develop hypertension compared with the whole population. On the contrary, this risk was raised in carriers of CYP11B2 TT genotypes, while CYP11B2 genotypes taken alone have no effect. In carriers of ACE DD compared to the other two ACE genotypes the incidence of hypertension is increased. However, this ACE DD effect is confined to carriers of ADD1 Trp allele or CYP11B2 CC genotype. The positive predictive value of the combination of these three genotypes is 40.1%. Carriers of ACE DD and ADD1 Trp allele tend to have an high 24 h urinary Na excretion (196 ± 8 mmol/day) compared with the carriers of the other genotypes (176 ± 5 mmol/day) $p = 0.03$. This implies a difference in Na intake. Parallel renal function studies in the Flemish population [108] (serum creatinine and urinary protein excretion in cross-sectional and follow up studies) provided consistent results with the development of hypertension. In fact the faster decline in renal function with age (with increased urine protein excretion) occurred in carriers of both the ACE D allele and ADD1 Trp alleles. The interaction between ACE and CYP11B2 on renal function was much less clear than that between ACE and ADD1. A very similar study [85] was carried out in a Han Chinese population involving 479 participants [108]. This study confirmed that in the carriers of the ACE D allele and ADD1 Trp genotypes, the level of BP was higher than in the carriers of other genotypes. This interaction between ACE and ADD1 was also present in family-based QTDT analysis. Only in the adducin Trp allele homozygotes offspring, the transmission of the ACE D allele was associated to an increase in BP. Moreover, only in the presence of the ADD1 Trp allele a significant interaction was found between the ACE genotype and urinary Na excretion in relation to plasma renin activity.

The influence of Trp allele on the arterial properties (femoral, carotid and brachial) of the Flemish population was also studied [109–112] both at a single gene level and in interaction with ACE I/D or AGT or angiotensin II receptor, type 1 (AGTR1). Again single gene effects were absent for ADD1 and CYP11B2 but present (although very modest on intima-media thickness) with ACE I/D only in femoral artery, but not in carotid artery. This effect was confined to carriers of the ADD1 Trp allele or the CYP11B2 T allele [109]. Family QTDT studies provided similar results. Cross-sectional compliance of carotid artery but not that of femoral artery decreased (compared with the population means) with the number of ACE D alleles [109]. No effect of ADD1 and CYP11B2 genotypes was detected on cross-sectional compliance and distensibility of carotid and femoral artery. Compared with the population mean, carriers of the ACE DD showed a decrease of cross-sectional compliance and distensibility (but not of diameters) of femoral artery in ADD1 Gly/Gly genotype. Conversely, in ACE DD carriers the distensibility coefficient and the diameter of the carotid artery were lower or higher respectively compared with the population mean. Interaction between ADD1 Trp and AGT C532T/G-6A or AGTR1 A1166C, including also brachial artery [111], provided not significant results in single gene analysis with the exception of a slightly larger brachial diameter in

AGTR1 C allele carriers compared to AA homozygotes. Conversely, the higher femoral cross-sectional compliance and distensibility in AGTR1 C allele carriers were confined only to Trp carriers. This effect on femoral distensibility was also found in family-based QTDT. A similar trend (not statistically significant, $p=0.11$) was detected for femoral cross-sectional compliance. The AGT polymorphism alone affects brachial diameter and distensibility but not femoral or carotid properties and does not interact with ADD1 on any of the three arteries [111].

2.3.2. Interaction between ADD1 and ADD3

ADD3 genotypes alone affect brachial diameter, distensibility and cross-sectional compliance, the first is lower and the other two are higher in ADD3 IVS11+386 GG vs. AA (rs3731566). This effect is confined to ADD1 Gly/Gly carriers and is blunted in ADD1 Trp allele carriers [111]. No genetic effect was detected on femoral or carotid artery by ADD3 or by ADD1 and ADD3 on the three arteries. The effect on brachial artery of ADD3 polymorphism was confirmed with family-based QTDT [111]. Below the age of 50 years the cardiac lateral Ea and the Ea/Aa ratio (indexes of diastolic relaxation) were higher in ADD1 Trp allele carriers particularly in presence of the ADD3 GG homozygotes [113]. In older subjects these associations were not significant. The transmission of the Trp allele to offspring (family-based QTDT) was associated with higher lateral Ea/Aa ratio [113] in ADD3 GG carriers. The interaction between ADD1 and ADD3, in EPOGH [114] included three populations (Belgium, Czech Republic and Poland) that were pooled together because no heterogeneity across countries was found. No effect of the single gene was found, but only in carriers of the ADD1 Trp allele the ADD3 GG carriers have an increased peripheral and central pressure and a tendency to a lower urinary aldosterone excretion. Conversely, in the ADD1 Gly/Gly carriers no influence of ADD3 genotypes in BP was detected, but subjects with ADD3 GG genotype have a lower urinary Aldosterone. Family study confirmed the effect of ADD3 GG genotype. This increase in diastolic relaxation detected only at a relatively young age may be consistent with an expansion of blood volume at the initial stage of a type of hypertension triggered by renal Na retention. In fact the slope of the pressure-natriuresis curve after saline infusion is consistent with increased renal sodium retention in carriers of ADD1 Trp and ADD3 GG genotypes (C. Lanzani and P. Manunta, personal communication). However, other mechanisms are possible. No interaction between ADD1 and ADD2 on pulse pressure was detected.

2.3.3. Interaction between ADD1 and ADD2

In women only the ADD2 1797T (rs4984) allele was associated with an increased risk to develop hypertension in Flemish population. This was confined to postmenopausal women and users of oral contraceptives. In men the plasma renin activity (PRA) and 24 h aldosterone values were lower in ADD2 T allele carriers. There is a significant interaction with ADD1 Trp allele: the above mentioned effect of ADD2 T allele was confined to women carrying the ADD1 Trp allele [102]. In the EPOGH study the Na excretion across the different European countries was included in the analysis of the relationship between adducin (or other genes) polymorphism and the phenotypes mentioned above [115]. Slavic population with high urinary Na excretion compared to Italian population, have a lower frequency of the ADD2 T allele. However, the association between this allele and 24 h systolic BP in the Slavic was not found in the Italian populations [116,117]. In both populations renal Na excretion was lower in T allele carriers than in the wild type allele carriers (CC homozygotes), implying some influence of the T allele on Na intake. The effect of the T allele on BP and Na excretion was confirmed by family-based QTDT.

2.3.4. Interaction between adducin and endogenous ouabain (EO)

A long list of experimental and clinical data supports the notion that EO may affect BP and renal Na excretion through the modulation of the Na–K pump either as a Na transport system or a signal transduction triggering mechanism in renal tubular cell or in vascular smooth muscle cells [66–69,118]. Mutated adducin affects these two functions of Na–K ATPase. Therefore, it is reasonable to study the interaction between adducin and EO. When the population of never treated and newly discovered hypertensive patients were subdivided into two subgroups, i.e. above and below the median values of plasma EO levels, only in the former subgroup a clear cut difference in the BP response to i.v. saline load was found between mutated ADD1 Trp allele carriers and wild type Gly/Gly ADD1 carriers ($+6.75\pm1.23$ mm Hg and $+1.61\pm0.88$ mm Hg, respectively) [69]. In presence of the mutated ADD1 Trp allele the plasma EO apparently increased in a predominantly normotensive population [119] or unchanged in new discovered and never treated essential hypertensive patients [69]. The reason for this discrepancy is not clear. Certainly, these interrelationship between EO and mutated adducin both at the Na–K pump and at the whole organism level are consistent with the notion that these two mechanisms are involved in the body sodium and BP regulation mainly throughout the modulation of the renal and vascular Na–K pump as mentioned above.

In the general population the ADD1 Trp allele does not affect the CV risk. However, when the level of BP is considered, this allele increases the CV risk in hypertensives and decreases the risk in normotensives [120] (Fig. 2). It is evident that the clinical symptoms associated to the mutated allele can be either on the right or on the left of the wild type carriers as shown in Fig. 3. Therefore, the classical genetic paradigm developed in monogenic diseases must be revisited for polygenic multifactorial diseases, where the same “culprit” allele also may be protective in a peculiar context.

When in the analysis the Trp allele effect is combined with that of the ACE DD genotype [121] an increase in CV risk is detected also in the general population. The demonstration of a higher membrane-bound ACE activity in fibroblasts removed from carriers of the ADD1 Trp allele may provide the cellular base of the association between ACE DD genotypes and ADD1 Trp allele on CV risk [121].

The attempt to accommodate the findings of the Staessen group with other previous findings within a comprehensive scientific is hampered by the difficulties to handle two layers of complexity:

1) the complexity of the pathophysiological data so far accumulated on the mechanisms triggering and sustaining hypertension in the different phases of hypertension, with the interrelationship among different tissues and regulatory systems;
2) the complexity underlying the cellular biochemical events at the single tissue level affected by the genetic mechanisms considered in these studies that may vary among the various type of cells (vascular, tubular or cardiac cells) involved in the core triggering RGN.

With these provisos, the Staessen clinical and epidemiological data may be reconciled with previous findings [10] by postulating an initial increase of renal sodium retention promoted by the interacting effects of adducin Trp allele, RAAS and EO. The secondary increase of blood volume and peripheral blood flow, in combination with others factors, particularly the increase in vascular tone (produced by EO), may trigger other structural CV changes on femoral artery and cardiac structure and function (diastolic relaxation). At present, it is not possible to dissect in humans the precise molecular mechanism of these sequential changes in each type of artery. Either physical factors (volume, blood flow, hydrostatic pressure, stretching etc.) or biochemical events (signal transduction or other humoral factors) may be involved.

In our opinion, the most clinically relevant contribution concerns the risk of hypertension and organ damage assessed within a defined epidemiological context. The increased CV risk may be the consequence of either an increase of body sodium [122,123] or of BP which, per se, may lead to organ damage [124]. Also the ability of RAAS, EO or

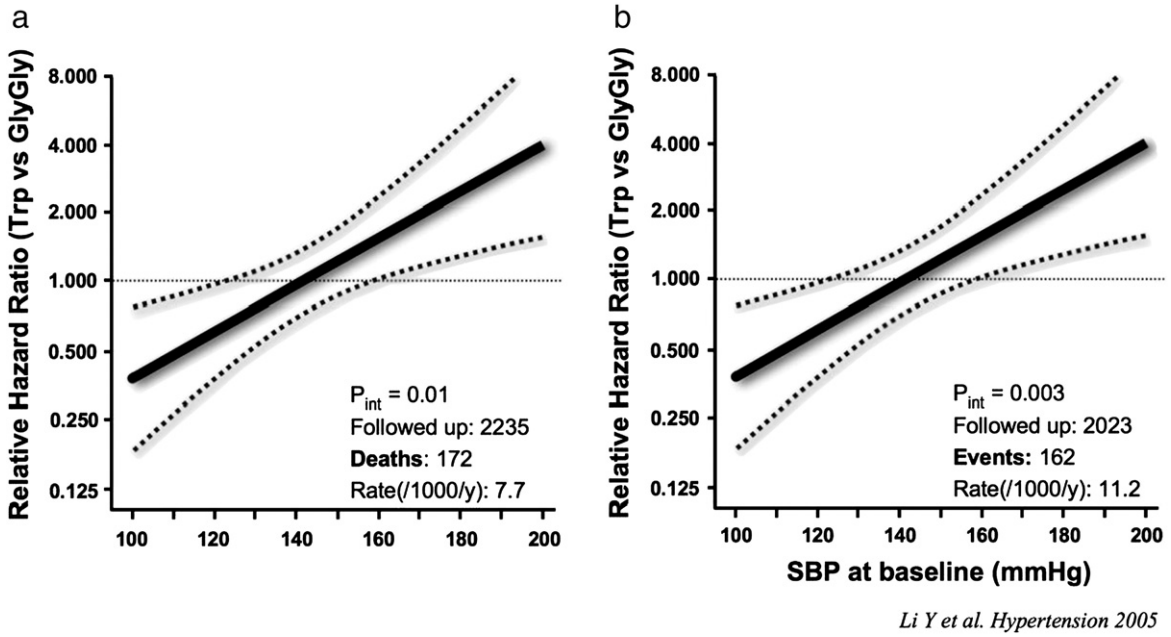

Fig. 2. Hazard ratios for total mortality (a) and all CV complications (b) in ADD1 Trp allele carriers relative to Gly/Gly homozygotes as a function of systolic BP at baseline. The continuous risk function with 95% confidence limits and the *P* values for interaction (P_{int}) were computed by multiple Cox regression.

ADD1 polymorphism to trigger a series of cellular changes in signal transduction and ion transport may favour both vascular remodelling [66,67,125] (M. Ferrandi, manuscript in preparation) and cardiac diastolic relaxation. In this respect, differences among populations or among subgroups of patients may be anticipated rather than surprising. Molecular and statistical sophistication are not adequate to cope with this complexity without the appropriate data.

2.4. Adducin polymorphisms and cardiovascular risk

A total of 14 articles discuss data regarding the possible effect of adducin polymorphisms on the CV risk, including stroke, coronary heart disease (CHD), ischemic heart disease (IHD), myocardial infarction (MI). These studies have been carried out in:

a) predominantly, normotensive populations where the development of CV events may or may not be preceded by the development of hypertension;
b) predominantly, normotensive populations with the distinction between the CV events developing in normotensive and those developing in hypertensive patients;
c) studies where subset of patients with and without hypertension and CV events are compared.

Including the articles already mentioned, 10 independent studies [120,126–134] evaluated the CV risk in hypertensives and showed that

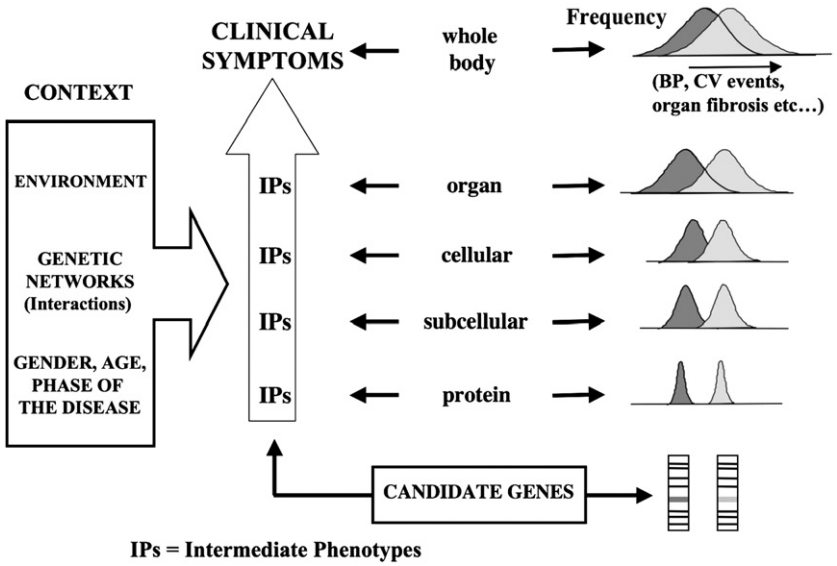

Fig. 3. Flowchart of the interaction of genetic and environmental backgrounds and biological factors on the sequence of events linking a DNA variation to a clinical symptom, mainly derived from monogenic diseases. *Right*: two hypothetical populations carrying the "wild type" (dark grey) and the "mutated" (light grey) gene variant are reported. Because of the progressive increase of the influence of various factors (*left*), the distribution of the values of the two populations tends to overlap as we move from the DNA level to the clinical symptom. Therefore, at this highest level of organization, the separation of the two populations is very modest. As explained in the text, the distribution of the clinical symptoms of carriers of mutant adducin may even be at the left of the distribution observed in homozygotes for the wild type allele. That is, the mutated allele may be "protective" in specific environmental or biological contexts as shown in Fig. 2.

this risk is higher in the Trp ADD1 than in the Gly/Gly ADD1 carriers. This increase of risk (OR or RR) ranged from 1.36 to 6 according to the different population subsets or biological characteristics. It is important to recognize that in the carriers ADD1 Trp allele and ACE DD genotypes one finds:

1) an increased risk to develop hypertension in a general population of Caucasian [107] and Chinese [85];
2) an increased CV risk either of CV events [121] or of arterial modification that may lead to CV events [110];
3) a faster decline of renal function with age associated to increased urinary protein excretion [108].

There are also data showing a protective effect of the Trp ADD1 allele in normotensives. In four studies the Trp allele increased the CV risk in hypertensives but not in normotensives [120,126,127,132].

Four Dutch studies were carried out on a primary care population aimed at assessing CV risk (Hippocrates Project). Two studies [135,136] addressed the relationship between ADD1 Gly/Trp polymorphism or other genes on arterial stiffness [135] or carotid intima-media thickness (CIMT) [136]. The only effect of ADD1 detected was a reduction of CIMT in women carrying the Trp allele. The two others studies evaluated potential influence of ADD1 Gly460Trp on CV risk. The frequencies of the ADD1 allele together with that of other alleles of 5 genes [137,138] were compared between two population subsets defined as high or low risk populations. The frequencies of Trp allele of ADD1 and the T allele of AGT (M235T) were lower in the high risk group; therefore, they were considered as protective. Also Tobin [139] found similar ADD1 data when compared patients surviving a MI with a control population without analyzing the influence of BP level. In both studies a huge difference in the traditional risk factors (smoking, age, cholesterol, diabetes, dislipidemia, hypertension, etc.) was present between the two groups. Conversely, when Gerard et al. [134] compared patients with MI and controls with the same levels of traditional risk factors, the Trp allele frequency was higher in cases. These findings raise the question of the competition among the various risk factors and the significance of the reduction of the ADD1 Trp allele in patients with MI. This reduction may be due either to a protective effect of this allele or to a premature death of these allele carriers. Four studies [140–143] evaluated the association between ADD1 with or without other four candidate gene polymorphisms and the diuretic effect on BP and on CV risk. No interaction analysis was carried out among these genes. This type of community-based studies rely upon general practitioner BP measurements, therapy assessed by pharmacy record and poor control of many other confounders as correctly admitted by the authors in their limitation section. Therefore, the negative results of these studies may enlighten the difficulties to assess the impact of genetics in medical practice. Certainly, the genetic complexity illustrated in the previous section of this review cannot be evaluated in this type of studies. Finally, the same group(s) [144] evaluated the contribution of four genes (ADD1, AGT, AGTR1 and GNB3) to the hereditability of the BP traits in a predominantly normotensive population with a substantial portion of hypertensives under treatment. As found in many other occasions, this contribution of ADD1 and GNB3 is very modest, 0.3% and 0.4%, respectively. The interactions among the four genes were not analyzed.

2.5. The pharmacogenomic strategy

At present the combination of the genetic technology with the selective inhibition of a given genetic mechanisms both in animal models (rodents) and humans is a promising approach able to provide information about the impact on hypertension of a given triggering RGN in both species. For adducin two types of drugs may be used to apply this strategy to humans. One is hydrochlorothiazide (HCTZ) that is not very specific but its action mechanism is certainly more suitable to affect the cellular effects of adducin than the action mechanisms of other drug. The other is rostafuroxin that it is discussed in another chapter of this issue (P. Ferrari).

2.5.1. Diuretics

The bulk of the data available supports the notion that the hypertension favouring effect of the Trp ADD1 allele is mediated by a constitutive increase of renal Na reabsorption. Therefore, the study of the association between the presence of the Trp allele alone, or in combination with other gene alleles modulating its effects in Na absorption together with the BP response to diuretics may provide further support to this notion or dispute it.

So far 10 articles dealing with this issue either showed an association [75,87,145,146] or its absence [142,147–151]. The differences between these two groups of studies are:

a) the former have been carried out by clinical trials in Italian populations, with one exception [87] in relatively young newly diagnosed and never treated patients;
b) the latter were carried out in previously treated patients with a variable period of washout (from 0 to 30 days) without any information of the duration of hypertension or other confounders. In one of these studies [150], the BP basal values were taken when the patients were switched from the previous therapy to diuretics, without a washout period. Moreover, diuretics were given together with lifestyle advice (reduction of Na intake) or cholesterol-lowering agents when needed [150]. One study [149] included 71 patients, and diuretics were given alone or "in addition to other antihypertensive agents". The two other studies [142,148] were observational population-based studies aimed at testing the influence of genetics on daily practice. Potential or real confounders mentioned by the authors were: data collection by self-reported questioners or from general practitioner or pharmacy records, uncertainties about the use of other antihypertensive agents 2 weeks before the BP measurements or of statins or of nonsteroidal anti-inflammatory drugs (NSAID), variations in the Na intake and so on. One study [140] addresses the question whether the diuretics could reduce the CV risk in hypertensive carriers of the ADD1 Trp allele. This reduction was suggested by a previous study [128] which compared patients, with the same levels of BP, receiving diuretic treatment either for 10 years or only 3 years. The interaction was present only in the former group of patients and the conclusion was that a long period of diuretic treatment is needed to unmask this interaction. In the study described by van Wieren–de Wijer [140], the analysis was carried out by comparing two groups of patients treated for 3.1 years and 1.5 years, respectively. Clearly, considering the data of the previous study, a negative finding could be anticipated.

2.5.2. Rostafuroxin

Recently, a very selective inhibitor of ouabain and the Trp ADD1 allele both in rodents and in humans, has been developed (rostafuroxin) and evaluated in two clinical trials [152]. The results obtained (M. Ferrandi, P. Ferrari and G. Valentini, manuscript in preparation) can be summarized as follow:

a) the BP effect over placebo was measured either in never treated patients or in previously treated patients after a washout period of 1 month before starting with the rostafuroxin administration;
b) in never treated patients a clear BP fall with rostafuroxin over placebo was detected in carriers of the gene variants previously predicted among the secondary end points of the study [152]. These gene variants were chosen because their influence on the EO activity, Trp ADD1 allele and rostafuroxin effects;
c) no effect of rostafuroxin over placebo was detected in previously treated patients carrying the alleles mentioned at point b).

Either the 1 month washout is not enough to remove the effects of the previous therapies as suggested by a series of previous observations [153–160] or the duration of hypertension in these patients was above the critical level that favours the development of secondary changes. Whatever is the reason of the clear cut differences between newly discovers and never treated on the one hand, and the previously treated patients on the other hand, the latter are not appropriate to study the interactions between the core RGN triggering hypertension and the selective inhibition of some constituents of this network.

3. Conclusions

The most important conclusion emerging from the results illustrated above is that the ADD1 Trp allele alone may be consistently associated to a given phenotype only in some specific conditions (i.e., 10 independent studies in hypertensives showed its association with increased CV risk). While in other conditions, or contexts, the ADD1 Trp allele must be associated with either genetic (other adducins, RAAS or EO genes), environmental (Na intake) or biological factors (gender, age, etc.) to unmask its involvement in the generation of a clinical phenotype, such as BP, risk to develop hypertension, arterial characteristics.

The lessons we can draw from these conclusions, which are also consistent with many previously known pathophysiological findings, are:

1) only the selective inhibition of the effect of ADD1 Trp allele may unmask its real genetic effect size which could be much larger than that measured effects applying the various genetic frequentistic approaches. Therefore, the current view that in polygenic multifactorial diseases a very large number of loci or genes may underlie the disease must be revised. Rather one must analyze the effect size of the various candidate genes with their appropriate interactions and inhibitors in newly discovered and never treated patients. For instance, the clinical impact of RAS and aldosterone gene polymorphisms may be evaluated by coupling the genotyping with BP response to RAS and aldosterone blockers.
2) the effect size of known genes or SNPs derived from GWA study may be affected by their transient effect in some specific phases of hypertension.
   Their detection may be limited to the subset of patients having the specific phase at work at the time of the study. Conversely, this phenomenon of phase-dependent gene activation may increase the effect size of a given set of some genes (RAAS or adrenergic genes), just because these systems may be activated in specific phases of different types of hypertension triggered by different RGNs. Paradoxically, the polymorphisms of these genes may be more "replicable" in different populations than that of genes involved in the triggering RGN. In fact the latter may require the interaction with other genes to shift or change the function of this RGN to produce the first BP increase. Genetics based on the differences among allele frequencies that do not account for this context dependency (hypertension phase dependency) is unable to provide the true allele effect size useful to develop an appropriate targeted therapy.

The data so far available on the clinical settings do not allow to propose a RGN organized with specific nodes and connections as it has been presented in various experimental settings. In human complex diseases the difficulties to establish precise relationship among protein interactions, functions, and clinical symptoms are overwhelming. We can only adopt the more realistic approach to apply the pathophysiological connections among the different nodes or gene products (regarding the adducin, EO or RAAS genes) that, in various combinations, are associated to the clinical phenotypes, discussed in this review. The hypothesis of the existence of "hubs proteins" in the RGN triggering hypertension can be tested in humans by a selective inhibitor of the function of these proteins. This trial must have a rigorous control of the many confounders (context) such as natural history of hypertension and CV events, age, gender, sodium intake, previous therapy, population ancestry, etc. The prediction is that the same few "hubs proteins" are at work in the various tissues in different contexts while other proteins may act as modifiers in some specific contexts. In rodents this complexity may be handled with quantitative network modelling tools to test RGNs, their hubs proteins and the effect of selective inhibitors [161,162]. An example of a successful prediction with a very selective inhibitor has been mentioned in this review. Similar studies with others inhibitors or drugs are in progress. It is likely that with this strategy we may "cure" the first phase of hypertension in the majority of patients and moreover we may obtain solid scientific information on the function of the proteins hit by this inhibitor. To provide the necessary experimental support to this hypothesis the following three approaches should be extended to the various general or hypertensive populations:

a) to assess the risk of developing hypertension in a normotensive population in carriers of the various triggering RGNs. The appropriate biological, epidemiological and genetic data have to be collected and included in the analysis. In all the four studies so far published addressing this issue [85,95,102,121], the CV risk was increased in carriers of ADD1 Trp allele either alone in a population of Japanese [95] or in combination with ACE DD in a Caucasian [121] and Chinese populations [85] or with ADD2 T allele in postmenopausal or Caucasian contraceptive user women [102];
b) to assess the risk of developing CV events in hypertensives or of the related intermediate phenotypes (negative data in this second type of risk may be expected for the previously given reasons). In all the 10 studies, where this risk has been assessed in hypertensives, the carriers of ADD1 Trp allele develop more CV events than the carriers of the wild allele;
c) to study the association between the various triggering RGNs with the phenotype response to a selective inhibitor of a specific gene (or set of genes) effect in new discovered and never treated hypertensive patients (the pharmacogenomic strategy). The advantages of this approach consist in the possibility to combine the gene structure (or DNA variations) with function measured as the degree of inhibition produced by the selective drug both in rodents and humans. In this way species-specific differences in triggering RGNs may also be assessed and the appropriate target for therapeutically intervention may be detected or envisaged [163,164].

References

[1] T.A. Manolio, F.S. Collins, N.J. Cox, D.B. Goldstein, L.A. Hindorff, D.J. Hunter, M.I. McCarthy, E.M. Ramos, L.R. Cardon, A. Chakravarti, J.H. Cho, A.E. Guttmacher, A. Kong, L. Kruglyak, E. Mardis, C.N. Rotimi, M. Slatkin, D. Valle, A.S. Whittemore, M. Boehnke, A.G. Clark, E.E. Eichler, G. Gibson, J.L. Haines, T.F. Mackay, S.A. McCarroll, P.M. Visscher, Finding the missing heritability of complex diseases, Nature 461 (2009) 747–753.
[2] A.W. Cowley, The genetic dissection of essential hypertension, Nat. Rev. Genet. 7 (11) (2006) 829–840.
[3] F.C. Luft, Present status of genetic mechanisms in hypertension, Med. Clin. N. Am. 88 (2004) 1–18.
[4] S. Sober, E. Org, K. Kepp, P. Juhanson, S. Eyheramendy, C. Gieger, P. Lichtner, N. Klopp, G. Veldre, M. Viigimaa, A. Doring, M. Putku, P. Kelgo, S. Shaw-Hawkins, P. Howard, A. Onipinla, R.J. Dobson, S.J. Newhouse, M. Brown, A. Dominiczak, J. Connell, N. Samani, M. Farrall, M.J. Caulfield, P.B. Munroe, T. Illig, H.E. Wichmann, T. Meitinger, M. Laan, Targeting 160 candidate genes for blood pressure regulation with a genome-wide genotyping array, PloS One 4 (2009) e6034.
[5] D. Levy, G.B. Ehret, K. Rice, G.C. Verwoert, L.J. Launer, A. Dehghan, N.L. Glazer, A.C. Morrison, A.D. Johnson, T. Aspelund, Y. Aulchenko, T. Lumley, A. Kottgen, R.S. Vasan, F. Rivadeneira, G. Eiriksdottir, X. Guo, D.E. Arking, G.F. Mitchell, F.U. Mattace-Raso, A.V. Smith, K. Taylor, R.B. Scharpf, S.J. Hwang, E.J. Sijbrands, J. Bis, T.B. Harris, S.K. Ganesh, C.J. O'Donnell, A. Hofman, J.I. Rotter, J. Coresh, E.J. Benjamin, A.G. Uitterlinden, G. Heiss, C.S. Fox, J.C. Witteman, E. Boerwinkle, T.J. Wang, V. Gudnason, M.G. Larson, A. Chakravarti, B.M. Psaty, C.M. van Duijn,

Genome-wide association study of blood pressure and hypertension, Nat. Genet. 41 (2009) 677–687.
[6] C. Newton-Cheh, T. Johnson, V. Gateva, M.D. Tobin, M. Bochud, L. Coin, S.S. Najjar, J.H. Zhao, S.C. Heath, S. Eyheramendy, K. Papadakis, B.F. Voight, L.J. Scott, F. Zhang, M. Farrall, T. Tanaka, C. Wallace, J.C. Chambers, K.T. Khaw, P. Nilsson, P. van der Harst, S. Polidoro, D.E. Grobbee, N.C. Onland-Moret, M.L. Bots, L.V. Wain, K.S. Elliott, A. Teumer, J. Luan, G. Lucas, J. Kuusisto, P.R. Burton, D. Hadley, W.L. McArdle, M. Brown, A. Dominiczak, S.J. Newhouse, N.J. Samani, J. Webster, E. Zeggini, J.S. Beckmann, S. Bergmann, N. Lim, K. Song, P. Vollenweider, G. Waeber, D.M. Waterworth, X. Yuan, L. Groop, M. Orho-Melander, A. Allione, A. Di Gregorio, S. Guarrera, S. Panico, F. Ricceri, V. Romanazzi, C. Sacerdote, P. Vineis, I. Barroso, M.S. Sandhu, R.N. Luben, G.J. Crawford, P. Jousilahti, M. Perola, M. Boehnke, L.L. Bonnycastle, F.S. Collins, A.U. Jackson, K.L. Mohlke, H.M. Stringham, T.T. Valle, C.J. Willer, R.N. Bergman, M.A. Morken, A. Doring, C. Gieger, T. Illig, T. Meitinger, E. Org, A. Pfeufer, H.E. Wichmann, S. Kathiresan, J. Marrugat, C.J. O'Donnell, S.M. Schwartz, D.S. Siscovick, I. Subirana, N.B. Freimer, A.L. Hartikainen, M.I. McCarthy, P.F. O'Reilly, L. Peltonen, A. Pouta, P.E. de Jong, H. Snieder, W.H. van Gilst, R. Clarke, A. Goel, A. Hamsten, J.F. Peden, U. Seedorf, A.C. Syvanen, G. Tognoni, E.G. Lakatta, S. Sanna, P. Scheet, D. Schlessinger, A. Scuteri, M. Dorr, F. Ernst, S.B. Felix, G. Homuth, R. Lorbeer, T. Reffelmann, R. Rettig, U. Volker, P. Galan, I.G. Gut, S. Hercberg, G.M. Lathrop, D. Zelenika, P. Deloukas, N. Soranzo, F.M. Williams, G. Zhai, V. Salomaa, M. Laakso, R. Elosua, N.G. Forouhi, H. Volzke, C.S. Uiterwaal, Y.T. van der Schouw, M.E. Numans, G. Matullo, G. Navis, G. Berglund, S.A. Bingham, J.S. Kooner, J.M. Connell, S. Bandinelli, L. Ferrucci, H. Watkins, T.D. Spector, J. Tuomilehto, D. Altshuler, D.P. Strachan, M. Laan, P. Meneton, N.J. Wareham, M. Uda, M.R. Jarvelin, V. Mooser, O. Melander, R.J. Loos, P. Elliott, G.R. Abecasis, M. Caulfield, P.B. Munroe, Genome-wide association study identifies eight loci associated with blood pressure, Nat. Genet. 41 (2009) 666–676.
[7] Y.S. Cho, M.J. Go, Y.J. Kim, J.Y. Heo, J.H. Oh, H.J. Ban, D. Yoon, M.H. Lee, D.J. Kim, M. Park, S.H. Cha, J.W. Kim, B.G. Han, H. Min, Y. Ahn, M.S. Park, H.R. Han, H.Y. Jang, E.Y. Cho, J.E. Lee, N.H. Cho, C. Shin, T. Park, J.W. Park, J.K. Lee, L. Cardon, G. Clarke, M.I. McCarthy, J.Y. Lee, J.K. Lee, B. Oh, H.L. Kim, A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits, Nat. Genet. 41 (2009) 527–534.
[8] E. Org, S. Eyheramendy, P. Juhanson, C. Gieger, P. Lichtner, N. Klopp, G. Veldre, A. Doring, M. Viigimaa, S. Sober, K. Tomberg, G. Eckstein, P. Kelgo, T. Rebane, S. Shaw-Hawkins, P. Howard, A. Onipinla, R.J. Dobson, S.J. Newhouse, M. Brown, A. Dominiczak, J. Connell, N. Samani, M. Farrall, M.J. Caulfield, P.B. Munroe, T. Illig, H.E. Wichmann, T. Meitinger, M. Laan, Genome-wide scan identifies CDH13 as a novel susceptibility locus contributing to blood pressure determination in two European populations, Hum. Mol. Genet. 18 (2009) 2288–2296.
[9] Y. Wang, J.R. O'Connell, P.F. McArdle, J.B. Wade, S.E. Dorff, S.J. Shah, X. Shi, L. Pan, E. Rampersaud, H. Shen, J.D. Kim, A.R. Subramanya, N.I. Steinle, A. Parsa, C.C. Ober, P.A. Welling, A. Chakravarti, A.B. Weder, R.S. Cooper, B.D. Mitchell, A.R. Shuldiner, Y.P. Chang, From the Cover: Whole-genome association study identifies STK39 as a hypertension susceptibility gene, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 226–231.
[10] G. Bianchi, Genetic variations of tubular sodium reabsorption leading to "primary" hypertension: from gene polymorphism to clinical symptoms, Am. J. Physiol. Regul. Integr. Comp. Physiol. 289 (6) (2005) R1536–R1549.
[11] C.R. Scriver, P.J. Waters, Monogenic traits are not simple: lessons from phenylketonuria, Trends Genet. 15 (7) (1999) 267–272.
[12] D.J. Weatherall, Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias, Nat. Rev. Genet. 2 (4) (2001) 245–255.
[13] Y. Chen, J. Zhu, P.Y. Lum, X. Yang, S. Pinto, D.J. MacNeil, C. Zhang, J. Lamb, S. Edwards, S.K. Sieberts, A. Leonardson, L.W. Castellini, S. Wang, M.F. Champy, B. Zhang, V. Emilsson, S. Doss, A. Ghazalpour, S. Horvath, T.A. Drake, A.J. Lusis, E.E. Schadt, Variations in DNA elucidate molecular networks that cause disease, Nature 452 (7186) (2008) 429–435.
[14] P.C. Phillips, Epistasis–the essential role of gene interactions in the structure and evolution of genetic systems, Nat. Rev. Genet. 9 (11) (2008) 855–867.
[15] R.B. Russell, P. Aloy, Targeting and tinkering with interaction networks, Nat. Chem. Biol. 4 (11) (2008) 666–673.
[16] H. Shao, L.C. Burrage, D.S. Sinasac, A.E. Hill, S.R. Ernest, W. O'Brien, H.W. Courtland, K.J. Jepsen, A. Kirby, E.J. Kulbokas, M.J. Daly, K.W. Broman, E.S. Lander, J.H. Nadeau, Genetic architecture of complex traits: large phenotypic effects and pervasive epistasis, Proc. Natl. Acad. Sci. U. S. A. 105 (50) (2008) 19910–19914.
[17] D.H. Erwin, E.H. Davidson, The evolution of hierarchical gene regulatory networks, Nat. Rev. Genet. 10 (2) (2009) 141–148.
[18] A.W. Cowley, Genomics and homeostasis, Am. J. Physiol. Regul. Integr. Comp. Physiol. 284 (3) (2003) R611–R627.
[19] W. Cookson, L. Liang, G. Abecasis, M. Moffatt, M. Lathrop, Mapping complex disease traits with global gene expression, Nat. Rev. Genet. 10 (3) (2009) 184–194.
[20] S.M. Williams, J.L. Haines, J.H. Moore, The use of animal models in the study of complex disease: all else is never equal or why do so many human studies fail to replicate animal findings? Bioessays 26 (2) (2004) 170–179.
[21] J. Tischler, B. Lehner, A.G. Fraser, Evolutionary plasticity of genetic interaction networks, Nat. Genet. 40 (4) (2008) 390–391.
[22] S.J. Dixon, Y. Fedyshyn, J.L. Koh, T.S. Prasad, C. Chahwan, G. Chua, K. Toufighi, A. Baryshnikova, J. Hayles, K.L. Hoe, D.U. Kim, H.O. Park, C.L. Myers, A. Pandey, D. Durocher, B.J. Andrews, C. Boone, Significant conservation of synthetic lethal genetic interaction networks between distantly related eukaryotes, Proc. Natl. Acad. Sci. U. S. A. 105 (43) (2008) 16653–16658.
[23] R. Bell, A. Hubbard, R. Chettier, D. Chen, J.P. Miller, P. Kapahi, M. Tarnopolsky, S. Sahasrabuhde, S. Melov, R.E. Hughes, A human protein interaction network shows conservation of aging processes between human and invertebrate species, PLoS Genet. 5 (3) (2009) e1000414.
[24] R.J. Sommer, Homology and the hierarchy of biological systems, Bioessays 30 (7) (2008) 653–658.
[25] C.G. Knight, J.W. Pinney, Making the right connections: biological networks in the light of evolution, Bioessays 31 (10) (2009) 1080–1090.
[26] A.L. Tyler, F.W. Asselbergs, S.M. Williams, J.H. Moore, Shadows of complexity: what biological networks reveal about epistasis and pleiotropy, Bioessays 31 (2) (2009) 220–227.
[27] F. Gross, G. Schaechtelin, H. Brunner, G. Peters, The role of the Renin-Angiotensin System in blood pressure regulatin and kidney function, Can. Med. Assoc. J. 90 (1964) 258–262.
[28] A.C. Guyton, T.G. Coleman, H.J. Granger, Circulation: overall regulation, Am. J. Physiol. 34 (1972) 13–46.
[29] J.H. Laragh, Renin, angiotensin, aldosterone and hormonal regulation of arterial pressure and salt balance, Fed. Proc. 26 (1967) 39–41.
[30] D.G. Beevers, J.J. Brown, R. Fraser, A.F. Lever, J.J. Morton, J.I. Robertson, P.F. Semple, M. Tree, The clinical value of renin and angiotensin estimations, Kidney Int. Suppl. (1975) S181–S201.
[31] E. Haber, George C. Griffith lecture. The role of renin in normal and pathological cardiovascular homeostasis, Circulation 54 (1976) 849–861.
[32] G. Bianchi, U. Fox, D. Pagetti, A.M. Caravaggi, P.G. Baer, E. Baldoli, Mechanisms involved in renal hypertension, Kidney Int. Suppl. (1975) S165–S173.
[33] J.M. Ledingham, R.D. Cohen, Changes in the extracellular fluid volume and cardiac output during the development of experimental renal hypertension, Can. Med. Assoc. J. 90 (1964) 292–294.
[34] G. Bianchi, L.T. Tenconi, R. Lucca, Effect in the conscious dog of constriction of the renal artery to a sole remaining kidney on haemodynamics, sodium balance, body fluid volumes, plasma renin concentration and pressor responsiveness to angiotensin, Clin. Sci. 38 (6) (1970) 741–766.
[35] C.M. Ferrario, Contribution of cardiac output and peripheral resistance to experimental renal hypertension, Am. J. Physiol. 226 (3) (1974) 711–717.
[36] G. Schultze, K. Kirsch, K. Preu, F.W. Lohmann, H.J. Stolpmann, R. Gotzen, T.H. Dibmann, Hamodinamik, Fiussigkeitshaushalt, plasmarenin und plasmakatecholamine in unterschiedlichen phasen der renovaskularen hypertonic des schales, Verh. Dtsch. Ges. Kreisl. Forsch 38 (1973) 1–11.
[37] J.F. Liard, A.W. Cowley, R.E. McCaa, C.S. McCaa, A.C. Guyton, Renin, aldosterone, body fluid volumes, and the baroreceptor reflex in the development and reversal of Goldblatt hypertension in conscious dogs, Circ. Res. 34 (4) (1974) 549–560.
[38] F.D. Gutmann, H. Tagawa, E. Haber, A.C. Barger, Renal arterial pressure, renin secretion, and blood pressure control in trained dogs, Am. J. Physiol. 224 (1) (1973) 66–72.
[39] A.C. Guyton, T.G. Coleman, A.V. Cowley, K.W. Scheel, R.D. Manning, R.A. Norman, Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension, Am. J. Med. 52 (1972) 584–594.
[40] J.O. Davis, The pathogenesis of chronic renovascular hypertension, Circ. Res. 40 (1977) 439–444.
[41] J.M. Ledingham, R.D. Cohen, Circulatory changes during the reversal of experimental hypertension, Clin. Sci. 22 (1962) 69–77.
[42] J.W. Funder, J.R. Blair-West, M.C. Cain, K.J. Catt, J.P. Coghlan, D.A. Denton, J.F. Nelson, B.A. Scoggins, R.D. Wright, Circulatory and humoral changes in the reversal of renovascular hypertension in sheep by unclipping the renal artery, Circ. Res. 27 (2) (1970) 249–258.
[43] M.E. Edmunds, G.I. Russell, R.F. Bing, Reversal of experimental renovascular hypertension, J. Hypertens. 9 (1991) 289–301.
[44] G. Bianchi, P.G. Baer, U. Fox, L. Duzzi, D. Pagetti, A.M. Giovannetti, Changes in renin, water balance, and sodium balance during development of high blood pressure in genetically hypertensive rats, Circ. Res. 36 (1975) 153–161.
[45] P. Lund-Johansen, P. Omvik, Hemodynamic patterns of untreated hypertensive disease, in: J.H. Laragh, B.M. Brenner (Eds.), Hypertension: pathophysiology, diagnosis, and management, Chapter 22, Raven Press Ltd, 1990.
[46] P.W. de Leeuw, W.H. Birkenhäger, The renal circulation in essential hypertension, J. Hypertens. 1 (1983) 321–331.
[47] G. Bianchi, D. Cusi, C. Barlassina, G.P. Lupi, P. Ferrari, G.B. Picotti, M. Gatti, E. Polli, Renal dysfunction as a possible cause of essential hypertension in predisposed subjects, Kidney Int. 23 (1983) 870–875.
[48] R.P. Lifton, A.G. Gharavi, D.S. Geller, Molecular mechanisms of human hypertension, Cell 104 (2001) 545–556.
[49] R.A. Shimkets, D.G. Warnock, C.M. Bositis, C. Nelson-Williams, J.H. Hansson, M. Schambelan, J.R. Gill, S. Ulick, R.V. Milora, J.W. Findling, et al., Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel, Cell 79 (1994) 407–414.
[50] R.P. Lifton, Genetic determinants of human hypertension, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 8545–8551.
[51] S.C. Thomson, R.C. Blantz, Glomerulotubular balance, tubuloglomerular feedback, and salt homeostasis, J. Am. Soc. Nephrol. 19 (2008) 2272–2275.
[52] J. Schnermann, J.P. Briggs, Tubuloglomerular feedback: mechanistic insights from gene-manipulated mice, Kidney Int. 74 (2008) 418–426.
[53] C. Grünberger, B. Obermayer, J. Klar, A. Kurtz, F. Schweda, The calcium paradoxon of renin release: calcium suppresses renin exocytosis by inhibition of calcium-dependent adenylate cyclases AC5 and AC6, Circ Res. 99 (2006) 1197–1206.
[54] S.M. Williams, M.D. Ritchie, J.A. Phillips III, E. Dawson, M. Prince, E. Dzhura, A. Willis, A. Semenya, M. Summar, B.C. White, J.H. Addy, J. Kpodonu, L.J. Wong, R.A.

Felder, P.A. Jose, J.H. Moore, Multilocus analysis of hypertension: a hierarchical approach, Hum. Hered. 57 (1) (2004) 28–38.
[55] A.V. Buchanan, S. Sholtis, J. Richtsmeier, K.M. Weiss, What are genes "for" or where are traits "from"? What is the question? Bioessays 31 (2) (2009) 198–208.
[56] E.D. Miller, A.I. Samuels, E. Haber, A.C. Barger, Inhibition of angiotensin conversion in experimental renovascular hypertension, Science 177 (54) (1972) 1108–1109.
[57] J.O. Davis, R.H. Freeman, J.A. Johnson, W.S. Spielman, Agents which block the action of the renin-angiotensin system, Circ. Res. 34 (3) (1974) 279–285.
[58] H.R. Brunner, J.D. Kirshman, J.E. Sealey, J.H. Laragh, Hypertension of renal origin: evidence for two different mechanisms, Science 174 (1971) 1344–1346.
[59] J.D. Swales, H. Thurston, Sodium restriction and inhibition of the renin-angiotensin system in renovascular hypertension in the rat, Clin. Sci. Mol. Med. 52 (1977) 371–375.
[60] B.E. Watkins, J.O. Davis, R.H. Freeman, J.M. DeForrest, G.A. Stephens, Continuous angiotensin II blockade throughout the acute phase of one-kidney hypertension in the dog, Circ. Res. 42 (1978) 813–821.
[61] K.G. Hofbauer, C. Huppertz, Pharmacotherapy and evolution, Trends Ecol. Evol. 17 (2002) 328–334.
[62] S. Ebrahim, G. Davey Smith, Mendelian randomization: can genetic epidemiology help redress the failures of observational epidemiology? Hum. Genet. 123 (2008) 15–33.
[63] P.R. Kamstrup, A. Tybjaerg-Hansen, R. Steffensen, B.G. Nordestgaard, Genetically elevated lipoprotein(a) and increased risk of myocardial infarction, JAMA 301 (2009) 2331–2339.
[64] G. Thanassoulis, C.J. O'Donnell, Mendelian randomization: nature's randomized trial in the post-genome era, JAMA 301 (2009) 2386–2388.
[65] S.A. Tishkoff, F.A. Reed, A. Ranciaro, B.F. Voight, C.C. Babbitt, J.S. Silverman, K. Powell, H.M. Mortensen, J.B. Hirbo, M. Osman, M. Ibrahim, S.A. Omar, G. Lema, T.B. Nyambo, J. Ghori, S. Bumpstead, J.K. Pritchard, G.A. Wray, P. Deloukas, Convergent adaptation of human lactase persistence in Africa and Europe, Nat. Genet. 39 (2007) 31–40.
[66] M. Ferrandi, I. Molinari, P. Barassi, E. Minotti, G. Bianchi, P. Ferrari, Organ hypertrophic signaling within caveolae membrane subdomains triggered by ouabain and antagonized by PST 2238, J. Biol. Chem. 279 (2004) 33306–33314.
[67] W. Schoner, G. Scheiner-Bobis, Endogenous and exogenous cardiac glycosides and their mechanisms of action, Am. J. Cardiovasc. Drugs 7 (2007) 173–189 (Review).
[68] J. Zhang, M.Y. Lee, M. Cavalli, L. Chen, R. Berra-Romani, C.W. Balke, G. Bianchi, P. Ferrari, J.M. Hamlyn, T. Iwamoto, J.B. Lingrel, D.R. Matteson, W.G. Wier, M.P. Blaustein, Sodium pump alpha2 subunits control myogenic tone and blood pressure in mice, J. Physiol. 569 (Pt 1) (2005) 243–256.
[69] P. Manunta, M. Maillard, C. Tantardini, M. Simonini, C. Lanzani, L. Citterio, P. Stella, N. Casamassima, M. Burnier, J.M. Hamlyn, G. Bianchi, Relationships among endogenous ouabain, alpha-adducin polymorphisms and renal sodium handling in primary hypertension, J. Hypertens. 26 (5) (2008) 914–920.
[70] P. Manunta, G. Lavery, C. Lanzani, P.S. Braund, M. Simonini, C. Bodycote, L. Zagato, S. Delli Carpini, C. Tantardini, E. Brioni, G. Bianchi, N.J. Samani, Physiological interaction between alpha-adducin and WNK1-NEDD4L pathways on sodium-related blood pressure regulation, Hypertension 52 (2008) 366–372.
[71] R. Efendiev, R.T. Krmar, G. Ogimoto, J. Zwiller, G. Tripodi, A.I. Katz, G. Bianchi, C.H. Pedemonte, A.M. Bertorello, Hypertension-linked mutation in the adducin alpha-subunit leads to higher AP2-mu2 phosphorylation and impaired Na+, K+-ATPase trafficking in response to GPCR signals and intracellular sodium, Circ. Res. 95 (2004) 1100–1108.
[72] M. Ferrandi, G. Tripodi, S. Salardi, M. Florio, R. Modica, P. Barassi, P. Parenti, A. Shainskaya, S. Karlish, G. Bianchi, P. Ferrari, Renal Na, K-ATPase in genetic hypertension, Hypertension 28 (1996) 1018–1025.
[73] P. Manunta, M. Burnier, M. D'Amico, L. Buzzi, M. Maillard, C. Barlassina, G. Lanella, D. Cusi, G. Bianchi, Adducin polymorphism affects renal proximal tubule reabsorption in hypertension, Hypertension 33 (1999) 694–697.
[74] P. Manunta, D. Cusi, C. Barlassina, M. Righetti, C. Lanzani, M. D'Amico, L. Buzzi, L. Citterio, P. Stella, R. Rivera, G. Bianchi, Alpha-adducin polymorphisms and renal sodium handling in essential hypertensive patients, Kidney Int. 53 (6) (Jun 1998) 1471–1478.
[75] N. Glorioso, P. Manunta, F. Filigheddu, C. Troffa, P. Stella, C. Barlassina, C. Lombardi, A. Soro, F. Dettori, P.P. Parpaglia, M.T. Alibrandi, D. Cusi, G. Bianchi, The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be excluded by a negative association study, Hypertension 34 (4 Pt 1) (1999) 649–654.
[76] S.L. Chu, D.L. Zhu, M.M. Xiong, G.L. Wang, W.Z. Zhang, H.F. Zhou, D. Shen, P.J. Gao, Y.M. Zhan, L. Jin, Linkage analysis of twelve candidate gene loci regulating water and sodium metabolism and membrane ion transport in essential hypertension, Hypertens. Res. 25 (4) (2002) 635–639.
[77] X. He, G. Wang, W. Huang, Z. Ding-Liang, Linkage analysis of five candidate genes and essential hypertension in 106 Chinese nuclear families, J. Hum. Hypertens. 17 (2003) 69–72.
[78] X. He, D.L. Zhu, S.L. Chu, L. Jin, M.M. Xiong, G.L. Wang, W.Z. Zhang, H.F. Zhou, S.Y. Mao, Y.M. Zhan, Q.N. Zhuang, X.M. Liu, Y. Zhao, W. Huang, alpha-Adducin gene and essential hypertension in China, Clin. Exp. Hypertens. 23 (7) (2001) 579–589.
[79] L.H. Lu, H. Chen, L. Yu, Association of alpha-adducin and angiotensin converting enzyme gene polymorphisms with salt-sensitive hypertension and early renal injury, Zhonghua Yi Xue Yi Chuan Xue Za Zhi 25 (2) (2008) 132–135.
[80] P.Y. Gong, G.M. Shen, H.M. Peng, Y. Luo, Y. Shen, X. Zhao, Association of the polymorphisms of sodium transport related genes with essential hypertension, Zhonghua Yi Xue Yi Chuan Xue Za Zhi 26 (1) (2009) 91–94.
[81] Z. Ju, H. Zhang, K. Sun, Y. Song, H. Lu, R. Hui, X. Huang, Alpha-adducin gene polymorphism is associated with essential hypertension in Chinese: a case-control and family-based study, J. Hypertens. 21 (10) (2003) 1861–1868.
[82] H.F. Guo, Y. Li, G.L. Wang, Y.G. Lu, H.F. Zhou, P.J. Gao, D.L. Zhu, J.G. Wang, Association of peripheral and central blood pressure with the alpha-adducin Gly460Trp polymorphism in a Chinese population, Zhonghua Xin Xue Guan Bing Za Zhi 33 (7) (2005) 608–612.
[83] W.Q. Niu, Y. Zhang, K.D. Ji, P.J. Gao, D.L. Zhu, Lack of association between alpha-adducin G460W polymorphism and hypertension: evidence from a case-control study and a meta-analysis, J. Hum. Hypertens. (2009) 1–8.
[84] M.H. Shin, E.K. Chung, H.N. Kim, K.S. Park, H.S. Nam, S.S. Kweon, J.S. Choi, Alpha-adducin Gly460Trp polymorphism and essential hypertension in Korea, J. Korean Med. Sci. 19 (6) (Dec 2004) 812–814.
[85] J.G. Wang, L. Liu, L. Zagato, J. Xie, R. Fagard, K. Jin, J. Wang, Y. Li, G. Bianchi, J.A. Staessen, L. Liu, Blood pressure in relation to three candidate genes in a Chinese population, J. Hypertens. 22 (5) (2004) 937–944.
[86] X.F. Dou, H.Y. Zhang, X.H. Huang, X.N. Liu, Z.Y. Ju, K. Sun, D.W. Wang, Y.H. Liao, A.Q. Ma, Z.M. Zhu, B.R. Zhao, J.Z. Zhao, Y. Song, L. Zhang, R.T. Hui, Alpha-adducin gene G/W460 polymorphism is associated with intracerebral hemorrhage in Chinese, Zhonghua Yi Xue Za Zhi 84 (3) (Feb 2 2004) 186–188 (Chinese).
[87] S.L. Wu, D.Q. Li, H.F. Li, Q. Yu, Y. Li, H.Y. Zhao, Association of polymorphism in alpha-adducin gene with antihypertensive effect of Hydrochlorothiazide, Zhao, Zhonghua Xin Xue Guan Bing Za Zhi 33 (10) (2005) 880–884.
[88] T.N. Kelly, T.K. Rice, D. Gu, J.E. Hixson, J. Chen, D. Liu, C.E. Jaquish, L.A. Bazzano, D. Hu, J. Ma, C.C. Gu, J. Huang, L.L. Hamm, J. He, Novel genetic variants in the alpha-adducin and guanine nucleotide binding protein beta-polypeptide 3 genes and salt sensitivity of blood pressure, Am. J. Hypertens. 22 (9) (Sep 2009) 985–992.
[89] X.Y. Song, S.Y. Lee, R.C. Ma, W.Y. So, J.H. Cai, C. Tam, V. Lam, W. Ying, M.C. Ng, J.C. Chan, Phenotype-genotype interactions on renal function in type 2 diabetes: an analysis using structural equation modelling, Diabetologia 52 (8) (2009) 1543–1553.
[90] N. Kato, T. Sugiyama, T. Nabika, H. Morita, H. Kurihara, Y. Yazaki, Y. Yamori, Lack of association between the alpha-adducin locus and essential hypertension in the Japanese population, Hypertension 31 (3) (1998) 730–733.
[91] K. Ishikawa, T. Katsuya, N. Sato, Y. Nakata, S. Takami, S. Takiuchi, Y. Fu, J. Higaki, T. Ogihara, No association between alpha-adducin 460 polymorphism and essential hypertension in a Japanese population, Am. J. Hypertens. 11 (4 Pt 1) (1998) 502–506.
[92] N. Iwai, S. Tamaki, Y. Nakamura, M. Kinoshita, Polymorphism of alpha-adducin and hypertension, Lancet 350 (9074) (1997) 369.
[93] K. Shioji, Y. Kokubo, T. Mannami, N. Inamoto, H. Morisaki, Y. Mino, N. Tagoi, N. Yasui, N. Iwaii, Association between hypertension and the alpha-adducin, beta1-adrenoreceptor, and G-protein beta3 subunit genes in the Japanese population; the Suita study, Hypertens. Res. 27 (1) (2004) 31–37.
[94] Y. Nakamura, Y. Tabara, T. Miki, S. Tamaki, Y. Kita, T. Okamura, H. Ueshima, Both angiotensinogen M235T and alpha-adducin G460W polymorphisms are associated with hypertension in the Japanese population, J. Hum. Hypertens. 21 (3) (2007) 253–255.
[95] Y. Watanabe, H. Metoki, T. Ohkubo, T. Katsuya, Y. Tabara, M. Kikuya, T. Hirose, K. Sugimoto, K. Asayama, R. Inoue, A. Hara, T. Obara, J. Nakura, K. Kohara, K. Totsune, T. Ogihara, H. Rakugi, T. Miki, Y. Imai, Accumulation of common polymorphisms is associated with development of hypertension: a 12-year follow-up from the Ohasama study, Hypertens. Res. 33 (2) (2010) 129–134.
[96] K. Yamagishi, H. Iso, T. Tanigawa, R. Cui, M. Kudo, T. Shimamoto, Alpha-adducin G460W polymorphism, urinary sodium excretion, and blood pressure in community-based samples, Am. J. Hypertens. 17 (5 Pt 1) (2004) 385–390.
[97] K. Sugimoto, A. Hozawa, T. Katsuya, M. Matsubara, T. Ohkubo, I. Tsuji, M. Motone, J. Higaki, S. Hisamachi, Y. Imai, T. Ogihara, alpha-Adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the Ohasama study, J. Hypertens. 20 (9) (2002) 1779–1784.
[98] S. Tamaki, Y. Nakamura, Y. Tabara, T. Okamura, Y. Kita, T. Kadowaki, Y. Tsujita, M. Horie, T. Miki, H. Ueshima, Combined analysis of polymorphisms in angiotensinogen and adducin genes and their effects on hypertension in a Japanese sample: The Shigaraki Study, Hypertens. Res. 28 (8) (2005) 645–650.
[99] I. Narita, S. Goto, N. Saito, J. Song, J. Ajiro, F. Sato, D. Saga, D. Kondo, K. Akazawa, M. Sakatsume, F. Gejyo, Interaction between ACE and ADD1 gene polymorphisms in the progression of IgA nephropathy in Japanese patients, Hypertension 42 (3) (2003) 304–309.
[100] N. Kato, T. Miyata, Y. Tabara, T. Katsuya, K. Yanai, H. Hanada, K. Kamide, J. Nakura, K. Kohara, F. Takeuchi, H. Mano, M. Yasunami, A. Kimura, Y. Kita, H. Ueshima, T. Nakayama, M. Soma, A. Hata, A. Fujioka, Y. Kawano, K. Nakao, A. Sekine, T. Yoshida, Y. Nakamura, T. Saruta, T. Ogihara, S. Sugano, T. Miki, H. Tomoike, High-density association study and nomination of susceptibility genes for hypertension in the Japanese National Project, Hum. Mol. Genet. 17 (4) (2008) 617–627.
[101] X. Zhou, W. Tang, H. Wu, Y. Cun, Y. Yang, J. Zhang, H. Yu, K. Li, L. He, C. Xiao, Are the beta-adducin C1797T polymorphism and gamma-adducin A386G polymorphism associated with essential hypertension in Yi and Hani populations? Clin. Chim. Acta. 374 (1–2) (2006) 153–154.
[102] J.G. Wang, J.A. Staessen, C. Barlassina, R. Fagard, T. Kuznetsova, H.A. Struijker-Boudier, L. Zagato, L. Citterio, E. Messaggio, G. Bianchi, Association between hypertension and variation in the alpha- and beta-adducin genes in a white population, Kidney Int. 62 (6) (2002) 2152–2159.
[103] H.M. Colhoun, P.M. McKeigue, G. Davey Smith, Problems of reporting genetic associations with complex outcomes, Lancet 361 (2003) 865–872 (Review).

[104] M.L. Freedman, D. Reich, K.L. Penney, G.J. McDonald, A.A. Mignault, N. Patterson, S.B. Gabriel, E.J. Topol, J.W. Smoller, C.N. Pato, M.T. Pato, T.L. Petryshen, L.N. Kolonel, E.S. Lander, P. Sklar, B. Henderson, J.N. Hirschhorn, D. Altshuler, Assessing the impact of population stratification on genetic association studies, Nat. Genet. 36 (2004) 388–393.
[105] L.R. Cardon, L.J. Palmer, Population stratification and spurious allelic association, Lancet 361 (2003) 598–604 (Review).
[106] L.C. Lazzeroni, C.A. Karlovich, Genotype to phenotype: associations, errors and complexity, Trends Genet. 18 (2002) 283–284.
[107] J.A. Staessen, J.G. Wang, E. Brand, C. Barlassina, W.H. Birkenhäger, S.M. Herrmann, R. Fagard, L. Tizzoni, G. Bianchi, Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population, J. Hypertens. 19 (8) (2001) 1349–1358.
[108] J.G. Wang, J.A. Staessen, L. Tizzoni, E. Brand, W.H. Birkenhäger, R. Fagard, S.M. Herrmann, G. Bianchi, Renal function in relation to three candidate genes, Am. J. Kidney Dis. 38 (6) (2001) 1158–1168.
[109] E.J. Balkestein, J.A. Staessen, J.G. Wang, J.J. van Der Heijden-Spek, L.M. Van Bortel, C. Barlassina, G. Bianchi, E. Brand, S.M. Herrmann, H.A. Struijker-Boudier, Carotid and femoral artery stiffness in relation to three candidate genes in a white population, Hypertension 38 (5) (2001) 1190–1197.
[110] E.J. Balkestein, J.G. Wang, H.A. Struijker-Boudier, C. Barlassina, G. Bianchi, W.H. Birkenhäger, E. Brand, E. Den Hond, R. Fagard, S.M. Herrmann, L.M. Van Bortel, J.A. Staessen, Carotid and femoral intima-media thickness in relation to three candidate genes in a Caucasian population, J. Hypertens. 20 (8) (2002) 1551–1561.
[111] J. Seidlerova, J.A. Staessen, T. Nawrot, E. Brand, S.M. Brand-Herrmann, N. Casamassima, L. Citterio, S. Hasenkamp, T. Kuznetsova, Y. Li, P. Manunta, T. Richart, H.A. Struijker-Boudier, R. Fagard, J. Filipovsky, Arterial properties in relation to genetic variation in alpha-adducin and the renin-angiotensin system in a white population, J. Hum. Hypertens. 23 (2009) 55–64.
[112] J. Seidlerová, J.A. Staessen, M. Bochud, T. Nawrot, N. Casamassima, L. Citterio, T. Kuznetsova, Y. Jin, P. Manunta, T. Richart, H.A. Struijker-Boudier, R. Fagard, J. Filipovský, G. Bianchi, Arterial properties in relation to genetic variations in the adducin subunits in a white population, Am. J. Hypertens. 22 (1) (2009) 21–26.
[113] T. Kuznetsova, L. Citterio, L. Herbots, S.D. Carpini, L. Thijs, N. Casamassima, T. Richart, R.H. Fagard, G. Bianchi, J.A. Staessen, Effects of genetic variation in adducin on left ventricular diastolic function as assessed by tissue Doppler imaging in a Flemish population, J. Hypertens. 26 (6) (2008) 1229–1236.
[114] M. Cwynar, J.A. Staessen, M. Tichá, T. Nawrot, L. Citterio, T. Kuznetsova, W. Wojciechowska, K. Stolarz, J. Filipovský, K. Kawecka-Jaszcz, T. Grodzicki, H.A. Struijker-Boudier, L. Thijs, L.M. Van Bortel, G. Bianchi, European Project On Genes in Hypertension (EPOGH) Investigators, Epistatic interaction between alpha- and gamma-adducin influences peripheral and central pulse pressures in white Europeans, J. Hypertens. 23 (5) (2005) 961–969.
[115] T. Kuznetsova, J.A. Staessen, E. Brand, M. Cwynar, K. Stolarz, L. Thijs, V. Tikhonoff, W. Wojciechowska, S. Babeanu, S.M. Brand-Herrmann, E. Casiglia, J. Filipovský, T. Grodzicki, Y. Nikitin, J. Peleska, H. Struijker-Boudier, G. Bianchi, K. Kawecka-Jaszcz, European Project on Genes in Hypertension Investigators. Sodium excretion as a modulator of genetic associations with cardiovascular phenotypes in the European Project on Genes in Hypertension, J. Hypertens. 24 (2) (2006) 235–242 (Review).
[116] V. Tikhonoff, T. Kuznetsova, K. Stolarz, G. Bianchi, E. Casiglia, K. Kawecka-Jaszcz, Y. Nikitin, L. Tizzone, J.G. Wang, J.A. Staessen, beta-Adducin polymorphisms, blood pressure, and sodium excretion in three European populations, Am. J. Hypertens. 16 (10) (2003) 840–846.
[117] V. Tikhonoff, T. Kuznetsova, K. Stolarz, G. Bianchi, E. Casiglia, K. Kawecka-Jaszcz, Y. Nikitin, L. Tizzone, J.G. Wang, J.A. Staessen, European Project on Genes in Hypertension Investigators, Blood pressure phenotypes in relation to the beta-adducin C1797T polymorphism in the European Project on Genes in Hypertension (EPOGH), Blood Press. Monit. 8 (4) (2003) 151–154.
[118] P. Manunta, M. Ferrandi, G. Bianchi, J.M. Hamlyn, Endogenous ouabain in cardiovascular function and disease, J. Hypertens. 27 (2009) 9–18.
[119] J.G. Wang, J.A. Staessen, E. Messaggio, T. Nawrot, R. Fagard, J.M. Hamlyn, G. Bianchi, P. Manunta, Salt, endogenous ouabain and blood pressure interactions in the general population, J. Hypertens. 21 (8) (2003) 1475–1481.
[120] Y. Li, L. Thijs, T. Kuznetsova, L. Zagato, H. Struijker-Boudier, G. Bianchi, J.A. Staessen, Cardiovascular risk in relation to alpha-adducin Gly460Trp polymorphism and systolic pressure: a prospective population study, Hypertension 46 (3) (2005) 527–532.
[121] Y. Li, L. Zagato, T. Kuznetsova, G. Tripodi, G. Zerbini, T. Richart, L. Thijs, P. Manunta, J.G. Wang, G. Bianchi, J.A. Staessen, Angiotensin-converting enzyme I/D and alpha-adducin Gly460Trp polymorphisms: from angiotensin-converting enzyme activity to cardiovascular outcome, Hypertension 49 (6) (2007) 1291–1297.
[122] N.R. Cook, Salt intake, blood pressure and clinical outcomes, Curr. Opin. Nephrol. Hypertens. 17 (2008) 310–314.
[123] P.W. Sanders, Dietary salt intake, salt sensitivity, and cardiovascular health, Hypertension 53 (2009) 442–445.
[124] A.J. Polichnowski, A.W. Cowley, Pressure-induced renal injury in angiotensin II versus norepinephrine-induced hypertensive rats, Hypertension 54 (2009) 1269–1277.
[125] A.Y. Bagrov, J.I. Shapiro, Endogenous digitalis: pathophysiologic roles and therapeutic applications, Nat. Clin. Pract. Nephrol. 4 (2008) 378–392.
[126] B.M. Psaty, C. Doggen, H.L. Vos, J.P. Vandenbroucke, F.R. Rosendaal, Association of the alpha-adducin polymorphism with blood pressure and risk of myocardial infarction, J. Hum. Hypertens. 14 (2) (2000) 95–97.
[127] A.C. Morrison, M.S. Bray, A.R. Folsom, E. Boerwinkle, ADD1 460 W Allele Associated With Cardiovascular Disease in Hypertensive Individuals, Hypertension 39 (2002) 1053–1057.
[128] B.M. Psaty, N.L. Smith, S.R. Heckbert, H.L. Vos, R.N. Lemaitre, A.P. Reiner, D.S. Siscovick, J. Bis, T. Lumley, W.T. Longstreth Jr., F.R. Rosendaal, Diuretic therapy, the alpha-adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension, JAMA 287 (2002) 1680–1689.
[129] X.F. Dou, H.Y. Zhang, X.H. Huang, X.N. Liu, Z.Y. Ju, K. Sun, D.W. Wang, Y.H. Liao, A.Q. Ma, Z.M. Zhu, B.R. Zhao, J.Z. Zhao, Y. Song, L. Zhang, R.T. Hui, Alpha-adducin gene G/W460 polymorphism is associated with intracerebral hemorrhage in Chinese, Zhonghua Yi Xue Za Zhi 84 (3) (Feb 2 2004) 186–188 (Chinese).
[130] M.J. van Rijn, Bos, M. Yazdanpanah, A. Isaacs, A. Arias-Vásquez, P.J. Koudstaal, A. Hofman, J.C. Witteman, C.M. van Duijn, M.M. Breteler, Alpha-adducin polymorphism, atherosclerosis, and cardiovascular and cerebrovascular risk, Stroke 37 (12) (2006) 2930–2934.
[131] M. Yazdanpanah, F.A. Sayed-Tabatabaei, A. Hofman, Y.S. Aulchenko, B.A. Oostra, B.H. Stricker, H.A. Pols, S.W. Lamberts, J.C. Witteman, J.A. Janssen, C.M. van Duijn, The alpha-adducin gene is associated with macrovascular complications and mortality in patients with type 2 diabetes, Diabetes 55 (10) (2006) 2922–2927.
[132] S.H. Cha, H.T. Kim, Y. Jang, S. Park, J.J. Kim, M.Y. Song, J.H. Park, H.J. Ryu, H.Y. Park, S.J. Yoon, K. Kimm, J.K. Lee, B. Oh, Association of alpha-adducin Gly460Trp polymorphism with coronary artery disease in a Korean population, J. Hypertens. 25 (12) (2007) 2413–2420.
[133] M.H. Zafarmand, Y.T. van der Schouw, D.E. Grobbee, P.W. de Leeuw, M.L. Bots, Alpha-adducin Gly460Trp variant increases the risk of stroke in hypertensive Dutch women, Hypertension 51 (6) (2008) 1665–1670.
[134] T. Gerhard, Y. Gong, A.L. Beitelshees, X. Mao, M.T. Lobmeyer, R.M. Cooper-DeHoff, T.Y. Langaee, N.J. Schork, M.D. Shriver, C.J. Pepine, J.A. Johnson, INVEST Investigators, Alpha-adducin polymorphism associated with increased risk of adverse cardiovascular outcomes: results from GENEtic Substudy of the INternational VErapamil SR-trandolapril STudy (INVEST-GENES) Am, Heart J. 156 (2) (2008) 397–404.
[135] A.W. Plat, H.E. Stoffers, P.W. de Leeuw, C.P. van Schayck, F.L. Soomers, A.D. Kester, K. Aretz, A.A. Kroon, The association between arterial stiffness and the angiotensin II type 1 receptor (A1166C) polymorphism is influenced by the use of cardiovascular medication, J. Hypertens. 27 (1) (2009) 69–75.
[136] A.W. Plat, H.E. Stoffers, P.W. de Leeuw, C.P. van Schayck, F.L. Soomers, A.D. Kester, K. Aretz, A.A. Kroon, Sex-specific effect of the alpha-adducin (G460W) and AGTR1 (A1166C) polymorphism on carotid intima-media thickness, J. Hypertens. 27 (11) (2009) 2165–2173.
[137] A.W. Plat, H.E. Stoffers, O.H. Klungel, C.P. van Schayck, P.W. de Leeuw, F.L. Soomers, P.M. Schiffers, A.D. Kester, A.A. Kroon, The contribution of six polymorphisms to cardiovascular risk in a Dutch high-risk primary care population: the HIPPOCRATES project, J. Hum. Hypertens. 23 (10) (2009) 659–667.
[138] A.W. Plat, H.E. Stoffers, P.W. de Leeuw, C.P. van Schayck, F.L. Soomers, A.D. Kester, K. Aretz, A.A. Kroon, The influence of six cardiovascular polymorphisms on a first event of ischemic heart disease is modified by sex and age, Coron. Artery Dis. 20 (8) (2009) 499–505.
[139] M.D. Tobin, P.S. Braund, P.R. Burton, J.R. Thompson, R. Steeds, K. Channer, S. Cheng, K. Lindpaintner, N.J. Samani, Genotypes and haplotypes predisposing to myocardial infarction: a multilocus case-control study, Eur. Heart J. 25 (6) (2004) 459–467.
[140] D.B. van Wieren-de Wijer, A.H. Maitland-van der Zee, A. de Boer, A.A. Kroon, P.W. de Leeuw, P. Schiffers, R.G. Janssen, B.M. Psaty, C.M. van Duijn, B.H. Stricker, O.H. Klungel, Interaction between the Gly460Trp alpha-adducin gene variant and diuretics on the risk of myocardial infarction, J. Hypertens. 27 (1) (2009) 61–68.
[141] H. Schelleman, O.H. Klungel, J.C. Witteman, M.M. Breteler, A. Hofman, C.M. van Duijn, A. de Boer, B.H. Stricker, Diuretic-gene interaction and the risk of myocardial infarction and stroke, Pharmacogenomics J. 7 (5) (2007) 346–352.
[142] H. Schelleman, B.H. Stricker, W.M. Verschuren, A. de Boer, A.A. Kroon, P.W. de Leeuw, D. Kromhout, O.H. Klungel, Interactions between five candidate genes and antihypertensive drug therapy on blood pressure, Pharmacogenomics J. 6 (1) (2006) 22–26.
[143] O. Bozkurt, A. de Boer, D.E. Grobbee, P.W. de Leeuw, A.A. Kroon, P. Schiffers, O.H. Klungel, Variation in Renin-Angiotensin system and salt-sensitivity genes and the risk of diabetes mellitus associated with the use of thiazide diuretics, Am. J. Hypertens. 22 (5) (2009) 545–551.
[144] M.J. van Rijn, A.F. Schut, Y.S. Aulchenko, J. Deinum, F.A. Sayed-Tabatabaei, M. Yazdanpanah, A. Isaacs, T.I. Axenovich, I.V. Zorkoltseva, M.C. Zillikens, H.A. Pols, J.C. Witteman, B.A. Oostra, C.M. van Duijn, Heritability of blood pressure traits and the genetic contribution to blood pressure variance explained by four blood-pressure-related genes, J. Hypertens. 25 (3) (2007) 565–570.
[145] D. Cusi, C. Barlassina, T. Azzani, G. Casari, L. Citterio, M. Devoto, N. Glorioso, C. Lanzani, P. Manunta, M. Righetti, R. Rivera, P. Stella, C. Troffa, L. Zagato, G. Bianchi, Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension, Lancet 349 (9062) (1997) 1353–1357.
[146] M.T. Sciarrone, P. Stella, C. Barlassina, P. Manunta, C. Lanzani, G. Bianchi, D. Cusi, ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy, Hypertension 41 (3) (2003) 398–403.
[147] S.T. Turner, A.B. Chapman, G.L. Schwartz, E. Boerwinkle, Effects of endothelial nitric oxide synthase, alpha-adducin, and other candidate gene polymorphisms on blood pressure response to hydrochlorothiazide, Am. J. Hypertens. 16 (10) (2003) 834–839.
[148] H. Schelleman, O.H. Klungel, J.C. Witteman, A. Hofman, C.M. van Duijn, A. de Boer, B.H. Stricker, The influence of the alpha-adducin G460W polymorphism and angiotensinogen M235T polymorphism on antihypertensive medication and blood pressure, Eur. J. Hum. Genet. 14 (7) (2006) 860–866.
[149] T. Matayoshi, K. Kamide, S. Takiuchi, M. Yoshii, Y. Miwa, Y. Takami, C. Tanaka, M. Banno, T. Horio, S. Nakamura, H. Nakahama, F. Yoshihara, T. Inenaga, T. Miyata, Y. Kawano, The thiazide-sensitive Na(+)-Cl(-) cotransporter gene, C1784T, and adrenergic receptor-

beta3 gene, T727C, may be gene polymorphisms susceptible to the antihypertensive effect of thiazide diuretics, Hypertens. Res. 27 (11) (2004 Nov) 821–833.
[150] B.R. Davis, D.K. Arnett, E. Boerwinkle, C.E. Ford, C. Leiendecker-Foster, M.B. Miller, H. Black, J.H. Eckfeldt, Antihypertensive therapy, the alpha-adducin polymorphism, and cardiovascular disease in high-risk hypertensive persons: the Genetics of Hypertension-Associated Treatment Study, Pharmacogenomics J. 7 (2) (2007) 112–122.
[151] T. Suonsyrjä, T. Hannila-Handelberg, H. Fodstad, K. Donner, K. Kontula, T.P. Hiltunen, Renin–angiotensin system and alpha-adducin gene polymorphisms and their relation to responses to antihypertensive drugs: results from the GENRES Study, Am. J. Hypertens 22 (2009) 169–175.
[152] J.A. Staessen, T. Kuznetsova, R. Acceto, A. Bacchieri, E. Brand, M. Burnier, H. Celis, L. Citterio, P.W. de Leeuw, J. Filipovsky, A. Fournier, K. Kawecka-Jaszcz, P. Manunta, Y. Nikitin, E.T. O'Brien, J. Redon, L. Thijs, P. Ferrari, G. Valentini, G. Bianchi, OASIS-HT: design of a pharmacogenomic dose-finding study, Pharmacogenomics 6 (2005) 755–775.
[153] A.E. Fletcher, P.J. Franks, C.J. Bulpitt, The effect of withdrawing antihypertensive therapy: a review, J. Hypertens. 6 (1988) 431–436.
[154] P.D. Levinson, I.M. Khatri, E.D. Freis, Persistence of normal BP after withdrawal of drug treatment in mild hypertension, Arch. Intern. Med. 142 (1982) 2265–2268.
[155] Y. Takata, T. Yoshizumi, Y. Ito, M. Ueno, A. Tsukashima, M. Iwase, K. Kobayashi, M. Fujishima, Comparison of withdrawing antihypertensive therapy between diuretics and angiotensin converting enzyme inhibitors in essential hypertensives, Am. Heart J. 124 (1992) 1574–1580.
[156] M.D. Blaufox, H.G. Langford, A. Oberman, C.M. Hawkins, S.W. Wassertheil-Smoller, G.R. Cutter, Effect of dietary change on the return of hypertension after withdrawal of prolonged antihypertensive therapy (DISH). Dietary Intervention Study of Hypertension, J. Hypertens. Suppl. 2 (1984) S179–S181.
[157] G.Y. Ho, M.D. Blaufox, S. Wassertheil-Smoller, A. Oberman, H. Langford, Plasma renin predicts success of antihypertensive drug withdrawal, Am. J. Hypertens. 7 (1994) 679–684.
[158] S. Swart, R.F. Bing, J.D. Swales, H. Thurston, Plasma renin in long-term diuretic treatment of hypertension: effect of discontinuation and restarting therapy, Clin. Sci. (Lond.) 63 (1982) 121–125.
[159] A. Zanchetti, G. Mancia, The dilemma of placebo controlled studies: scientific evidence, guidelines, ethics and regulatory recommendations, J. Hypertens. 27 (2009) 1–2.
[160] S.A. Dukacz, M.A. Adams, R.L. Kline, The persistent effect of long-term enalapril on pressure natriuresis in spontaneously hypertensive rats, Am. J. Physiol. 273 (1997) F104–F112.
[161] M. Liang, N.H. Lee, H. Wang, A.S. Greene, A.E. Kwitek, M.L. Kaldunski, T.V. Luu, B.C. Frank, S. Bugenhagen, H.J. Jacob, A.W. Cowley Jr., Molecular networks in Dahl salt-sensitive hypertension based on transcriptome analysis of a panel of consomic rats, Physiol. Genomics 34 (2008) 54–64.
[162] L. Lu, P. Li, C. Yang, T. Kurth, M. Misale, M. Skelton, C. Moreno, R.J. Roman, A.S. Greene, H.J. Jacob, J. Lazar, M. Liang, A.W. Cowley Jr., Dynamic convergence and divergence of renal genomic and biological pathways in protection from Dahl salt-sensitive hypertension, Physiol. Genomics 41 (2010) 63–70.
[163] A.L. Hopkins, Network pharmacology: the next paradigm in drug discovery, Nat. Chem. Biol. 4 (2008) 682–690.
[164] P. Vallance, M. Levick, Drug discovery and development in the age of molecular medicine, Clin. Pharmacol. Ther. 82 (2007) 363–366.