Modulation of Genetic Cardiovascular Risk by Age and Lifestyle

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Cardiovascular disease remains the leading cause of death worldwide. In this review, we briefly summarize new insights on the modulation of genetic cardiovascular risk by host factors such as sex and age and lifestyle factors such as salt intake. As phenotypes (genotypes) of interest, we considered left ventricular structure and function *(ADD1 Gly460Trp; AGTR2 G1675A; ACE D/I),* the incidence of heart failure *(ADD1 Gly460Trp),* heart rate variability *(CYP11B2 C–344T; AGTR1 A1166C),* carotid distensibility *(IL6 G–174C),* and serum lipid levels *(APOE ε2/ε3/ε4; APOA1 A75G).* In each case, the associations with the genetic cardiovascular risk factor were modulated by sex, age, sodium intake, or a combination thereof. These interactions highlight that genetic risk factors and phenotype–genotype associations can only be interpreted within their ecogenetic context. Perhaps a better understanding of these phenomena will lead researchers to target in a more specific way the pathophysiologic mechanisms operating within each individual.

Introduction

Cardiovascular disease remains the leading cause of death worldwide. In 1990, ischemic heart disease and stroke accounted for 6.26 and 4.38 million deaths, respectively [1]. In developing regions, cardiovascular disorders are fast replacing infectious diseases and malnutrition as the primary cause of disability and premature death. High blood pressure, hypercholesterolemia, and smoking explain 85% of the modifiable cardiovascular risk.

As a consequence of heritability or the shared environment, risk factors and cardiovascular disorders cluster within families [2]. Cardiovascular disease has a complex multifactorial etiology. Until now, geneticists have failed to identify single common genes with large effects on cardiovascular disease. It is conceivable that such genes do not exist and that cardiovascular diseases are dependent on a mosaic of many loci, each with a small influence or with a contribution differing according to host factors or lifestyle [3]. Throughout life, genetically determined host factors (eg, sex and age) continuously interact with environmental influences including lifestyle (Fig. 1). Any resulting change in cardiovascular phenotypes is initially counteracted by self-organizing homeostatic mechanisms, which encompass intracellular signaling, metabolic and hormonal regulation at cell and tissue levels, and systemic feedback loops involving the whole body [3]. Disease finally develops as a consequence of interactions between the innate condition, as coded in the genotype, and exposures to environmental agents indexed by time and space that are integrated by dynamic, regulatory networks at levels above the genome [4].

In this review, we briefly summarize new insights and recent data on the modulation of genetic cardiovascular risk by host factors and lifestyle. We use age and sex as examples of host factors and sodium intake as an example of a lifestyle characteristic.

Host Factors

Age

Among host factors, aging is an overriding determinant of cardiovascular risk; it operates in all ethnicities and in women as well as men. Most risk factors behave as age-related quantitative traits. Advancing age decreases insulin sensitivity [5], increases salt sensitivity [6], steepens the relationship between blood pressure and exchangeable body sodium [7], reduces renal perfusion [8], and compromises the buffering capacity of the large elastic arteries, leading to an increase in systolic and a decrease in diastolic blood pressure [9]. Aging also increases the exposure time to an unhealthy lifestyle or to detrimental environmental factors.

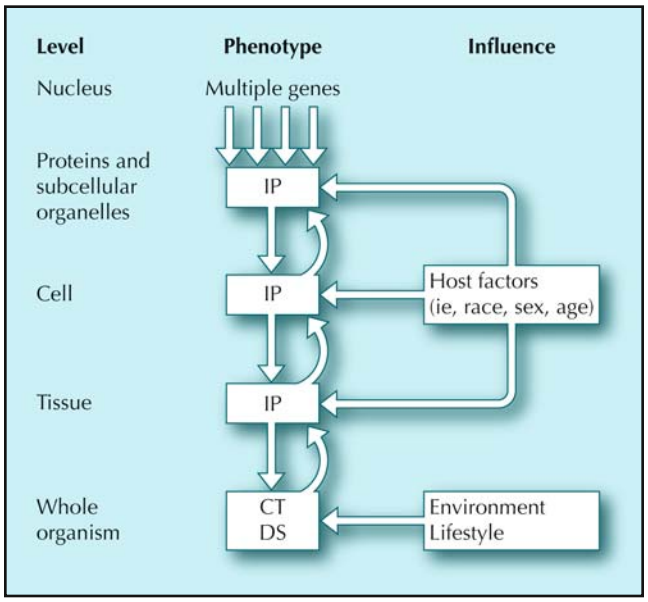

Figure 1. Representation of a complex multigenic trait, affected by host factors and lifestyle. *Curved arrows* represent feedback loops. CT—complex trait; DS—disease; IP—intermediate phenotypes. (*From* Staessen et al. [3]; with permission.)

Mutation of α-adducin is a risk factor predicting cardiovascular morbidity and mortality [10,11••]. Adducin is a heterodimeric cytoskeleton protein [12]. Investigations in rats [12,13], in vitro transfection studies [14], interventions in never-treated hypertensive patients [15], and epidemiologic studies [16,17] have shown a logical sequence of events leading from a point mutation in the α-subunit [13] to a cellular dysfunction characterized by higher activity of the sodium pump [14], hence increased tubular sodium reabsorption in the kidney [15] and, ultimately, hypertension [16].

Several mechanisms might explain how aging modulates the influence of genetic risk factors. One expectation is that by accumulation of environmental exposures throughout life, phenotype–genotype associations weaken in older people [18]. We recently investigated in a family-based population study the possible association of left ventricular diastolic function with genetic variation in the subunits of adducin [19••]. We measured early (Ea) and late (Aa) diastolic peak velocities of the mitral annulus by tissue Doppler imaging. In multivariable-adjusted analyses, the lateral Ea/Aa ratio, an index of left ventricular diastolic function, was higher in carriers of mutated α-adducin *(ADD1 Gly-460Trp)* compared with wild-type homozygotes (*Trp* allele carriers vs *GlyGly* homozygotes, 1.51 vs 1.4; $P = 0.005$). The best-fitting model for the lateral Ea/Aa ratio included an interaction term with age. In subjects younger than 50 years of age, the lateral Ea/Aa ratio was higher in *ADD1 Trp* allele carriers than in *GlyGly* homozygotes (1.91 vs 1.73; $P = 0.006$). In older subjects, this phenotype–genotype association was not significant. Also, transmission of the *ADD1 Trp* allele to offspring was associated with higher Ea/Aa ratio (+0.23; $P = 0.0008$). In this illustration of an association of a trait with a genetic risk factor, the association was more prominent in younger subjects, in whom long-standing environmental factors were less likely to mask the genetic effect.

An alternative explanation of genetic risk factor modulation by age rests on the mutation–accumulation theory [20]. Mutations with late-onset deleterious effect should be more common in the population the later the age at which they take effect. According to this theory, mutations with late-onset effects can be passed to offspring and therefore increase their frequency within the population. However, the phenotypic variation due to mutations with late-onset effect is larger in older than younger subjects.

In multivariable adjusted analyses of a Flemish population, we noticed an interaction between the α-adducin genotype and age at recruitment analyzed as a continuous variable [10]. For cardiac end points, which included coronary events and heart failure, the hazard ratio associated with the *ADD1 Trp* allele increased by 39.7% (95% CI, 3%–87.7%; $P = 0.03$) for each 10-year increment in age. The age-related heart failure risk in carriers of the *Trp* allele is not inconsistent with the better diastolic function at the younger age (higher Ea/Aa ratio). Indeed, the point mutation in α-adducin leads to higher sarcolemmal sodium–potassium pump activity [14,21]. Variation in the Na^+, K^+-ATPase activity and intracellular Na^+ concentration might influence the Na^+-dependent transmembranous Ca^{2+} transport in cardiomyocytes [22]. Changes in the transmembranous Ca^{2+} fluxes might underlie the faster left ventricular relaxation at young age and also explain the higher susceptibility to heart failure at old age, because intracardiomyocyte Ca^{2+} plays an essential role in the excitation–contraction coupling [19••].

Telomeres are tandem repeats of DNA sequences that cap the ends of chromosomes and shield them from DNA damage repair pathways [23]. With each replication of somatic cells, telomeres become progressively shorter [24]. Several studies showed that telomere length is a heritable trait [25]. Population studies associated shorter telomere length in leukocytes with cardiovascular disease and possibly with a shorter lifespan [26]. In a random population sample (mean age, 42.5 years; 55.8% women; 22.2% smokers), we investigated the association among biologic and vascular aging, oxidized low-density lipoprotein (LDL), smoking, and the functional interleukin-6 *(IL6) C–174G* gene polymorphism [27]. In all subjects, the sex- and age-adjusted telomere length was independently and inversely correlated with plasma oxidized LDL ($P < 0.001$). In nonsmokers, telomere length, oxidized LDL, and carotid artery distensibility were not associated with the IL6 polymorphism. In contrast, in smokers, telomere length ($P < 0.02$) and carotid distensibility ($P < 0.05$) decreased, whereas plasma oxidized LDL ($P < 0.05$) increased with the number of copies of the *IL6 –174C* allele. These findings exemplify how genetic risk factors, vascular aging, and lifestyle (in this case, smoking) are intertwined.

Sex

Although women and men share most genetic information, they have substantially different susceptibilities to common diseases such as atherosclerosis and diabetes and their preceding risk factors [28,29,30••]. Apolipoprotein E (apoE) is a constituent of circulating chylomicrons, very low-density lipoproteins, and high-density lipoproteins (HDLs) and serves as a ligand for the LDL receptor and the LDL receptor–related protein [31]. The most commonly studied genetic variation at the *APOE* locus results from three common alleles in the population: ε4 (representing genotypes ε4/ε3 and ε4/ε4), ε3 (representing ε3/ε3), and ε2 (representing ε2/ε2 and ε2/ε3). These *APOE* alleles may account for up to 7% of the variation in the serum concentrations of total and LDL cholesterol in the general population [32]. In view of these data, the Framingham Heart Study [33] and the Copenhagen City Heart Study [34] investigated how sex modulates the cardiovascular risk associated with genetic variation in *APOE*.

In the Framingham Offspring Study, the period prevalence of cardiovascular disease (366 events) between examinations 1 and 5 was related to the *APOE* genotype [33]. The age-adjusted period prevalence of cardiovascular disease in men was 19% for ε4, 13% for ε3, and 18% for ε2 ($P = 0.004$). In women, these rates were 10%, 7%, and 5%, respectively ($P = 0.04$). After adjustment for all risk factors, the relative odds of cardiovascular disease in men with the ε2 allele compared with men with the ε3/ε3 genotype were 1.94 ($P = 0.004$), and in men with ε4, this estimate was 1.51 ($P = 0.03$). Therefore, the presence of the ε2 or ε4 allele in men was associated with a significantly greater risk of cardiovascular disease. In women, the ε4 allele was also associated with an increased prevalence of cardiovascular disease, whereas in contrast to men, the ε2 allele was protective.

The investigators of the Copenhagen City Heart Study determined the *APOE* genotypes of 5112 women and 4129 men from the general population and those of 247 women and 693 men with ischemic heart disease [34]. After adjustment for age and other risk factors, the odds ratios were 0.38 (95% CI, 0.18–0.79) for women with ε2/ε3, 1.35 (95% CI, 1.02–1.78) for men with ε4/ε3, and 1.58 (95% CI, 0.8–3.08) for men with ε4/ε4. Therefore, the presence of the ε2/ε3 genotype was protective in women (9% lower relative risk of ischemic heart disease) compared with ε3/ε3, whereas in men, ε4/ε3 and ε4/ε4 were associated with an increased coronary risk (8% and 2%, respectively).

Apolipoprotein A1 (apoA1) is the major protein of HDL, an in vivo activator of the enzyme lecithin-cholesterol acyltransferase, and constitutes a key component of the reverse cholesterol transport process [35]. The *APOA1* gene clusters with the *APOC3, APOA4,* and *APOA5* genes on the long arm of human chromosome 11 [36]. This DNA region has been analyzed extensively. Multiple reports have shown associations of some common genetic variants with lipid abnormalities as well as with an increased risk of coronary heart disease [37,38]. A common variant due to an adenine (A)-to-guanine (G) transition (G/A) was described more than two decades ago 75 bp upstream of the *APOA1* gene transcription starting site. Carriers of the *A* allele (15%–20% of whites) have higher serum concentrations of HDL cholesterol than do *GG* homozygotes [39]. A meta-analysis examined the associations of this polymorphism with plasma lipid profiles and concluded that there may be a mild association with the plasma *APOA1* concentration that is more apparent in men than in women [39]. In the Framingham Offspring Study, an inverse association between the dietary intake of polyunsaturated fatty acids (PUFA) and serum HDL cholesterol occurred in female *GG* homozygotes but not in female *A* allele carriers [40]. Furthermore, female *GG* homozygotes had higher HDL cholesterol concentrations than did female *A* allele carriers when dietary PUFA was less than 4% of total energy intake, but the opposite was true when dietary PUFA was more than 8% of total energy. In men, the interaction between PUFA and the *A–75G* polymorphism was statistically significant only when alcohol consumption and smoking were factored into the regression model, confirming the importance of analyzing women and men separately. In this setting, higher PUFA intake benefited female *A* allele carriers by increasing their HDL cholesterol concentration and therefore reducing their cardiovascular risk.

Lifestyle

Petersen et al. [41•] investigated the genetic influence on the rate of dying before age 70 years in Danish adoptees and their biologic full and half siblings. They selected 1552 case adoptees who died before April 1993 and 1710 noncase adoptees who were alive at that date. Compared with the mortality of the biologic siblings of noncase adoptees, the mortality of biologic siblings of dead adoptees was approximately twofold higher for death from infectious and vascular causes and about 45% for death from natural or all causes. The Danish investigators suggested that before 70 years old, there is a genetic effect on the rate of death, whereas there is little indication of an influence of shared sibling environment. However, most available evidence suggests that phenotype–genotype associations in complex diseases can only be interpreted if one accounts for lifestyle and environment [3] (Fig. 1). Underestimation of this complexity was a major hurdle in dissecting genetic variation in relation to cardiovascular disease. In this section, we illustrate this principle using left ventricular structure and heart rate variability as the phenotypes of interest in relation to genetic variation in the renin-angiotensin-aldosterone system (RAAS) and sodium intake as lifestyle factor.

Left ventricular structure

Left ventricular hypertrophy is a risk factor for developing ischemic heart disease and heart failure, two conditions

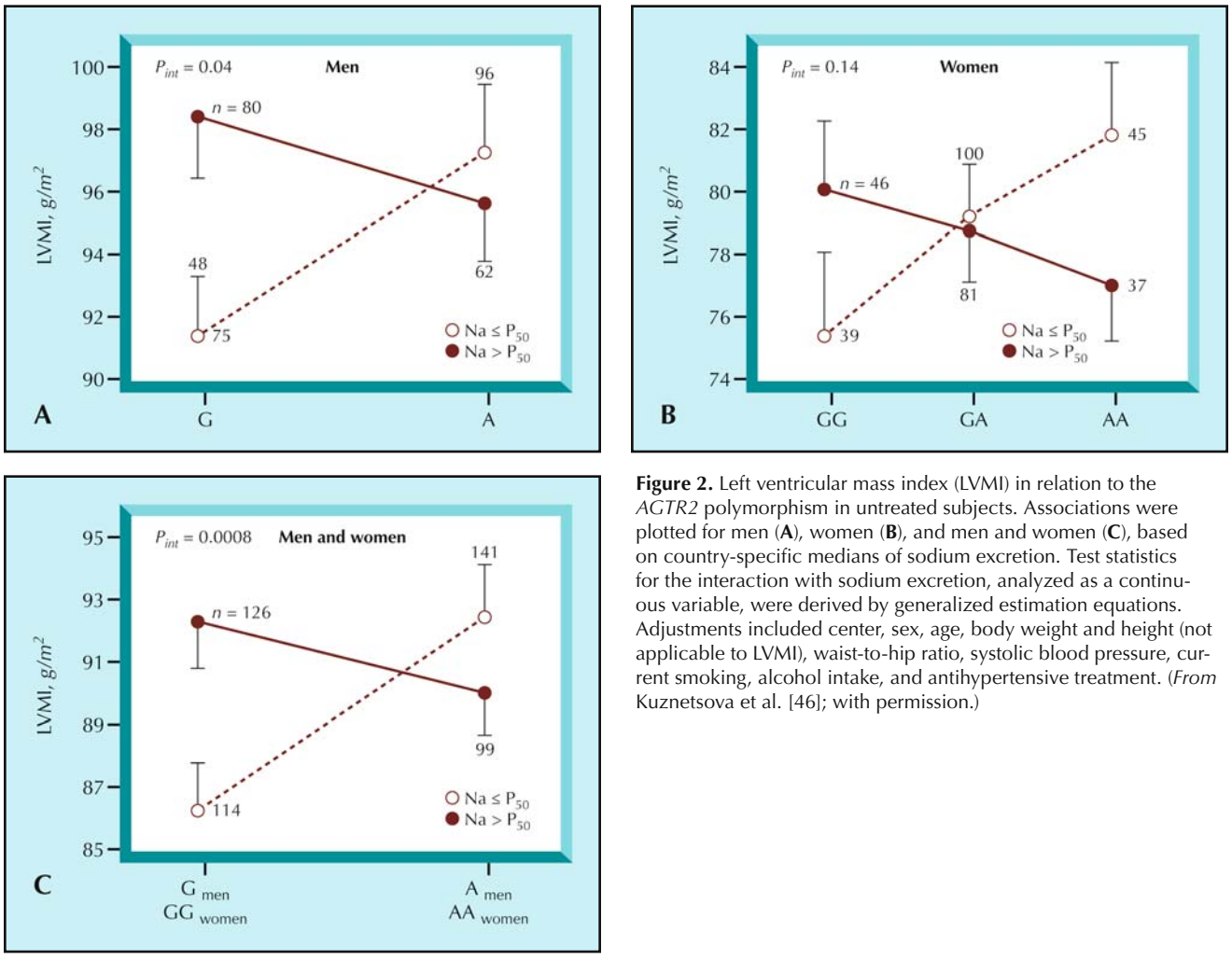

Figure 2. Left ventricular mass index (LVMI) in relation to the *AGTR2* polymorphism in untreated subjects. Associations were plotted for men (**A**), women (**B**), and men and women (**C**), based on country-specific medians of sodium excretion. Test statistics for the interaction with sodium excretion, analyzed as a continuous variable, were derived by generalized estimation equations. Adjustments included center, sex, age, body weight and height (not applicable to LVMI), waist-to-hip ratio, systolic blood pressure, current smoking, alcohol intake, and antihypertensive treatment. (*From* Kuznetsova et al. [46]; with permission.)

associated with high morbidity and mortality in developed and developing regions. The RAAS not only regulates sodium and water homeostasis but also emerges as a pivotal player in cardiovascular remodeling [42]. Its activity modulates the development and regression of left ventricular hypertrophy. Angiotensin II mediates many effects of the RAAS via stimulation of angiotensin II type 1 (AT1) receptor. The angiotensin II type 2 (AT2) receptor counterbalances the vasoconstrictor and antinatriuretic effects produced by angiotensin II via the AT1 receptor [43]. The AT2 receptor gene *(AGTR2)* maps to the X chromosome [43]. Regulatory elements of the transcription of *AGTR2* are located in the promoter area and the first intron [44]. The *AGTR2 G1675A* polymorphism, which is located in intron 1, is likely to be functional, in that the *G* allele is associated with increased *AGTR2* transcription and therefore with increased expression of the AT2 receptor [44]. In animal experiments, dietary sodium depletion also enhanced the expression of the AT2 receptors [45].

One might speculate that the more pronounced expression of the AT2 receptor in *G* allele carriers, particularly in the presence of sodium depletion, might exert protective effects. Indeed, we observed that in men the effect of the *AGTR2 G1675A* polymorphism on left ventricular mass (LVM) differed according to sodium excretion [46]. In women, this gene–environment interaction did not reach statistical significance. Continuous analyses demonstrated that in male *G* allele carriers, the LVM index and left ventricular internal diameter (but not wall thickness) increased with higher sodium excretion. Further analyses, involving only untreated men and dichotomized according to the median sodium excretion, showed that when sodium excretion was less than 240 mmol/d (median), LVM index was lower in *G* than *A* allele carriers (Fig. 2).

The hypothesis that the AT2-mediated effects of angiotensin II on LVM are modulated by sodium intake is plausible. LVM is calculated from the left ventricular internal diameter and wall thickness. To some degree, left ventricular diameter reflects the circulating fluid volume, whereas wall thickness might be more indicative of processes confined to the myocardium itself. We found that the interaction between the *G1675A* polymorphism and sodium excretion in relation to LVM was mediated via the left ventricular internal diameter [46]. This suggests that renal AT2 receptors—via their influence on sodium

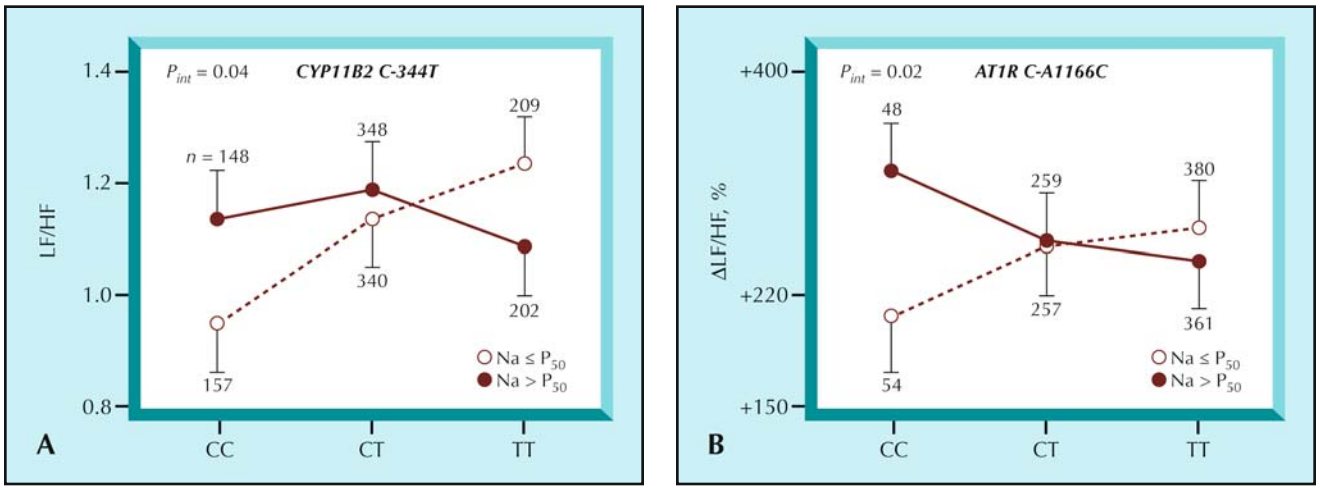

Figure 3. Low-to-high frequency ratio in the supine position (LF/HF) and orthostatic change in this ratio expressed as a percentage of its value in the supine position (ΔLF/HF) in relation to the *CYP11B2* (**A**) and *AT1R* (**B**) polymorphisms, respectively. Associations were plotted for two groups, based on country- and sex-specific medians of sodium excretion. The probability of the interaction *(P_{int})* between genotype and sodium excretion analyzed as a continuous variable was derived by generalized estimation equations and accounts for nonindependence within families and covariables. (*From* Stolarz et al. [49]; with permission.)

balance and the circulating plasma volume—might contribute to the explanation of the above findings. On the other hand, we also confirmed an independent effect of the angiotensin-converting enzyme *(ACE) D/I* genotype on LVM [47]. This effect was mediated via wall thickness, and it was also dependent on sodium intake.

Heart rate variability

Sympathetic tone increases with RAAS stimulation and is influenced by salt intake [48]. Measurement of heart rate variability (HRV) in the frequency domain provides information on the autonomic nervous modulation of the cardiovascular system. The high frequency (HF) component of HRV depends on vagal activity, whereas the low frequency (LF) component predominantly reflects sympathetic modulation. Therefore, we investigated whether polymorphisms in the genes encoding aldosterone synthase *(CYP11B2 C–344T)* and the type-1 angiotensin II receptor *(AGTR1 A1166C)* affect the autonomic modulation of HRV at varying salt intake levels [49]. We measured the LF and HF components of HRV and their ratio (LF/HF) in the supine and standing positions in 1797 participants (401 families) who were randomly recruited from six European populations and whose average urinary sodium excretion ranged from 163 to 245 mmol/d. When we did not account for sodium excretion, neither the population-based nor the family-based analyses revealed any significant association between HRV and the genetic polymorphisms. However, in subjects with sodium excretion less than 190 mmol/d, supine heart rate, LF, and LF/HF increased and HF decreased with the number of *CYP11B2 –344T* alleles. The orthostatic changes in LF, HF, and LF/HF were blunted in carriers of the *AGTR1 1166C* allele (Fig. 3). In subjects with sodium excretion greater than 190 mmol/d, these associations were not significant (with the *CYP11B2* polymorphism) or were in the opposite direction (with the *AGTR1* polymorphism) (Fig. 3).

Our findings, in keeping with other reports in the literature [50,51], support the hypothesis that genetic polymorphisms and lifestyle factors leading to expansion of the circulating plasma volume might affect the autonomic nervous regulation of the cardiovascular system. An excessive salt intake might be associated with an expanded circulating plasma volume [7], which in turn might mask the genetic influence of the *CYP11B2 C–344T* polymorphism on heart rate variability. Salt intake modulates the expression of AT1 receptors [52]. Depending on salt intake, the *AGTR1 1166CC* genotype may or may not be associated with a tendency for volume expansion, which might explain the differential autonomic modulation of heart rate variability in *CC* homozygotes.

Conclusions

Genotype–phenotype associations can only be interpreted within their ecogenetic context by accounting for interactions with host factors (eg, age and sex) and lifestyle (eg, salt intake). The notion that the discovery of major genes or interference with a sole pathophysiologic mechanism will substantially advance prevention and treatment is an oversimplification that ignores the heterogeneous nature of cardiovascular disease. Integration of genetic, molecular, clinical, and epidemiologic research could disclose the interactive way in which genetic, host, and environmental factors lead to different expression of proteins controlling the disease processes. Such integrated multidisciplinary research might identify subsets of patients in whom specific combinations of genetic and host factors cause disease and, in the long run, might lead to individualized treatment.

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Disclosures

No potential conflicts of interest relevant to this article were reported.

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