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Renin–angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases

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Abstract

Since the first description of the angiotensin-converting enzyme insertion/deletion polymorphism more than a decade ago, many hundreds of investigations have reported associations between this polymorphism and cardiovascular diseases. Subsequently, similar studies were performed in relationship with several other renin–angiotensin system gene polymorphisms, most notably the angiotensinogen M235T polymorphism and the angiotensin AT_1 receptor A1166C polymorphism. Surprisingly however, especially in view of the many contradictory results that have been obtained, very little attention has been paid to the mechanism(s) that may link these genetic variants and respective diseases. Here, we review the limited evidence that is currently available on the functional consequences (including compensatory mechanisms) of the above three renin–angiotensin system gene polymorphisms, in order to provide an explanation for the reported associations (or lack thereof) between these polymorphisms and cardiovascular diseases.

Keywords: Angiotensin; Angiotensin-converting enzyme; Angiotensinogen; Angiotensin AT_1 receptor; Polymorphism; Renin

1. Introduction

In the past decade, associations between several cardiovascular diseases and polymorphisms of renin–angiotensin system genes have been reported, in particular for the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene, the M235T polymorphism of the angiotensinogen gene and the A1166C polymorphism of the angiotensin AT_1 receptor gene. Although many of these associations were supported or disputed in hundreds of subsequent investigations, little attention has been paid to the mechanisms that may link these genetic variants and respective diseases. Specifically, the intermediate phenotypes that may result from these polymorphisms should be identified in much more detail in order to understand why contradictory results have been obtained. In this respect, the only observations that have been made consistently are elevated angiotensin-converting enzyme levels in carriers of the angiotensin-converting enzyme D allele and elevated angiotensinogen levels in carriers of the angiotensinogen T235 variant (Fig. 1). Convincing evidence for a relationship between angiotensin AT_1 receptor density and/or function and the angiotensin AT_1 receptor C allele has not yet been obtained.

In the present study, we review the limited evidence that is currently available on the functional consequences (including compensatory mechanisms) of the above three renin–angiotensin system gene polymorphisms, in order to provide an explanation for the reported associations (or lack thereof) between these polymorphisms and cardiovascular diseases.

2. Renin–angiotensin system gene polymorphisms and biochemical levels of renin–angiotensin system components

2.1. Angiotensin-converting enzyme gene I/D polymorphism

Angiotensin-converting enzyme is an ectoenzyme found in most mammalian tissues on the external surface of the

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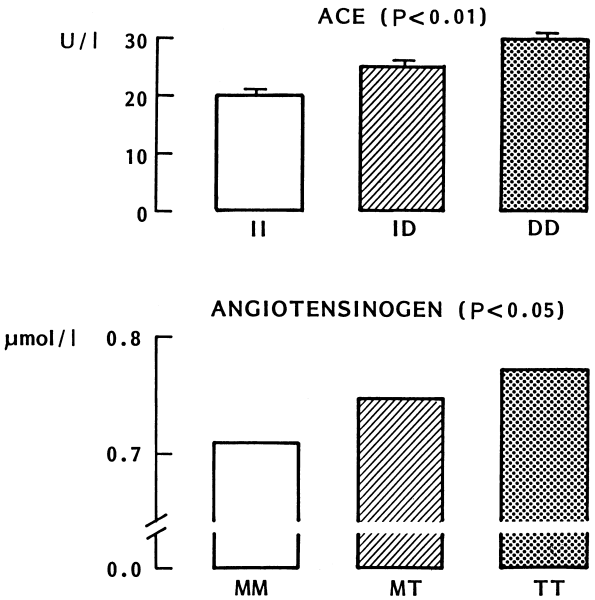

Fig. 1. Plasma angiotensin-converting enzyme (ACE) levels (mean ± S.E.M.) according to angiotensin-converting enzyme I/D genotype (top), and plasma angiotensinogen levels (geometric mean) according to angiotensinogen M235T genotype (bottom) in 396 subjects who had participated in the MONICA population survey (Danser et al., 1998a).

plasma membrane of endothelial and epithelial cells. In addition, an active, soluble form of angiotensin-converting enzyme, derived from endothelial cells, is present in circulating blood plasma. Angiotensin-converting enzyme cleaves a number of substrates, most notably, angiotensin I and bradykinin. Angiotensin I is activated to the vasoconstrictor angiotensin II, while bradykinin, a vasodilating peptide, is degraded to inactive metabolites.

The most prominent polymorphism in the angiotensin-converting enzyme gene is defined by the presence (insertion; I) or absence (deletion; D) of a 287 base pair insert in intron 16 of the gene. This insert harbors a sequence very similar to a silencer element (Yoshida et al., 1997), which may explain why subjects with one or two D alleles have approximately 25% and 50% higher angiotensin-converting enzyme levels than subjects with the II genotype. These higher angiotensin-converting enzyme levels are observed both in plasma (Rigat et al., 1990; Schunkert, 1997) and at tissue sites (Danser et al., 1995; Mizuiri et al., 1998). The enzymatic activity (K_m) of angiotensin-converting enzyme is not affected by the genotype (Faure-Delanef et al., 1998).

Membrane-bound angiotensin-converting enzyme, rather than soluble angiotensin-converting enzyme, is responsible for the regional conversion of angiotensin I into angiotensin II (Danser et al., 1992; Admiraal et al., 1993). This process follows first-order kinetics, since the angiotensin I levels in blood plasma or the interstitium ($\approx$ 20 pM; Admiraal et al., 1993; Schuijt et al., 1999) are approximately six orders of magnitude below the K_m for angiotensin I ($\approx$ 16 μM). First-order kinetics will apply even at angiotensin I levels that are 10,000-fold higher than normal. Accordingly, in humans, pigs and rats, angiotensin I–II conversion was found to be similar over a wide range of arterial angiotensin I levels (Admiraal et al., 1990; Danser et al., 1992; Müller et al., 1998; Van Dijk et al., 2000). Remarkably, a recent study on the human forearm circulation documented that angiotensin I–II conversion at high and low flow rates is indistinguishable (Saris et al., 2000) (Fig. 2). In contrast, forearm angiotensin I degradation (i.e., all angiotensin I metabolism other than conversion) showed the expected inverse correlation with blood flow (Fig. 2). The latter is in agreement with the concept that, at lower flow rates, more time is available for metabolism. The fact that this does not apply to angiotensin I–II conversion suggests that conversion occurs more efficiently than degradation, with maximum result even at high flow rates. An alternative explanation might be that not all arterially delivered angiotensin I is exposed to angiotensin-converting enzyme, for instance because angiotensin-converting enzyme is only present in a limited part of the vascular bed (e.g., the arterioles). Degradation involves many enzymes (the so-called angiotensinases), and most likely occurs across the entire vascular bed.

Evidence for increased angiotensin I–II conversion at increased angiotensin-converting enzyme levels (such as found in carriers of the angiotensin-converting enzyme DD genotype or in subjects with cardiac hypertrophy) is scarce. In the few animal studies that investigated this phenomenon, the authors did not correct for regional an-

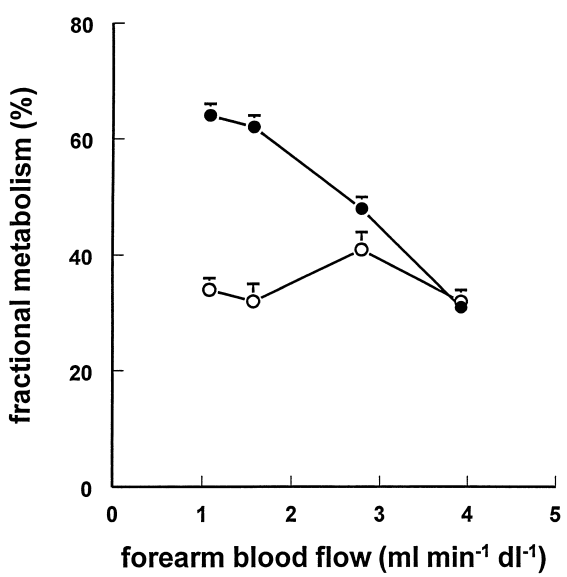

Fig. 2. Fractional conversion (open symbols; mean ± S.E.M.) and degradation (closed symbols) of arterially delivered angiotensin I in the forearm (i.e., the percentage of arterially delivered angiotensin I that is metabolised into angiotensin II or other metabolites, respectively, during passage of the forearm vascular bed). Metabolism was quantified at varying blood flow rates, during infusion of angiotensin I into the brachial artery of 14 healthy volunteers (Danser et al., 1999; Saris et al., 2000).

giotensin II degradation (by angiotensinases) when calculating regional angiotensin I–II conversion on the basis of the angiotensin I and angiotensin II levels in the venous effluent (Schunkert et al., 1990; Schunkert et al., 1993; Müller et al., 1998). Nevertheless, some of these studies demonstrated increased angiotensin I–II conversion rates when tissue angiotensin-converting enzyme activity was induced. By contrast, a recent study observed similar angiotensin I-induced blood pressure responses in inbred rats of two strains with high and low angiotensin-converting enzyme levels, despite the two- to threefold differences in plasma and tissue angiotensin-converting enzyme levels in the F2 progeny groups (Challah et al., 1998). Moreover, transgenic rats overexpressing cardiac angiotensin-converting enzyme 40-fold had normal cardiac angiotensin II levels (Pinto et al., 1998).

Remarkably, despite the many hundreds of studies investigating associations between the angiotensin-converting enzyme I/D polymorphism and cardiovascular diseases, the number of studies investigating whether the D allele-related increase in angiotensin-converting enzyme concentration actually affects the levels of angiotensin II is very limited. None of these studies reported differences in plasma angiotensin II levels between II, ID and DD subjects (Table 1) (Lachurié et al., 1995; Ueda et al., 1995; Chadwick et al., 1997; Danser et al., 1999; Van Dijk et al., 2000). Thus, the genetic variability of angiotensin-converting enzyme concentration related to the I/D polymorphism (1) is either too small to affect circulating angiotensin II levels, (2) may have no functional consequences, for instance because the excess angiotensin-converting enzyme is located at a site that does not equilibrate with circulating angiotensin I, or (3) is compensated via an increase or reduction of renal renin release. The first explanation is unlikely, since a 50% increase in angiotensin-converting enzyme levels should theoretically result in a 50% increase in angiotensin I–II conversion, and consequently in a rise in the levels of angiotensin II. The second explanation fits with our observation that regional angiotensin I–II conversion is flow-independent. Indeed, if all angiotensin I that is capable of interacting with angiotensin-converting enzyme is converted to angiotensin II, a 50% increase in angiotensin-converting enzyme concentration will have no further consequences. The third explanation will be discussed below. Taken together, the present data do not support the idea that a 25–50% increase in angiotensin-converting enzyme levels results in higher plasma angiotensin II concentrations under physiological conditions. As far as tissue angiotensin II levels are concerned, no data are currently available in relationship with the angiotensin-converting enzyme I/D polymorphism.

The implications of the angiotensin-converting enzyme I/D polymorphism for bradykinin-mediated effects have not been investigated in great detail. Brown et al. (1998) observed a significantly longer half life of bradykinin in sera of subjects with the II genotype. By contrast, Van Dijk et al. (2000) found no differences in the bradykinin-induced changes in forearm blood flow between healthy individuals with the angiotensin-converting enzyme II or DD genotype. Similar observations were made in rats with high and low angiotensin-converting enzyme levels during infusion of bradykinin (Challah et al., 1998). Future studies should explore this issue, in particular because the involvement of bradykinin in the beneficial actions of angiotensin-converting enzyme inhibitors has become more apparent in recent investigations.

2.2. Angiotensinogen gene M235T polymorphism

Angiotensinogen is an extracellular glycoprotein that is synthesized and released by hepatocytes, adipocytes, and astrocytes. It is constitutively secreted, thereby ruling out rapid changes in its concentration. Most, if not all, angiotensinogen in the circulation and in tissues that do not synthesize angiotensinogen in large quantities (such as the heart) is liver-derived (Danser et al., 1994; De Lannoy et al., 1997; Heller et al., 1998). The plasma concentrations of angiotensinogen (≈ 1 μM) approximate the Michaelis–Menten constant of the renin reaction, so that plasma angiotensin production is sensitive to small changes in angiotensinogen concentration. Indeed, angiotensinogen levels correlate inversely with renin levels, both in the circulation (Danser et al., 1998b) and at tissue sites (Danser et al., 1997).

Table 1
Baseline plasma levels of angiotensin II, angiotensin I-II conversion during infusion of angiotensin I and blood pressure response to angiotensin I and/or II in subjects with the angiotensin-converting enzyme II, ID or DD genotype

	Lachurié et al., 1995	Ueda et al., 1995	Chadwick et al., 1997	Danser et al., 1999	Van Dijk et al., 2000
n (II/ID/DD)	12/0/12	10/0/10	8/0/8	8/16/5	8/0/8
Plasma angiotensin II at baseline	−	−	−	−	−
Angiotensin I-II conversion	−	+	ND	−	−
Response to angiotensin I	−	+	−	ND	+
Response to angiotensin II	ND	−	ND	ND	−

+, enhanced effect in DD vs. II; −, no difference between DD and II; ND, not determined.

The angiotensinogen M235T polymorphism consists of a missense mutation located in exon 2 of the gene, corresponding to a change from methionine to threonine at position 235 of mature angiotensinogen. Inoue et al. (1997) recently demonstrated a very tight linkage disequilibrium between T235 and a molecular variant in the proximal promoter of the angiotensinogen gene (an adenine instead of a guanine six nucleotides upstream from the site of transcription initiation, A(−6)). The A/G substitution at nucleotide −6 affects specific interactions between at least one trans-acting nuclear factor and the promoter of angiotensinogen, thereby influencing the basal rate of transcription of the gene. This finding most likely explains why T235 homozygotes have plasma angiotensinogen levels that are 10–20% higher than M235 homozygotes (Fig. 1) (Jeunemaître et al., 1992; Schunkert et al., 1997). Such differences in angiotensinogen level may also occur in angiotensinogen-generating tissues, since the expression of the T235 allele in decidual spiral arteries of heterozygous women was elevated twofold compared to the M235 allele (Morgan et al., 1997).

The M235T polymorphism alone does not alter the K_m for renin (Inoue et al., 1995). However, Gimenez-Roqueplo et al. (1998) recently demonstrated that a cysteine residue at position 232 (i.e., very close to the M235T site) is crucial for the formation of a complex with the proform of the eosinophilic major basic protein (proMBP) in pregnant women. Hydrolysis of the angiotensinogen-proMBP complex by human renin was seven times slower than hydrolysis of the monomeric form, whatever the M235T genotype. The complex/monomeric angiotensinogen ratio was greater for M235 than for T235 angiotensinogen, thus raising the possibility that angiotensin I generation could be significantly lower in pregnant women with the MM genotype.

In the only study that related plasma angiotensin II levels to the M235T polymorphism, no differences were found between the three genotype groups (Hopkins et al., 1996). This is not what one would predict based upon the fact that the levels of circulating angiotensinogen resemble the K_m for the renin reaction. Thus, as previously discussed with regard to the angiotensin-converting enzyme I/D polymorphism, compensation may have occurred via a reduction of renal renin release.

2.3. Angiotensin AT_1 receptor gene A1166C polymorphism

Angiotensin AT_1 receptors mediate most of the known effects of angiotensin II, i.e., vasoconstriction, stimulation of Na^+ reabsorption and aldosterone biosynthesis, induction of cellular growth and hypertrophy, and facilitation of sympathetic neurotransmission. Angiotensin AT_1 receptors are expressed on a variety of cell types, including vascular smooth muscle cells, myocardial cells, mesangial cells and proximal tubule cells of the kidney, and glomerulosa cells of the adrenal gland.

The angiotensin AT_1 receptor A1166C polymorphism is located in the 3′ untranslated region of the gene. Since its first description more than 5 years ago (Bonnardeaux et al., 1994), little progress has been made in unraveling the functional significance of this polymorphism. The binding of AUF1, a protein that affects angiotensin AT_1 receptor mRNA stability through binding to AU-rich regions of the 3′ untranslated region of the mRNA, is not affected by the A1166C polymorphism (Pende et al., 1999). Paillard et al. (1999) did not detect a genotype effect on either the B_{max} or K_d of angiotensin AT_1 receptors on platelets of 114 normotensive subjects. In agreement with this lack of C allele-related changes in angiotensin AT_1 receptor density or affinity, these authors, as well as Miller et al. (1999) found no genotype-related alterations in the plasma levels of angiotensin II.

Interestingly, CC homozygotes ($n = 13$) displayed significantly greater alpha-adrenoceptor and serotonin receptor stimulation in distal coronary vessels than subjects with the AA ($n = 67$) or AC ($n = 60$) genotype (Amant et al., 1997). This enhanced vasoconstriction was confirmed in vitro, as the contractile response to phenylephrine of internal mammary arteries obtained from 20 AA homozygotes was larger than the response of arteries from 30 C allele carriers (Henrion et al., 1998).

In summary, evidence for a C allele-related change in angiotensin AT_1 receptor density and/or affinity could not be obtained. In agreement with this observation, plasma angiotensin II levels were unaffected by the genotype. The CC genotype is associated with enhanced vasoconstriction through an unknown mechanism.

2.4. Feedback regulation and renin–angiotensin system genotypes

Within a feedback-regulated system such as the renin–angiotensin system (Fig. 3) one should consider compensatory mechanisms that may neutralise alterations of one

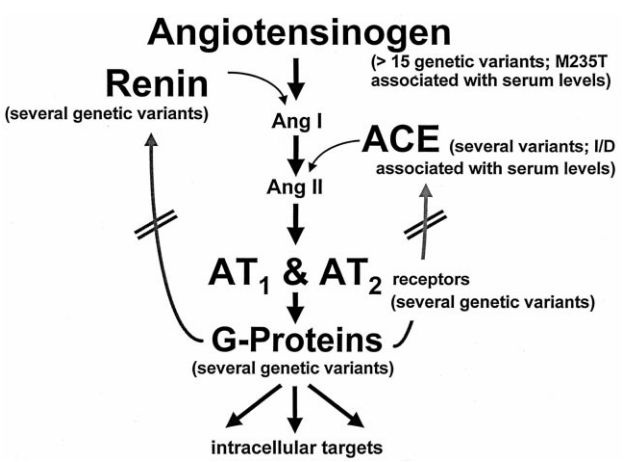

Fig. 3. Feedback mechanisms within the renin-angiotensin system. ACE, angiotensin-converting enzyme; Ang, angiotensin; AT_1, angiotensin AT_1 receptor; AT_2, angiotensin AT_2 receptor.

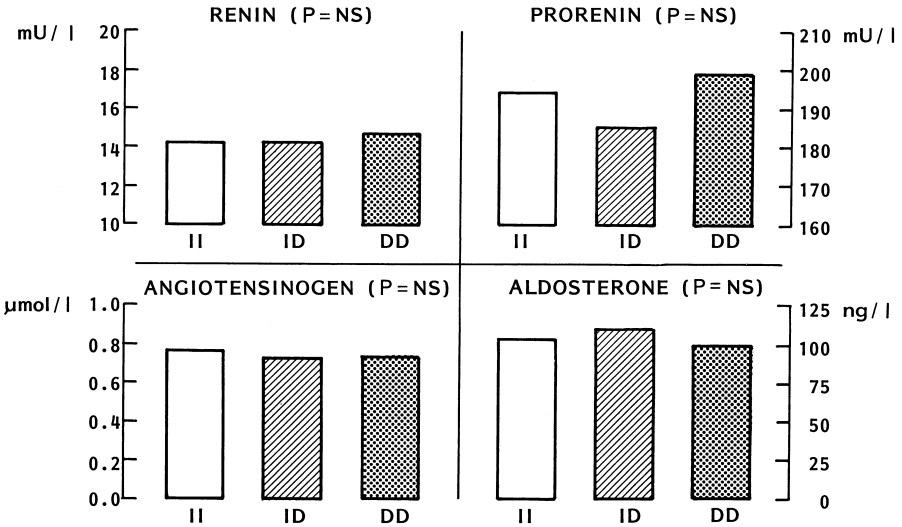

Fig. 4. Plasma levels of renin, prorenin, angiotensinogen and aldosterone (geometric means) according to angiotensin-converting enzyme I/D genotype in 396 subjects who had participated in the MONICA population survey (Danser et al., 1998a).

individual component. We asked the question whether a potential rise in the plasma levels of angiotensin II, due to the genetically elevated angiotensin-converting enzyme or angiotensinogen levels in carriers of the D and T235 allele, is compensated by a reduction in renal renin release. Such a mechanism would return the rate of angiotensin II generation to normal levels, thereby explaining why no D allele- or T235 allele-related changes in plasma angiotensin II levels have been observed (Lachurié et al., 1995; Ueda et al., 1995; Hopkins et al., 1996; Chadwick et al., 1997; Danser et al., 1999; Van Dijk et al., 2000). The results, obtained in a large epidemiological study (Danser et al., 1998a), are shown in Figs. 4 and 5. Plasma renin levels were downregulated in carriers of the angiotensinogen T235 allele, but not in those with the angiotensin-converting enzyme D allele. Changes in the plasma levels of prorenin, the inactive precursor of renin, paralleled those in renin. Interestingly, no angiotensinogen genotype-related differences were observed for either plasma angiotensin-converting enzyme or plasma aldosterone. This supports the contention that the decreased renin levels that accompany the genetically increased angiotensinogen levels result in similar angiotensin II generation rates in MM, MT and TT subjects. The lack of angiotensin-converting enzyme genotype-related changes in the plasma levels of (pro)renin, angiotensinogen and aldosterone argues against the concept of elevated angiotensin II generation in carriers of the D allele.

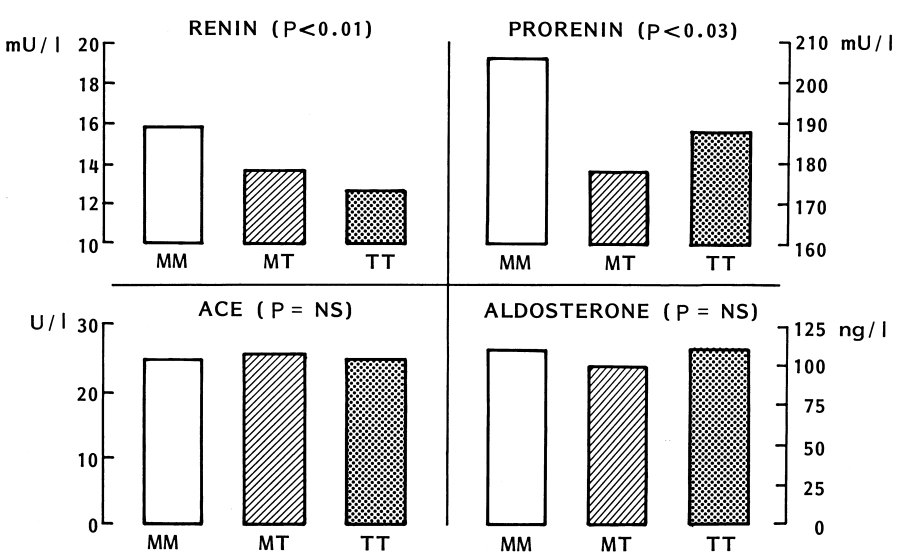

Fig. 5. Plasma levels of renin, prorenin, angiotensin-converting enzyme (ACE) and aldosterone (geometric means) according to angiotensinogen M235T genotype in 396 subjects who had participated in the MONICA population survey (Danser et al., 1998a).

Taken together, our findings fully support the notion that an increase in angiotensinogen, but not an increase in angiotensin-converting enzyme, affects plasma angiotensin II levels. Feedback regulation, however, allows renal renin to compensate for such elevated angiotensin II levels, thereby normalising the overall activity of the circulating renin–angiotensin system in T235 allele carriers. It is currently not known whether such feedback regulation also compensates for elevated angiotensinogen levels at tissue sites, in particular in renin- and/or angiotensinogen-producing tissues. Alternatively, if such compensation does not occur, long-term exposure to elevated angiotensin II levels should eventually result in a decreased angiotensin AT_1 receptor density, thereby still normalising the effects of angiotensin II.

In view of the latter concept, it is not surprising that the angiotensin AT_1 receptor A/C polymorphism, which has no effect on angiotensin AT_1 receptor density or affinity, does not affect the plasma levels of angiotensin II (Miller et al., 1999; Paillard et al., 1999). Moreover, no angiotensin AT_1 receptor genotype-related alterations in the plasma levels of renin, angiotensinogen or angiotensin-converting enzyme were observed by Paillard et al. (1999) and Miller et al. (1999). In contrast, in a group of 104 patients with hypertrophic cardiomyopathy, Osterop et al. (1998) found elevated renin levels in carriers of the C allele. However, the latter finding should be interpreted with care, since the majority of these patients was using drugs (β-adrenoceptor antagonists, Ca^{2+} channel blockers and diuretics) that are known to affect plasma renin (Danser et al., 1998b).

3. Renin–angiotensin system gene polymorphisms and biological responsiveness to angiotensins I and II

3.1. Angiotensin-converting enzyme gene I/D polymorphism

Functional angiotensin-converting enzyme genotype-related differences in angiotensin I–II conversion have been studied in vivo either by quantifying regional angiotensin I–II conversion during infusion of angiotensin I or by measuring potential differences in pressor responses to angiotensin I (Lachurié et al., 1995; Ueda et al., 1995; Chadwick et al., 1997; Danser et al., 1999; Van Dijk et al., 2000). Only one of these studies (Ueda et al., 1995) provided evidence for enhanced angiotensin I–II conversion in DD subjects as compared to subjects with the II genotype (Table 1). Statistical significance for the difference between II and DD subjects was obtained, however, only at the highest angiotensin I dose (20 ng/kg/min) that was tested, leading the authors to conclude that D allele-related effects on conversion will only be observed under conditions in which the renin–angiotensin system is stimulated. As discussed above, the first-order kinetics of angiotensin-converting enzyme over a wide range of angiotensin I levels, even at levels in the nanomolar range, do not support this concept.

Surprisingly, despite the lack of clear D allele-related effects on conversion rates, enhanced angiotensin I-induced vasoconstriction in DD subjects was observed in two studies (Table 1). Simultaneous investigation of the pressor response to angiotensin II ruled out the possibility that the difference in angiotensin I pressor response was due to differences in angiotensin AT_1 receptor density and/or affinity between II and DD subjects (Table 1). In contrast to these in vivo studies, Buikema et al. (1996) observed a diminished response to angiotensin II in isolated internal mammary arteries obtained from subjects with the DD genotype.

Furthermore, the possibility of angiotensin I–II conversion by enzymes other than angiotensin-converting enzyme (e.g., chymase (Urata et al., 1990)) should be taken into consideration, although evidence for the importance of these enzymes is largely based on in vitro findings rather than in vivo studies (Maassen van den Brink et al., 1999; Saris et al., 2000). Remarkably, despite the major contribution of chymase to angiotensin I–II conversion in isolated vascular preparations, Buikema et al. (1996) were able to demonstrate enhanced angiotensin I–II conversion (calculated as the area between the angiotensin I and angiotensin II concentration–response curves) in isolated internal mammary arteries of DD subjects. Steeds et al. (1999), studying angiotensin I-induced vasoconstriction of mesenteric resistance arteries in the presence of the chymase inhibitor chymostatin, as well as the contractile response to the angiotensin-converting enzyme-specific angiotensin I analogue, Proline^{10}-angiotensin I, failed to confirm this observation.

Taken together, the angiotensin I and II infusion studies that have been performed so far do not provide convincing evidence for D allele-related differences in angiotensin I–II conversion. This conclusion is in full agreement with the absence of differences in the levels of plasma renin–angiotensin system components between II, ID and DD subjects. The enhanced vascular responsiveness to angiotensin I that was observed in two in vivo studies, if not related to enhanced angiotensin I–II conversion, may be due to other mechanisms, for instance an increased potentiating effect of angiotensin II on the response to other vasoconstrictors (Henrion et al., 1999).

Finally, it should be taken into account that the studies that have been performed so far are limited to selected vascular beds and thus do not necessarily reflect the situation at tissue sites where enhanced formation of angiotensin II, if present, may have the strongest functional implications (e.g., the coronary circulation).

3.2. Angiotensinogen gene M235T polymorphism

Angiotensin responsiveness in relationship with the M235T polymorphism has been evaluated in one study

only (Hopkins et al., 1996). In this study, angiotensinogen T235 homozygotes ($n = 18$) on a high-salt diet (i.e., with a suppressed renin–angiotensin system) were found to have blunted renal vascular responses to angiotensin II in comparison with MT heterozygotes ($n = 51$) and MM homozygotes ($n = 51$). This effect became apparent only after adjustment for baseline renal plasma flow, body mass index, and sex. The authors speculated on the presence of high local angiotensin II concentrations in the kidneys of T235 homozygotes, thereby drawing a parallel with hypertensive patients not responding to angiotensin II while on a high-salt diet ('non-modulators'; Hollenberg and Williams, 1990).

3.3. Angiotensin AT_1 receptor gene A1166C polymorphism

Young male healthy volunteers (56 AA, 47 AC and 13 CC) did not display C allele-related differences in their blood pressure-, renal hemodynamic-, or aldosterone responses to angiotensin II (Hilgers et al., 1999). Similarly, angiotensin II infusion in 66 healthy subjects resulted in similar renal blood flow- and renal vascular resistance changes in AA homozygotes and C allele carriers (Miller et al., 1999). Despite the angiotensin II-induced increase in renal vascular resistance in the latter study, C allele carriers maintained glomerular filtration rate, while AA homozygotes showed the expected decline in glomerular filtration rate. This led the authors to speculate on larger increases in efferent arteriolar resistance in C allele carriers, based on the greater "angiotensin II activity" (i.e., angiotensin AT_1 receptor density) that they assume to exist in these subjects. However, in vitro studies in 30 mesenteric resistance arteries by Steeds et al. (1999) did not reveal C allele-related differences in angiotensin I or angiotensin II responsiveness (a measure for angiotensin AT_1 receptor density), nor did Henrion et al. (1998) observe differences in the response of internal mammary arteries to angiotensin II between 20 AA homozygotes and 30 C allele carriers. In contrast, Van Geel et al. (2000) found an increased maximal response to angiotensin II in internal mammary arteries of 17 CC homozygotes as compared to 95 subjects with the AC and AA genotype. Interestingly, in the latter study EC_{50} values (a measure for K_d) were slightly higher in CC subjects. Moreover, prior angiotensin-converting enzyme inhibitor treatment increased the maximal response to angiotensin II in all subjects to the same degree, which indicates a normal relationship between angiotensin II levels and angiotensin AT_1 receptor density in CC subjects. Taken together, with the exception of one in vitro study, no evidence has been obtained for increased responsiveness to angiotensin II in carriers of the C allele. This conclusion is in agreement with the lack of C allele-related differences in angiotensin AT_1 receptor density or affinity.

4. Renin–angiotensin system gene polymorphisms and the effect of renin–angiotensin system blockade

Studying the effects of renin–angiotensin system blockade in relationship with renin–angiotensin system gene polymorphisms provides an alternative approach of unraveling the importance of these polymorphisms. However, the interpretation of such studies is complex. At first sight, one would predict that subjects with elevated levels of a certain renin–angiotensin system component need higher doses of renin–angiotensin system blockers to obtain equally sufficient blockade. For instance, patients with high endogenous angiotensin-converting enzyme levels (i.e., subjects with the angiotensin-converting enzyme DD genotype), when given a fixed dose of an angiotensin-converting enzyme inhibitor, will have more residual angiotensin-converting enzyme activity than patients with low endogenous angiotensin-converting enzyme levels. Consequently, patients with the DD genotype might show a diminished response to angiotensin-converting enzyme inhibitor treatment. In this respect, it is important to realise that residual angiotensin-converting enzyme activity does not equal the degree of angiotensin-converting enzyme inhibition (i.e., the percentage of pretreatment angiotensin-converting enzyme activity that is blocked), since the latter will be the same in all subjects. This has been confirmed in two studies in healthy volunteers (Todd et al., 1995; Ueda et al., 1998). An additional complicating factor is that long-term renin–angiotensin system blockade, because of its interference with feedback mechanisms, usually results in an increase of the level of one or more renin–angiotensin system components (e.g., angiotensin-converting enzyme or angiotensin AT_1 receptor upregulation). It is currently not known whether renin–angiotensin system upregulation is genotype-dependent, but if so, this should be taken into consideration.

The above line of reasoning results in the expectation that subjects with the angiotensin-converting enzyme II genotype (respectively, the angiotensinogen MM- and the angiotensin AT_1 receptor AA genotype) will respond more strongly to renin–angiotensin system blockade than those with the DD, TT or CC genotype. However, the opposite might also be predicted. Based upon the positive associations between cardiovascular diseases and renin–angiotensin system gene polymorphisms, one would expect subjects with the DD, TT and/or CC genotype to have a higher chance of developing chronic structural alterations in blood vessels, heart, kidney, or other organs (possibly at an earlier age). Consequently, these subjects may benefit to a larger extent from chronic renin–angiotensin system blocker treatment, even under conditions of incomplete renin–angiotensin system blockade. In other words, the relative risk reduction with renin–angiotensin system blockade obtained in these subjects will be larger, simply because their condition is worse. Thus, when studying the importance of renin–angiotensin system gene polymor-

phisms in relationship with renin–angiotensin system blocker treatment, one should correct for differences in the severity of the disease at baseline, even if such differences are small and difficult to quantify.

The latter problem might be avoided by studying healthy volunteers. Moreover, if the study is limited to a single dose, renin–angiotensin system upregulation will probably not occur. This approach has been followed in three small studies (Table 2), all investigating the blood pressure response to an angiotensin-converting enzyme inhibitor in relationship with the angiotensin-converting enzyme I/D polymorphism (Todd et al., 1995; Mizuiri et al., 1997; Ueda et al., 1998). Two of these studies confirm the concept of a better and longer lasting blood pressure response in healthy subjects with the II genotype, whereas the third study did not find a difference in response between healthy II and DD subjects. Interestingly, a fourth study (Mondorf et al., 1998) in subjects with hypertension that also investigated the response to a single dose of an angiotensin-converting enzyme inhibitor found more subjects with the DD genotype among those who showed a blood pressure fall of >5 mmHg, in agreement with the concept that diseased subjects with the DD genotype will respond better to treatment than diseased subjects with the II genotype. Similarly, Prasad et al. (2000), who studied the effect of enalaprilat on acetylcholine-induced coronary vasodilation in 56 subjects with atherosclerosis or its risk factors, observed the greatest improvement in the response to acetylcholine (i.e., in endothelial function) in those with the DD or ID genotype. Nakano et al. (1997), however, were unable to demonstrate differences in the hypotensive response to 50 mg captopril between hypertensive subjects with the DD and II genotype.

All other studies investigating the blood pressure response to angiotensin-converting enzyme inhibition according to angiotensin-converting enzyme genotype were long-term studies, ranging from 4 weeks to 5 years, and involved a wide variety of patients (Table 2). Although most of these studies did not observe angiotensin-converting enzyme genotype-related differences in blood pressure response, a few observed a better response in II subjects. At present, it is too early to draw any conclusions from these studies, since they were largely retrospective, involved small numbers of patients ($n<100$) with varying backgrounds, had different treatment regimens (sometimes involving other antihypertensive drugs as well) and did not take into account dietary Na^+ intake and the state of renin–angiotensin system activation.

Similar inconclusive results have been obtained in studies investigating the angiotensin-converting enzyme I/D polymorphism and the response to angiotensin-converting enzyme inhibitor therapy in patients with renal disease. In a recent review, Navis et al. (1999) reported that, as a general trend, Japanese studies find the D allele to be associated with a better antiproteinuric response, while European studies report either no effect of the genotype or a poor response associated with the D allele in both diabetic and non-diabetic patients. Remarkably, a high Na^+ intake was associated with a poor renal response to angiotensin-converting enzyme inhibition in DD homozygotes, but not in subjects with the ID or II genotype (Van der Kleij et al., 1997). This underlines the importance of accounting for differences in Na^+ intake in future studies.

As far as the effect of angiotensin-converting enzyme inhibition on left ventricular hypertrophy and remodeling is concerned, patients with the DD genotype appeared to

Table 2
Blood pressure response to angiotensin-converting enzyme inhibition according to angiotensin-converting enzyme I/D genotype

Reference	*n*	Population	Treatment duration	Largest blood pressure decrease in II subjects	Largest blood pressure decrease in DD subjects	No difference between II and DD subjects
Hingorani et al., 1995	125	Hypertensive patients	4 weeks			×
Moriyama et al., 1995	36	Patients with non-diabetic proteinuria	3 months			×
Todd et al., 1995	27	Healthy men	Single dose			×
Dudley et al., 1996	91	Hypertensive patients	4 weeks			×
Mizuiri et al., 1997	27	Healthy volunteers	Single dose	×		
Nakano et al., 1997	82	Hypertensive patients	Single dose			×
Ohmichi et al., 1997	57	Hypertensive patients	6 weeks	×		
Cannella et al., 1998	30	Hemodialyzed uremic patients	5 years			×
Haas et al., 1998	36	Patients with non-diabetic proteinuria	6 months	×		
Jacobsen et al., 1998	60	Insulin-dependent diabetes mellitus patients	6 months	×		
Mondorf et al., 1998	121	Hypertensive patients	Single dose		×	
O'Toole et al., 1998	34	Patients with heart failure	6 weeks	× (captopril)		× (lisinopril)
Ueda et al., 1998	23	Healthy men	Single dose	×		
Van der Kleij et al., 1997	88	Patients with non-diabetic proteinuria	4–12 weeks			×
Kohno et al., 1999	54	Hypertensive patients	> 2 years			×

benefit most according to two studies (Pinto et al., 1995; Sasaki et al., 1996), whereas the opposite was observed in two other investigations (Cannella et al., 1998; Kohno et al., 1999). The number of patients in these studies was low (ranging from 30 to 60), and this may explain the inconsistent results.

Finally, a limited number of studies investigated the role of the angiotensinogen M235T polymorphism and/or the angiotensin AT_1 receptor A1166C polymorphism in the response to renin–angiotensin system blockade. Hingorani et al. (1995) found the largest decrease in blood pressure during angiotensin-converting enzyme inhibitor treatment in angiotensinogen T235 carriers, and observed no effect of the angiotensin AT_1 receptor C allele on blood pressure response. In contrast, Dudley et al. (1996) and Mondorf et al. (1998) were unable to show a T235-related effect on blood pressure response to angiotensin-converting enzyme inhibition, while Miller et al. (1999), studying the effect of 25 mg losartan in Na^+-replete healthy volunteers, found blood pressure to decrease and glomerular filtration rate to increase in angiotensin AT_1 receptor C allele carriers, but not in AA homozygotes. The latter finding argues against the concept of healthy volunteers with the C allele needing larger renin–angiotensin system blocker doses to obtain complete blockade.

In summary, at present it is impossible to provide clear statements about the relationship between renin–angiotensin system gene polymorphisms and the effect of renin–angiotensin system blockade. The results obtained so far vary according to the population (healthy/diseased) that is studied, the duration of treatment, the dosage regimen, Na^+ status and the use of other drugs. Therefore, in the future large prospective studies are required, most likely involving hundreds or even thousands of well-defined patients, and taking into consideration all renin–angiotensin system gene polymorphisms at the same time, as well as all of the aforementioned issues.

5. Renin–angiotensin system gene polymorphisms and complex cardiovascular diseases

Given the uncertainty as to whether the angiotensin-converting enzyme I/D polymorphism has any implications for the rates of angiotensin I–II conversion or bradykinin degradation, it may be of surprise how intensively the association with this genetic variant and complex disorders has been studied (for review see Samani et al., 1996 and Schunkert et al., 1997). Indeed, one of the most extensively studied associations between any genetic polymorphism and a cardiovascular disease is that of the angiotensin-converting enzyme I/D polymorphism and myocardial infarction. A recent meta-analysis that included $>10{,}000$ subjects came to the conclusion that individuals who carried the DD genotype are exposed to a 10% increased risk to suffer from myocardial infarction (Keavney et al., 2000). Unfortunately, the interpretation of a meta-analysis as such suffers from publication bias (positive findings have a higher chance of being published), heterogeneity of patient and control populations, retrospective nature of patient ascertainment, and differences in the definition of end points. Thus, definitive proof of an association between angiotensin-converting enzyme I/D polymorphism and myocardial infarction is still lacking. Instead of counting even larger numbers of patients and genotypes, we wish to give several reasons that may explain the difficulty of such genetic association studies within the renin–angiotensin system:

5.1. Renin–angiotensin system genes are highly polymorphic

The genetics of the renin–angiotensin system are more diverse than just the one polymorphism for each component that we discussed here. In fact, with respect to the angiotensin-converting enzyme gene, 78 polymorphisms have been described (Rieder et al., 1999). Similar diversity applies to the other components (Jeunemaître et al., 1992; Zhang et al., 2000). Thus, a more complete investigation of a given gene would distinguish families of haplotypes (or polymorphisms) rather than a single marker polymorphism (Rieder et al., 1999). Of course, such studies are more complex and do not guarantee success.

5.2. The renin–angiotensin system is a complex physiological system

As has been discussed in Section 2.4, the renin–angiotensin system is a complex physiological system that comes with a powerful negative feedback mechanism. Future studies may be more successful when this fact is considered and genetic implications are integrated for the entire system. Moreover, the physiological role of the renin–angiotensin system varies with Na^+ intake, age and disease state. Thus, a precise definition of the study group may be a challenge for future studies. Finally, the actions of angiotensin II (and bradykinin) are not always easy to define. Specifically, chronic effects on vascular or cardiac hypertrophy may precipitate even when differences in plasma levels are minute and barely detectable (Griffin et al., 1991). Such structural alterations may, in turn, affect acute measurements. In this respect, the wide spectrum of clinical benefits observed in the HOPE study (HOPE Study Investigators, 2000) illustrates the versatile but subtle actions of the system that are accessible to angiotensin-converting enzyme inhibition and, on the other hand, to the induction of the system.

5.3. Other physiological systems interact with the renin–angiotensin system

A physiological system such as the renin–angiotensin system not only has multiple components and genes that

define its activity, its regulatory actions (e.g., on vascular growth or blood pressure) may also be enhanced or counteracted by multiple other systems (Fig. 6). Thus, there may be competition between multiple systems for a given phenotype (or risk factor for that matter).

A clarification of the association between renin–angiotensin system gene polymorphisms and myocardial infarction might evolve therefore from studies that elucidate less complex phenotypes. Specifically, it is of interest as to whether the renin–angiotensin system gene polymorphisms increase the prevalence of traditional risk factors such as arterial hypertension, diabetes mellitus, or hypercholesterolemia by genetic means. Alternatively, these gene polymorphisms might affect the coronary clot formation via interaction with pro- or anti-coagulant factors, plaque stability, formation of oxidized low density lipoproteins, endothelial dysfunction or vascular smooth muscle growth. However, none of these precipitating steps towards myocardial infarction nor any risk factor has been conclusively related to renin–angiotensin system gene polymorphisms. Right now, the best evidence in favor of any clinically relevant association is that for the association between the angiotensin-converting enzyme I/D polymorphism with arterial hypertension in men (Fornage et al., 1998; O'Donnell et al., 1998; Higaki et al., 2000).

5.4. Gene–gene and gene–environment interaction

In different genetic or environmental backgrounds, a given genetic polymorphism or, for that matter, genetically increased angiotensin-converting enzyme activity or angiotensinogen levels may come with different pathophysiological implications. For example, studies in various ethnic backgrounds such as African-Americans vs. Caucasians vs. Japanese came to different conclusions with respect to the association of genotypes and complex disorders. This point has utmost importance when the polymorphism under investigation is by itself not causal and rather a marker for other mutations in close chromosomal proximity. Thereby, polymorphisms may produce positive associations by linkage disequilibrium that, of course, may not exist in populations that lack the causal mutation.

5.5. Quantitative issues

The Framingham Heart Study demonstrated a 1.6-fold risk increase for hypertension in male carriers of the DD genotype (O'Donnell et al., 1998). Our group found a twofold increased risk for left ventricular hypertrophy with the DD genotype, again only in men (Schunkert et al., 1994). Although many studies confirmed these observations (Fornage et al., 1998; Schunkert, 1998; Higaki et al., 2000; Nakahara et al., 2000), one should ask the question whether such associations are quantitatively powerful enough to explain a subsequent increase in the risk to suffer from myocardial infarction. More specifically, given that the risk of hypertension is 1.6-fold higher in men with the DD genotype (O'Donnell et al., 1998), and hypertension by itself carries a 1.67-fold increased risk for myocardial infarction (Wilson et al., 1998), the hypertension mediated-risk increase in the DD group can be estimated as follows.

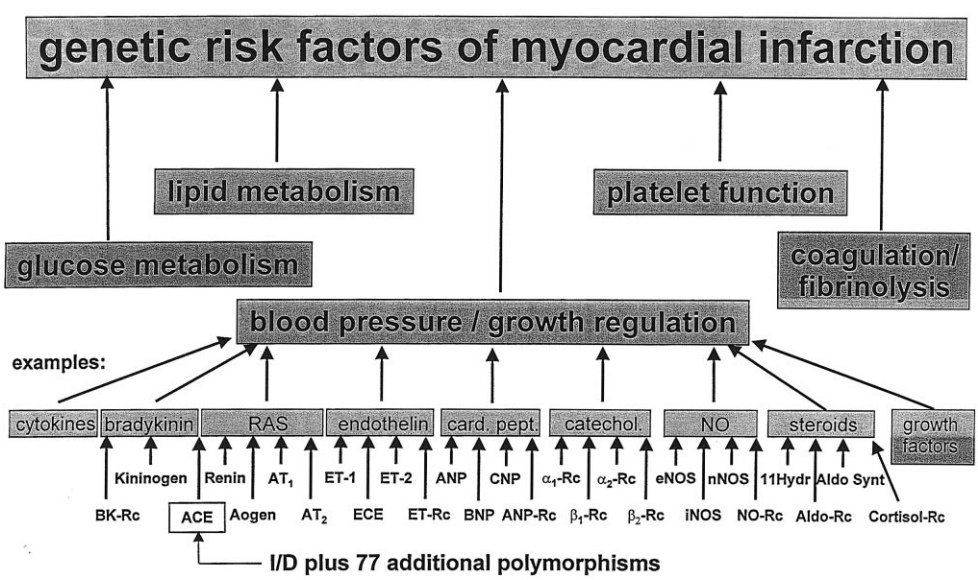

Fig. 6. Genetic risk factors of myocardial infarction. ACE, angiotensin-converting enzyme; Aldo, aldosterone; Aldo Synt, aldosterone synthase; ANP, atrial natriuretic peptide; Aogen, angiotensinogen; AT_1, angiotensin AT_1 receptor; AT_2, angiotensin AT_2 receptor; BK, bradykinin; BNP, brain natriuretic peptide; card. pept., cardiac peptides; catechol., catecholamines; CNP, C-type natriuretic peptide; ECE, endothelin-converting enzyme; eNOS, endothelial nitric oxide synthase; ET, endothelin; 11Hydr, 11β-hydroxylase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; Rc, receptor.

Table 3
Male participants of the Framingham Heart Study ($n = 1444$) subdivided according to angiotensin-converting enzyme genotype and classified by the presence or absence of arterial hypertension. Data are from O'Donnell et al. (1998)

	Arterial hypertension	No arterial hypertension	Total
DD genotype	$n = 232$	$n = 457$	$n = 689$
ID/II genotypes	$n = 205$	$n = 550$	$n = 755$

Step 1. Individuals with and without the angiotensin-converting enzyme DD genotype are classified by presence or absence of arterial hypertension (O'Donnell et al., 1998): see Table 3. To calculate the relative risk of myocardial infarction in individuals carrying the DD genotype, the numbers of all individuals with arterial hypertension (DD genotype, $n = 232$; ID/II genotypes, $n = 205$) are multiplied by 1.67 (DD genotype, $x_1 = 387$; ID/II genotypes, $x_2 = 342$) and the numbers of those individuals without arterial hypertension are multiplied by 1.0 (no risk increase; DD genotype, $y_1 = 457$; ID/II genotypes, $y_2 = 550$).

Step 2. For determination of the risk ratio for myocardial infarction (RR_{MI}), the calculated products of individuals carrying the angiotensin-converting enzyme DD genotype are added ($x_1 + y_1 = 844$) and are divided by the actual sum of individuals carrying the DD genotype ($n = 689$):

$$RR_{MI}\,(\text{DD genotype}) = 844/689 = 1.225.$$

The same is done for those individuals carrying the ID/II genotypes ($x_2 + y_2 = 892$ and actual sum of individuals with the ID/II genotypes $n = 755$):

$$RR_{MI}\,(\text{ID/II genotypes}) = 892/755 = 1.181.$$

Step 3. The relative risk of individuals carrying the angiotensin-converting enzyme DD genotype to develop myocardial infarction attributable to the phenotype arterial hypertension is now calculated as:

$$RR_{MI}\,(\text{DD genotype})/RR_{MI}\,(\text{ID/II genotypes}) = 1.0337.$$

Thus, the mildly elevated risk for hypertension in DD males translates to a trivial risk increase for myocardial infarction (+3.37%). This matches perfectly with the number obtained by Keavney et al. (2000) in their recent large study. One can further calculate that such risk increase requires >48,000 individuals with and without myocardial infarction to achieve statistical significance of the DD group versus the ID/II group (least significant number [power 50%] at an alpha error of 0.05). If one asks for a prospective study design in a population with an incidence of 250 cases in 100,000 per year (i.e., the incidence in Germany and The Netherlands) one has to study at least this number of people for many years to come.

6. Conclusions and directions for future investigations

This review summarises initial data from a young discipline: genetics of complex diseases. In fact, the angiotensin-converting enzyme I/D polymorphism was the first to be associated with myocardial infarction. While data on this topic are fashionable and provocative, we must realise that answers from associations with complex diseases will not come easily. Right now, we learn more on how we should perform studies properly rather than about specific questions on the genes that cause hypertension, hypertrophy, or myocardial infarction. Likewise, renin–angiotensin system pharmacogenomics will be of clinical relevance only when reliable and consistent data that, ideally, should improve the treatment of an individual patient, are at hand. Recently, criteria have been discussed on how these data should be generated (Editorial, 1999). These include a large sample size, small *P*-values, biological sense, alleles that affect gene products and studies that are replicated in additional samples with different genetic strategies (e.g., association plus linkage design). These hurdles are high but nevertheless mandatory in order to obtain reproducible and clinically relevant data. Thus, a first step towards a clearer picture will be a focus on better and more reliable studies.

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