This Review is part of a thematic series on Angiotensin-Converting Enzyme, which includes the following articles:

Six Truisms Concerning ACE and the Renin-Angiotensin System Eueducd from the Genetic Analysis of Mice
ACE II in the Heart and the Kidney
Signaling by the Angiotensin-Converting Enzyme

ACE Polymorphisms

F.A. Sayed-Tabatabaei, B.A. Oostra, A. Isaacs, C.M. van Duijn, J.C.M. Witteman

Abstract—Angiotensin converting enzyme (ACE) plays an essential role in two physiological systems, one leading to the production of angiotensin II and the other to the degradation of bradykinin. The wide distribution and multifunctional properties of these peptides suggest that ACE could be involved in various pathophysiological conditions. The discovery that ACE levels are under genetic control ushered in a new era of investigation; most studies focused on an insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene as a marker for a functional polymorphism. Recently, many single nucleotide polymorphisms were detected in the gene and the search for the locations of functional polymorphisms became a topic of extensive investigation. Nevertheless, association studies on the I/D polymorphism and clinical outcomes continued, mostly with conflicting results. This article reviews the current state of knowledge regarding ACE polymorphisms and suggests that a functional polymorphism is most likely located between intron 18 and the 3’ UTR. The potential existence of another functional polymorphism in the 5’ UTR, however, cannot be excluded. This review also presents an overview of ACE function in different pathophysiological systems, and summarizes previous reports on ACE and clinical outcomes. Although findings on the I/D polymorphism and disorders like diabetic nephropathy and Alzheimer disease can be considered conclusive, reports on most of the cardiovascular phenotypes are still controversial. Genotypic and phenotypic misclassifications, insufficient power in some studies, and the presence of interaction with other genes or environmental factors are possible explanations for the contradictory findings. (Circ Res. 2006;98:1123-1133.)

Key Words: angiotensin ■ polymorphism ■ genetics

The ACE Protein

Angiotensin converting enzyme (ACE) is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. Several different names refer to this enzyme in the scientific literature (Table). ACE converts the inactive decapeptide, angiotensin I (Ang I or Ang 1-10), to the active octapeptide and potent vasoconstrictor angiotensin II (Ang II or Ang 1-8), which is the main active product of the renin–angiotensin system (RAS).1 Long-term regulation of blood pressure and blood volume in the body is controlled by RAS. Figure 1 shows schematically the relationships between different proteins in this hormonal system. Renin is released by the juxtaglomerular cells in the kidneys under conditions of salt, volume loss, or sympathetic activation. It cleaves the inactive peptide angiotensinogen (synthesized by the liver), producing angiotensin I, which is a vasoinactive protein. In turn, angiotensin I is converted to angiotensin II through the action of angiotensin converting enzyme (Figure 1).

Angiotensin II is a potent vasoconstrictor. It also acts on the adrenal cortex, causing the release of aldosterone, which
stimulates tubules in the kidneys, allowing them to reabsorb more sodium and water from the urine. These effects directly act to increase the amount of fluid in the blood, making up for a loss in volume, and to increase blood pressure. Angiotensin II also mediates cell growth and proliferation by stimulating various cytokines and growth factors. Furthermore, angiotensin II may induce endothelial dysfunction by reducing nitric oxide bioavailability. These findings emphasize the importance of angiotensin II in cardiovascular pathophysiology and motivate exploration of the role of RAS in atherosclerosis and other cardiovascular outcomes.

ACE also plays an important role in another hormonal system, the kinin–kallikrein cascade (Figure 1). ACE metabolizes bradykinin, which is a strong vasodilator, forming the inactive metabolite bradykinin 1-5. ACE, therefore, plays a prominent role in blood pressure regulation through this pathway as well. Neurokinins are a family of neurotransmitters in the central nervous system (CNS) that play a key role in the transmission of pain, regulation of emotions, and alteration of inflammatory and immune responses. Although the role of ACE in degenerating these proteins was not always replicated in vivo, these findings triggered studies on neurological diseases such as Parkinson disease, depression, and other affective disorders. The role of ACE in CNS, however, is not limited to these neurotransmitters. It has been demonstrated that ACE degrades amyloid beta peptide in vitro, one of the primary adverse biological agents implicated in Alzheimer disease (AD) pathogenesis. Thus, if present in sufficient levels in vivo, ACE may preclude or lessen the formation of senile plaques, a hallmark of AD.

### The ACE Gene

Although ACE is deemed to be a mammalian enzyme, close sequence homologues with very similar activities are found in

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a wide range of animal species. The Table summarizes these homologues. In *Homo sapiens*, the gene encoding ACE is located on the long arm of chromosome 17 (17q23). The gene is 21 kilo bases (kb) long and comprises 26 exons and 25 introns. In the National Center for Biotechnology Information (NCBI) records, more than 160 ACE gene polymorphisms are listed, most of which are single nucleotide polymorphisms (SNPs). Only 34 of those polymorphisms are located in coding regions; 18 of them are missense mutations.

The gene encodes 2 isoforms of ACE: the somatic form (sACE), with a molecular mass of 170 kDa, which is expressed in somatic tissue; and the testicular form (testis ACE [tACE], also named germinal ACE [gACE]), with a smaller molecular mass of 100 kDa, expressed in germinal cells in the testes.16 These 2 forms result from initiation by 2 different promoters. Somatic ACE is transcribed from a promoter located on the 5'-side of the first exon (Spro), and leads to the transcription of all exons. In mature sACE mRNA, exons 1 to 26 are transcribed, except for exon 13, which is spliced. Germinal ACE, however, is transcribed from a specific internal promoter, a 91-bp fragment in intron 12 (Gpro); germinal mRNA includes exons 13 to 26.17 The two forms differ in that sACE has 2 active sites (N and C terminus), whereas tACE has a single active site analogous to the C-terminal portion of sACE.5 The detailed function of tACE is unknown, but it appears to play a role in male reproduction.18

The structure of the ACE gene may be the result of the duplication of an ancestral gene. Exons 4 to 11 and 17 to 24, encoding the 2 homologous domains of the ACE molecule, are very similar both in size and in sequence.19 In all mammalian species in which the gene was cloned (humans, mice, and rabbit), it appears to be duplicated. In *Drosophila melanogaster*, however, the ACE-like enzyme gene is not duplicated; this form may resemble the ancestral form of the gene before duplication.20 Speculation suggests that the duplication might have happened around 300 million years ago.

A homologue of ACE, ACE2, was also discovered in humans. Cloned from human heart failure and lymphoma cDNA libraries,21,22 analysis of the genomic sequence of ACE2 revealed that the gene contains 18 exons and maps to Xp22.22 Discovery of this enzyme led to an increasing awareness that angiotensin peptides other than Ang II have biological activity and physiological importance (Figure 1). The reported vasodilatory actions of Ang 1-7,23 along with the potential involvement of ACE2 in both Ang II degradation and Ang 1-7 production, add another level of complexity to RAS.24,25 In vitro studies indicate that the catalytic efficiency of ACE2 for Ang II is 400-fold greater than for Ang I,26 indicating that the major role of ACE2 is the conversion of angiotensin II to angiotensin 1-7. The potential role of angiotensin 1-7 as a cardioprotective peptide with vasodilator, antigrowth, and antiproliferative actions was recognized relatively recently.23,27 ACE2, however, has a much more restricted distribution than ACE. In humans, ACE2 transcripts were identified in the heart, kidney and testis.21,22 In this review, we focus on the genetics of ACE, and would like to refer readers interested in ACE2 to the vast number of articles available on that topic.

**Genetic Control of ACE Levels**

Plasma ACE levels are stable when measured repeatedly in the same individual, whereas large interindividual differences are observed.28 This suggests strong long-term control of plasma levels, possibly with genetic origins. In 1988, Cambien et al29 performed segregation analysis in 87 healthy nuclear families (the Nancy Study) and demonstrated that a possible major gene effect accounts for 29%, 29%, and 75% of the variance of age-adjusted ACE levels in fathers, mothers, and offspring, respectively.29 In 1990, Rigat and coworkers30 published an important report that provided the impetus to further study polymorphisms in this gene. They found a polymorphism involving the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of the gene (NCBI ref. SNP ID: rs1799752). Mean ACE activity levels in DD carriers were approximately twice that found in II genotype individuals.30 Subjects with the ID genotype had intermediate levels indicating codominancy. The I/D polymorphism accounted for approximately half (47%) of the observed variance in ACE levels in this study group. Later studies showed that the involve-
The ACE I/D polymorphism was initially detected by restriction fragment length polymorphism (RFLP) analysis. The first polymerase chain reaction (PCR)-based detection of this polymorphism was reported by Rigat et al., who used a set of primers flanking the insertion sequence. Family based studies performed by Shanmugam et al., however, showed the possibility of mistyping ID heterozygotes with this PCR method. Preferential amplification of the shorter D allele may cause the misclassification of approximately 4 to 5% of ID genotypes to DD. An additional PCR amplification reaction was, therefore, formulated for the confirmation of DD genotypes obtained in the first standard PCR, including a new sense primer that is insertion-specific. This combination of standard and confirmatory PCR reactions was subsequently used in multiple studies.

Functional Polymorphisms in the ACE Gene

Although the ACE I/D polymorphism is often studied with respect to cardiovascular and other complex disorders, it's location in a noncoding region makes it unlikely to be a functional variant. Because the physiological importance of the I/D polymorphism was discovered through its association with plasma ACE levels, many investigators followed the same line of inquiry, studying the association between other variants in the gene and plasma levels, to find functional polymorphisms. In 1996, Villard et al. suggested 2 quantitative trait loci (QTLs) controlling ACE levels. They argued that one QTL was in close linkage disequilibrium (LD) with the I/D polymorphism, and the second QTL was yet to be identified, possibly in the 5' region of the gene.

Keavney and coworkers analyzed plasma ACE levels and 10 polymorphisms (including the famous I/D polymorphism) spanning 26 kb of the gene in Caucasian British families (Figure 2a). Because of strong linkage disequilibrium operating over this small chromosomal region, they revealed a limited number of haplotypes. They constructed a haplotype tree (cladogram) with three main branches (clades A through C), which account for 90% of the observed haplotypes. They observed that clade C very closely resembles clade B in its influence on ACE levels, and concluded, in contrast to Villard et al., that the functional variant is located downstream of the ancestral breakpoint and that there is no significant variant in the promoter or other upstream sequences (Figure 2b). The last three polymorphisms, 4656(CT)3/2 (also named 23945(CT)2/3), 2350G/A (14521G/A, rs4343), and I/D, showed no substitutions within the clades; consequently, no differential inferences could be made regarding their influence on ACE levels.

The location of this putative ancestral breakpoint was refined later, in 1999, by the same group of investigators. The results of this analysis, combined with their previous results, indicated that the ancestral breakpoint is downstream of the 06435 polymorphism (rs4305, located in intron 5). Based on these new findings, exons 1 to 5, introns 1 to 4, and part of intron 5 were also excluded from the region that may contain the functional polymorphism.
Because of high LD between the polymorphisms in this area, ACE haplotypes needed to be determined in other populations with different recent evolutionary histories to search for additional ancestral breakpoints. Several studies demonstrated a greater genetic diversity among populations of African descent, which should increase the likelihood of observing recombinant haplotypes and assist in fine mapping. Implementing this idea, Zhu et al. focused on the remaining 3’ region of the ACE gene, genotyping 7 bi-allelic polymorphisms across a 13-kb segment in 159 Afro-Caribbeans from Jamaica (Figure 3a). Of 28 haplotypes, eight occurred with a frequency >4.4%, accounted for >80% of the chromosomes, and could be grouped into three main clades (Figure 3b). Plasma ACE levels were similar within each clade, indicating that the segment including the last 2 polymorphisms was unlikely to contain the functional polymorphism. Further analysis revealed that clades A and B resulted in similar phenotypes, whereas there was a significant difference between clades A and C. These results indicated that the functional polymorphism might be located in the region between 15214G/A (rs4344) in intron 18 and 23945(CT)2/3 in the 3’ UTR, excluding the well known I/D polymorphism in intron 16 (Figure 3).41

In a further attempt, through sequencing of the transcribed regions of the ACE gene (including 11 kb in the 5’ region and 3.5 kb in the 3’ region), Soubrier and coworkers noted 62 polymorphic sites. Their analysis confirmed previous findings by Zhu et al. that revealed a second ancestral breakpoint close to polymorphism 23945(CT)2/3. Additionally, Cox et al. identified 4 polymorphic sites associated with ACE levels using a sample of Nigerian individuals. Three of those polymorphisms were located in the previously described 3’ region. The other polymorphism (A6138C) was located in the upstream noncoding sequence. The presence of a potential functional polymorphism in the 5’ region was also confirmed by McKenzie et al. in 2005. They found 2 polymorphisms from the 5’ UTR were associated with plasma ACE levels. These findings are in line with previous reports supporting the possibility that 2 ACE-linked QTLs control ACE activity.

The presence of yet another QTL, located on chromosome 4, was recently suggested. In a multipoint linkage analysis performed in 29 pedigrees from the San Antonio Family Heart Study, Kammerer et al. showed strong evidence that a QTL located on chromosome 17 near the ACE structural locus, and a second QTL on chromosome 4 near marker D4S1548, were both associated with plasma ACE levels. The evidence for the presence of this new QTL remained strong, even after adjustment for the first QTL near the ACE gene. The nature of this new locus remains unknown. If the new QTL represents a regulatory gene, this discovery might help explain some of the paradoxical results regarding the relation between the ACE gene, ACE activity, and clinical outcomes, and might help in the development of alternative means of prophylaxis and treatment.

Pathophysiological Impacts of the ACE I/D Polymorphism

Although the nature and location of the functional polymorphism, or polymorphisms, responsible for plasma ACE
levels remain a mystery, all of the studies performed in Caucasian populations indicated that the majority of the polymorphisms in the candidate region of the gene are tightly linked with the I/D polymorphism. Researchers, therefore, continued to use the I/D polymorphism as a valid marker for studying the associations between the unknown functional polymorphism(s) and pathophysiological conditions. In an experiment on normotensive men, by Ueda et al., after infusion of Ang I, venous levels of Ang II and increases in blood pressure were higher in DD carriers compared with II carriers. This finding, however, was not replicated in another study published the same year. Alterations in the other function of the ACE enzyme, degradation of bradykinin, were also studied in vivo. Significant correlations were observed between the number of D alleles and BK1-5 (the inactive product) concentrations, and the ratio of BK1-5 to bradykinin.

The evidence favors the hypothesis that functional polymorphisms, linked with the I/D polymorphism, are involved in pathophysiological conditions through the renin–angiotensin and kinin–kallikrein systems. Naturally, associations between the I/D polymorphism and disease are also expected, and an immense number of studies published thus far investigated the association between the I/D polymorphism and numerous clinical outcomes. These investigations included not only the presence or incidence of disease, but also covered other aspects, such as symptoms and manifestations, efficacy of drugs and therapies, interaction with other genetic or environmental factors, recovery rates, disease progression, and survival. Each of these topics requires a detailed review of its own. Here, we will limit the review to the most frequently studied outcomes.

ACE I/D Polymorphism and Clinical Outcomes

ACE I/D Polymorphism and Blood Pressure

In a linkage study, Jeunemaitre et al. found no evidence to support linkage between the ACE locus and essential hypertension. Likewise, in the Dutch Hypertension and Offspring Study, Schmidt et al. failed to find a significant association between the I/D polymorphism and blood pressure status in subjects with high or low blood pressure and in their offspring. This lack of association was repeatedly found in later studies. Several other studies, however, reported a positive association between the D allele and high blood pressure. The first meta-analysis on this topic, published by Staessen et al., included 23 studies through October 1996 and consisted of 28 case–control groups with a total of 6923 subjects. A pooled odds ratio (OR) of 1.10 [95% CI: 0.95, 1.27] indicated a 10% increased risk of hypertension in DD versus II genotype, which was not a significant association because of the confidence interval covering the null value of one. There was, however, a strong indication of heterogeneity among the reports (P for heterogeneity <0.01). Sensitivity analyses were performed in subgroups based on gender, ethnicity, mean age (with a cut-off point of 50 years), and genotyping method (with or without the insertion-specific primer). There was a significant relationship between the D allele and hypertension in women and in Asians. In all other subgroups, no association existed.

Another meta-analysis, published by Agerholm-Larsen et al., was restricted to Caucasians and analyses of blood pressure as a continuous variable. The selection criteria resulted in a collection of 19 studies with very little overlap with the previous meta-analysis. The pooled results of more than 15,942 individuals also indicated that blood pressure was not influenced by genotype (weighted mean difference = 0.5 mm Hg [95% CI: −0.5, 1.8] for DD versus II genotype). In 2005, in a review of the genetics of human hypertension, Agarwal et al. listed a completely new set of 26 association studies, of which 12 published positive and 14 published negative results.

The causal relationship between the function of the ACE gene and blood pressure was tested in mice having 1, 2, or 3 functional copies of the gene at its normal chromosomal location. Although serum ACE activity increased progressively from the 1-copy animals to the 3-copy animals, the blood pressures of the mice did not differ significantly. The authors concluded that quantitative changes in ACE expression would observably affect blood pressures when accompanied by additional environmental or genetic factors.

The bulk of this evidence indicates that any effect of the D allele on hypertension must be very small. The findings on mice, and data on possible associations in human studies among Asians or in women, triggered many investigations on the interaction between ACE and other genes or environmental factors. Despite some promising findings, those results remain to be replicated.

ACE I/D Polymorphism and Atherosclerosis

Numerous studies investigated the association between the ACE I/D polymorphism and atherosclerosis using carotid artery intima media thickness (IMT) measurements. We reported a positive association between the D allele and common carotid IMT in a meta-analysis containing 9833 subjects from 23 published articles through October 2002. In this meta-analysis, the overall results were concordant between Caucasians and Asians, but the association was stronger among high-risk populations (ie, subjects with underlying diseases such as cerebrovascular disease, diabetes, or hypertension). In low-risk/general populations, the weighted mean difference between DD and II was 0.01 mm [95% CI: 0.00, 0.02], whereas in high-risk populations this difference was 0.07 mm [95% CI: 0.03, 0.12]. This finding suggested the existence of a possible interaction between cardiovascular risk factors and the ACE polymorphism with respect to carotid IMT. The association between ACE genotype and atherosclerosis was also assessed using coronary calcification as a measure of coronary atherosclerosis and autopsy measurements of aortic atherosclerosis, but the results were discordant. In general, a modest positive association between the D allele and atherosclerosis is expected, particularly in those who carry other (genetic or environmental) cardiovascular risk factors.

ACE I/D Polymorphism and Coronary Heart Disease and Stroke

The first study reporting a positive association between the D allele and myocardial infarction (MI) was published in 1992.
by Cambien et al. In a multi-center case–control study of 610 cases and 733 controls (Etude Cas-Temoin de l’Infarctus du Myocarde [ECTIM]), they found that the DD genotype was significantly more frequent in male patients with MI than in controls, particularly among low-risk individuals (subjects with low body mass index and low plasma levels of apolipoprotein B). This result, however, was not replicated in a large association study by Agerholm-Larsen et al. which investigated the association in a case–referent study (n=10 150) as well as in a retrospective cohort study (n=7263); no significant differences in incident MI, or any other manifestation of ischemic heart disease, between genotype classes were found.

Three years later, the same research group published a meta-analysis, in which they included their own study and 21 other association studies. Five of these studies were large (n>600), and the remainder were small. Although the overall result was positive (pooled OR=1.21 [95% CI: 1.11, 1.32]) for DD versus ID/II genotypes, Agerholm-Larsen et al. indicated that small studies showed a more pronounced effect on risk of MI, and suggested that those studies may even be responsible for the overall estimate of a significant odds ratio. In another study published the same year by Keavney and coworkers, the authors also demonstrated the difference between small and large studies. There were 48 small studies (each with fewer than 200 cases), which totaled 5092 cases and yielded a pooled odds ratio for MI and other coronary heart disease associated with the DD genotype of 1.43 [99% CI: 1.28, 1.60]; 29 larger published studies with a total of 5092 cases and yielded a combined odds ratio for MI and other coronary (each with fewer than 200 cases), which totaled 5092 cases and yielded a pooled odds ratio for MI and other coronary heart disease associated with the DD genotype of 1.43 [99% CI: 1.28, 1.60]; 29 larger published studies with a total of 14 868 cases yielded a combined odds ratio of 1.04 [99% CI: 1.00, 1.08].

Despite the idea that small studies might be prone to bias because of a lower level of quality control or study design, the pattern of small versus large studies might indicate the importance of the ACE polymorphism in particular subgroups. In other words, although representative samples of the general population do not indicate an association between the D allele and coronary heart disease, an association is usually detected in smaller nonrepresentative samples. This is in line with the findings of high-risk versus low-risk populations in regards to atherosclerosis, as selective samples are usually derived from particular study populations, such as hospitalized or high-risk groups of patients. In relation to coronary heart disease, the D allele is not clinically important in the general population, but may play an important role in certain groups of patients.

In a different approach, Katzov et al. analyzed clades using multiple SNPs in the ACE gene, instead of only genotyping the I/D polymorphism. They found a number of significant phenotype associations (including MI) in a sample of more than 4000 Swedish individuals that may not have been detectable by typing only the I/D polymorphism. This highlights the importance of enhanced classification of genotypes to avoid genotypic misclassification and to gain a better insight on the genetics of complex diseases such as MI.

Both MI and ischemic stroke commonly result from thrombosis superimposed on atherosclerotic plaques. This pathophysiological similarity prompted investigators to study the role of ACE gene polymorphisms in stroke. Although 2 meta-analyses reported significant positive associations between the D allele and ischemic stroke, it was not confirmed in a later prospective matched case–control study. In the Physicians’ Health Study, 348 subjects who had been healthy at enrollment and experienced a stroke during 12 years of follow-up were matched with the same number of controls. The odds ratios associated with the D allele under recessive and dominant models were 1.10 [95% CI: 0.80, 1.51] and 1.22 [95% CI: 0.83, 1.79], respectively. There were more negative than positive findings in later studies. Any effect of the ACE gene, therefore, is very small in relation to ischemic stroke. Although the role of gene–gene–environmental interactions cannot be ruled out, studies with larger sample sizes will be needed to detect them.

**ACE Polymorphism and Diabetic Nephropathy**

Because of the central role of ACE in the renin–angiotensin system, numerous studies have addressed the role of the I/D polymorphism in microvascular disorders, particularly in diabetes. In a literature search through October 1996, Stassen and coworkers found an association between the I/D and diabetic nephropathy using 11 published studies (pooled OR=1.56 [95% CI: 1.27, 1.91]) for DD versus II genotype). In sensitivity analysis based on ethnicity, age, genotyping method (with or without insertion specific primers), and impact factor of the journals, the D allele was significantly associated with an increased risk of diabetic nephropathy in all of the subgroups except for the studies in which the subjects had been genotyped in the absence of insertion-specific primers (pooled OR=1.23 [95% CI: 0.96, 1.58]). This, on one hand, confirms the association between the I/D polymorphism and diabetic nephropathy, and, on the other hand, demonstrates once more the importance of genotypic misclassification in association studies that are designed to detect a relatively small effect size.

The most recent meta-analysis on this topic was performed on studies published between 1994 and 2004, comprising 14 727 subjects. Fifty-eight studies were identified that covered all of the publications used in previous meta-analyses, although 11 of them were excluded because of reasons such as duplicated data or insufficient available details. There was a significantly higher risk of diabetic nephropathy in carriers of the D allele than the II genotype group (pooled OR=1.28 [95% CI: 1.14, 1.45]). This association was confirmed in a well established cohort (the DCCT/EDIC Genetics Study) for the I/D polymorphism, as well as 2 other markers (rs1800764 and rs9896208).

Causality underlying the association between the ACE gene and diabetic nephropathy was addressed in an animal study by Huang et al. They induced diabetes in mice having 1, 2, or 3 copies of the gene. In 12 weeks, the 3-copy diabetic mice developed higher blood pressures and proteinuria. Proteinuria was correlated to plasma ACE levels in the 3-copy diabetic mice. This study proves that a modest genetic increase in ACE levels is sufficient to cause nephropathy. Altogether, there is solid evidence for a causal relationship between ACE gene function and diabetic nephropathy.
ACE Polymorphism and Muscle Performance
Montgomery and coworkers\(^7\) reported changes in left ventricular mass associated with the D allele. One hundred fifty-six male military recruits underwent 10 weeks of physical training; mean left ventricular mass increased by 2.0, 38.5, and 42.3 g in II, ID, and DD carriers, respectively (\(P<0.01\)). In a later study, Montgomery et al\(^7\) also found an association between the ACE polymorphism and physical performance. The genotype distribution of 25 elite British mountaineers was compared with that of 1906 British males free of cardiovascular disease. Although the genotype frequencies were in HWE in both samples, there were relative excesses of the II genotype and commensurate deficiencies of the DD genotype in the climbers.\(^7\) This observation was replicated when it was tested with 64 Australian national rowers,\(^8\) 91 British Olympic caliber runners,\(^9\) 217 Russian athletes,\(^10\) 60 Spanish elite athletes,\(^11\) 120 swimmers from the European and Commonwealth championships,\(^12\) and 35 swimmers from the World Championships,\(^13\) particularly in very long distance athletes.

The association of the I allele with improved endurance is in line with the association of the D allele with left ventricular hypertrophy. Carriers of the D allele may develop hypertrophy attributable to lower metabolic efficiency.

ACE Polymorphism and Alzheimer Disease
Because ACE degrades amyloid beta peptide in vitro,\(^14\) higher ACE levels may play a protective role in AD pathophysiology. Kehoe et al\(^15\) reported an association between the I/D polymorphism and AD for the first time. In their case–control study, a positive association was found between presence of the I allele and AD (OR = 2.43 [95% CI: 1.35, 4.39] for II/ID versus DD genotypes). Subsequently, they tested the same association in two independent case-control samples and successfully replicated their finding. The authors stated that, given the replicated findings and the high levels of statistical significance after Bonferroni correction, these results were unlikely to be false-positives arising from ethnic stratification or multiple testing.\(^16\) This association was subsequently examined in several studies. A cumulative meta-analysis revealed that, after only 4 studies, the pooled odds ratio (OR = 1.25) reached significance and remained stable thereafter as other studies were added.\(^17\)

To better characterize the nature of the observed association, Kehoe et al\(^18\) conducted a haplotype analysis by genotyping 7 SNPs in 5 independent case–control samples, which included more than 3100 individuals. They found that, in AD patients, there was an excess of one of the haplotypes (containing the I allele) in 4 different sets of cases and controls; this result was concordant with the meta-analysis data.\(^18\) Additionally, they found that SNP rs4291, which showed the strongest effect on AD of the 7 SNPs, was also the SNP most weakly linked with the I/D polymorphism \((r^2 = 0.46)\). In contrast, SNP rs4343, which showed the weakest effect on AD, was strongly linked with the I/D polymorphism \((r^2 = 0.91)\). This suggests that reliance on the I/D polymorphism likely contributed to many of the negative findings previously reported. Kehoe et al\(^18\) estimated that if rs4291 was in complete LD with the “true” functional variant or variants, using the I/D as a marker would require doubling the sample size to detect the same effect size. In general, the association between the I allele and AD is well established. Knowing that the I/D polymorphism is not the functional variant, low statistical power could be the main reason for inconsistencies between the studies.

ACE Polymorphism and Longevity
The associations of the ACE polymorphism with the development of, prognosis for, or survival from multiple diseases triggered questions regarding the influence of ACE on longevity. In 1994, in the first attempt to answer these questions, Schachter et al\(^19\) genotyped 338 centenarians and 164 control individuals, aged 20 to 70 years, from the cohort ascertained by the Centre d’Etude du Polymorphisme Humain (CEPH) in Paris, France. Surprisingly, there was an increased frequency of the DD genotype in the centenarian group compared with the control group (0.40 versus 0.26, \(P<0.01\)). This finding was unexpected, given the published reports on the adverse effects of the DD genotype on cardiovascular disease. The authors speculated that the cardiovascular risk conferred by the D allele is offset by a possible long-term protective effect. Such an effect may give some early selective advantage or a late reversal of its negative survival influence.\(^19\)

Subsequent studies, however, did not confirm Schachter’s finding. In 2000, Blanchet et al\(^20\) tested the quality of the first report.\(^20\) Retyping the 1994 centenarian cohort revealed 17 inconsistencies, confirmed by a second experiment, leading to a 5% difference with the original data. The majority of the discordances resulted from the detection of I alleles in the new data, which were not present in the 1994 data.\(^20\) Furthermore, the authors used 560 additional French centenarians, each paired with a younger individual of the same sex and geographic origin. Comparison of allelic and genotypic frequencies failed to reveal a difference between the centenarian and the control populations.\(^20\)

In a study of 6968 elderly individuals from the Rotterdam Study by Arias-Vazquez et al,\(^21\) an increased risk of early mortality (below age 65) was associated with the DD genotype (hazard ratio [HR] = 1.8 [95% CI: 1.1, 2.9] for DD versus II genotype). There was no evidence of an association with mortality in subjects older than 65 year of age. In stratified analyses, this association was highly significant in smokers (HR = 3.1 [95% CI: 1.5, 6.3]) and was not observed in nonsmokers (HR = 1.2 [95% CI: 0.7, 2.1]).\(^21\) Interaction between smoking and the I/D polymorphism was also reported with respect to blood pressure\(^22\) and carotid and coronary atherosclerosis.\(^23,24\) Altogether, although the association between the ACE gene and longevity was not confirmed, there is evidence that this gene plays a role in early mortality. The association with mortality was observed for both cardiovascular and noncardiovascular causes of death, indicating the importance of ACE in various pathophysiological systems.\(^21\)

Summary
Research on ACE polymorphisms began when Cambien et al\(^25\) reported that circulating ACE levels are under substantial genetic control. Attention was drawn to an insertion/deletion
polymorphism in intron 16 when Rigat et al\(^{30}\) stated that it accounts for approximately half of the observed variance in plasma levels. The location of this polymorphism in a noncoding region of the gene, however, makes it unlikely to be a functional variant. Despite considerable effort, the precise location of the functional polymorphism, or polymorphisms, is still unknown; it is most likely located in the region between intron 18 and the 3′ UTR\(^{41}\). The possibility of another functional polymorphism in the 5′ region cannot be excluded,\(^{35,44}\) and the presence of yet another QTL, located on chromosome 4, was recently suggested.\(^{45}\)

An immense number of studies were published investigating associations of the I/D polymorphism with different pathophysiological conditions. Many studies found significant associations, whereas others did not confirm those findings. Meta-analyses did not always resolve these controversies, and study limitations should be taken into consideration in searching for an explanation for contradictory findings. Cumulative meta-analyses, as presented by Lehmann et al,\(^{87}\) may give us a better insight into underlying effects.

Based on the meta-analyses and large population-based association studies discussed here, the expectations are that the effect sizes of any possible associations between the ACE polymorphism and complex disorders are relatively small. To detect incremental associations, it is necessary to consider both the validity and the precision of planned studies. Misclassifications in both genotypic and phenotypic data may lead to false-negative or false-positive results. To avoid genotypic misclassifications, the use of haplotypes, instead of the I/D polymorphism, is strongly recommended for future association studies. Such an attempt was made by Katzov et al\(^{71}\) with promising results. With respect to phenotypic misclassification, the complexity of the diseases should be considered. Inclusion of uncertain diagnoses must be avoided, and, when appropriate, subclasses of a phenotype should be evaluated separately. The complexity of the phenotypes also includes the possibility of multiple interactions between genes, or genes and environmental factors. Many promising findings about such interactions with the ACE polymorphism were reported, but remain to be replicated. Last, but not least, the power of any future association study should always be evaluated before commencing data collection. Underpowered studies, in the long run, may lead to publication bias scenarios, which cause confusion and may mislead future investigations.

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