Pleotropni učinci polimorfizma ACE

Pleiotropic effects of ACE polymorphism

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Sažetak

Angiotenzin-konvertirajući enzim (engl. *angiotensin converting enzyme*, ACE) ima vitalnu ulogu u normalnoj ljudskoj fiziologiji zbog svojeg izravnog djelovanja u sustavu renin-angiotenzin-aldosteron (RAAS), sustavu kinin-kalikrein, *in vitro* razgradnji amiloid-beta-peptida, aktivnosti GPI-aze (glikozilfosfatidilinozitol, GPI) te u signalnoj transdukciji. Budući da na aktivnost ACE snažno utječe insercijsko-delecijski (I/D) polimorfizam gena *ACE*, prikupljeno je mnoštvo podataka, kako bi se razjasnila udruženost I/D polimorfizma s kardiovaskularnim i ostalim bolestima poput šećerne bolesti, dijabetičke nefropatije, dijabetičke retinopatije, ateroskleroze, koronarne bolesti srca, moždanog udara, hipertenzije, Alzheimerove bolesti, karcinoma i Parkinsonove bolesti. Ovaj će se pregled ograničiti na učinak I/D polimorfizma gena *ACE* na dugovječnost s obzirom na patofiziologiju nekih bolesti.

Ključne riječi: polimorfizam ACE; dugovječnost; prerana smrtnost; kardiovaskularne bolesti

Abstract

Angiotensin converting enzyme (ACE) has vital role in normal functioning of the human body due to its direct involvement in the renin-angiotensin-aldosterone system (RAAS), kinin-kallikrein system, *in vitro* degradation of amyloid beta-peptide, GPlase (glycosylphosphatidylinositol, GPI) activity and in signal transduction. As ACE activity level is strongly influenced by ACE insertion/deletion (I/D) polymorphism, a huge body of data has been generated to elucidate the association of I/D polymorphism with cardiovascular and non cardiovascular diseases like diabetes, diabetic nephropathy, diabetic retinopathy, atherosclerosis, coronary heart diseases and stroke, hypertension, Alzheimer's disease, cancer and Parkinson's disease. This review will be limited to the effect of ACE I/D polymorphism on longevity considering the pathophysiology of several diseases.

Key words: ACE polymorphism; longevity; early mortality; cardiovascular diseases

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Angiotenzin-konvertirajući enzim (ACE)

Angiotenzin-konvertirajući enzim (engl. *angiotensin converting enzyme*, ACE) je karboksipeptidaza ovisna o kloridima i cinku, prisutna na površini epitelnih i endotelnih stanica. Kod ljudi se mogu naći njegove dvije izoforme. Jedna je veći protein sastavljen od 1300 aminokiselina (150–180 kDa) i zove se somatski ACE (sACE), zbog prisutnosti u somatskim tkivima. sACE se može usidriti u plazmatskoj membrani preko transmembranske domene ili može biti prisutan u krvi u topljivom obliku (1). Druga je izoforma manji protein sastavljen od 730 aminokiselina (100–110 kDa), prisutan samo u testisima te se stoga i zove

Angiotensin converting enzyme

Angiotensin converting enzyme (ACE) is a chloride and zinc dependent carboxypeptidase enzyme present on the surface of epithelial and endothelial cells. In humans, two isoforms exist. One is larger protein, composed of 1300 amino acids (150–180 kDa) and is called somatic ACE (sACE), due to its presence in somatic tissues. sACE can be anchored in plasma membrane through transmembrane domain, or be present in plasma in the soluble form (1). The other isoform is a smaller protein composed of 730 amino acids (100–110 kDa), present only in testicles and called germinal form or testicular form (tACE). Its function

germinalni ili testikularni oblik ACE (tACE). Njegova funkcija još nije sasvim jasna, no čini se da je uključen u fiziologiju muške plodnosti (2). Ove se dvije izoforme razlikuju u broju aktivnih mjesta: sACE ima dva aktivna mjesta, dok tACE ima samo jedno (3).

Glavne funkcije ACE su slijedeće:

- pretvorba dekapeptida angiotenzina I (neaktivna komponenta) u oktapeptid angiotenzin II (aktivna komponenta). Angiotenzin II uzrokuje suženje krvnih žila (vazokonstrikciju), otpuštanje aldosterona, posreduje u staničnom rastu i proliferaciji te inducira disfunkciju endotela (4-7);
- razgradnja bradikinina (neurokinin koji uzrokuje proširenje krvnih žila) omogućujući time sužavanje krvnih žila (3);
- put signalne transdukcije (8);
- *in vitro* razgradnja amiloid-beta-peptida (9);
- aktivnost GPI-aze čime se omogućuje otpuštanje glikozilfosfatidilinozitola (GPI) vezanog za membranu (10).

Genetika angiotenzin-konvertirajućeg enzima

Gen odgovoran za ekspresiju oba oblika sACE i tACE nalazi se na lokusu 17q23, dugačak je 21 kb i sastavljen od 26 eksona i 25 introna. Za ekspresiju pojedinih izoformi odgovorni su različiti promotori. Promotor za sACE leži u 5′ bočnoj regiji prvog eksona i odgovoran je za transkripciju eksona 1-12 i 14-26, dok promotor za tACE leži unutar introna 12 i regulira transkripciju eksona 13-26 (11,12). Mjesta inicijacije za transkripciju dviju mRNA koje dekodiraju te izoforme udaljena su 5,7 kb jedno od drugoga, a mjesta poliadenilacije su udaljena 628 bp (13).

Polimorfizam I/D u genu *ACE*

Rigat i sur. su 1990. uočili da u intronu 16 gena *ACE* dolazi do umetanja sekvence od 287 bp (NCBI ref. SNP ID: rs1799752) (14), rezultirajući intronskim (I) alelom, dok je delecijski (D) alel prisutan ako nema te insercije. Taj je polimorfizam odgovoran za razinu aktivnosti ACE, koja se udvostručava kod D/D homozigota, u odnosu na I/I homozigota. Kod I/D heterozigota, prisutna je srednja aktivnost gena ACE. Ta je kodominacija primijećena u aktivnosti gena ACE i u plazmi i u tkivu (15).

Otkrivanje polimorfizma I/D

Rigat i sur. su za otkrivanje I/D polimorfizma pomoću lančane reakcije polimeraze (engl. *polymerase chain reaction*, PCR) rabili set početnica na bočnoj strani regije insercije (14). Problem s uporabom ove metode je 5-10% pogrešaka pri genotipiziranju, pri čemu dolazi do predominan-

is not clear, but it appears to be involved in male fertility (2). These isoforms differ in active sites: sACE has two active sites, whereas tACE has only one active site (3).

Major functions of ACE are as follows:

- conversion of decapeptide angiotensin I (inactive) to octapeptide angiotensin II (active compound). Angiotensin II causes vasoconstriction, release of aldosterone, mediation of cell growth, and proliferation and induction of endothelial dysfunction (4-7);
- inactivation of bradykinin (a vasodilating neurokinin) allowing for vasoconstriction (3);
- signal transduction pathway (8);
- *in vitro* degradation of amyloid beta peptide (9); and
- GPI-ase activity which allows for the release of membrane glycosylphosphatidylinositol (GPI) – anchored protein (10).

Genetics of angiotensin converting enzyme

A single gene is responsible for the expression of both sACE and tACE. It is located at locus 17q23. *ACE* gene is 21 kb long and composed of 26 exons and 25 introns. Alternative promoters are responsible for the expression of each isoform. The promoter for sACE lies in the 5′ flanking region of the first exon and transcribes exon 1-12 and 14-26, whereas the promoter for tACE lies within intron 12 and transcribes exon 13-26 (11,12). The initiation sites for the transcription of two mRNAs encoding these isoforms are 5.7 kb apart and polyadenylation sites are 628 bp apart (13).

Insertion/deletion (I/D) polymorphism in ACE gene

In 1990, Rigat *et al.* observed a polymorphism involving insertion of 287 bp sequence (NCBI ref. SNP ID: rs1799752) resulting in insertion (I) allele, whereas deletion (D) allele is present in the absence of insertion (14). This polymorphism is responsible for the ACE activity level, which increases 2-fold in homozygous deletion carriers (D/D), as compared to homozygous insertion carriers (I/I). I/D carriers show intermediate ACE activity. This codominance was observed both, in plasma and tissue ACE levels (15).

Detection of I/D polymorphism

Rigat *et al*. (1990) used a set of primers flanking the insertion region for the detection of I/D polymorphism through polymerase chain reaction (PCR) (14). In this method, there was a problem of 5%-10% of mistyping, causing preferred amplification of the D allele depicting I/D heterozygotes as D/D carriers (16). For accurate genotyping, this mistyping had to be eliminated. Different strategies

tnog umnožavanje D alela, pa se jedan dio I/D heterozigota pogrešno prikazuje kao D/D heterozigoti (16). Za točnu genotipizaciju takvu je pogrešku bilo potrebno ukloniti. U tu su svrhu razni znanstvenici usvojili razne strategije. Shanguman i sur. su 1993. rabili 5%-nu otopinu dimetil-sulfoksida (DMSO) i *sense* početnicu na 5′ insercijskom kraju sekvence zajedno sa standardnom *antisense* početnicom (17). Kasnije je točnost metode poboljšana modifikacijom PCR, koja podrazumijeva postupno sniženje temperature prijanjanja (engl. *step down PCR modification*) (18).

Višestruka PCR metoda (engl. *multiplex PCR*) i PCR u stvarnom vremenu (engl. *real-time PCR*) također su se upotrebljavale u otkrivanju I/D polimorfizma, no niti jedna od njih nije uspjela zamijeniti Shangumanovu metodu, zbog problema povezanih s obradom nakon same metode PCR, kao što su elektroforeza na agaroznom gelu kod višestruke PCR i visoki troškovi PCR u stvarnom vremenu (19,20). Nedavno su Koyama i sur. (2008.) opisali brzu i jednostavnu tehniku koja obuhvaća denaturirajuću tekućinsku kromatografiju visoke djelotvornosti (engl. *denaturing high performance liquid chromatography*, DHPLC). U uvjetima koji nisu denaturirajući, ona analizira PCR produkt za probiranje I/D polimorfizama u epidemiološkim genetskim istraživanjima (21). Ta metoda otklanja mogućnost pogrešnog određivanja i nudi 100% točnost kod I/D heterozigota, no zbog visokih troškova kromatografije nije isplativa.

Učinci polimorfizma I/D na ljudsko zdravlje

Utjecaj I/D polimorfizma na patofiziologijske uvjete kroz aktivnost ACE rezultirao je mnoštvom podataka koji svjedoče o njegovoj povezanosti s nekoliko bolesti (Tablica 1.). U ovom se pregledu o njegovoj ulozi u ljuskom zdravlju raspravlja u svjetlu prethodnih istraživanja o nekoliko pokazatelja zdravlja populacije.

Rast i polimorfizam ACE

Unutarmaterična okolina ima očigledan učinak na ekspresiju gena ACE koji naposlijetku utječe na trudnoću i početnu težinu novorođenčadi. Kajantie i sur. (2004.) su primijetili povezanost I/I genotipa s kraćim trajanjem trudnoće i većom težinom kod poroda, što kod odraslih neizravno znači da postoje manji izgledi za razvoj koronarne bolesti srca, šećerne bolesti tipa 2, inzulinske rezistencije i metaboličkog sindroma (22-24). Tako je, dakle, razvijen koncept da je alel I odgovoran za veću tjelesnu težinu pri porodu. Taj je koncept bio prihvatljiv sve dok Hindmarsh i sur. (2007.) nisu ustvrdili kako ne postoji povezanost genotipa ACE novorođenčeta ili njegovih roditelja sa porođajnom težinom (25). Međutim, I/I genotip pokazao je svoju pozitivnu ulogu u ranom rastu djece koja su unutar

were adopted by different scientists. Shanguman *et al.* (1993) used 5% dimethyl sulfoxide (DMSO) and sense primer from the 5′ end of the insertion sequence, along with the standard antisense primer (17). Later on, step down PCR modification in this method improved the accuracy of the method (18).

Multiplex PCR method and use of real time PCR were also devised for the detection of I/D polymorphism, but both methods failed to replace Shangumans method, due to problems related to post PCR handling, such as agarose diagonal gel electrophoresis in case of multiplex PCR and expensive nature of real time PCR (19,20). Recently, Koyama *et al*. (2008) have described a quick and easy technique involving DHPLC (denaturing high performance liquid chromatography) in non denaturing conditions to analyze the PCR product for screening for I/D polymorphisms in genetic epidemiological studies (21). This method removes the chances of mistyping and yields 100% accuracy in I/D heterozygotes, but expenses of chromatography do not prove it to be cost effective.

Effects of I/D polymorphism on human health

The influence of I/D polymorphism on pathophysiological conditions mediated through ACE activity has generated a lot of data showing its association with several diseases (Table 1). In the present review, its role in deciding on the health status is discussed in the light of previous studies on several health indicators.

Growth and ACE polymorphism

Intrauterine environment have obvious effects on the gene expression of ACE, which ultimately influences the period of gestation and birth weight of the newborn. Kajantie *et al.* (2004) noticed the association of I/I genotype with shorter gestation duration and higher birth weight, which indirectly means less chances for development of coronary heart diseases, type 2 diabetes, insulin resistance and metabolic syndrome in adults (22-24). So, a concept was developed on I allele to be responsible for higher birth weight. This concept remained acceptable until Hindmarsh *et al*. (2007) have claimed that there is no association of ACE genotype of the newborn or his/her parents with birth weight (25). However, I/I genotype exhibited advantageous role in the early growth of babies, showing more gain in weight, body mass index and midarm circumference in one fiscal year as compared to the babies with D/D genotype, the majority of which showed no change or catch-down. I/D genotype was distributed equally across all the categories. These effects were more prominent in males. So, I/I genotype has positive effects on the early growth after birth.

Tablica 1. Udruženost bolesti s polimorfizmom gena *ACE*

Table 1. Diseases in association with ACE polymorphism

Association present	Association absent	Controversial
Diabetic nephropathy	Type 2 diabetes	Hypertension
Atherosclerosis	Diabetic retinopathy	Coronary heart disease and stroke
Alzheimer disease	Gastric cancer	Colorectal cancer
Parkinson's disease	Systemic lupus erythematosus	
Breast cancer		
Oral cancer		

jedne fiskalne godine dobila na težini, povisio im se indeks tjelesne mase i povećao opseg nadlaktice u usporedbi s djecom s D/D genotipom, gdje većina ispitanika nije pokazivala promjene ili su pokazivali sniženje mjerenih parametara. Distribucija I/D genotipa bila je ravnomjerna u svim kategorijama. Ti su učinci bili istaknutiji kod muške populacije. Dakle, I/I genotip ima pozitivne učinke na rani rast nakon poroda.

Šećerna bolest i polimorfizam ACE

Postoje mnoga istraživanja koja pokazuju utjecaj I/D polimorfizma gena ACE na pojavu šećerne bolesti (26-29). No, nedavno objavljeni rezultati ne potvrđuju prethodne nalaze. Primjerice, opsežno praćenje u trajanju od 10,2 godina izvršeno kod 24.309 bjelkinja bez šećerne bolesti na početku ispitivanja nije uspjelo dokazati bilo kakvu povezanost ACE genotipa i šećerne bolesti (30). Taj se rezultat ponavljao u mnogim drugim istraživanjima na ispitanicima iz različitih etničkih skupina te na ispitanicima s nefropatijom i bez nje (31-33). Dakle, polimorfizam gena *ACE* ne može se predodrediti kao nezavisan čimbenik odgovoran za šećernu bolest. U svakom su slučaju potrebna daljnja istraživanja ostalih djelotvornih genetskih i okolišnih čimbenika, kako bi se utvrdila uloga polimorfizma ACE kod pojave šećerne bolesti.

Dijabetička nefropatija i polimorfizam ACE

Dijabetička nefropatija je najčešći razlog smrtnosti kod kroničnih bolesnika sa šećernom bolešću. Klinički uzrok bolesti varira od bolesnika do bolesnika. Meta-analiza Nga i sur. (2005.) na 14.727 bolesnika pokazala je značajno viši rizik od dijabetičke nefropatije kod nosilaca alela D, nego kod skupine sa genotipom divljeg tipa (I/I) (OR = 1,28; 95% CI = 1,14-1,45) (34). Rezultati Movvea i sur. (2007.) također su dokazali da su nosioci alela D koji boluju od šećerne bolesti tipa 2 podložniji dijabetičkoj nefropatiji (35).

Dijabetička retinopatija i polimorfizam ACE

Dijabetička retinopatija je sljepoća zbog oštećenja mrežnice uslijed komplikacija šećerne bolesti. Dosada se pretpostavljalo da je *ACE* glavni gen kandidat s predispozicija-

Diabetes and ACE polymorphism

There are many association studies showing influence of ACE I/D polymorphism on the onset of diabetic mellitus (26-29). However, recent findings do not support this statement. For example, a large follow up of 10.2 years in 24,309 Caucasian women free from diabetes at baseline failed to show any association of ACE genotype with diabetes (30). This result was replicated in many other studies in different ethnic groups, both in patients with and without nephropathy (31-33). So, ACE polymorphism cannot be supposed as an independent factor responsible for diabetes. Anyhow, further research can investigate other effective genetic factors and environmental factors to find out the possible role of ACE in the onset of diabetes.

Diabetic nephropathy and ACE polymorphism

Diabetic nephropathy is a major cause of mortality in chronic diabetic patients. The clinical course of the disease is variable among patients. A meta-analysis by Ng *et al*. (2005) comprising 14,727 subjects showed a significantly higher risk of diabetic nephropathy in the carriers of D allele as compared with the I/I genotype group (OR = 1.28; 95% CI = 1.14-1.45) (34). Findings of Movva *et al*. (2007) also demonstrated D allele carriers with type 2 diabetes to be more vulnerable to the development of diabetic nephropathy (35).

Diabetic retinopathy and ACE polymorphism

Diabetic retinopathy is blindness due to retinal damage as a complication of diabetes mellitus. The *ACE* gene has been the main probable candidate gene predisposing the development of diabetic retinopathy. Findings reported by Globocnik-Petrovic *et al*. (2003) show no association of ACE genotype with diabetic retinopathy, non-proliferative, proliferative or severe proliferative type (36). However, Matsumoto *et al.* (2000) and Feghhi *et al.* (2008) observed frequent occurrence of D/D allele in patients with proliferative diabetic retinopathy in Japanese and Iranian population, respectively (37,38). Contradictory results reported by Wiwanitkit (2008) also reject involvement of ACE po-

ma za razvoj dijabetičke retinopatije. Rezultati Globocnik-Petrovica i sur. (2003.) pokazali su da nema povezanosti ACE genotipa s neproliferativnom, proliferativnom ili teškom proliferativnom dijabetičkom retinopatijom (36). Međutim, istraživačke skupine Matsumota (2000.) i Fenghhija (2008.) promatrale su česte pojave genotipa D/D kod bolesnika koji boluju od proliferativne dijabetičke retinopatije u populaciji Japana (37) i Irana (38). Rezultati istraživanja Wiwanitkita i suradnika (2008.) također su zanijekali povezanost polimorfizma gena *ACE* s razvojem dijabetičke retinopatije (39). Zbog tih proturječnih rezultata teško je definirati ulogu polimorfizma gena *ACE* u razvoju dijabetičke retinopatije.

Ateroskleroza i polimorfizam gena ACE

Ateroskleroza se može dijagnosticirati mjerenjem debljine intime medije (engl. *intima-media thickness*, IMT), prilikom obdukcije i određivanjem stupnja koronarne kalcifikacije. Sayed-Tabatabaei i sur. (2003.) objavili su meta-analizu 23 objavljena članka do listopada 2002. s 9.833 ispitanika na temu ispitivanja povezanosti polimorfizma gena *ACE* s aterosklerozom, koja se temeljila na mjerenjima IMT (40). Podaci su pokazali jaku povezanost između alela D i uobičajene IMT karotidnih arterija. Ta je povezanost bila jača kod ispitanika s cerebrovaskularnim bolestima, šećernom bolešću ili povišenim krvnim tlakom. Slične su rezultate objavili Pawel i sur. (2008.) te Kretowski i sur. (2007.) (41,42). Treba primijetiti da pokusi koji uključuju dijagnostiku za vrijeme obdukcije i koronarnu kalcifikaciju za otkrivanje ateroskleroze nisu pokazali povezanost polimorfizma gena *ACE* s aterosklerozom (43,44). Nepodudarni rezultati dobiveni različitim dijagnostičkim metodama zahtijevaju pouzdanije dijagnostičke metode za potvrdu prisustva bolesti, što će pomoći pri pronalaženju uloge polimorfizma ACE kod ateroskleroze.

Povišeni krvni tlak i polimorfizam gena ACE

Za D/D genotip se vjerovalo da je povezan s povišenim krvnim tlakom, zbog povećane aktivnosti ACE. Kod različitih su varijanta gena ACE postojali različiti izgledi za pojavu povišenog krvnog tlaka, uslijed starenja i poremećenog noćnog krvnog tlaka (45,46). Prva je meta-analiza temeljena na 23 istraživanja s 28 kontrolnih skupina i 6.923 ispitanika pokazala 10%-no, ali statistički neznačajno, povećanje rizika od pojave povišenog krvnog tlaka. U svakom slučaju, postojala je statistički značajna povezanost između D/D genotipa i povišenog krvnog tlaka kod ispitanica i Azijata (47). Druga se meta-analiza ograničila na bijelce, no niti ona nije pokazala povezanost između ACE genotipa i povišenog krvnog tlaka (48). Rezultati Miyame i sur. (2007) i Glavnika i Petrovica (2007.) također ne pokazuju utjecaj alela genotipa *ACE* na pojavu povišenog krvnog tlaka (49,50). Postojanje proturječnih rezultata ukazuje na složenu interakciju polimorfizma gena *ACE* s čimbenicima

lymorphism in the development of diabetic retinopathy (39). So, because of contradictory findings, it is difficult to define the role of ACE polymorphism in the expression of diabetic retinopathy.

Atherosclerosis and ACE polymorphism

Atherosclerosis can be diagnosed by measuring intima media thickness (IMT), on autopsy and by determining coronary calcification. Sayed-Tabatabaei *et al.* (2003) conducted a meta-analysis of 23 articles published until October 2002, including 9,833 subjects to study the association of ACE polymorphism with atherosclerosis based on IMT measurement (40). Data suggested strong relation between D allele and common carotid IMT. This association was more prominent in subjects with cerebrovascular disease, diabetes or hypertension. Similar results have been reported by Pawel *et al*. (2008) and Kretowski *et al*. (2007) (41,42). It is noteworthy that experiments involving autopsy measurements and coronary calcification for the detection of atherosclerosis showed no association of ACE polymorphism with atherosclerosis (43,44). Discordant findings due to different diagnostic methods pose the need of more reliable diagnostic methods to confirm the presence of disease, which will help identify the role of ACE polymorphism in atherosclerosis.

Hypertension and ACE polymorphism

D/D genotype is predicted to be associated with hypertension, because of the increased level of ACE activity. Different ACE variants showed different chances to develop hypertension, due to aging and abnormality in nocturnal blood pressure (45,46). The first meta-analysis based on 23 studies consisting of 28 case-control groups with 6,923 subjects showed a 10% increased, but statistically non-significant risk, of hypertension in D/D *versus* I/I genotype. Anyhow, there was a significant correlation between D genotype and hypertension in women and in Asians (47). Another meta-analysis restricted to Caucasians, also failed to show any association between ACE genotype and hypertension (48). Findings by Miyama *et al*. (2007) and Glavnik and Petrovic (2007) also showed no influence of ACE genotype on hypertension (49,50). These contradictory results indicate the complex interaction of ACE polymorphism with the environmental and other genetic factors for the expression of hypertension. Recently, Bautista *et al.* (2008) have reported on D/D genotype as an independent factor for developing hypertension among Hispanics. Similar findings have been reported for the Chinese population and male population of Bangladesh, Japan and Argentina (51-55). However, Napoles *et al*. (2007) found no association of ACE genotype and hypertension in Cuban population (56). It is also noteworthy that the effects of ACE polymorphism in different ethnic groups are different. It predicts the effects

okoline i ostalim genetskim čimbenicima za pojavu povišenog krvnog tlaka. Nedavno su Bautista i sur. (2008.) objavili da je genotip nezavisan čimbenik kod povišenog krvnog tlaka među Latinoamerikancima. Sličan je rezultat objavljen kod populacije Kineza, kod muške populacije u Bangladešu, Japanu i Argentini (51-55). Međutim, Napoles i sur. (2007.) nisu pronašli povezanost ACE genotipa i hipertenzije u Kubanskoj populaciji (56). Dakle, treba primijetiti da je utjecaj polimorfizma gena *ACE* različit kod različitih etničkih skupina. On predskazuje utjecaj različitih načina života i čimbenika okoline koji uzrokuju povišeni krvni tlak kod različitih etničkih skupina.

Koronarne srčane bolesti i polimorfizam gena ACE

Uloga D/D genotipa kod infarkta miokarda koju su promatrali Cambien i sur. (1992.) pobudila je veliko zanimanje znanstvenika, no proturječni su je rezultati učinili spornom (57). Meta-analiza koja je uključivala 1.918 ispitanika bijelaca (1.196 ispitanika eksperimentalne i 722 ispitanika kontrolne skupine) nije pokazala razliku u genotipu (P > 0,05), niti u alelima ACE (P > 0,05) između ispitanika eksperimentalne i ispitanika kontrolne skupine. Ukupni omjer rizika (OR) za alel D kao nezavisan čimbenik kod pojave moždanog udara bio je 1,31 u recesivnom modelu i 1,14 u dominantnom modelu. Taj rezultat pokazuje da je učinak alela D u recesivnom modelu skroman, ali nezavisan čimbenik rizika za pojavu moždanog udara (58). Međutim, te rezultate nije potvrdila opsežna meta-analiza sastavljena od 46 istraživanja uključujući ukupno 32.175 ispitanika bijelaca (48). Njeni su rezultati pokazali povezanost polimorfizma gena ACE s aktivnosti enzima u plazmi, no ne i s kardiovaskularnim bolestima. Istraživanja meta-analize na populaciji Azijata također su pokazala nepostojanje povezanosti genotipa ACE i pojave bolesti (59). Slijedećih su godina ponovljeni isti rezultati (60,61). Može se zaključiti da ACE genotip ne pokazuje jaku udruženost s infarktom miokarda.

Alzheimerova bolest i polimorfizam ACE

Zbog *in vitro* razgradnje amiloid-beta-peptida koju provodi ACE, pretpostavljalalo se da ACE ima zaštitnu ulogu protiv Alzheimerove bolesti. Prvo su Kehoe i sur. (1999.) promatrali pozitivnu povezanost između I alela i Alzheimerove bolesti (62). Nakon toga su otkrili da je polimorfizam SNP rs 4343 pokazuje jaču povezanost s Alzheimerovom bolesti od SNP rs 4291, za koji se mislilo da je genetska varijanta odgovorna za nastanak Alzheimerove bolesti (63). Učinci polimorfizma gena ACE na Alzheimerovu bolest su nadalje potvrđeni meta-analizom Lehmanna i sur. (2005.) (64). Ta je meta-analiza uključivala 39 istraživanja, 6.037 bolesnika s Alzheimerovom bolesti i 12.099 kontrolnih ispitanika. Rezultati su ukazali na to da nosioci D/D polimorfizma imaju smanjeni rizik (OR = 0,81; 95% CI = 0,72-0,90; P < 0,001) za razvoj Alzheimerove bolesti. Kod

of different lifestyles and environmental factors causing hypertension in different ethnic groups.

Coronary heart disease and ACE polymorphism

The role of D/D genotype in myocardial infarction observed by Cambien *et al*. (1992) generated huge scientific interest, but contradictory results make its role controversial (57). A meta-analysis involving 1,918 white subjects (1,196 cases and 722 controls) showed no difference in ACE genotype (P > 0.05) or allele frequency (P > 0.05) between cases and controls. The overall OR for D allele as an independent risk factor in ischemic stroke was 1.31 under a recessive model, and 1.14 under a dominant model. This result indicates that D allele, acting recessively, is a modest but independent risk factor for ischemic stroke onset (58). These results, however, were not confirmed by a large meta-analysis of 46 studies including a total of 32,715 white individuals (48). Results showed an association of ACE polymorphism with plasma activity level, but not with cardiovascular diseases. Meta-analysis studies in Asian population also showed the absence of any role of ACE polymorphism in disease occurrence (59). Later on, similar results were replicated in the next years (60,61). It can be concluded that ACE genotyping has no prominent association with myocardial infarction.

Alzheimer's disease and ACE polymorphism

In vitro degradation of amyloid beta-peptide by ACE predicted its possible protective role against Alzheimer's disease (AD). First of all, Kehoe *et al*. (1999) observed positive association between I allele and AD (62). Later on, they found that SNPrs 4343 was more associated with AD in spite of SNPrs 4291, which was previously thought to be a strong genetic variation responsible for AD (63). Effects of ACE polymorphism on AD were further verified by a meta-analysis by Lehmann *et al*. (2005) (64). The meta-analysis included 39 studies comprising 6,037 AD cases and 12,099 controls. Findings suggested that D/D carriers were at a reduced risk (OR = 0.81; 95% CI = 0.72-0.90; P < 0.001); I/I homozygotes exhibited no association with AD, while heterozygotes were more vulnerable to AD. Similar results were seen among North Europeans, South Caucasians, and East Asians. Anyhow, in North Europeans, both association and Hardy-Weinberg analysis indicated partial heterogeneity, due to unknown reason. These results were also replicated in other studies (65,66). Some discordant results have also been reported, thus opening way to further investigations to confirm the role of ACE in disease manifestation (67-69). The greater body of data confirming association as compared to those denying association appears to suggest the influence of several factors on deciding about ACE polymorphism as a candidate gene variation responsible for AD.

I/I homozigota nije nađena povezanost, dok su heterozigoti bili skloniji razvoju Alzheimerove bolesti. Slični su rezultati zabilježeni među sjevernim Europljanima, bijelcima koji žive u južnijim krajevima i istočnim Azijatima. U svakom slučaju, kod sjevernih Europljana su obje analize, analiza povezanosti i Hardy-Weinbergova analiza, iz nepoznatog razloga ukazivale na djelomičnu heterogenost. Ti su rezultati ponovljeni i u drugim istraživanjima (65,66). Također su objavljeni neki nepodudarni rezultati koji otvaraju put daljnjim istraživanjima u potvrđivanju uloge gena *ACE* u manifestaciji bolesti (67-69). Postojanje većeg broja rezultata koji potvrđuju udruženost, nego onih koji ju negiraju, postavlja pretpostavku da nekoliko čimbenika igra ulogu u odluci je li polimorfizam gena *ACE* varijanta gena kandidata odgovorna za Alzheimerovu bolest.

Parkinsonova bolest

Malo se napravilo u pronalaženju bilo kakve povezanosti polimorfizma gena *ACE* s Parkinsonovom bolesti. Lin i sur. (2003.) su proveli istraživanje parova koje je obuhvaćalo 127 sporadično odabranih bolesnika s Parkinsonovom bolesti i 198 zdravih kontrolnih ispitanika. Primijetili su da je homozigotni D/D genotip bilo učestaliji kod bolesnika s Parkinsonovom bolesti, nego kod kontrolnih ispitanika (P = 0,048). Unatoč tome, nije bilo statistički značajne razlike u raspodjeli alela (P = 0,133) (70). Stupnjevita logistička regresija potvrdila je nezavisnu ulogu D/D genotipa kao čimbenika rizika za razvoj Parkinsonove bolesti (OR = 1,32; 95% CI = 1,12-2,16).
Lin i sur. (2007.) također su primijetili da je I/D polimorfizam gena *ACE* primarni predskazatelj pojave psihoze potaknute L-dopom kod bolesnika s Parkinsonovom bolesti (71). Dakle, preporuča se ACE genotipizacija kod bolesnika s Parkinsonovom bolesti, kako bi se identificirali bolesnici kod kojih postoji rizik od psihoze potaknute L-dopom te kako bi se umanjili izgledi razvoja psihoze. Ta istraživanja nisu dovoljna za donošenje krajnjeg zaključka o ulozi polimorfizma gena *ACE*, već traže potvrdu jednim kohortnim istraživanjem za praćenje kasne faze Parkinsonove bolesti.

Karcinom i polimorfizam gena ACE

Uključenost polimorfizma gena ACE u pojavu nekoliko malignih bolesti, proliferaciju i migraciju tumorskih stanica, angiogenezu i razvoj metastaza posredovana je angiotenzinom I/I, što dokazuje da je angiogenetski čimbenik i čimbenik rasta (72). Ta je informacija postala temeljem mnogih istraživanja povezanosti koja su se odnosila na različite vrste karcinoma. Spomenut ćemo rezultate samo nekih.

Karcinom dojke

Godine 2003. Koh i sur. su u okviru kohortnog istraživanja ispitali 189 slučajno odabranih bolesnica s karcinomom

Parkinson's disease

Little work has been done to find out any relation of ACE polymorphism with Parkinson's disease (PD). Lin *et al.* (2003) conducted a case-control study comprising of 127 sporadic PD patients and 198 healthy controls, and observed the presence of homozygote D/D genotype to be more frequent in patients with PD than in controls (P = 0.048), although there was no significant difference in the allelic frequency (P = 0.133) (70). A stepwise logistic regression analysis verified the independent role of D/D genotype as a risk factor for PD (OR = 1.32; 95% CI = 1.12-2.16).
Lin *et al*. (2007) also observed that ACE I/D polymorphism was primary predictor for the occurrence of psychosis in L-dopa patients (71). So, ACE genotyping is recommended in PD patients for identification of subjects at risk and for minimizing the chances of L-dopa induced psychosis. These studies are not sufficient for conclusive role of ACE polymorphism but seek confirmation through a cohort followed until the late phase of PD.

Cancer and ACE polymorphism

ACE polymorphism involvement in the occurrence of several malignancies, tumor cell proliferation, tumor cell migration, angiogenesis and metastatic behavior is mediated by angiotensin I/I which has been proven to be an angiogenic and growth factor (72). This information has become a basis for many association studies related to different types of cancer. We shall discuss the outcomes of some of them.

Breast cancer

In 2003, Koh *et al*. analyzed 189 incident breast cancer cases and 671 female cohort control subjects for sorting out any impact of ACE polymorphism on breast cancer in Singapore (73). It was observed that I allele carriers were at a low risk as compared to D allele carriers, suggesting that the renin-angiotensin system may serve as a therapeutic target for breast cancer treatment and prevention. This finding was also supported by many other studies (74-76).

Oral cancer

Vairaktaris *et al*. (2007) recorded a three-fold risk in I/I homozygotes for developing oral cancer, regardless of smoking habit or alcohol consumption, early or advanced stage of cancer, and presence or absence of a family history of cancer or thrombophilia (77). For confirmation of the above mentioned results, additional analyses with a larger sample size are required.

Gastric cancer

No relation of ACE polymorphism with gastric cancer was observed by Röcken *et al*. (2005) but in the same year, dis-

dojke i 671 kontrolnu ispitanicu, kako bi ispitali povezanost polimorfizma gena *ACE* sa karcinomom dojke u Singapuru (73). Primijećeno je da su nositeljice alela I imale manji rizik od karcinoma dojke od nositeljica alela D, što ukazuje na činjenicu da renin-angiotenzinski sustav može služiti kao terapijski cilj za liječenje i prevenciju karcinoma dojke. Taj su rezultat poduprla i mnoga druga istraživanja (74-76).

Karcinom usne šupljine

Vairaktaris i sur. (2007.) su primijetili da kod I/I homozigota postoji trostruko viši rizik od karcinoma usne šupljine, bez obzira na to je li bolesnik pušač ili konzumira alkohol, je li bolest u ranom ili uznapredovalom stadiju te ima li u obiteljskoj anamnezi slučajeva karcinoma ili trombofilije (77). Za potvrdu gore navedenih rezultata potrebno je provesti više analiza na većem uzorku.

Karcinom želuca

Röcken i sur. (2005.) nisu našli udruženost polimorfizma gena ACE s karcinomom želuca, no iste su godine Goto i sur. dobili nepodudarne rezultate (72,78). Njihovi su se rezultati temeljili na istraživanju na 454 Japanca koji su prošli sistematski pregled i 202 bolesnika s karcinomom želuca. Nije pronađena udruženost polimorfizma sa seropozitivnosti na *Helibacter pylori* ili atrofijom želuca. Međutim, I/D nosioci imaju povećani rizik od karcinoma želuca (OR = 1,59; 95% CI = 1,02-2,48).

Karcinom debelog crijeva

Röcken i sur. (2007.) proveli su pokuse u kojima su metodama kvantitativne lančane reakcija polimerazom nakon reverzne transkripcije (engl. *reverse transcription-polymerase chain reaction*, RT-PCR) i imunohistokemije provjeravali lokalnu ekspresiju gena *ACE* kod karcinoma i adenoma debelog crijeva (79). Rezultati su pokazali veću proizvodnju proteina ACE kod adenoma (17 [81%]) i epitelnih stanica raka (22 [100%]) nego kod odgovarauće kripte koja nije maligna i površinskog epitela (2 [10%] kripta i 2 [9%] epitel). Štoviše, pronađena je povezanost polimorfizma gena *ACE* sa spolno specifičnim razlikama kod prvotne veličine tumora i stope preživljavanja bolesnika. U usporedbi s bolesnicima, kod bolesnica s rakom debelog crijeva češće je ustanovljen I/D genotip nego genotipovi I/I i D/D. Tumori kod I/D i D/D nosilaca (muškaraca) bili su veći nego kod muškaraca s genotipom I/I. Iste su godine rezultati Nikiteasa i sur. negirali gore navedene rezultate te postavili pretpostavku da polimorfizam gena *ACE* može biti povezan sa sklonosti ka karcinomu debelog crijeva (80).

Rad mišića i polimorfizam ACE

Proširenje krvnih žila ovisno o endotelu može se povećati aerobnim vježbama kod zdravih osoba uslijed povišenja

cordant results were obtained by Goto *et al*. (72,78). Their results were based on a study including 454 Japanese subjects undergoing health checkup and 202 gastric cancer patients. There was no effect of the polymorphism on *Helicobacter pylori* seropositivity or gastric atrophy. However, I/D carriers were at an increased risk of gastric cancer (OR = 1.59; 95% CI = 1.02-2.48).

Colorectal carcinoma

Röcken *et al*. (2007) conducted experiments to assess local expression of ACE by using quantitative reverse transcription-polymerase chain reaction and by immunohistochemistry in colorectal carcinomas and adenomas (79). Results showed greater production of ACE protein in adenomas (17 [81%]) and cancer epithelial cells (22 [100%]) than in the corresponding non-neoplastic crypt and surface epithelium (2 [10%] and 2 [9%], respectively). Moreover, I/D polymorphism was found to be associated with gender specific differences in primary tumor size and patient survival. Female colorectal carcinoma patients were found to more frequently have I/D genotype and less frequently I/I and D/D genotypes as compared to male patients. Tumors of I/D and D/D male carriers were larger than those of I/I genotype. In the same year, the findings reported by Nikiteas *et al.* negated the above results suggesting controversial predisposition of ACE polymorphism for colorectal cancers (80).

Muscle performance and ACE polymorphism

Endothelium-dependent vasodilation can be increased with aerobic exercise in healthy individuals due to the increase in nitric oxide (NO) production and decreased NO inactivation, leading to an increase in NO bioavailability. Improvement of vasodilation by regular isotonic exercise varies with different alleles of ACE carriers. ACE I/I carriers can better improve this vasodilation as compared to I/D and D/D carriers (81). So, I/I genotype promotes the chances for improvement of vasodilation during aerobic exercise.

Frequency distribution of ACE genotype indicates that I/I and I/D genotypes are frequent in endurance athletes, long distance runners, rowers and mountaineers, whereas D/D genotype is found mostly among top level professional French cyclists (82,83). Moran *et al*. (2006) observed a relation of I allele with phenotypes related more to strength than to endurance in 1,027 teenage Greeks (84). It suggests a more complicated role for the *ACE* gene in human physical performance than previously described. This finding apparently opposes the results of previous experiments. However, those studies are not comparable due to high selection and relatively small population. So, a modest influence of *ACE* gene on physical performance is clear in general population. Now, the only challenge is

stvaranja dušik-oksida (NO) i smanjenja inaktivacije NO, što dovodi do povećanja bioraspoloživosti NO. Poboljšanje stanja vazodilatacije izotoničnim vježbanjem varira kod polimorfizma gena ACE. Kod nosilaca I/I genotipa stanje se proširenih krvnih žila može značajnije poboljšati, nego kod I/D i D/D nosilaca (81). Dakle, genotip I/I pojačava izglede za poboljšanje stanja proširenih krvnih žila tijekom aerobnog vježbanja.

Raspodjela učestalosti genotipa ACE ukazuje na činjenicu da su genotipovi I/I i I/D česti kod vrhunskih sportaša, trkača na duge staze, veslača i planinara, dok se genotip D/D može najčešće naći među vrhunskim francuskim biciklistima (82,83). Moran i sur. (2006.) su primijetili vezu alela I s fenotipovima povezanim, prije sa snagom, nego s izdržljivošću kod 1.027 grčkih adolescenata (84). To ukazuje na složeniju ulogu gena *ACE* u ljudskom fizičkom radu, nego što se o tome prethodno izvještavalo. Taj rezultat očigledno nije u skladu s rezultatima prethodnih pokusa. Međutim, ta se istraživanja ne mogu uspoređivati zbog visoke selekcije i relativno male populacije na kojoj su izvedena. Jasno je, dakle, da postoji skroman utjecaj gena *ACE* na fizički rad kod opće populacije. Ostaje jedini izazov uočiti mehanizam utjecaja polimorfizma gena ACE na rad u odnosu na fenotipove.

Imunitet i polimorfizam ACE

Učinci genotipa ACE na imunitet i imune poremećaje bio je također temom od interesa u prethodnom desetljeću. Temeljitije će se obraditi nekoliko istraživanja ističući važnost polimorfizma gena *ACE*.

Sepsa

Cogulu i sur. (2008.) su detaljno istražili ulogu I/D polimorfizma gena ACE u obrani od sepse kod djece (85). Primijetili su da nosioci alela I (genotipa I/I ili I/D) imaju povećani rizik u usporedbi s D/D nosiocima. Taj rezultat ukazuje na zaštitnu ulogu genotipa D/D kod sepse. Budući da se taj rezultat temelji na istraživanju s malim brojem ispitanika (N = 287) potrebno ga je ponoviti na većoj populaciji kako bi se bez ikakve sumnje mogao stvoriti zaključak.

Astma i alergijski rinitis

Pojava simptoma astme kod nekih bolesnika i alergijskog rinitisa kod drugih postavlja pretpostavku o uključenosti genetskih čimbenika. Lue i sur. (2006.) pokušali su saznati genetsku osnovu za dva različita fenotipa te su zbog toga istraživali polimorfizam gena *ACE* (86). Proveli su genotipizaciju ACE kod 106 djece s alergičnim rinitisom bez astme, 105 djece iste dobi i spola s alergičnim rinitisom i astmom te 102 zdrave djece. Za svaki je uzorak izmjerena i ukupna koncentracija imunoglobulina u serumu (IgE), IgE osjetljivost na specifične alergene i broj eozinofilnih granulocita. Češća pojava genotipa D/D kod djece koja su imala i alergijski rinitis i astmu nego kod djece s alergijskim rinitisom

to trace out the mechanism of ACE influence on performance related phenotypes.

Immunity and ACE polymorphism

Effects of ACE genotype on immunity and immune disorders have also been a topic of interest during the previous decade. We shall precisely discuss a few of them pointing out the importance of ACE polymorphism.

Sepsis

The role of ACE I/D polymorphism in defense against sepsis in children was studied in detail by Cogulu *et al.* (2008) (85). They noted that I allele carriers (I/I or I/D genotype) were at an increased risk as compared to D/D carriers. This finding speculates the protective role of D/D genotype against sepsis. As this finding is based on the experiment with a small number of subjects (N = 287), it is needed to repeat the experiment in a large population to make a doubt free conclusion.

Asthma and allergic rhinitis

Development of symptoms of asthma in some patients and of allergic rhinitis in other patients conjectures the involvement of genetic factors. Lue *et al.* (2006) tried to find out the genetic reason for the two different phenotypes and speculated the ACE polymorphism for it (86). They performed genotyping for ACE in 106 children with allergic rhinitis but no asthma, 105 age- and gender-matched children with allergic rhinitis and asthma, and 102 healthy children. Serum level of total immunoglobulin E (IgE), allergen-specific IgE sensitivity, and eosinophil count were also measured for each sample. A more frequent occurrence of D/D genotype in children with both allergic rhinitis and asthma than in children with allergic rhinitis but no asthma showed the protective role of D/D genotype in the development of asthma phenotype in children with allergic rhinitis.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) manifestation due to ACE polymorphism was confirmed by Lee *et al.* (2006) in a meta-analysis study of 13 comparison studies including 1,411 patients with SLE and 1,551 controls (87). No effect of ACE I/D polymorphism was observed on SLE either in total sample or according to ethnic groups. A trend for association of D/D genotype (OR = 1.212; 95% CI = 0.966-1.520; P = 0.097) and D allele with SLE was observed in Caucasian patients (OR = 1.157; 95% CI = 0.991-1.349; P = 0.064); however, it was not statistically significant. So, it is obvious that there is no relation of ACE polymorphism with SLE.

no bez astme ukazuje na zaštitnu ulogu genotipa D/D u razvoju fenotipa astme kod djece s alergijskim rinitisom.

Sistemski eritemski lupus

Manifestacija sistemskog eritemskog lupusa (engl. *systemic lupus erythematosus*, SLE) uslijed polimorfizma gena *ACE* potvrđena je istraživanjem Leeja i sur. (2006.) u meta-analizi u kojoj su usporedili 13 različitih istraživanja na 1.411 bolesnika oboljelih od SLE i 1.500 kontrolnih ispitanika (87). Primijećen je trend povezanosti genotipa D/D (OR = 1,212; 95% CI = 0,966-1,520; P = 0,097) i alela D sa SLE kod bolesnika bijelaca (OR = 1,157; 95% CI = 0,991-1,349; P = 0,064); međutim, rezultat nije bio statistički značajan. Očito je, dakle, da nema udruženosti između polimorfizma ACE i SLE.

Kosti i polimorfizam gena ACE

Osteoporoza, bolest kostiju, je multifaktorski problem kod muškaraca i žena u starijoj životnoj dobi. Postoji malo podataka koji razjašnjavaju ulogu I/D polimorfizma gena *ACE* u manifestaciji i liječenju osteoporoze. Postoje podaci koji ukazuju na povezanost polimorfizma ACE s osteoporozom (88). Rezultati pokazuju da mala aktivnost enzima povezana s I/I genotipom ima pozitivnu ulogu u liječenju osteoporoze. U presječnom istraživanju 3.887 Kineza (N = 1.958) i Kineskinja (N = 1.929) Lynn i sur. (2006.) su primijetili da I/I nosioci imaju bolje učinke liječenja ACE inhibitorima od I/D ili D/D nosilaca (89,90). O sličnim su učincima I/I genotipa polimorfizma ACE izvijestili Woods i sur. (2001.) tijekom hormonske nadomjesne terapije (91). Može se zaključiti da je I/D polimorfizm ACE također povezan i s koštanom patofiziologijom.

Dugovječnost i polimorfizam ACE

Povezanost različitih bolesti s polimorfizmom ACE (Tablica 1.) daje mu ulogu pokazatelja dugovječnosti. Kako bi potvrdili tu hipotezu, Schachter i sur. (1994.) su napravili genotipizaciju 338 osoba koji su navršili 100 godina i 164 kontrolnih ispitanika u dobi od 20 do 70 godina u okviru kohorte koju je potvrdio Centar za istraživanje ljudskih polimorfizama (franc. *Centre d'Etude du Polymorphisme Humain*, CEPH) u Parizu, Francuska (92). Njihovi su rezultati bili neočekivani te su ukazali na češću pojavu genotipa D/D u skupini stogodišnjaka nego u kontrolnoj skupini (40% prema 26%, P = 0,01). Isti je pokus ponovljen 2000. s 560 dodatnih francuskih stogodišnjaka, od kojih je svaki stavljen u par s mlađim ispitanikom istoga spola i zemljopisnog podrijetla. No, ti rezultati nisu pokazali razliku između stogodišnjaka i kontrolnih ispitanika (93). Slični su zaključci dobiveni rezultatima istraživanja Nacmiasa i sur. (2007.) (67).

U populacijskoj studiji Aras-Vasqueza i sur. (2003.) je kod 6.968 starijih osoba provedena genotipizacija i nije pronađena udruženost polimorfizma ACE s dugovječnošću,

Bones and ACE polymorphism

Osteoporosis, a bone disease, is a multifactorial problem reported in elderly subjects of both sexes. Scarce data are available showing the role of ACE I/D polymorphism in the manifestation of osteoporosis and its treatment. There is evidence showing an association of ACE polymorphism with osteoporosis (88). Findings suggest that low ACE activity associated with ACE I/I genotype has a beneficial role in its treatment. In a cross-sectional study of 3,887 Chinese men (N = 1958) and women (N = 1,929), Lynn *et al*. (2006) observed that I/I carriers showed better results with ACE inhibitor therapy as compared to I/D or D/D carriers (89,90). Similar advantageous effects of ACE I/I genotype have been reported by Woods *et al*. (2001) for hormone replacement therapy (91). So, ACE I/D polymorphism is also important in relation to bones.

Longevity and ACE polymorphism

The status of several diseases in association with ACE polymrophism (Table 1) predicts it as a marker of longevity. For confirmation of this hypothesis, Schachter *et al*. (1994) genotyped 338 centenarians and 164 control individuals aged 20 to 70 years, from a cohort ascertained by the Centre d'Etude du Polymorphisme Humain (CEPH) in Paris, France (92). Their findings were unexpected showing frequent occurrence of D/D genotype in the centenarian group compared with the control group (40% *vs*. 26%, P = 0.01). In 2000, the experiment was repeated with 560 additional French centenarians, each paired with a younger individual of the same sex and geographic origin. However, the results now failed to reveal a difference between the centenarian and control populations (93). A similar conclusion has been reported by Nacmias *et al*. (2007) (67).

In a population based study, Arias-Vasquez *et al*. (2003) genotyped 6,968 elderly individuals and found no relation of ACE polymorphism with longevity but with early mortality, which was common in smokers with D/D genotype, whereas frequency distribution was not significantly different in non smokers (94). So, ACE polymorphism has significant relation with mortality at early age due to cardiovascular and non-cardiovascular diseases in the presence of other physical and environmental factors.

Conclusion

Aging is due to a complex interaction of genetic, epigenetic, and environmental factors, but a strong genetic component appears to have an impact on survival to extreme ages. ACE polymorphism was thought to be responsible for longevity because of its role in the expression of many diseases. In the beginning, I/I genotype was thought to be associated with high birth weight, which is a sign of healthy future in old ages. But later on, it was found that

već s preranom smrtnošću, što je uobičajeno kod pušača s D/D genotipom, dok raspodjela učestalosti nije bila statistički značajno različita kod nepušača (94). Prema tome, polimorfizam ACE pokazuje statistički značajnu povezanost sa smrtnošću u ranoj životnoj dobi uslijed kardiovaskularnih bolesti i onih koje nisu kardiovaskularne naravi u prisutnosti ostalih fizičkih i okolišnih čimbenika.

Zaključak

Starenje je posljedica složene interakcije genetskih i epigenetskih čimbenika te čimbenika okoline, ali se čini da još jedna jaka genetska komponenta ima jak utjecaj na život do duboke starosti. Za polimorfizam gena *ACE* se smatralo da je odgovoran za dugovječnost zbog svoje uloge u razvoju mnogih bolesti. U početku se mislilo da je I/I genotip povezan s povećanom porođajnom težinom, što je bio znakom zdrave budućnosti u starijoj životnoj dobi. Međutim, kasnije je ustanovljeno da nema povezanosti između porođajne težine i genotipa, već da na rani rast nakon rođenja utječu I/I ili I/D genotip. Zaštitni učinci alela I/I u slučaju kardiovaskularnih bolesti i komplikacija uslijed šećerne bolesti nisu jednoznačno dokazani i stoga su još uvijek sporni. Te nam činjenice pokazuju da polimorfizam gena *ACE* ima malu ulogu u predskazivanju dugovječnosti.

there is no relation between birth weight of the newborn but that early growth after birth is influenced by I/I or I/D genotype. The protective effects of I/I allele against cardiovascular diseases and diabetic complications are not obvious and are still controversial. These facts show that ACE polymorphism has a low-level role in determining longevity.

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