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Relationship Between Ace Insertion/Deletion Genotypes And Response To Anti-Hypertensive Medications In T2dm Patients With Hypertension

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Abstract: The objective of the present study was to investigate, if there exists a relationship between ACE insertion/ deletion genotypes and anti-hypertensive treatment being received by type-2 diabetes mellitus (T2DM) patientswith hypertension. This is a non-interventional study where a total of 46 T2DM cases with hypertension were recruited and 45 healthy normotensive subjects served as controls. The anti-hypertensive medications being received were Angiotensin Receptor Blockers (ARBs 40mg, OD), Calcium Channel Blockers (5mg, OD), Beta-Blockers (25mg, OD) and Diuretics (12.5mg, OD) either alone or in combination. It was observed that 70% of the patients with II-genotype responded to angiotensin receptor blockers (ARBs) whereas, nearly 92% of the patients of DD-genotype responded to calcium channel blockers (50%)/ beta-blocker (42.35%) either alone or in combination. In 66% patients with ID-heterozygous genotype ARBs were found to be effective whereas the remaining cases responded to calcium channel blockers/ beta-blockers. If confirmed these results appear to be of clinical significance as prior knowledge of ACE I/D-genotype appears to be useful in prescribing appropriate anti-hypertensive treatment in T2DM cases with hypertension. The possibility of prescribing ARBsin combination with other classes of anti-hypertension may be considered especially in patients with DD-genotype (at early stages of T2DM), in view of reno-protective effects of ARBs.

Keywords: Hypertension, Type2 Diabetes Mellitus, ACE gene I/D-Genotypes, Anti-Hypertensive Treatment.

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I. INTRODUCTION

Hypertension also known as high blood pressure is reported to affect nearly 29.2% of the males and about 24.8% of females[1,2]. Important factors that contribute to predisposition to hypertension are non-specific life-style and genetic factors apart from excess salt intake and high body mass index (BMI) [3]. Hypertension is responsible for high morbidity and mortality. It is reported that hypertension is responsible for about 51% of cerebro-vascular disease and is the cause of ischemic heart disease in 45% of hypertensive subjects. People living in developing countries have about double the risk of death due to hypertension compared to those in developed countries [1,2].

A large proportion of patients suffering from T2DM also have co-existent hypertension. In a cross-sectional study carried out on incidence of hypertension in T2DM, it was reported that nearly 70.4% patients with T2DM have hypertension. It was demonstrated by logistic regression analysis that hypertension was positively associated with duration of diabetes mellitus, advancing age of patients and body mass index (BMI). Thus, hypertension is considered asa common co-morbid condition inT2DM patients [4]. There is a causal relationship between hypertension and diabetes mellitus which is estimated to be about 3 million persons suffering from the combination of the two in United States. It has been estimated that 35-75% of diabetic complications can be attributed to hypertension [5].

The relevance of selecting ACE I/D-genotypesto assess response to various anti-hypertensives can be realized from the observation that angiotensin II raises blood pressure by various actions; the most important ones are by vasoconstriction, sympathetic nervous stimulation and increase in aldesteron biosynthesis. These actions are mediated by binding of AngII to typeI receptor (AT_1) [6].

II. OBJECTIVES

- The first objective of the study was to identify ACE I/D genotypes in patients with hypertension who are suffering from T2DM.
- The second objective of the study was to record the treatment details for hypertension in the above patients; this was done in order to analyze whether a pattern emergesshowing a relationship of ACE I/D genotypes and response to anti-hypertensive treatment.
- Individuals with different ACE I/D genotypes (DD, ID, and II) differ significantly with regards to concentration of serum angiotensin II. Difference between II and DD genotype individuals is around 47% in terms of activity of angiotensin II. In case of T2DM patients because of elevated levels of oxidative stress it is assumed that Angiotensin II may induce/ over stimulate NADPH oxidase causing excessive release of reactive oxygen thereby contributing to excess oxidative stress [7]. ROS thus released is reported to cause intracellular rise in calcium, thereby contributing more to hypertension. Interaction of ROS with nitric oxide results in the formation of peroxynitrite, thus causing reduced bioavailability of muscle relaxant nitric oxide. It is for these reasons the cases of T2DM with hypertension have been selected. To our knowledge there are no earlier reports in the literature reporting response to anti-hypertensive treatment in T2DM patients with hypertension having different ACE I/D genotypes. The reports available on this aspect are mainly restricted to cases of T2DM with nephropathy. Therefore, we evaluated response to anti-hypertensives inT2DM patients with hypertension having different ACE insertion/deletion genotypes and assume hypertension to be more severe in individuals with DD-genotype.

III. METHODOLOGY

The study protocol was approved by Institutional Review Board (Ethical Committee, Deccan College of Medical sciences and Owaisi Group of Hospitals, Hyderabad). A total of 46 cases suffering from T2DMwith hypertension were recruited. The cases were consecutively attending the diabetic clinic, department of Medicine Princes Esra Hospital (Deccan College of Medical science, Hyderabad). All the cases selected were clinically confirmed as suffering from T2DM (as per the criteria of American diabetic association) and were also suffering from hypertension. A case is defined as suffering from hypertension when the systolic blood pressure (SBP) was greater than or equal to 140mmhg and a diastolic blood pressure (DBP) of greater than or equal to 90mmhg or those who were currently receiving anti-hypertensive therapy. All the patients were on anti-hypertensive therapy at the time of inclusion in the study. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) of each patient was determined twice with a gap of 5 minutes and the average of the two readings was considered as the final SBP and DBP.

For ACE I/D genotyping, 2ml of intravenous blood was drawn aseptically in Tris-EDTA vacutainers and stored at -20^0 C till further use. Genomic DNA was extracted from the stored blood samples according to rapid salting out method as described by Lahiriet.al[8]. PCR amplification was performed in a 25 micro litres mixture that contained 1 microgram of genomic DNA, 20 picomole of each primer and 12.5 micro liter of Tag DNA master mix (2X Takara, Japan). The sequences of forward and reverse primers were respectively as follows:

(F) 5-CTGGAGACCACTCCATCCTTTCT-3 and

(R) 5-GATGTGGCCATCACATTGTCAGAT-3.

PCR was performed in a T100 Thermal Cycler (BIORAD, USA) according to the method described by Seckin et.al [9]. The thermal programming was as follows: an initial denaturation step of 5 minutes at 94^0C, followed by 30 cycles of denaturation at 94^0C for 1 minute, annealing at 58^0C for 1 minute and extension at 72^0C for 2 minutes and a final extension for 15 minutes at 72^0C. PCR products were separated by electrophoresis on a 3% Agarose gel. Amplified DNA fragments were then visualized under UV light in a GelDoc-BioRad (USA). The PCR fragments corresponding to 3 genotypes were a 490bp band (II), a 190bp band (DD) and both 490 and 190bp bands (ID).

The Endocrinologists who were treating the patients were not aware of the ACE I/D genotype of the patients, and the basic aim of the study was to carry out ACE I/D genotype of these patients in order to identify responders to various anti-hypertensives in each genotype group. The patients selected were suffering from T2DM and hypertension for variable durations. A few newly diagnosed cases were also selected but predominantly the cases were chronic hypertensives generally in the range of 5-20 years. These patients were on different anti-hypertensive treatments including ACE-Inhibitors (5mg OD), Angiotensin Receptor Blockers (ARBs 40mg, OD), Calcium Channel Blockers (5mg, OD), Beta-Blockers (25mg, OD) and Diuretics (12.5mg, OD) with or without Diuretics(12.5mg, OD).

The advantage of this study is that it is likely to provide interesting information on personalized medicine based on ACE I/D genotypes, if a specific pattern of response to anti-hypertensives drugs in relation to ACE I/D genotypes is observed.

IV. RESULTS

Table-1: Baseline clinical characteristics of Type-II Diabetes Mellitus patients with Hypertension and controls

	Clinical Category	
	T2 DM + HTN	Controls
Mean Age	56.97± 10.99	42±5.20
Hypothyroidism	9 (19.56)	Nil
CAD	10 (21.73)	Nil
CKD	4 (8.69)	Nil

The mean age of the test group was 57.08± S.D years while it was 42±5.2 years in case of controls. Out of the 46 patients suffering from T2DM with hypertension, 9 were also found to be suffering from hypothyroidism, 10 were found to have history of CAD, 5 of which were of DD-genotype, 3 with ID-genotype and 2 with II-

Clinical Category	Total No. Of Patients (Percentage)	Genotype	Allelelic Frequencies

genotype. Four patients were suffering from CKD, 1

Table-2: Age distribution Analysis T2DM of the patients with hypertension

Class intervals in years	No.of cases	Percentage
35-40	5	10.90
41-50	8	17.40
51-60	17	37.00
61-70	13	26.08
71-80	2	4.35
81-90	1	2.17

Details of the age distribution analysis of the patients are given in table-2. It was observe that nearly 63% cases were found in the age range 51-70 years.

with ID-genotype with creatinine value as 1mg/dl, one with II-genotype with creatinine value as 0.8mg/dl, and 2 cases with DD-genotype; of whichone had serum creatinine levels 1.5mg/dl and the other 3.3mg/dl.

The genotypic and allelic frequencies of ACE I/D-Polymorphism in the two study groups are

shown in Table-3. In the test group the frequencies of homozygous II and heterozygous ID-genotypes were 14(30.43%) and 18 (39.13%) respectively. The number of cases with DD

genotype were 14 (30%). In contrast to this the genotype frequencies of II an ID were 40% (18/45)and 44.4% (20/45) in the control group. It was interesting to note that the frequencies of DD-homozygotes in controls was 15% which wasnearlyhalf of that observed in patients (30%) ($p<0.05$). A significant increase was observed in the frequency of D-allele in the patients 46 (50%) compared to that of control 34(44%) indicating a significant increase in the frequency of D-allele in the patients group.

Table 3: Distribution of Genotypic and Allelic Frequencies in the Two Study Groups

		II	ID	D	I	D
T2 DM + HTN	46	14 (30.43%)	18 (39.13%)	14* (30.43%)	46 (50%)	46 (50%)
Controls	45	18 (40%)	20 (44.4%)	7 (15.5%)	56 (56%)	34 (44%)

*p<0.05

Table-4: Correlation between ACE Gene I/D-Genotypes and Anti-Hypertensive Treatment

Genotype	Total Cases (N=46)	ARBs	Ca Channel blockers	Beta blockers	Diuretics
II	**14**	**10 (70%)**	**6 (42.35%)**	**5(35.71%)**	**7 (50%)**
		*ARB + D.U =4 ARB alone =2 ARB+ Ca C.B=3 ARB + B.B=1	Ca.C.B+ARB=2 Ca.C.B+ARB+D.U=1 Ca.C.B.B =2 Ca.C.B+ARB+D.U=1	B.B+ D.U=1 B.B+Ca.C.B=3 ARB+B.B=1 B.B+ Ca.C.B+D.U=1	ARB+D.U=4 B.B + D.U=1 Ca.C.B+B.B+D.U=1 Ca.C.B+ARB+D.U=1
ID	**18**	**12 (66.6%)**	**5 (27.7%)**	**3 (16.6%)**	**7 (38.8%)**
		ARB alone=5 ARB+D.U =5 ARB+Ca.C.B=1 ARB+B.B+D.U=1	ARB+Ca.C.B=1 Ca.C.B=2 Ca.C.B+B.B=1 Ca.C.B+D.U=1	B.B=2 ARB+B.B+D.U=1	ARB+DU=5 Ca.C.B+D.U=1 ARB+B.B+D.U=1
DD	**14**	**3 (14.28%)**	**7 (50%)**	**6(35.62%)**	-
		ABR=2 ACEi =1	Ca.C.B=5 Ca.C.B+B.B=2	B.B=4 B.B+Ca.C.B=2	-

*ARBs: Angiotensin Receptor Blockers, *Ca^{+} ch. Blockers: Calcium Channel Blockers.
ARB= Angiotensin receptor blockers; D.U=Diuretics; Ca.C.B=Ca channel blockers; B.B=Beta blockers

*Since combination of different hypertensives were prescribed the total number of cases shown under columns of each hypertensive drug for each genotype exceeds the no.of patients shown in column2 of table

Aperusal of table-3 reveals that out of 14 II-genotype patients 10 (70%) were receiving ARBs while 2 each were receiving a combination of ARBs plus calcium channel blockers, andARBs plus beta-blocker. In contrast to this only 2 (14.28%) cases were receiving ARBs in the group of patients with DD-genotype. While in the remaining DD group 7(50%) patients - responded to calcium channel blockers and 6(42.35%) to beta-blockers. In the group with ID-genotype out of 18, 12 (66.6%) were receiving ARBs while 5 (27.7%) and3 (16.6%) were receiving calcium channel blockers and beta-blockers respectively.

There appears to be a similarity of response to ARBs in ID and II genotype patients'presumablybecause of presence of I-allele as a common allele.

The figure-1 reveals that 70% of the patients with II-genotype responded to ARBs whereas, nearly 92% of the patients of DD-genotype responded to calcium channel blocker (50%) beta-blocker (42.35%) either alone or in combination. In 66% patients with ID-heterozygous genotype ARBs were found to be effective whereas in the remaining cases were on calcium channel blocker/ beta-blockers.

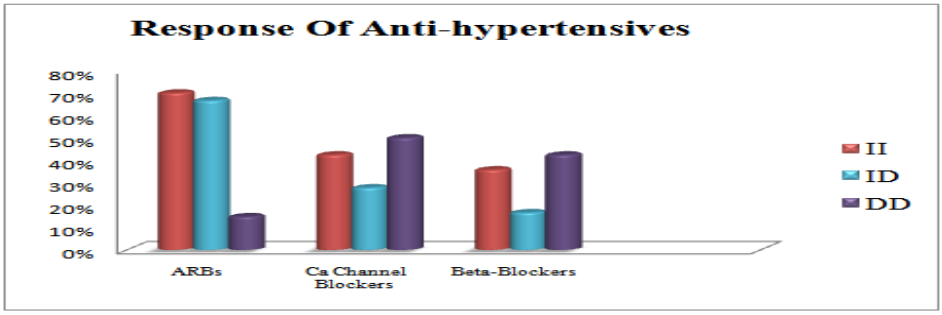

Figure: 1 Shows percentage of cases with different ACE I/D genotypes responding to various anti-hypertensive medications.

V. DISCUSSION

Recent developments in the field of pharmacogenomics have led to the evolution of personalized medicine, where clinicians make the best therapeutic choice according to the patient's genotype thus optimizing therapy and improving outcomes. Variations are observed in response of individuals to drug treatment due to differences in DNA sequence that alter expression or function of the proteins targeted by the drugs. This may be considered as bases for evaluating drug response in relation to genotype variation of genes. An example of this is low NO (Nitric oxide) producer genotype of endothelial nitric oxide synthase (e-NOS) which may result inhypertension[10]. As mentioned in the introduction, the relevance of selecting ACE I/D-genotypes to assess response to anti-hypertensives is based on the significant variation in serum Angiotensin-II concentration in DD and II individuals.It is well-known that angiotensin-II is not only a vasoconstrictor but also a potent inducer of NADPH- oxidase an enzyme involved in the generation of free radicals of oxygen (ROS) [7]. It is known that ROS interacts with nitric oxide (NO) and converts a proportion of it into peroxy-nitrite thereby reducing the bioavailability of nitric oxide to a certain degree. This reduces vasodilating effect of nitric oxide. Moreover, intra-cellular ROS signaling results in intracellular rise in calcium ions (Ca^{+2}) contributing to additional vasoconstriction and resulting in rise in blood pressure .ROS increases intracellular free calcium ion concentration , a major determinant of vascular reactivity [11-14]. It is presumed that the basal rates of ROS production are high in DD genotype patients. Therefore, II-genotype patients responded well to ARBs and patients with DD-genotype responded well to calcium channel blockers/ and beta-blockers.

In experimental studies carried out in ratsCrowley, et.al demonstrated that angiotensin-II causes rise in hypertension by binding angiotensin-I receptor (AT-1). The stimulation of AT-1 receptor causes potent vasoconstriction. In the adrenal cortex, the activation stimulated the release of aldosterone that in turn promotes sodium reabsorption in the minor corticoid responsive segment of the distal nephron. The ARBs are Reno-protective because dilation of efferent arteriolesreduce intra glomerular hypertension and also reduces protenuria in both essential hypertension and type-II diabetes mellitus with hypertension [15].

Several studies carried out earlier concluded that the DD-genotype and the ‘D’ allele predispose not only to essential hypertension but also to hypertension in cases of type-II diabetes mellitus [16]. Association studies carried out in populations in other countries like Taiwan[17], Turkey [18], Malaysia [19] and Chinese[20]also confirmed these observations. One of these studies observed that the frequency for II,ID and DD-genotype of the ACE gene to be 30.77%, 53.85%, and 15.38% in T2DM+HTN; in the controls it was 57.14%, 40.00% and 2.50% respectively. The frequency for ‘D’ allele 42.31% in T2DM+HTN compared to 22.86% in controls[19].

It is concluded that the ARBs might have proved more effective in 70% cases of II genotype because of significantly reduced serum angiotensin-II concentration in these patients, which might have resulted in effective blocking of angiotensin-II mediated AT_1-type 1 receptor stimulation. As the prescription of ARBs initially in DD- individuals might have proved less effective in more than 90% of the cases due to significantly increased level of angiotensin-II and so calcium channel blockers/beta-blockers might have been prescribed. Another important outcome of the present study is that in patients with DD-genotype if the calcium channel blocker / beta blocker are used in combination with adequate dose of ARBs (in initial stage of disease) it may prove to have beneficial effects in terms of reno-protection and reduction in proteinurea by lowering glomerular filtration rate. However for such a combination therapy prior knowledge of patients ACE I/D genotype is required.

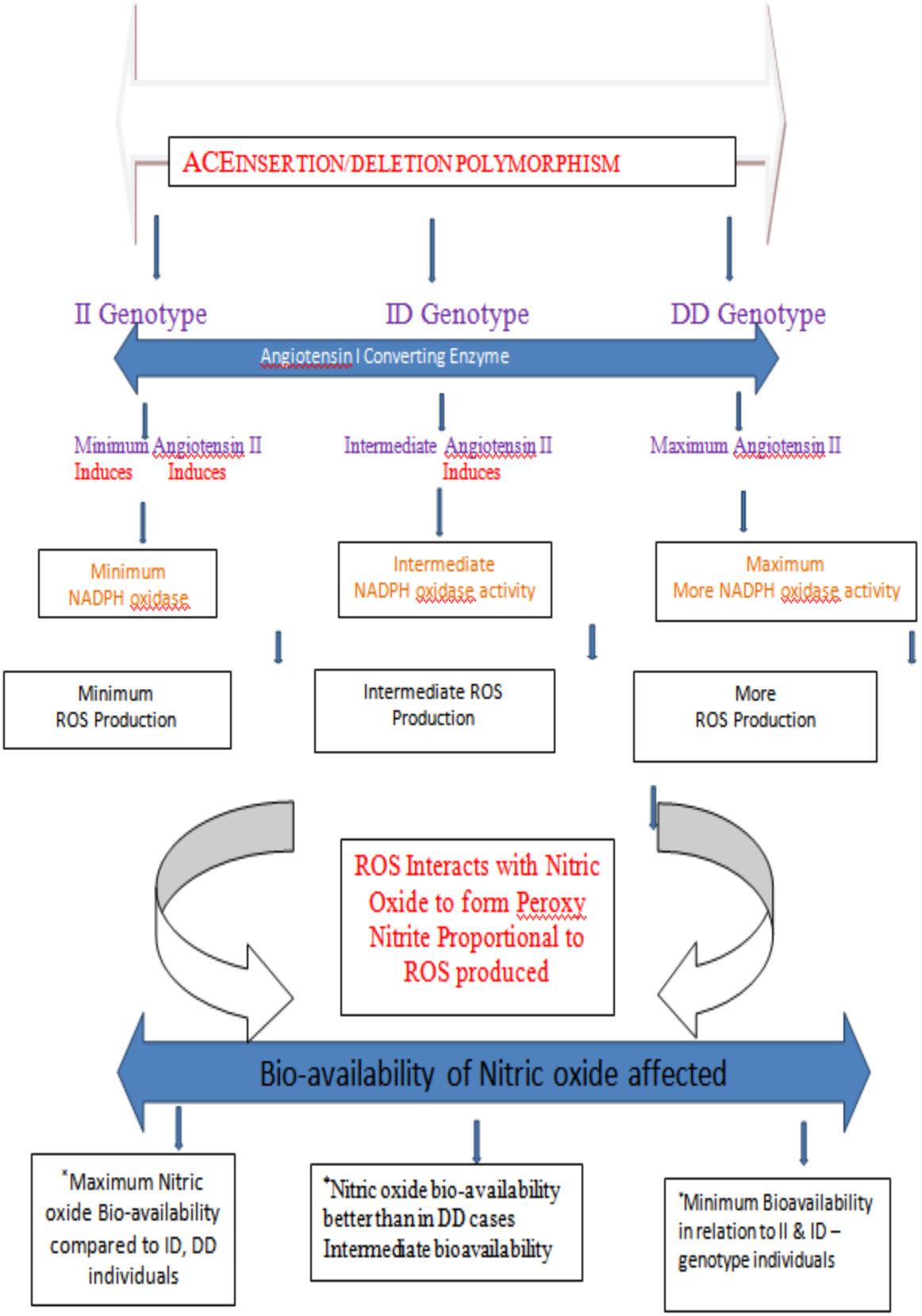
ACE INSERTION/DELETION POLYMORPHISM
II Genotype
ID Genotype
DD Genotype
Angiotensin I Converting Enzyme
Minimum Angiotensin II Induces
Intermediate Angiotensin II Induces
Maximum Angiotensin II
Minimum NADPH oxidase
Intermediate NADPH oxidase activity
Maximum More NADPH oxidase activity
Minimum ROS Production
Intermediate ROS Production
More ROS Production
ROS Interacts with Nitric Oxide to form Peroxy Nitrite Proportional to ROS produced
Bio-availability of Nitric oxide affected
*Maximum Nitric oxide Bio-availability compared to ID, DD individuals
*Nitric oxide bio-availability better than in DD cases Intermediate bioavailability
*Minimum Bioavailability in relation to II & ID – genotype individuals

Figure 2: Depicts inter-individual variations with regards to susceptibility to severity of hypertension based on ACE I/D genotypes. The DD individuals are at a higher risk of developing hypertension based on highest serum concentration of Ang.II compared to II-genotype individuals. High ROS production and reduced bio-availability of serum Nitric oxide are additional predisposing factors. Intermediate risk is likely in individuals with ID genotype and minimal risk may exist for II genotype persons.

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Conflicts of Interest: None

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