SA gene polymorphism in Saudi Hypertensive Patients

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Introduction

- During the early 1990's, studies in rats showed that a newly identified gene known as the SA-gene shows markedly higher levels of expression in the kidneys of spontaneously hypertensive rats (SHR) than in their non-hypertensive reference strain, the Wistar-Kyoto (WKY) rat. (Lindpaintner et al 1993; Iwai & Inagami 1992; Samani et al 1992).
- Based on the important role of the kidney in blood pressure regulation, the possibility was raised that this gene or an area linked to it, the translational product of which remains unknown, may participate in the pathogenesis of primary hypertension (Samani& Lodwick 1995; Frantz et al 1998; Lodwick et al 1998; Ishinaga et al 1997) .
- Several studies in rats showed the presence of polymorphic sites of various restriction endonuclease (e.g.pst 1), closely linked to the development of hypertension in rat models and it was proposed that either the SA gene, or a gene closely linked to it, has a capacity to influence the blood pressure values. Further studies showed that in rats the SA gene locus co-segregates with elevation in BP, accounting for up to 25% of the genetic variation in systolic and diastolic BP(Samani et al 1993; Harris et al 1993).
- These studies were extended to the human populations and several contradictory results were obtained.

- During studies in some populations including the Japanese and Polish populations, significant differences in the frequency of the A2 allele between the hypertensive and control groups (P = .0001) was reported (Iwai et al 1994a,b; Iwai et al 2002; Nalogowska-Glosnicka 2002; Narita et al 2002). However, in other populations including French, British and Han Chinese populations such association were not identified. (Nabika et al 1995; Harrap et al 1995; Chen et al 2001).
- These contradictory reports in the human populations aroused our interest to investigate the SA gene polymorphism in Saudi population, where hypertension, a multi-factorial disorder, occurs at a high prevalence and shows a significant positive correlation with age, body weight and body mass index in both males and females.
- We believe that if markers of hypertension could be identified, then pre-symptomatic identification of genetically susceptible individuals, followed by appropriate interventions and changes in life style could be an important step towards control and prevention of hypertension. This could also be a significant step towards prevention of several complications, frequently associated with the hypertensive state.
- This paper presents results of our studies on SA gene polymorphism.

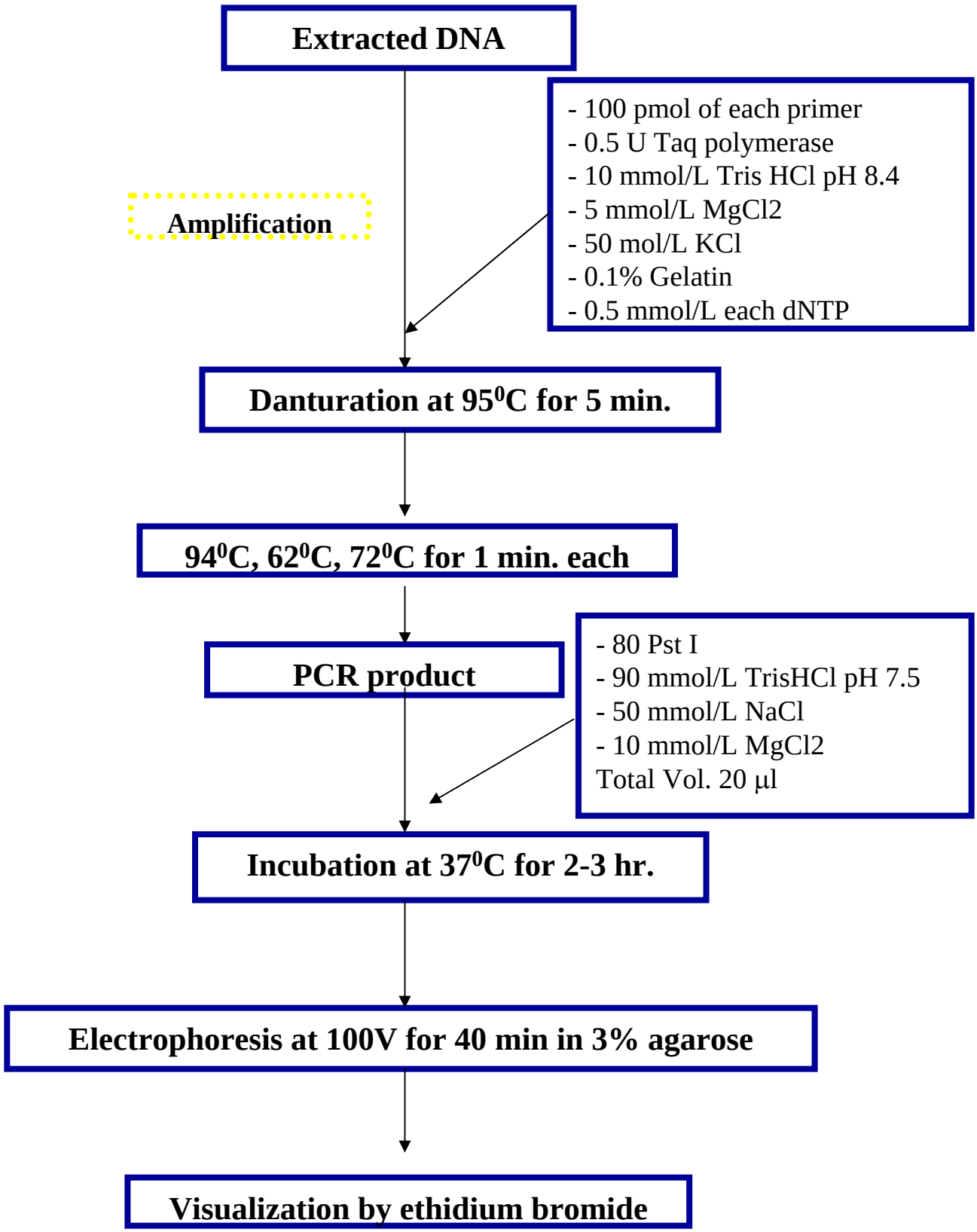
Objectives

To study SA gene polymorphism in Saudi hypertensive patients

Methods and Materials

- **Subjects males and females:**
  - **Hypertensive: 157**
  - **Controls: 369**
- **Measurements:**
  - **Height, Weight, BMI**
  - **Systolic and diastolic BP**
- **Fasting Blood collection for:**
  - **Estimation of biochemical parameter:**
    - **Creatinine and urea**
    - **Electrolytes**
    - **Uric Acid**
  - **DNA extraction from buffy coat:**
    - **SA gene polymorphism studies (Figure 1)**
      - **PCR**
      - **Pst I digestion of PCR product**

Figure 1: Outline of steps involved in study of Pst polymorphic site in S_A gene



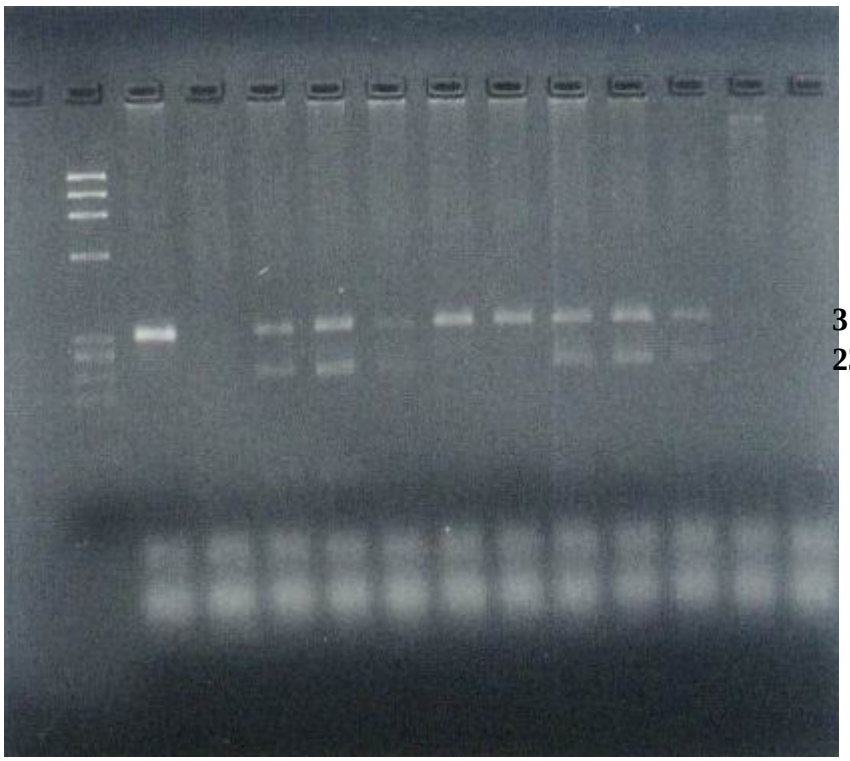
Results

- The anthropometric data of the individuals investigated during this study is presented as Table 1.
- An electrophoretogram showing the SA genotypes A1A1, A1A2, and A2A2 is presented as Figure 2. After restriction with PstI, the A1 allele gave a fragment 315 bp, while the A2 allele gave a 230 bp fragment. Those heterozygous had both 315 and 230 bp fragments.
- Figure 3 presents the frequency of A1A1, A1A2 and A2A2 genotypes in the hypertensive patients and the control group. The difference in the genotype frequencies was statistically significant [$\chi 2 = 5.260$; $p = 0.0218$].
- The frequency of A1 and A2 alleles were calculated (Figure 4), and the results in the patients and control group did not differ significantly [$\chi 2 = 1.864$; $p = 0.1722$].
- The results of renal function tests, electrolytes and uric acid were separately analyzed in individuals with different genotypes, and the results are presented as Table 2.

Table 1: Anthropometric data of the controls and hypertensive patients

		Hypertensive Mean ±SD	Normal Mean ±SD	*p*
Age (yrs)	F	51.13 ± 14.95	26.69 ±12.72	<0.05
	M	52.40 ±17.31	28.26 ±11.26	<0.05
Height (cm)	F	153.98 ± 4.68	155.21 ± 8.18	NS
	M	159.18 ± 11.7	165.86 ± 7.51	NS
Weight (Kg)	F	76.55 ±18.17	52.46 ± 12.00	<0.05
	M	75.15 ±17.79	66.33 ± 52.84	<0.05
Systolic (mmHg)	F	143.84 ± 25.0	114.45 ±14.20	<0.05
	M	146.42 ±20.42	115.86 ±9.37	<0.05
Diastolic (mmHg)	F	95.07 ± 14.97	74.45 ±8.10	<0.05
	M	93.19 ±18.89	75.63 ±7.33	<0.05
BMI (Kg/m2)	F	32.07 ±7.75	21.95 ± 6.49	<0.05
	M	30.32 ± 7.29	21.39 ±2.97	<0.05

Figure 2 Agarose gel electrophoretic pattern of S_A gene A1 and A2 alleles



315 bp= A1
230 bp= A2

Figure3: SA gene Genotype frequency in Hypertensive patients and Controls

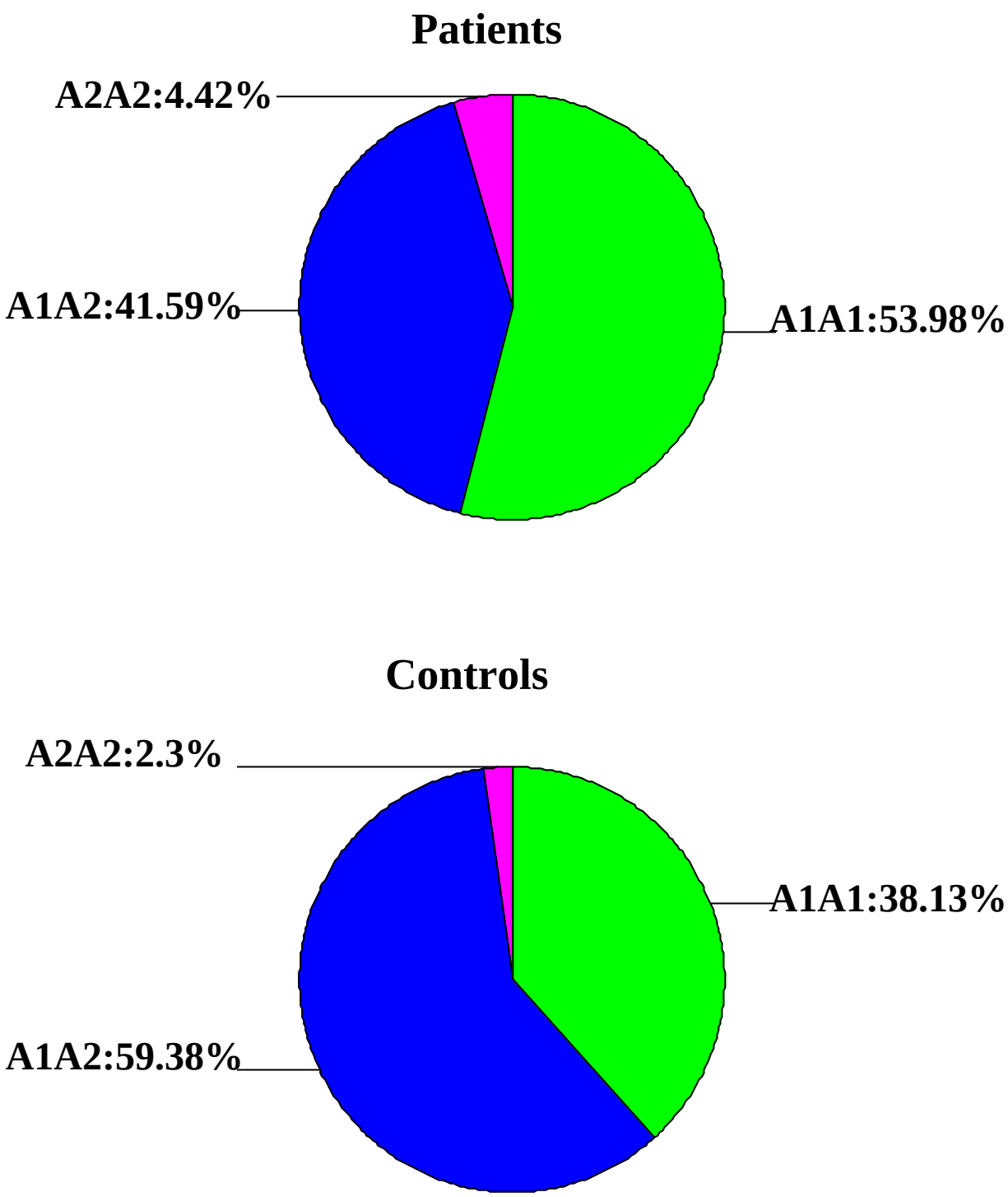


Figure4: SA gene Allele Frequency in Hypertensive Patients and Controls

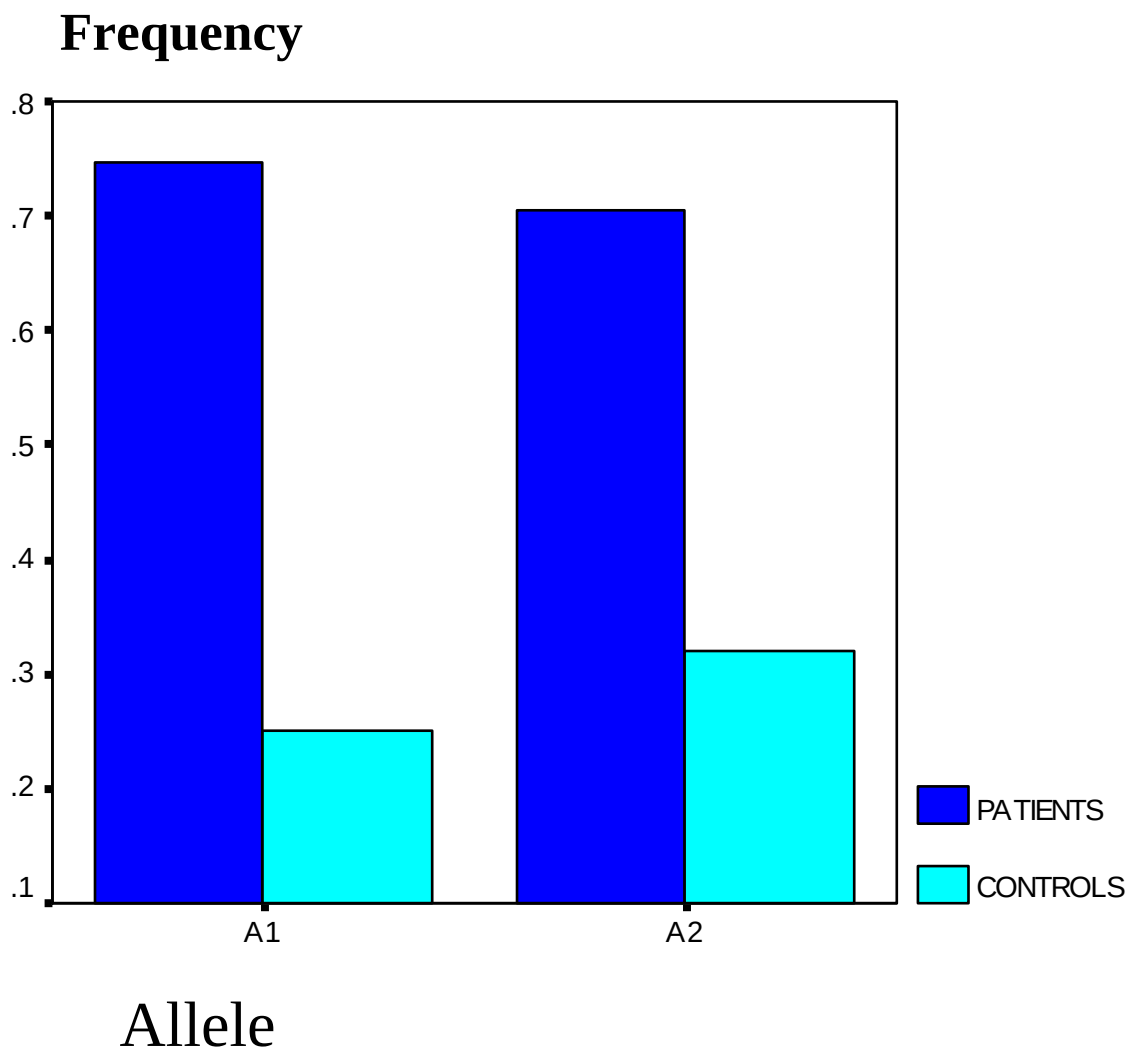


Table 2: Biochemical parameters in the different SA gene genotypes

	SA genotype	Mean±SD	*p*
Urea (mmol/l)	**A1A1**	**7.12 ± 4.23**	<0.05
	A1A2	**6.02 ±3.05**	
Creatinine (µmol/l)	**A1A1**	**155.15 ±202.18**	<0.05
	A1A2	**150.19 ± 276.18**	
Na^+ (mmol/l)	**A1A1**	**136.05 ± 20.36**	NS
	A1A2	**138.04 ± 6.46**	
K^+ (mmol/l)	**A1A1**	**5.25 ±6.53**	<0.05
	A1A2	**4.34 ±.72**	
Cl^- (mmol/l)	**A1A1**	**103.95 ±4.90**	NS
	A1A2	**105.25 ± 3.60**	
Uric Acid (µmol/l)	**A1A1**	**290.43 ± 103.38**	<0.05
	A1A2	**238.40 ±61.96**	

Discussion

- It is well established that essential hypertension is not a distinct, single gene disorder, but that it is polygenic with environmental factors contributing to its development, i.e. it is a complex multifactorial disorder (Kurtz and Spence, 1993).
- It is estimated that approximately 20% - 60% of population variability in blood pressure is genetically determined (Williams et al, 1988, 1991; Kurtz and Spence, 1993). This has been confirmed based on the results of twin studies, adoption studies and statistical analysis of blood pressure.
- Several genes have been implicated in the pathogenesis of hypertension.
- The S_A gene is one such genes and it has been implicated in rat genetic models and in Japanese hypertensive patients (Zee et al, 1997; Iwai et al, 1992, 1994; Nabika et al, 1995) while in certain white populations e.g. the British and French, no such association has been found and it has been suggested that such studies should be carried out on other racial groups.
- The human S_A gene is located on chromosome 16 (Samani et al, 1994), in the vicinity of the renal epithelial sodium channel ß-subunit gene, and displays several restriction fragment length polymorphism (RFLPs).

- Interestingly it is differentially expressed in the kidney of the spontaneously hypertensive rat and cosegregates with an increase in blood pressure in F2 rats derived from a cross of the spontaneously hypertensive rat with normotensive Wistar-Kyoto rats, accounting for 28 and 21% of the genetic variability in systolic and diastolic blood pressures, respectively. This has led to several interesting studies in human populations.
- These findings raise the exciting possibilities that: (i) SA represents a major component of an important novel system regulating blood pressure, and (ii) it underlies a primary renal mechanism predisposing to hypertension (Samani NJ, Lodwick D. 1995). The SA gene is expressed in the kidneys and is associated with hypertension in man and experimental animal models. Predisposition to hypertension is associated with renal haemodynamic abnormalities and increased renal SA gene expression (Harrap et al 1995)
- Our study in Saudis shows that there is a genotype difference in the healthy non-hypertensive individuals compared to the hypertensive ones. No differences are seen in the genotype frequencies in the Saudi males and females. Additionally when the allele frequencies of the normotensive and hypertensive individuals are compared the difference is not found to be statistically significant.

- These results suggest that despite significant differences in the genotype frequency between the hypertensive and the control group, since all three genotypes occur at a high though frequency even in the normotensive individuals, it will not be possible to include it as a marker for the early detection of high blood pressure in Saudi population.
- Interesting slight differences are observed in biochemical parameter values in individuals carrying different SA gene genotypes, where creatine and urea occur at a higher level in the A1A1 genotype and this may play a role in renal pathophysiology as suggested in earlier studies. Other parameters do not differ significantly.
- One major problem with the study of markers of multifactorial disorders in normal individuals, is the fact that these individuals may develop the disorder later on in their life.
- Hence more extensive studies are necessary to identify more specific markers of hypertension in human populations.