UNIVERSITY OF SOUTHAMPTON

Genetic Studies of the Renin Angiotensin Pathway Genes and Quantitative Studies of Transcribed Haplotypes of the Angiotensin II Type I Receptor Gene

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ABSTRACT

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Genetic Studies of the Renin Angiotensin Pathway Genes and Quantitative Studies of Transcribed Haplotypes of the Angiotensin II Type I Receptor Gene

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The Renin Angiotensin pathway has very important physiological roles. The most important member of this pathway is angiotensin II. It has many functions in the cardiovascular system and in fluid and electrolyte balance. There is extensive evidence of the association of polymorphisms of this pathway, e.g. *ACE* I/D, with cardiovascular and metabolic traits. Most of the angiotensin II effects are mediated by the *AGTR1* which is expressed in blood vessel walls, heart and many other organs. The *AGTR1* A1166C has been reported to associate with hypertension and myocardial infarction.

We have studied the association of the *AGTR1* A1166C with cardiovascular and metabolic traits in the north and east Hertfordshire UK populations. The results represent that CC genotype is a strong determinant of anthropometric measures in men and also shows associations with traits of insulin resistance. Another SNP (L191L) in exon 5 and two more (C-521T and A-153G) in the 5′ UTR have also been typed to resolve the structure of haplotype blocks in the *AGTR1*. The possible association of these SNPs with the above phenotypes was also studied. L191L, to a lesser extent, was also significantly associated with some of the above phenotypes. C-521T and A-153G has much less strong association. However, there were some associations in haplotype phenotype study. It was found that there are two main haplotype blocks separated by a gap in the *AGTR1* gene.

There are also association between *GH* polymorphisms (A5157G, C5187A and microsatellite) and metabolic traits. Therefore a study was undertaken of linkage disequilibrium (LD) between these polymorphisms and those of the *ACE* gene (A-5466C, C1237T and I/D). This study showed that there are relatively significant levels of LD between the above polymorphisms. Detailed haplotype analysis has been undertaken in the *ACE* gene, and haplotype frequencies accord well with published literature. The *AGT* M235T was also typed and studied in the NH and EH populations, but no significant association was observed.

To examine the functional effects of the *AGTR1* A1166C and L191L polymorphisms, a ratiometic analysis was developed to compare the ratio of band intensities following restriction digestion of cDNA derived from L191L and A1166C heterozygotes. Additional to these, within-individual-between-allele assays and between-individual comparisons were made using TaqMan assays applied to both homozygous and heterozygous genotype and haplotype. No significant effect appeared from alleles of the L191L, but the C allele of the A1166C and haplotypes carrying it downregulated the *AGTR1* mRNA.

These findings underscore the importance of the RAS polymorphisms in terms of metabolic and cardiovascular traits, and provide an explanation for the mechanism of the *AGTR1* effects.

Table of Contents

Table of figures

Table of tables

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I would like to dedicate this to my father who passed away last year,
while I was too busy with my project to be at his bedside on his last days of life,
May the Almighty God bless him

Abbreviations

AA or aa	= Amino Acid
ACE	= Angiotensin Converting Enzyme
AD	= Alzheimer's Disease
AGA	= Appropriate for Gestational Age
AGT	= Angiotensinogen
AGT I, II…	=Angiotensin I, II,…..
AGTR 1 (AT1R)	= Angiotensin II Type 1 Receptor
AGTRa	= Angiotensin II Type 1 Receptor Subtype a
AGTRb	= Angiotensin II Type 1 Receptor Subtype b
AGTR 2 (AT2R)	= Angiotensin II Type 2 Receptor
App.	= Appendix
ARMS	= Amplification Refractory Mutation System
bp	= Base Pair
BMD	= Bone Mineral Density
BMI	= Body Mass Index
CAD	= Coronary Artery Disease
cDNA	= Complementary DNA
CHD	= Coronary Heart Disease
DN	= Diabetic Nephropathy
DNA	= Deoxyribonucleic Acid
DOP	= Degenerate Oligo Primer
DR	= Diabetic Retinopathy
EDTA	= Ethylene Diamine Tetraacetic Acid
EH	= Essential Hypertension or East Hertfordshire
Exp.	= Experiment
GFR	= Glomerular Filtration Rate
GH	= Growth Hormone
HW eq.	= Hardy-Weinberg Equilibrium
IDDM	= Insulin Dependent Diabetes Mellitus

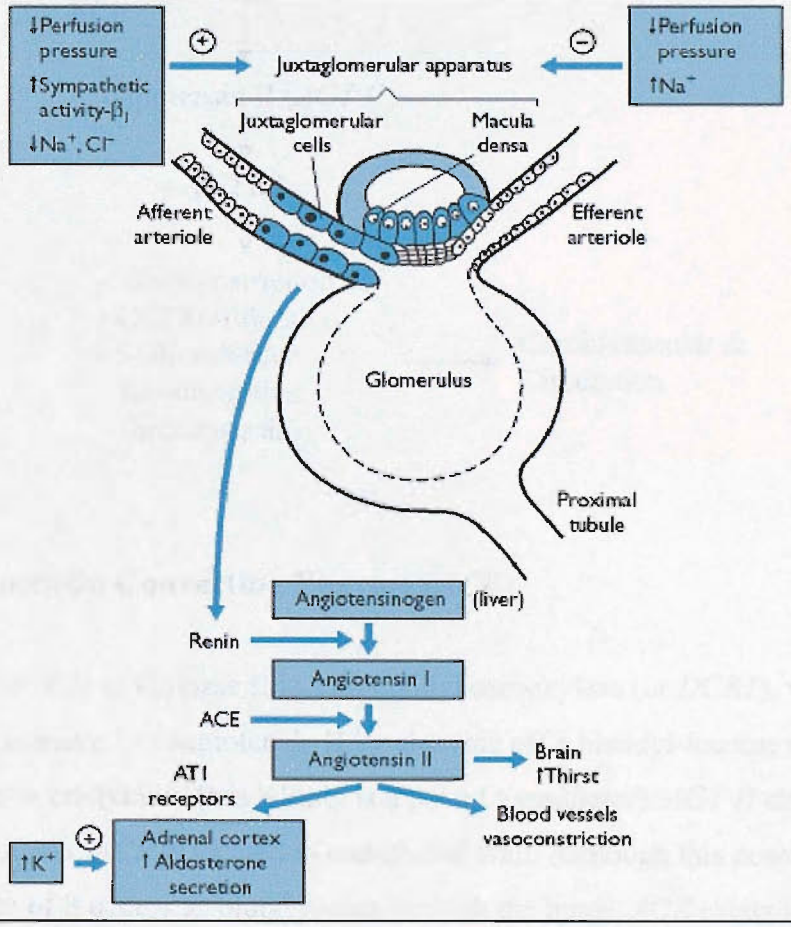
IGF1or 2	= Insulin Like Growth Factor 1 or 2
IGF2R	= Insulin Like Growth Factor 2 Receptor
IRE	= Iron-responsive Element
IRP	= Iron Regulatory Protein
Kb	= Kilo Base Pair
kDa	= Kilo Dalton
LVH	= Left Ventricular Hypertrophy
LD	= Linkage Disequilibrium
MADGE	= Microtiter Array Diagonal Gel Electrophoresis
Mb	= Megabase
mRNA	= Messenger Ribonucleic Acid
NH	= North Hertfordshire
NIDDM	= Non Insulin Dependent Diabetes Mellitus
NO	= Nitric Oxide
OGTT	= Oral Glucose Tolerance Test
PCR	= Polymerase Chain Reaction
RAS	= Renin Angiotensin System
RNA	= Ribonucleic Acid
RNAbp	= RNA Binding Protein
SBP	= Systolic Blood Pressure
Sec	= Second
SGA	= Small for Gestational Age
SNP	= Single Nucleotide Polymorphism
TBE	= Tris-Boric Acid EDTA
UTR	= Untranslated Region

1. Introduction

1.1 Physiological Importance of the Renin Angiotensin System

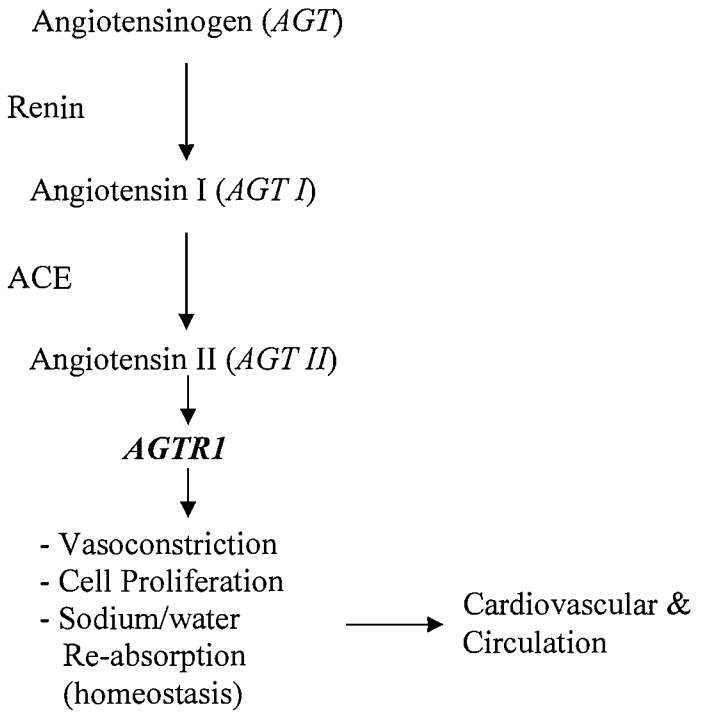
Renin is secreted by highly specialized cells (macula densa) in the juxtaglomerular apparatus [5]. It has a half life of about 80 minutes in circulation and its major role is conversion of angiotensinogen (*AGT*, renin substrate) to angiotensin I (*AGT I*) (Figures 1.1 &1.2).

Figure 1.1: Physiology and Mechanism of the RAS



Juxtaglomerula cells are modified smooth muscle cells which secrete renin. The macula densa are tubular cells of the thick ascending limb of the loop of Henle which can detect circulating concentrations of sodium. In response to the indicated stimuli renin is secreted and converts angiotensinogen (synthesized in the liver) to angiotensin I. Angiotensin-converting enzyme (*ACE*) converts antiotensin I →II which acts on *AGTR1* receptors in the adrenal cortex (glomerulosa), blood vessels and brain (via circumventricular organs). In addition to angiotensin II high circulating concentrations of K^+ also stimulate aldosterone secretion. Adopted from Endocrinology, an integrated approach; S. S. Nussey and S. A. Whitehead, Oxford. UK: BIOS Scientific Publishers. Ltd: 2001.

Figure 1.2: Physiologic Pathway of the RAS



1.1.1 Angiotensin Converting Enzyme (*ACE*)

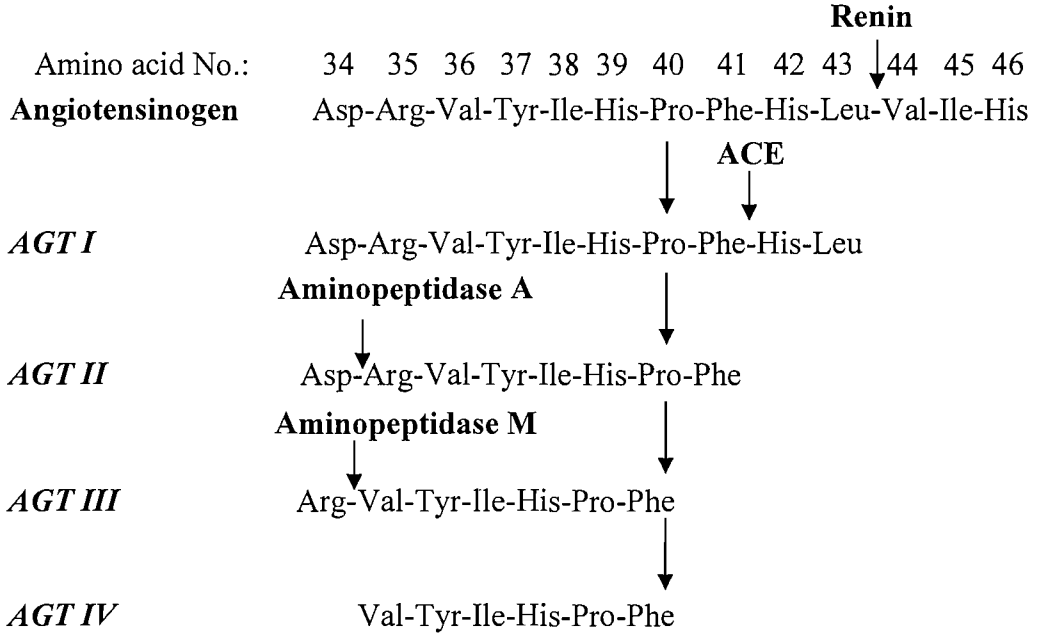
ACE (or *ACE1*) or kininase II is a dipeptidyl carboxylase (or *DCR1*), which converts inactive angiotensin I to angiotensin II by cleaving off a histidyl-leucine dipeptide (Figure 1.3), and inactivates bradykinin (bradykinin is a potent vasodilator). *AGT II* also decrease the production of NO. *ACE* is located in endothelial wall. Although this conversion occurs in many organs, much of it occurs as blood passes through the lungs. *ACE* exists in two forms, a somatic form found throughout the body and a germinal form found in post-meiotic spermatogenic cells and spermatozoa. Both are encoded by the same gene but with two different promoters producing two different mRNAs. *ACE2* has recently been mapped to X chromosome in mice. Its disruption leads to increased *AGT II* level and impaired cardiac contractility. However, the relationship between *ACE* and *ACE2* is not entirely clear yet [39].

1.1.2 Angiotensin II (*AGT II*)

AGT II has a half-life of about 1-2 minutes in circulation. It is metabolised by an aminopeptidase resulting in *AGT III*, which is physiologically active too [146].

As explained in Figure 1.3, *AGT II* would be converted to *AGT III* and *AGT IV*. Although the role of last two is not known clearly yet; however, *AGT IV* has been identified in different tissues, such as: brain, heart kidney, cultured coronary microvascular, aortic and lung endothelial cells. It increases blood flow in brain and kidney and activates the lung endothelial cell isoform of nitric oxide (NO) synthase (eNOS), leading to increases in NO release, production of guanosine 3, 5 cyclic monophosphate (cGMP), and NO-cGMP mediated pulmonary artery vasodilation [8].

Figure 1.3: Biochemical Steps of the Angiotensins Synthesis



Shown are the biochemical processes of the synthesis of the RAS elements. Adopted from LADENS Bioscience (www.eurekah.com).

1.1.3 Functions of Angiotensins

AGT I: The known role of *AGT I* is acting as precursor of *AGT II* and it has no other known function.

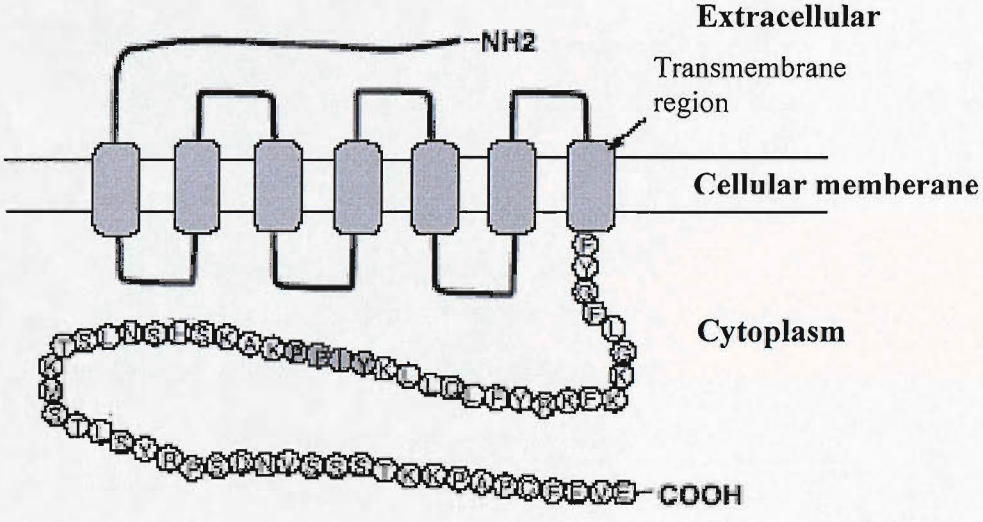
AGT II: *AGT II* causes arteriolar constriction and an increase in systolic and diastolic blood pressure (Figures 1.1&1.2). Its pressor activity is 4-8 times stronger than that of norepinephrine (NE). It was previously called hypertensin or angiotonin [146]. It acts on adrenal cortex and increases the secretion of aldosterone, which increases reabsorption of sodium in the distal nephron. It also has other roles:

1) Facilitation of the release of NE by a direct effect on post-ganglionic sympathetic neurons.
2) Contraction of mesangial cells with a final decrease in GFR.
3) Action on brain to increase blood pressure, water intake and the secretion of vasopressin and ACTH (Figure 1.1).
4) Locally released *AGT II* acts within the vascular walls as a paracrine peptide to promote both the proliferation and the contraction of vascular smooth muscle cells.
5) It also has a proliferation effect on fibroblasts, which contributes to fibrosis evident in cardiomyopathies and cardiac hypertrophy. The hypertrophic effect of *AGT II* may somehow contribute to remodelling, which occurs in surviving ventricular muscle following myocardial infarction [50].
6) *AGT II* decreases the excretion of both salt and water, which is performed through intrarenal vascular constriction. This effect functions over a period of hours and days (long term effect) [16].
7) It has direct effects on coagulation & fibrinolytic pathways.
8) It also has a positive inotropic effect on atrial & ventricular muscle, which is mediated by *AGTR1* [50].

1.1.4 Angiotensin II Receptors

At least two classes of angiotensin receptors have been known: angiotensin II type I receptor and type II (*AGTR1* & 2). *AGTR1* receptors are GTP-binding protein-linked integral membrane proteins with seven transmembrane-spanning domains that are characterised by binding the diphenylimidazole derivative losartan. It shares the 7-transmembrane region motif with the G protein-coupled receptor super family (Figure 1.4). *AGTR1* has two subtypes (a & b) in mouse and one in human. Angiotensin II exerts its physiological effect on binding to the *AGTR1* by increasing the cytosolic free Ca2+ level [50]. Activation of the *AGTR1* results in cell growth, vascular contraction, inflammation responses and salt and water retention [140].

Figure 1.4: Structure of *AGTR1* Receptor



Schematic representation of the *AGTR1* receptr. Adopted from Inada Y et al. (2003) [75].

*AGTR1*a, which mediates most of the known effects of angiotensin II, is located in blood vessel walls, brain and many other organs; while *AGTR1*b is located in anterior pituitary and the adrenal cortex [50].

Both *AGTR1 & AGTR2* have the same affinity to *AGT II. AGTR2*s are serpentine receptors with seven transmembrane domains too, but they do not appear to act via G protein and their second messengers remain unknown. The activation of the *AGTR2* leads to apoptosis, vasodilation and natriuresis [140]. They are mainly found in fetal and neonatal life but they remain in the brain and other organs during adulthood.. It has been shown that *AGTR2*, through counteracting *AGTR1*, has antiproliferative and proapoptotic effects in vascular smooth muscle cells (VSMC) of mouse in the process of neointimal formation after vascular injury [1].

[1] Suzuki J, Iwai M, Nakagami H, Wu L, Chen R, Sugaya T, Hamada M, Hiwada K, Horiuchi M. Role of angiotensin II-regulated apoptosis through distinct AT1 and AT2 receptors in neointimal formation. Circulation. 2002 Aug 13;106(7):847-53.

1.2 An Overview of the Genetics of the Renin Angiotensin System

1.2.1 Angiotensinogen (*AGT*)

Angiotensin I (*AGT I*) is derived from angiotensinogen during a proteolytic cleavage by renin; angiotensinogen (*AGT*) is synthesised in liver. *AGT* can be found in the alpha globulin fraction of plasma [3]. Its molecular weight is about 50 kDa.

Kageyama R et al. (1984) reported the complete sequence of the human angiotensinogen mRNA, and Gaillard I et al. (1989) [58] showed that the gene contains 5 exons with similarities with alpha 1-antitrypsin gene. The angiotensinogen gene was mapped to 1q42-q43 by Isa M N et al. (1989) [76] using nonisotopic *in situ* hybridisation.

Tanimoto K et al. (1994) [135] made angiotensinogen-deficient mice, which did not express *AGT* in liver and displayed no angiotensin I in plasma. These mutant mice had blood pressures of 66.9 ± 4.1 in comparison with 100.4 ± 4.4 in wild type mice.

The expression of the angiotensinogen in adipose tissue along with other elements of the RAS was reported by Karlsson C et al. (1998) [85]. The presence of the local (cardiac) RAS was demonstrated in an in vitro model [128]. It was shown that mechanical stress causes release of angiotensin II from cardiac myocytes, which would function as an initial mediator of the hypertrophic reaction.

Jeunemaitre X et al. (1992) [82] in a study on American and French hypertensive sibpairs showed the linkage between hypertension and *AGT*. The association was strongly significant with two variants of the *AGT* gene: T174M and M235T which are in complete LD.

Hata A et al. (1994) [68] found the association of the T235 variant with essential hypertension in Japanese population. They also found that T235 frequency was higher in Japanese than Caucasian individuals.

The association of the *AGT* M235T with coronary heart disease was reported by Katsuya T et al. (1995) [86], in which T235 was an independent risk factor with two fold susceptibility to the disease. In another study [144], the association of the *AGT* T235 with pregnancy induced hypertension in Caucasian women was reported.

Although there is evidence to show the linkage of the *AGT* locus and association of the *AGT* M235T with hypertension [68,82,86], there are also reports showing no association of the *AGT* M235T with hypertension [29,30].

1.2.2 Angiotensin Converting Enzyme (*ACE*)

The *ACE* (See Figures 1.1, 1.2, 1.3) gene encodes two isoenzymes: the somatic *ACE* isoenzyme, which is expressed in many tissues such as vascular endothelial cells, renal epithelial cells and testicular Leydig cells; and the testicular or germinal *ACE* isoenzyme, which is only expressed in sperm. *ACE* not only circulates in plasma but also is present as a membrane-bound enzyme on the surface of vascular endothelial cells. The plasma enzyme may be synthesized in vascular endothelium [2].

Mattei M G et al. (1989) [99] using *in situ* hybridisation assigned the *ACE* gene to 17q23 and Jeunemaitre X et al. (1992) [81] mapped *ACE* to 17q22-q24 which was consistent with 17q23.

Plasma *ACE* level shows an inter-individual variation. Based on a study, which was undertaken by Cambien F et al. (1988) [26], plasma *ACE* level was uncorrelated with age, height, weight or blood pressure in parents but a negative correlation with age was observed in offspring. It was shown that 50% of inter-individual variability of plasma *ACE* concentration is determined by a major gene effect.

After cloning of the *ACE* gene, it was suggested that 50% of interindividual variability of plasma *ACE* is determined by an insertion deletion (I/D) polymorphism representing presence or absence of a 287bp [109]Alu element situated in intron 16 of the *ACE* gene [28].

In 1990, Rigat B et al. (1990) [123] studied 80 healthy Caucasians in 10 nuclear families. They detected the I/D polymorphism (250bp) in the *ACE* gene using an endothelial *ACE* cDNA probe. In this study, Mendelian inheritance of the *ACE* I/D was investigated, and frequency of alleles were 0.6 and 0.4 for D and I alleles respectively. It was observed that mean serum *ACE* levels are related to the *ACE* I/D genotype: people with DD genotype had the highest (494.1 ± 88.3 μg/l) and II the lowest (299.3 ± 49 μg/l) and I/D individuals had intermediate level. This accounted for the genetic effect of 47% of the total phenotypic variance of the *ACE* plasma level in adults. Several hypotheses could account for this phenotypic variance, including biochemical mechanisms; however, it is perhaps easier to consider that control of the *ACE*

concentration is made at genetic level. A polymorphism in an intron might not cause a direct functional change, but may impact on *ACE* concentration through LD with some regulatory elements. The authors could not exclude that a part of this sequence might be present in mature messenger RNA due to some difference in *ACE* pre-messenger RNA splicing. They also suggested that the I allele might change the splicing process in the *ACE* gene.

1.2.2.1 Association Studies

1.2.2.1.1 With Anthropometric and Metabolic Traits

Factors involved in the pathogenesis of atherosclerosis, thrombosis and vasoconstriction contribute to the development of coronary heart disease. In a study comparing male patients after myocardial infarction (MI) with controls recruited from different European countries (all Caucasians), Cambien F et al. (1992) [28] found association between coronary heart disease and *ACE* I/D polymorphism. They reported that the DD genotype, which is associated with higher level of circulating *ACE* than I/D and I/I genotypes, is significantly more frequent in patients (n = 610) with MI ($p = 0.007$) than in controls (n = 733) especially among subjects with low body mass index and low plasma levels of Apo B ($p < 0.0001$). The Low risk group was defined as individuals with plasma level of Apo B lower than 125 mg/dl, those not taking hypolipidaemic drugs and those with BMI < 26 kg/m^2. In the high-risk group, there was a highly significant and also homogeneous association between *ACE* I/D and MI; the overall Odds Ratio after adjustment for population was 3.2 ($p < 0.0001$). The DD genotype and its association with risk status were still highly significant with MI ($p < 0.0005$ and $p < 0.02$ respectively) even after adjustment for age, smoking, plasma Apo AI, plasma Lp (a), blood pressure and social class in the whole population. Thus the *ACE* I/D polymorphism looks to be a strong risk factor for coronary artery disease (CAD) in individuals formerly considered to be at low risk according to common criteria.

They worked out that in low risk group the *ACE* D/D genotype may account for 35% of the MI cases. The results of this study are consistent with those of Pfeffer M et al. (1992) [120],

which showed that administration of *ACE* inhibitor not only decreased the risk of developing heart failure but also reduced the risk of recurrent MI. Experimental studies had shown that *ACE* gene expression is increased in myocardial tissue after coronary artery occlusion.

Cambien F et al. (1998) [27] suggested that *ACE* gene polymorphism as a genetic modulator might influence the effect of the small for gestational age (SGA) on insulin resistance in adulthood. It was proposed that insulin resistance in adult might be a consequence of low birth weight, which could be reflected by an increased fasting level of plasma insulin or an abnormal insulin response to a glucose load. They had previously reported that adults born with SGA would have reduced height and elevated plasma insulin (especially 30 minutes after glucose load) and pro-insulin. Thus, it could have been proposed that SGA might be a predisposing factor to insulin resistance. It was also reported that the *ACE* polymorphism might make people susceptible to insulin resistance and is implicated in cardiovascular and renal disorders especially in diabetes. To study the possible effects of this polymorphism on plasma glucose and insulin level, they investigated a group of adult born as SGA (n = 172) and a control group (n = 207) born as appropriate for gestational age (AGA). They observed a significant correlation between fasting plasma glucose and insulin level in SGA (n =165, R = 0.196, p <0.015); this finding was restricted to the *ACE* II homozygotes (R = 0.539, p <0.0009, 0.021 and 0.098 in II, ID and DD genotypes respectively); they did not find this correlation in AGA group. The interaction between gestational age and *ACE* genotype on insulin levels were described as: the insulin level increased with shorter gestational age in II homozygotes (p <0.0005) and in ID (p <0.005) but it did not in DD homozygotes (p >0.05). The above results could suggest that DD genotype subjects might be resistant to the effect of short gestational age on adult plasma insulin. The observed association does not necessarily imply that I/D polymorphism is a functional one. It could be in LD with a nearby functioning polymorphism, perhaps, where the human growth gene is located (17q23). Finally, it was concluded that if the suggested idea (that the consequences of SGA on insulin resistance in the young adult may be modulated when the D allele is present which might attenuate sequels of low birth weight and short gestational age on cardiovascular gene expression) is correct, it would be the first instance of a genetic variant affecting the late consequences of intrauterine growth retardation. This could perhaps be a partial genetic explanation of programming [17].

High activity of *ACE* in skeletal muscle of guinea pig and rat and heart of guinea pig [45] was an encouraging point to test the plausible involvement of the *ACE* I/D in muscle metabolism and performance. In a study undertaken by Montgomery H et al. (1997) [108], 460 normotensive Caucasian male military recruits undergoing an intensive 10-week physical training course were investigated. Left ventricular mass increased by 18% ($p < 0.0001$) and this increase was more significant in persons with the *ACE* D allele: mean LV mass altered by +2.0, +38.5 and +42.3 g in II, ID and DD, respectively ($p < 0.0001$). Thus, it was concluded that exercise-induced left ventricular growth in young Caucasian males is strongly associated with the *ACE* I/D polymorphism.

Montgomery H et al. (1997) [108] showed that local RAS may regulate myocardial growth through the local generation of angiotensin II, and it is known that the D allele is associated with elevated *ACE* levels in ventricular tissue as well as in circulation [2]. Therefore, if cardiac RAS is an important myocardial growth factor, then subjects with higher *ACE* may be expected to exhibit a greater response to a hypertrophic stimulus such as physical training.

The association of D allele of the *ACE* I/D with training induced LV (left ventricle) mass was also reported by Fatini C et al. (2000) [54].

Montgomery H et al. (1998) [109] found that the *ACE* I allele was associated with improved endurance performance. This association was investigated in 2 parallel experiments. A relative excess of II genotype and a deficiency of DD genotype were found in 25 elite unrelated male British mountaineers (mean age 40.6 ± 6.5) with a history of ascending beyond 7000m without using supplementary oxygen as compared with 1906 British males (mean age 55.6 ± 3.2) without clinical cardiovascular disease. Among 15 climbers who had ascended beyond 8000m without oxygen, nobody was homozygous for the D allele (6 II and 9 ID: frequency of allele I = 0.65). They also determined *ACE* genotype in 123 Caucasian (mean age 19.0 ± 0.2) males recruited to the UK army. The maximum duration (in seconds) for which they could perform repetitive elbow flexion while holding a 15kg barbell was assessed both before and after the

[2] Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, Schunkert H. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation. 1995 Sep 15;92(6):1387-8.

training period (ten weeks). Pre-training performance was independent of the I/D genotype. Duration of exercise improved significantly for 66 individuals of II and ID genotype but not for the 12 of DD genotype. Improvement was 11-fold greater ($p = 0.001$) for those of II than that of DD genotype.

As discussed earlier, the insertion (I) rather than deletion (D) allele is associated with lower *ACE* activity in tissues and improved response to physical training. Montgomery H et al. (1999) [107] studied the association between the *ACE* I/D polymorphism in relation to an intensive exercise programme, to survey the metabolic effects of local RAS. They studied changes in body composition and performance in 81 young male army recruits (mean age 19. years, body mass index 22.2 kg/m^2) over 10 weeks of intensive physical training using three independent methods (bioimpedance, multiple skinfold-thickness assessment of whole body composition, MRI of the mid-thigh). It was found that participants with II genotype had a greater anabolic response in proportion to others who had one or more D allele for fat mass (0.55 vs. – 0.20 kg, $p = 0.04$ by bioimpedance) and not fat mass (1.31 vs. – 0.15 kg, $p = 0.01$ by bioimpedance). Body mass indexes were also allele dependent (II 0.48 g/m^2, D+ - 0.14 g/m, $p = 0.03$).

They concluded that II genotype, as a marker of low *ACE*, might keep a positive energy balance during rigorous training that suggests enhanced metabolism efficiency. The association of body fat stores with improved physical performance might suggest an effect of the *ACE* I/D genotype on energy balance and on the nature and efficiency of the use of oxidative fuel for metabolism. There are several possible explanations for these observations. Firstly, local adipose RAS may alter substrate mobilisation from fat stores and affect the metabolism. Secondly, a local musculoskeletal RAS may modify the use of energy substrate. Thirdly, through involvement of systemic endocrine or nutritional mechanisms, the polymorphism could be in LD with a functional variation in the neighbouring growth hormone gene, which is the closest identified gene. Through the first two mechanisms *ACE* inhibitors might improve the survival and performance of myocardial cells during ischemia.

Regarding the association of the I allele of the *ACE* I/D with greater endurance performance and increased mechanical efficiency, Zhang B et al. (2003) [154] hypothesised that it might be associated with an increased slow-twitch fiber, which is more efficient in low-velocity contraction compared with fast-twitch one. It was found that homozygotes of II had higher percentage of slow-twitch fiber type I, and there was a linear trend for reduction in type I fiber and increase in fast-twitch fiber (type IIb). This finding, to some extent, can explain the association found between the *ACE* I/D and endurance performance.

In the division of Human Genetics at the University of Southampton, Day I N M et al (unpublished) have studied the effects of *ACE* I/D on different metabolic phenotypes in the populations from North and East Hertfordshire areas (NH and EH respectively, see 2.1.1.1, 2.1.1.1.1 and 2.2.1.4 for population and datasets). In the NH group, they found the association of allele I of *ACE* I/D with obesity (central), higher creatinine concentration ($p = 0.04$ in men and 0.06 in women), thicker skin ($p = 0.02$ in men and 0.006 in women) and paradoxically heart attack (in men but not so closely in women) ($p = 0.03$). No significant association was found the *ACE* I/D and blood pressure in the women of this area. In the EH, they found the association of allele I of *ACE* I/D with increased peripheral obesity in men and decreased central obesity in women and decreased insulin response during the OGTT. Additionally, there was a lower fasting apolipoprotein A in subjects carrying the allele I and they also suggested a trend for decreased Lp (a) lipoprotein in heterozygotes and some homozygotes. A list of phenotypic effects of the *ACE* I/D polymorphism are provided in Table-1.1.

The significant role of the *ACE* I/D in overweight and abdominal adiposity in men was reported in the Olivetti Prospective Heart Study [134]. In this study, DD genotype was associated with overweight and abdominal adiposity, especially among older people compared with ID or II genotype (relative risk = 2.34).

Table 1.1: Distribution of phenotypic variation regarding the *ACE* I/D

I allele	Improved endurance performance	Montgomery H et al. (1998)
	Increased plasma level of insulin in SGA	Cambien F et al. (1998)
	Positive energy balance	Montgomery H et al. (1999)
	The association of the D allele with: Obesity, higher creatinine, thicker skin, increased peripheral obesity in men, decreased obesity in women, decreased fasting Apo A, increased response during OGTT, heart attack in men,	Day I et al. (unpublished)
D allele	Increased level of circulating *ACE*	Rigate B et al. (1990)
	Association with MI esp. in low BMI and Apo B	Cambien F et al. (1992), Keavney B et al. (2000) [87]
	Exercise-induced LV growth	Montgomery H et al. (1997)
	Attenuation of consequences of SGA on adult plasma insulin	Cambien F et al. (1998)

1.2.2.1.2 With Cardiovascular Disorders

The role of the *ACE* I/D in progression of coronary heart disease (CHD) in non-insulin dependent diabetes (NIDDM) was reported by Ruiz J et al. (1994) [126]. It was shown that D allele is associated with early onset CHD in NIDDM. This effect was independent of hypertension and lipid values.

It has been shown that DD genotype is associated with higher *ACE* mRNA levels [110]. The effect of the *ACE* I/D on *ACE* mRNA level in the kidney of healthy Japanese people was studied by Mizuiri S et al. (2001) [105], and it was found that the mRNA level was significantly high in DD individuals compared with II ones, and ID subjects had intermediate values.

The association of D allele of the *ACE* I/D with the risk of ischemic heart disease [59] and poor prognosis in patients with congestive heart failure [12] have been reported.

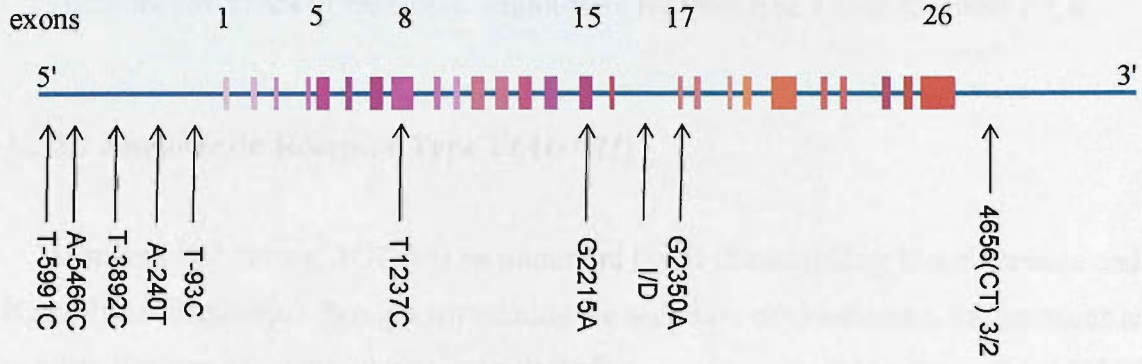
It is generally believed that *ACE* inhibitors are very useful agents for the treatment of hypertension [43] and heart failure [66] which decreases the mortality rate by 25% in the latter case. They are among the first choices for treatment of hypertension, heart failure, MI and DN [112]. Furthermore, the pharmacogenetic interaction between the *ACE* I/D and treatment with β-blockers in the outcome of heart failure was reported, in which patients with systolic dysfunction and carrying D allele and not using β-blockers had a poorer survival compared with those taking them [101].

1.2.2.2 Haplotype Studies

Keavney B et al. (1998) [88] studied ten polymorphisms (T-5991C, A-5466C, T-3892C, A-240T, T-93C, T1237C, G2215A, I/D, G2350A, 4656[CT] 3/2) (Figure 1.5) in 26 kb of the *ACE* gene and found multiple haplotypes in Caucasian British families. They made a haplotype tree (cladogram: a tree diagram used to illustrate phylogenetic relationships) with three branches (clades A to C), which consists 90% of the observed haplotypes. They assumed that haplotype 3 is derived from an ancestral recombination in subjects with genotype A/2. Using nested measured haplotype analyses, they concluded that a major variant influencing the *ACE* plasma level is somewhere downstream of the ancestral breakpoint. Upstream sequence, including the *ACE* promoter, was excluded from harbouring it (Figure 1.6). This result was confirmed later with a refined mapping of the ancestral breakpoint excluding a further 4 kb of the *ACE* gene consisting exons 1-5 and introns 1-4 from harbouring the major *ACE* linked quantitative variant [53]. Moreover, it has been reported that there are two quantitative trait loci (QTL) affecting the ACE level: one is somewhere inside the gene (perhaps I/D or a marker in

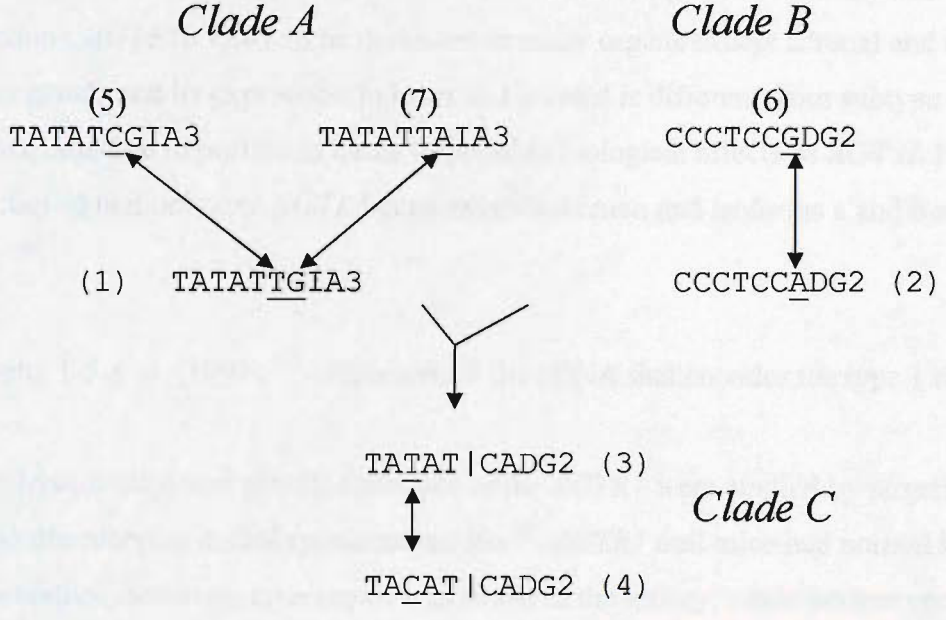
complete LD with it), and the other one is nearby to the gene [100,142], likely in the 3′ region [155]. However, Zhu X et al. (2003) [156] in a study on blacks (African-American) and whites (European-Americans) found one haplotype block in the both populations including three main haplotypes in whites and four main haplotypes in blacks.

Figure 1.5: Polymophisms in the *ACE* Gene



Shows the schematic representation of the *ACE* gene consisting ten known polymorphisms.

Figure 1.6: Cladogram of the *ACE* Gene



Evolutionary tree (cladogram) for ten *ACE* polymorphisms, adopted from Keavney's et.al. (1998) [88] report.

1.2.3 Angiotensin Receptors

There are two kinds of receptors: angiotensin receptor type I & II, See also 1.1.4.

1.2.3.1 Angiotensin Receptor Type 1 (*AGTR1*)

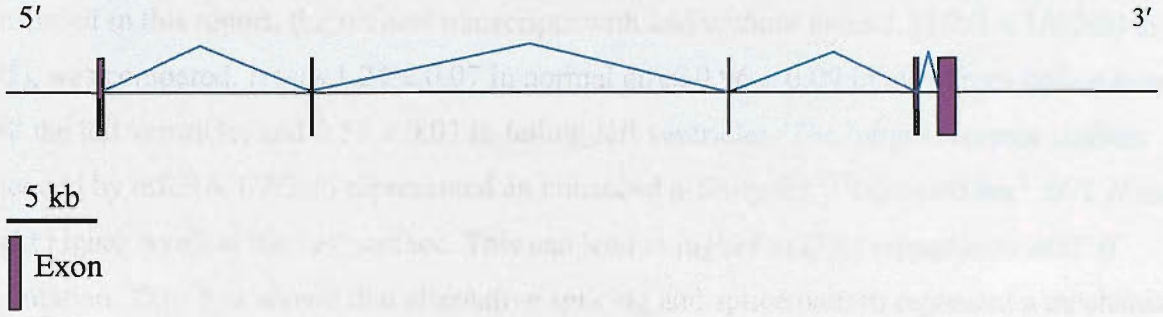
As mentioned before, *AGT II* is an important factor in controlling blood pressure and electrolytes distribution through stimulating the secretion of aldosterone. Its functions are mediated by two separate subtypes of cell surface receptors: type 1 and type 2 (*AGTR2*). It is generally believed that the major cardiovascular effect of *AGT II* is mediated by type 1 [4]. The receptor is included in the guanyl nucleotide binding protein (G-protein) coupled receptor super family and intracellular messengers are phospholipases, calcium signalling and protein kinase [46].

The two subtypes of *AGTR1*, a and b have been found in human, rat and mouse. Ito M et al. (1995) [78] reported that these two genes have substantial sequence similarity and wide tissue distributions. *AGTR1a* looks to be dominant in many organs except adrenal and anterior pituitary glands and its expression in heart and adrenal is different from subtype 2. This difference could be important in terms of possible biological effects of *AGT II*. However, it is now believed that only one *AGTR1* gene exists in human and isoforms a and b are present in rodents [46].

Murphy T J et al. (1991) [111] characterised the cDNA that encodes the type 1 receptor.

The physiological and genetic functions of the *AGTR1* were studied by targeting the gene encoding this receptor in embryonic stem cells [78]. *AGTR1* null mice had normal kidneys, heart and vasculature; however, no receptor was found in the kidney, while heterozygotes had a reduction in binding to *AGT II* about 50%. In null homozygous mice, no pressor response emerged following *AGT II* infusion. In comparison with wildtype mice, systolic blood pressure was decreased by 12 mmHg in heterozygous and by 24 mmHg in homozygous ones.

Figure 1.7: Structure of the *AGTR1* Gene



The structure of the *AGTR1* gene on 3q. Adopted from Guo D et al. (1994) [65] and Antonellis A et. al. (2002) [13].

Gemmill R M et al. (1991) [60] mapped *AGTR1* to 3q21-q25 using a somatic cell hybrid. Guo D F et al. (1994) [65] using cDNA & genomic DNA comparison found that *AGTR1* gene has 5 exons and its length is more than 55kb (Antonellis A et al. (2002) [13] have measured about 60.5 kb) of genomic DNA. Exon sizes range from 59 to 2014 bp, four of them encode 5′ UTR and there are also multiple transcription initiation sites (Figure 1.7).

Curnow K M et al. (1995) [36] studied splicing patterns for *AGTR1*. Exon 2 was found to have an inhibitory effect on translation. One third of *AGTR1* mRNA in tissues examined, had transcripts containing exon 3 and 5, encoding a receptor with an amino-terminal extension of 32-35 amino acids, emphasising the presence of the translation initiation in frame with *AGTR1* ORF (open reading frame). These transcripts were translated into a larger receptor isoform which was functional in terms of ligand binding and signalling characteristics in vitro. Transcripts containing exons 1/2/5 were predominant in the atria from normal hearts, similarly in human lung and liver, whereas those containing exons 1/5 were most abundant in the ventricles and atria of failing hearts, as in the adrenals, kidneys, fibroblasts, placenta, spleen, colon and gonads [36,145].

The hypothesis of modulation of translation of the *AGTR1* by differential splicing of the 5′ UTR exons was tested by Warnecke C et al. (1999) [145]. They studied the *AGTR1* and mRNAs in normal and diseased human heart. Transcripts containing exons 1/2/5 and 1/5 represented

about 93-98% of all *AGTR1* mRNA, while those containing 1/2/3/5 were 8% in the atria and 2% in ventricles. Considering the inhibitory effect of exon 2 on translation which was also confirmed in this report, the ratio of transcripts with and without exon 2, (1/2/5 + 1/2/3/5) to (1/5), was compared. It was 1.24 ± 0.07 in normal atria, 0.96 ± 0.09 in atria from failing hearts, 0.68 the left ventricle, and 0.58 ± 0.03 in failing left ventricles. The longer receptor isoform (encoded by mRNA 1/2/3/5) represented an enhanced affinity for ^{125}I-labeled Sar^{1} *AGT II* and slight higher levels at the cell surface. This can lead to higher *AGTR1* response to *AGT II* stimulation. Thus it is shown that alternative splicing and splice pattern represent a mechanism for *AGTR1* regulation, which has also been outlined and discussed by Martin M M et al (2001) [98].

It should be noted in this discussion of splicing patterns for *AGTR1* that *AGTR1* expression (not *AGTR2*) is decreased in ventricles of failing heart [69]; while *AGTR1* is over-expressed in the atria of failing heart [69,84].

1.2.3.1.1 Regulation of *AGTR1*

Evidence shows the down regulation of the *AGTR1* expression (reduced mRNA level) by growth factors (epidermal growth factor, basic fibroblast growth factor and platelet derived growth factor –BB) in rat thoracic aorta vascular smooth muscle cells (VSMC) [115]. This caused the loss of membrane associated receptors and *AGT II* stimulated inositol phosphate production, and also a marked decrease in the half-life of the *AGTR1*, suggesting post transcriptional destabilization of the *AGTR1* mRNA which is susceptible to pre-treatment with transcription inhibitors (e.g. Actinomycin D). It was then suggested that the effect of growth factors on the *AGTR1* is mediated through an unknown gene or genes; an idea which has also been suggested by Nickenig G et al (1996) [116]. They indicated that *AGT II* stimulation may induce a complex of polyribosomal proteins that bind specifically in the distal 350 bp of the *AGTR1* mRNA.

Stimulation of *AGTR1* by *AGT II* causes rapid desensitization of intracellular signal and a decrease in the number of cell surface receptor [72]. Using immunoflurescence microscopy, it

was shown that *AGTR1* was internalized into endosome after stimulation by *AGT II*. Then *AGT II* and *AGTR1* (as a complex) are directed into the lysosom pathway where *AGT II* is delivered and *AGTR1* returns to the cell membrane (i.e. recycled). It was observed that there is a dynamic equilibrium between the sequestration of the receptors into endosomes and recycling to the plasma membrane (this did not happen to *AGTR2*).

Ishihata A et al. (1998) [77] reported inhibition of the *AGTR1* expression in rat adrenal gland following *AGT II* stimulation. They excluded the involvement of the promoter in this suppression and suggested other mechanisms such as destabilisation of mRNA.

Similar result was observed in vascular smooth muscle cells [9], in which *AGT II* caused internalization of *AGTR1* and induced suppression of the *AGTR1* expression. Down-regulation of the *AGTR1* by *AGT II* in rat aortic smooth muscle cells has also been reported [93]. *AGTR1* mRNA and protein decreased by 70% and 35% respectively. This effect was blocked by transcription and translation inhibitors, suggesting the importance of protein factors (e.g. trans-acting elements) and both transcriptional and posttranscriptional mechanisms.

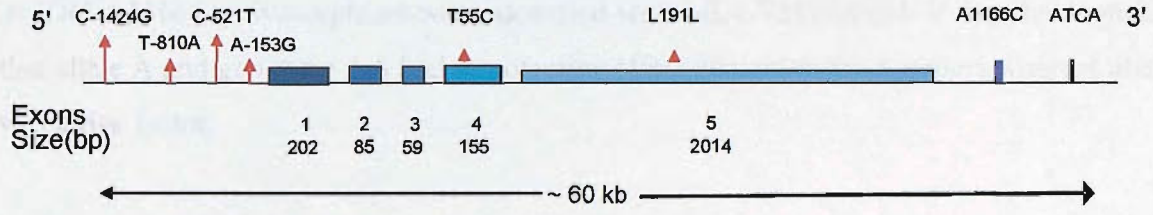
Another important issue worth mentioning here is the influence of environmental factors e.g. dietary salt, in the expression of *AGTR1*. Schmid C et al. (1997) [130] showed that this effect might be in a tissue, time and subtype specific. They fed mice with three diets: low, normal and high in salt. It was seen that the expression of the *AGTR1* (subtype a) decreased in kidney, liver and lung, but not adrenal between days five and ten after beginning the regimen (in low salt group). In adrenal, where *AGTR1* (subtype b) is prominent, the expression of the *AGTR1* (subtype b) increased. In another study, the expression of the *AGTR1* in rats kidneys exposed to low protein during intra-uterine life was studied [129]. It was observed that the *AGTR1* expression increased by 24%.

1.2.3.1.2 *AGTR1* Polymorphisms

Bonnardeaux A et al. (1994) [25] found five polymorphisms through the coding region (exon 5) and 3′ UTR: T573C (L191L), +1062 (A to G), +1166 (A to C), +1517 (G to T) and +1878

(A to G). Some other polymorphisms were found within the 5′ UTR: -1424, -810 and -521, -153 [121] (Figure 1.8). Bonnardeaux A et al. (1994) [25] showed that an A to C variant in 3′ UTR at nucleotide 1166 has an association with essential hypertension.

Figure 1.8: Polymorphisms of the *AGTR1* Gene



Schematic representation of the *AGTR1* gene and the positions of the SNPs and microsatellite (ATCA).

No interaction was found between this A1166C and variant M235T of the *AGT*. It was also shown that *AGTR1* A1166C variant is associated with more severe and earlier onset of essential hypertension. It was then suggested that because this A/C transversion does not change a potential mRNA polyadenylation or destabilisation segment, i.e. not functional, it must be in linkage disequilibrium with an unknown functional polymorphism. The frequency of A1166C was described as 0.36.

To find out the possible functional polymorphism Poirier O et al. (1998) [121] in the ECTIM (Etude Cas-Temoin de l'Infarctus du Myocarde) cohort (a multicentre study comparing 651 patients who had survived a MI and 728 controls from Belfast and Lille, Strasbourg and Toulouse) studied the region with the aforementioned polymorphisms. None of them was in linkage disequilibrium with *AGTR1* A1166C. However, they suggested that *AGTR1* -810 T/A or other polymorphisms that are in complete association with it might increase the risk of myocardial infarction. Lists of the *AGTR1* polymorphisms and haplotypes are presented in Inserm website: http://genecanvas.idf.inserm.fr/.

Wang W Y et al. (1997) [143] undertook a case control study of A1166C polymorphism in 108 Caucasian hypertensive individuals with a strong family history and early onset hypertension. The frequency of C allele was 0.4 in hypertensives and 0.29 in normotensives.

Chistiakov D A et al. (2000) [33] studied the *AGTR1* A1166C polymorphism in patients from Moscow with MI (n = 32), LVH (n = 38) and EH (n = 178). The low frequency of allele A in all three affected groups was found in comparison to healthy controls. It was also found that *AGTR1* A1166C polymorphism was associated with MI, LVH and EH. It was then concluded that allele A and genotype AA had a protective effect against these disorders whereas allele C was a risk factor.

In a study in Norway by Berge K E (1997) [24] the association of CC homozygosity with MI in Norwegian males was found. This association was stronger among low-risk phenotype.

Alvarez R et al. (1998) [11] studied *ACE* I/D and *AGTR1* A1166C polymorphisms in Spain. They found the synergistic effect of these two polymorphisms on CAD, i.e. CC + DD genotype might have higher risk for CAD. This synergistic effect of these two polymorphisms on cardiovascular phenotype has also been reported by Ye S et al. (2003) [149].

Chistiakov D A et al. (1999) [32] in a study of a Moscow population described the relationship between *AGTR1* A1166C with diabetic nephropathy and retinopathy. No association was found between *AGTR1* A1166C with diabetic nephropathy but they found the association with diabetic retinopathy (DR). In this association C allele is a risk factor for DR, in contrast to allele A, which is a protective factor.

Moczulski D K (1998) [106] using linkage study on pairs of siblings with type 1 diabetes who were discordant for diabetic nephropathy (DN) showed that a 20 cM region on chromosome 3 harbouring *AGTR1* is susceptible for DN.

Because there are many other papers about the effects of A1166C, a group of them have been listed in Table 1.2.

Table 1.2: Associations of the A1166C with different phenotypes in various studies

Polymorphism	Phenotype	Effect/ Allele	Country	Ethnic Group	Date	Author
A1166C	Essential Hypertension	+/C	France	Caucasian	1994	Bonnardeaux, A. et al .
A1166C	Pulse Wave Velocity & High Density Lipoprotein	+/C	France	Caucasian	1995	Benetos, A. et al .
A1166C	Low Systolic BP	+/CC	Italy	Caucasian	1996	Castellano, M. et al .
A1166C	Pulse Wave Velocity	+/C	France	Caucasian	1996	Benetos, A. et al .
A1166C	Essential Hypertension	+/CC	Australia	Caucasian	1997	Wang, W. Y. et al .
A1166C	Coronary artery disease	+/C A is supportive	France	Caucasian	1998	Tiret, L. et al .
A1166C	Essential Hypertension	+/C	Finland	Caucasian	1999	Kainulainen, K. et al .
A1166C	LVH, EH, MI	+/AC	Moscow	Caucasian	2000	Chistiakov et al.
A1166C	CAD & Hypercholest-rolemia	+/C	England	Caucasian	2000	Wierzbicki, A. S. et al .
A1166C	Stroke & number of lacunae	+/C?	Japan	Japanese	2000	Takami, S. et al .

1.2.3.1.3 Haplotypes

To search the haplotypes structure of the *AGTR1*, Zhu X et al. (2003) [156] studied the genes of the RAS and resolved the haplotypes of each gene for 193 blacks (African-American) and 160 whites (European-American). In the *AGTR1* gene, there were three haplotypes and two haplotype blocks in both populations; one block extending through the promoter region and the other one including the coding region and 3′ UTR. It is worth mentioning that the frequencies of haplotypes were slightly different in the two populations.

We also know that ACE inhibitors and *AGTR1* receptor antagonists (e.g. losartan) have significant medical benefit in the treatment of hypertension and the above evidence suggests an association of *AGTR1* polymorphism with hypertension. Bonnardeaux A et al. (1994) [25] has shown that it is possible that the gene product of *AGTR1* may not only be a good target for treatment but might also display genotype-specific drug response effect.

1.2.3.2 Angiotensin Receptor Type II (*AGTR2*)

The human type II angiotensin receptor gene, which has an intronless coding region, was analysed by Koike G et al. (1994) [90]. It has 363 amino acid residues and is similar to the rat and mouse proteins. They mapped it to chromosome X and Chassagne C et al (1995) [31] assigned it to Xq22-Xq23. The activation of the *AGTR2* leads to apoptosis, vasodilation and natriuresis [140].

Ichiki T et al. (1995) [74] and Hein L et al. (1995) [71] generated mice lacking the gene encoding *AGTR2*. Hein L et al. (1995) [71] observed that mutant mice had normal development but there was an impaired drinking response to water deprivation. They had normal baseline blood pressure with increased pressure response to *AGT II* injection. They concluded that it has an effect on central nervous system and cardiovascular functions. Ichiki L et al. (1995) [74] observed that these mutant mice had high blood pressure and high sensitivity to pressor function of *AGT II*. Thus, they reported that *AGTR2* might have a depressor effect (antagonist to *AGTR1*) and also may have a modulatory effect on central nervous system functions, such as behaviour.

Hunley T E et al. (2000) [73] reported that *AGTR2* actions decrease the *ACE* activity.

1.3 Growth Hormone

Growth hormone (*GH* or *somatotropic hormone or somatotropin*) is a small protein molecule consisting 191 amino acids, which is secreted from acidophilic cells in anterior pituitary. Its gene has been mapped to 17q22-24 [117]. Its secretion is under control of hypothalamus hormones:

Growth hormone releasing hormone (GHRH), which causes release of *GH.*
Growth hormone inhibitory hormone (GHIH), which inhibits release of *GH.*

It induces the growth of almost all tissues of the body that are capable of growing. It also induces hypertrophy, hyperplasia, mitosis and differentiation of some specific cells like bone and early muscle cells. Besides to the above general functions, it has some special metabolic effects:

1 - Stimulation of protein synthesis through:

a - Facilitation of intake of amino acids into cells.

b - Increasing RNA translation.

c - Enhancement of DNA transcription (a relatively long effect, 24 - 48 hours).

d - Decreased breakdown of proteins.

2 - One of its important metabolic functions is mobilization of fatty acids from adipose tissue resulting in higher free fatty acid in circulation and switching the energy consumption to fatty acids as substrate.

3 - It diminishes the rate of glucose utilization throughout the body. This is done by:

a - Reduction in glucose uptake in tissues e.g. skeletal muscle and fat.

b - Higher level of glucose production in the liver.

c - Stimulation of insulin secretion. The final effect of these actions is induction of "insulin resistance", high level of blood glucose and increased insulin secretion. These are the same metabolic disorders of diabetes type II, for this reason, it is said that growth hormone is diabetogenic.

In summary, it makes protein, uses fat reservoirs and conserves carbohydrates. Its protein anabolic effect in addition to the promotion of fat utilization increases the lean body mass. *GH* secretion is decreased after adolescence, and in old age it is about 25% of the adolescent level. Physiologically, the secretion of *GH* increases in starvation, hypoglycaemia, exercise, excitement, deep sleep and trauma. Some other factors such as catecholamines, dopamine and serotonin stimulate the secretion of the *GH* [67].

It has been discovered that *GH* induces liver and to a much lesser extent other tissues to produce several small proteins called *somatomedins*. Many of their effects on growth are similar to those of insulin. Therefore, they are called insulin - like growth factors (IGF). At least four somatomedins are found, however, the most important one is *somatomedin C* (also called insulin - like growth factor I [IGF1]). It is presumed that most of *GH* functions, if not all of them, are mediated by *somatomedins* especially C type.

Growth hormone gene along with four other nearby genes is considered as *GH* cluster. The order from 5' to 3' is GH1- CSL – CSA – GH2 – CSB [117].
CSL (CSHP1): *Chorionic somatomammotrophin CS-5 pseudogene (chorionic somatomammotropin-like gene).*
CSA (CSH1): *Chorionic Somatomammotrophin 1 gene.*
CSB (CSH2): *Chorionic Somatomammotrophin 2 gene.*

The relation between the RAS, *GH* and *IGF1* in rat has been studied by van Eickels M et al. (2000) [141]. They showed that mitogenic action of *IGF1* is partly mediated by the RAS (mainly *AGT II*) activation and *IGF1R* expression is increased by the RAS activation. They also presented data showing that *ACE* and *AGTR1* inhibition block the proliferation effects of *IGF1* and expression of *IGF1R* expression in rat cardiac fibroblasts. Finally, they showed that *IGF1* activates the RAS in neonatal rat cardiac fibroblasts. There are some other studies suggesting the similar actions of the RAS on the *IGF1* system in vascular smooth muscle cells [14].

Based on the trophic properties of *GH* and *IGF1* which increase body weight and cardiac mass and also their positive effects on vasodilatation and myocardial contractility, and on the

other hand, the benefits of *ACE* inhibitors in inhibition of cell hypertrophy, some researches have suggested that patients with congestive heart failure may take advantage of administration of *GH*, *IGF1* and *ACE* inhibitors [124].

These data show the relations between the RAS and *IGF1*. Further studies are required to establish certain effects of them in terms of metabolic and cardiovascular diseases, especially that *GH* and *ACE* genes are located on the same chromosome (17q 23), and are close together i.e. *GH* gene is located about 400-500 kb in 3′ side of the *ACE* gene (http://www.ncbi.nlm.nih.gov/) or about 200kb according to ensembl data (http://www.ensembl.org). In this case, they may have some functional effects on each other.

In a study in the Human Genetic Division at the University of Southampton, King T H et al (2002) (unpublished data) have studied the LD between the *ACE* I/D and two SNPs and one microsatellite in the *GH* gene. They have found the D′ value of 0.19-0.55 between those two markers. Their results suggest the possible association between different alleles of the *GH* microsatellite with lower birth weight (alleles 4 and 29), higher birth weight (allele 16), high adult height (alleles 4 and 19) and lower weight (allele 1) in men, and lower birth weight (alleles 3, 31 and 11), high birth weight (alleles 19 and 32), increased adult height (allele 4), lower adult weight (alleles 8, 11, 24, 19 and 31) and increased weight (allele 32) in women (see also [3]).

[3] Day IN, King TH, Chen XH, Voropanov AM, Ye S, Syddall HE, Sayer AA, Cooper C, Barker DJ, Phillips DI. Insulin-like growth factor-I genotype and birthweight. Lancet. 2002 Sep 21;360(9337):945; author reply 945-6.

1.4 Linkage Disequilibrium

In a linkage equilibrium condition, a marker with two alleles of equal frequencies is linked to a disease allele. It will be associated with each of the marker alleles in 50% of affected families. In other words, a condition will be considered in linkage equilibrium in which proportions of the likely allelic combinations can be predicted by the product of population frequencies of alleles at the given loci.

On the contrary, when a disease-causing mutation is in association with only one particular allele at a given locus, it is considered to be in linkage disequilibrium (LD) [113]. LD is defined as non-random association of alleles at linked loci. This phenomenon is a useful tool for localization of genes and studying recombination events over past generations. It takes advantage of using hundreds of thousands of new polymorphic markers that have mainely been discovered over last decade [83].

When a mutation occurs for the first time in a population, it is in a haplotype with particular linked markers. At this stage, there is a complete LD between the markers and disease mutation, i.e. the disease mutation could be found only in the presence of a specific set of marker alleles. Over time, recombination occurs between disease and marker alleles and LD lessens gradually. The rate at which it decays is predominantly dependent on recombination. However, it is also subject to other factors such as mutation, selection and genetic drift [48]. There are some other factors that influence the strength of LD like population growth and population structure [15].

Richard Lewontin (1964) was the first to develop the commonly used LD measure, D. For a pair of diallelic loci A and B, D shows the difference between the co-occurrence of an allele of A (A_1) and an allele of B (B_1) and the expected frequency of their co-occurrence in linkage equilibrium.

The observed gametic frequency (p_{11}) is the proportion of chromosomes on which A_1 and B_1 alleles co-occur in a population. The expected value of p_{11} assuming linkage equilibrium is the product of allele frequency of A_1 and B_1 in the population. Hence:

$D = p_{11} - q_1 p_1$

Where:

$p_1 = f(A_1)$; $p_2 = 1-p_1 = f(A_2)$

$q_1 = f(B_1)$; $q_2 = 1- q_1 = f(B_2)$

If D is significantly higher than zero, it is said that LD exists. It is clear that D is dependent on allele frequencies in the population [83]. The magnitude of LD between two loci depends on the recombination fraction, θ, and time in generations, t (i.e. since a disease causing mutation has happened at time 0). Hence:

$D_t = D_0 (1- \theta)^t$

It can be concluded that D decreases when two loci are far apart and through generations as a result of recombination. Thus it shows the frequency of recombination and/or the physical distance between two loci. Overall, if θ is determined, we will be able to measure the age of a disease-causing mutation. A more useful measure of LD is D′, which varies between -1 and 1 . The range of D′ is independent of allele frequencies [70,95]. Thus allowing comparison of the intensity of LD between pairs of loci, for testing the relationship between LD and physical distance and for testing homogeneity of LD between different populations [151].

D′ is defined as $D' = D/Dmax$

Where:

$Dmax = \min [p_1q_1, (1-p_1)(1-q_1)]$ when $D<0$ or $\min [p_1(1-q_1), (1-p_1)q_1]$ when $D>0$

$D' = 1$ if, and only if, two SNPs have not been separated by recombination or mutation or gene conversion during history. Values of $D' < 1$ indicate that the complete ancestral LD has been disrupted. The relative magnitude of values $D' < 1$ have no clear interpretation and should

not be used for comparison of the strength of LD between studies or to measure the extent of LD when sample sizes are different [15]. Other properties of LD can be found in papers published by Lonjou C et al. (1999) [96] and Zapata C et al. (2001) [150].

1.4.1 Extent of LD

The extent of LD varies in different populations and regions of the genome. Some investigators mention that LD could be found over long distances of about 30-50 kb or more [34]; however, LD has been found over distances of up to 2 Mb in recently founded populations such as the Finnish [118]. Ardlie K G et al. (2002) [15] have proposed the range of 10-30 kb for northern European populations with lower nucleotide diversity; this is higher than that found in African populations. It has also been mentioned that LD has block-like pattern across the genome and chromosomes, which may or may not be compatible with recombination hotspots [38].

If these ideas extend to populations, it will be observed that those with different demographic histories display different LD patterns. Higher levels of LD could be detected in recently founded populations such as Finnish compared with Africans, as discussed above. In recently founded populations, LD may be detected over several Megabases (Mb). These results could suggest that younger populations are more suitable for primary detection of a disease locus using LD at large distances and older populations which have more recombinations may be useful for fine-scale mapping LD [83]. It should also be mentioned that the extent of LD may not be a suitable tool for mapping, because there is considerable variability in the extent of LD from one part of the genome to the another one, and also even in high LD regions, some pairs of loci do not represent significant level of LD. The latter could be caused by gene conversion, allele frequency differences or some other factors [15].

1.4.2 Haplotype Structure

The number of discovered SNPs is increasing significantly (2.4 million in SNP consortium [6], and it is believed that 99.9% of DNA sequence of people are identical [7], i.e. one difference in

1250 nucleotides [122]. Regarding the above issues, one idea has been emerged that SNPs are present in sets called haplotype and are also inherited in the same format. Recent investigations suggest that haplotypes are located in different blocks separated by gaps, which reflect recombination sites (hotspots). The size of haplotypes and the pattern of distribution across the human genome are not quite clear yet.

Daly M J et al. (2001) [38] completed a high-resolution analysis of haplotype structure across 500 kb on chromosome 5q31 (containing genetic risk factor for Crohn's disease). In this study, 103 SNPs were used in European-derived population with a marker density of 1 SNP every 5kb. They found discrete haplotype blocks ranging 10-100kb; each block had few common haplotypes. These blocks are separated by gaps in which multiple independent historical recombination events might have occurred suggesting hotspots of inhomogenity of recombination, while the LD in each block is maintained without significant deterioration.

Jeffreys A J et al. (2001) [79] analysed 216 kb of the class II region of the histocompatibility complex (MHC) on chromosome 6 and found long extended LD interrupted with six gaps. The study on sperm typing showed that these gaps corresponded exactly to meiotic crossover hotspots. These hotspots occur in clusters and reflect almost all recombination in that region of MHC.

In another study performed for determination of haplotype blocks in the human genome, Gabriel S B (2002) [57] chose 54 autosomal regions, spanning 13.4 Mb, from different parts of the genome with the same SNP density as that of the SNP consortium (one SNP every 2 kb); overall, 3738 SNPs. 275 subjects were selected from different populations: Nigeria (Yoruba), Europeans, Japanese, Chinese and African Americans. Their data showed that the extension of LD is similar between Asian and European samples and also between African-American and Yoruban samples. 928 blocks were found in these four populations. The size of each block varied significantly from <1 to 94 kb in the African-Americans and Yoruban samples and <1 to 173 kb in the European and Asian samples. Haplotype diversity was higher in Yoruban and African-American samples, with an average of 5 common haplotypes; this variation was 4.2 in Europeans and 3.5 in Asians. Using different analyses, it was estimated that half of the human

genome lies in blocks of ≥ 22 kb in African and African-American samples and in blocks of ≥ 44 kb in European and Asian ones. In each block, there are only three to five common haplotypes, which represent about 90% of all chromosomes in each population. Both haplotype variations and block limits are similar to a considerable extent in different populations. It should also be mentioned that the D′ was generally constant in each haplotype but it decays in intervals between blocks reflecting the site of historical recombination.

Reich D E et al. (2002) [122] using analysis of data from The SNP Consortium (TSC) and the "BAC (Bacterial Artificial Chromosome) overlap" project, emphasized the presence of (haplotype block) in the human genome. Furthermore, it is generally believed that the main determinant factor of these variations is shared genealogical history. At any locus, the rate of polymorphism is governed by two factors: the local gene history and the local mutation rate. It was also concluded that gene history (e.g. recombination) is a more important force than mutation rate in creating polymorphisms.

LD analyses, which describe the haplotype block structure have many advantages [38]. Within a block, a single SNP or SNP pair may used to represent all diversity within that block, thereby reducing the typing needed for a genome scan. This method is statistically and theoretically more powerful and economically viable than traditional association studies in which one SNP is studied with a disease. Thus, haplotype blocks open a new field for association studies and finding complex disease genes. However, in the case of haplotype tagging SNPs (htSNPs), there might be one or more SNPs in a haplotype which are more informative compared to the others (Zeggini E et al. 2002) [153]. If the haplotype map of the human genome is created as proposed by the National Human Genome Research Institute, it will be a framework and reference set for association studies and population genetics. Using this map, it will be easier to compare a patient's haplotype pattern with the reference one and find out the possible risks for a particular disease, different responses to drugs and environmental factors (http://www.hapmap.org/).

1.5 Hypothesis and Plan

Previous studies (internationally) implicated *ACE* genotype in a wide variety of metabolic traits. Day et al (unpublished) have demonstrated a series of metabolic associates of the *ACE* I/D genotypes in the locally developed East and North Hertfordshire cohorts. If these associations involve the RAS, as most groups surmise, then other human polymorphisms could also be studied in similar way.

Therefore, the goal of these studies is to extend analyses systematically to human polymorphisms of the RAS genes. The first marker for which I am going to develop an assay is the A1166C in the *AGTR1*, for which there is a strong literature concerning cardiovascular risk traits. This experience will enable me to systematise this work in silico and in vitro for a more comprehensive examination of the RAS gene variations in relation to metabolic and cardiovascular risk traits with Hertfordshire cohorts. I will then study other SNPs in the *AGTR1* to investigate their associations with the above phenotypes and also resolve haplotype structures in the gene. Eventually, it is aimed to study the effects of the *AGTR1* SNPs and haplotypes on the expression of the gene to find out the appropriate explanation for observed associations.

Simultaneously, I am going to investigate SNPs in the *ACE* gene to scrutinise its haplotypes by itself and along with the *GH* polymorphisms (in collaboration with colleagues in the department). In addition to these, I would also intend to study the *AGT* M235T in the EH and NH populations.

2 Materials and Methods

2.1 Materials

2.1.1 Materials Used for PCR and Genotyping

2.1.1.1 DNA Bank

DNAs of 1112 individuals (aged 59-71) born in the East and North Hertfordshire (EH & NH) were extracted from 5ml K-EDTA venous blood, quantitated by picoGreen assay with concentrations then being equalised. Long term stock DNA aliquots were laid down and working 96-well plates of DNA dilutions to 7-10ng/μl prepared. Their anthropometric criteria were registered at birth and through their first year of life by the attending midwife. Thus this bank includes anthropometric and cardiovascular phenotypes of these individuals (http://www.mrc.soton.ac.uk/project.asp?proj=34). The number of subjects in the EH bank was: 255 Men and 146 women; and in the NH was: 408 men and 303 women [18-21,51,52].

2.1.1.1.1 DNA Samples

A) Standard DNA samples:

These samples which have been used as template for experiments and cohorts, are provided from DNA bank of NH and EH populations stored at –72°C in the Human Genetics Division at the University of Southampton. The concentration of these DNAs is about 7-10 ng/μl. These banks are really valuable and can not be replaced again, and therefore significant efforts are undertaken not to use these DNAs unnecessarily. Thus, for typing SNPs of the *ACE* gene, the products of degenerate oligo primer (DOP) amplification have been used as template.

B) Control DNA:

These DNAs are extracted from blood samples of volunteers in the department then normalised to the same concentration. These samples are used as templates for optimising reactions.

2.1.1.2 Primers

All primers have been supplied by MWG-Biotech; Ebersberg, Germany (www.mwgdna.com). The primer for the long PCR has been designed using primer 3 software and those of the ARMS reactions using TIXIS (designed by Dr. Manolis Spanakis, Human Genetics Division, University of Southampton). A List of primers is provided in Appendix 1.

2.1.1.3 Enzymes

- *Taq DNA polymerase (5u/μl)*: purchased from Promega Company; MADISON, WI, USA, catalogue number: M1665. This enzyme has been used for all PCR reactions.

- *Pwo DNA polymerase*: purchased from Roche Diagnostics, Lewes, UK. This enzyme has been used in the long PCR reaction due to its 3′→5′exonuclease activity.

- *Ttth111I*: this restriction enzyme was purchased from New England Biolabs: http://www.neb.com/neb/; catalogue number: #R0185S. It was used for genotyping *AGT* M235T.

2.1.1.4 PCR Reagents

The following reagents have been used in PCRs:

- *Deoxynucleotide Triphosphates (dNTPs),* 100mM: pH 7.5. Purchased from Promega, MADISON, WI, U.S.A. catalogue number: U1240.

- *Magnesium Chloride*, 50mM: provided with Taq DNA polymerase from Promega.

- *Betaine*: purchased from SIGMA Chemical Co. U.S.A. B-2629, [107-43-7], EC NO 203-490-6.

- *10 × PCR Buffer (minus Magnesium)*: provided with Taq DNA polymerase from Invitrogen Ltd. Paisley. U.K. (www.invitrogen.com).

- *Primers 100pmol/µl*: purchased from MWG-Biotech, Ebersberg, Germany (www.mwgdna.com).

- *10 × Long PCR Buffer*: homemade reagent using the following formula:

  - 500mM Tris-HCl, pH 8.9
  - 140mM Ammonium Sulphate

- *Trisbase, Tris (hydroxymethyl) methylamine*: purchased from BDH Lab. Supplies, Poole, UK. Product number 10315.

- *Ammonium Persulphate*: purchased from Fisher Scientific International Co. UK. Ltd. Code No A/6160/53.

2.1.1.5 MADGE Kit

Microtiter array diagonal gel electrophoresis (MADGE) is a method developed by Day I N M et al. (1994) [41].

- *96 (8×12) well "industry-standard" plates*: purchased from Thermo-Fast®, Abgene house, UK (www.abgene.com), catalogue number: AB-0800. MJ Research Inc, U.S.A. Catalogue number: MSA-5001.

- *384 well plates*: purchased from Robbins Scientific Corporation, CA. Catalogue number: 1047-00-0.

- *Microseal™ 'A' Film*: Provided by MJ Research Inc, U.S.A (www.mjr.com). Catalogue number: MSA-5001. These were used for sealing 384 well plates.

- *Glass plates*: were cut from standard glass to 110mm × 170mm sizes; purchased from local suppliers.

- *MADGE Former*: purchased from MADGEBIO Ltd, Nottingham. UK.

- *Sticky silane*: homemade; made of Ethanol 99%, Glacial acetic acid 0.5%, γ-methylacryloxypropyltrimetoxy silane 0.5%.

- *96-pin passive replicator*: purchased from MADGEBio.

2.1.1.6 Chemicals and Reagents for Making Gel

The following reagents have been used for making gels:

- *Acrylamide (Acrylamide-bis-acrylamide) solution, ratio 19:1*: purchased from Severn Biotech Ltd. UK. Catalogue number 003661.

- *Agarose (pure)*: purchased from Life Technologies, Paisley. Scotland. 15510-027.

- *Ammonium Persulphate*: purchased from Fisher Scientific International Co. UK. Ltd. Code No /6160/53.
- *TEMED (N, N, N′,N′– Tetramethylethylendediamine)*: purchased from Sigma Chemical Co. U.S.A. EC No 203-744-6.

- *10 × TBE*: consisting of the following chemicals:
    - *Trisbase, Tris (hydroxymethyl) methylamine*: purchased from BDH Lab. Supplies, Poole, UK. Prod. No 10315.

- *Boric Acid*: purchased from Fisher Chemicals UK Ltd, UK. Code B/3760/60.
- *Ethylenediamietetra-acetic acid sodium salt (EDTA)*: purchased from GDH Lab Supplies, Poole, UK. Prod. 10315.

2.1.1.7 Chemicals for Staining the Gels

- *Ethidium Bromide 10mg/ml*: purchased from Sigma Chemical Co. MO. USA. EEC No 214-984-6.

- *Vistra Green™ 10000 × concentrate*: purchased from Amersham Pharmacia Biotech UK Ltd. Buchinghamshire, UK. RPN 57860L/00/02.

2.1.1.8 Instruments

- *Fluorimager*: Model FI595, Molecular Dynamics, Sunvyvale, CA.

- *Thermal Cycler*: DNA Engine Tetrad. MJResearch Inc. USA.

- *Centrifuge*: International Equipment Company Inc, Centra® MP4, Model PTC-225; Watertown, Massachusetts, USA.

- *Microcentrifuge*: 1.5ml tube, International Equipment Company (IEC), centra® MP4, Model 230, USA.

- *Output power supply*: 200 V/A, 50-60 Hz, BIO-RAD laboratories, Inc., CA. USA.

- *Output power supply*: 250 V/A, HOWE (PowerPac Junior).

2.1.1.9 Commonly Used Materials and Chemicals

- *Pipette*: pipetman (10µl, 20µl, 200µl, 1000µl), GILSON S.A. Villiers-le-Bel, France.

- *Disposable lab tips*: 5-200µl, Cat No 94300120; 200-100µl, catalogue number: 94300220; purchased from Life Science International Ltd., Hampshire, UK. 0.1-10µl, provided by Thermolife Sciences, 012560817282, product code tp46.

- *Multiple channel finnpipette (8 channel)*: purchased from Labsystem, Helsinki, Finland.

- *Formamide dye mix (MADGE Dye)*: composed of 98% deionised formamide (SIGMA Chemical Co, USA), 10mM EDTA (pH 8.0, 0.5M), Xylene Cyanol FF (SIGMA Chemical Co, USA).

- *100 bp DNA Ladder*: purchased from Promega, Madison, USA. Catalogue number: G2101.

- *1 kb DNA Ladder*: purchased from Promega, Madison, USA. G571A 12864704.

- *Microtubes (0.5ml)*: purchased from Alpha Laboratories Ltd.Catalogue number: LW2372 and 1.5ml, catalogue number: LW 2375, Hampshire, UK.

2.1.1.10 Software

- *Phoretix software 1D*: version 4.0 (www.phoretix.com), Phoretix Intl. Newcastle UK.

- *Microsoft Excel*: http://www.microsoft.com/office/excel/.

- *TIXIS © 2001*: designed by Dr. Manolis Spanakis in the Human Genetics Division at the University of Southampton.

- *Primer 3*: http://www.genmewi.mit.du/cgi-bin/priemr/primer3_www.cgi.

- *Arlequin ver1.1 © LGB*: to calculate structure and frequency of haplotypes downloaded from the University of Geneva website.

- *2LD (two-locus LD calculator)*: http://linkage.rockefeller.edu.

- *RNA structure version 3.71*: downloaded from http://rna.chem.rochester.edu.

- *EMBOSS Software*: Used to measure the GC content of the *AGTR1* gene: http://bioweb.pasteur.fr/seqanal/interfaces/isochore.html#_data

- *LD Unit map*: http://cedar.genetics.soton.ac.uk/public_html/.

- *Phase programme*: to calculate the structure and frequency of haplotype http://www.stat.washington.edu/stephens/.

2.1.1.11 Buffers and Solutions

Long PCR Buffer

Long PCR buffer was made using 140mM Ammonium acetate (Sigma-Aldrich Company Ltd, Poole, UK) and 500Mm Tris-HCl, pH 8.9 (BDH).

PCR Buffer

Containing: 10mM Tris-HCl (pH 9.0 at 25°C), 50mM KCl and 0.1% Triton® X-100.

10×TBE

A stock solution of 10×TBE was made using Trisbase 108g, Boric acid 55g, EDTA 9.3 g, which were mixed with sufficient dH_2O and then the volume was increased to 1litre, pH 8.3. This solution was kept at room temperature in the laboratory for further use.

Betaine 5M

A stock solution of Betaine ($C_5H_{11}NO_2$) 5M was prepared using 58.55g in 100ml distilled water. It was kept at 4°C not more than two weeks.

Sticky Silane

Sticky Silane was prepared using pure ethanol 99ml, glacial acetic acid 500µl and 500µl of γ-methylacryloxypropyltrimetoxy silane. It was applied to the glass surface before placing on the gel former.

Ethidium Bromide Staining Solution

It was prepared using following formula: 20ml of 10×TBE, 20µl of ethidium bromide and 180ml of water. It was then kept in a dark tank as light degrades ethidium bromide. Gels were left for 30 minutes in this solution before undertaking electrophoresis.

Ammonium Persulphate (APS)

A 25% solution was prepared by dissolving 2.5g of Ammonium persulphate in appropriate water and then increasing the volume to 10ml.

2.1.1.12 Gels

Agarose Gel

0.7g of pure agarose was added to 100ml water and then heated in a microwave for 3 minutes on high power. Appropriate water was then added to compensate for the evaporated water.

Polyacrylamide Gel

To make a 5% polyacrylamide gel, 8.3ml of 30% acrylamide was mixed with 5ml 10×TBE, 36.7ml of H_2O, 150µl of APS (25%), 150µl of TEMED. These materials should be added to 36.7ml of water. Immediately after mixing, the solution was poured into the MADGE former

very gently to prevent bubble formation. In the next step, a glass plate, silane side downward, was overlaid. It should be left for five minutes to set.

2.1.2 Materials Used for Ratiometric Analysis

2.1.2.1 Placenta

Placenta samples were kindly provided by Dr. Rohan Lewis and Professor Iain Cameron, Princess Anne Hospital, University of Southampton, U.K. Placental samples have been collected since 2001 as a part of the Southampton Women's Study (SWS). The SWS focuses on the effect of different aspects of life style on women's health and fetal development (http://www.swsurvey.soton.ac.uk/).

2.1.2.2 DNA Extraction

- *Wizard® SV Genomic DNA Purification System*: used for DNA extraction, purchased from Promega Co., catalogue number: A2360. 2800 Woods Hollow Road. Madison, WI 53711-5399 U.S.A (www.promega.com).

- *Proteinase K*: purchased from Promega Co., catalogue number: V3021. 2800 Woods Hollow Road. Madison, WI 53711-5399 U.S.A (www.promega.com).

2.1.2.3 RNA Extraction

- *TRI REAGENT*™: purchased from SIGMA® (www.sigmaaldrich.com/), 3050 Spruce St. Saint Louis, Missouri 63103 USA; product number: T 9424.

- *Chloroform*: purchased from SIGMA® (www.sigmaaldrich.com/), 3050 Spruce St. Saint Louis, Missouri 63103 USA; product number: C 2432.

- *Isopropanol*: purchased from SIGMA® (www.sigmaaldrich.com/), 3050 Spruce St. Saint Louis, Missouri 63103 USA; product number: I 9516.

- *DNA-free™*: purchased from Ambion® (www.ambion.com); catalogue number: 1906. It contains DNase I, RNase-free (2units/µl), 10× DNase Buffer, DNase Inactivation reagent, Nuclease-free Water/0.1 mM EDTA.

- *RNA storage solution (50ml)*: purchased from Ambion® (www.ambion.com), pH = 6.4; catalogue number: 7001.

- *Homogenization Pestle (1.5ml)*: purchased from Bioquote limited, The Royal Centre, James St. York, YO10 3DW, UK. catalogue number: PMT-15.

- *Biofuge fresco (Heraeus)*: Fabr.-Nr.: 40364779, Bestell-Nr.: $75005521^{/01}$, purchased from Kendro Laboratory Products; D-37520 Osterode, Germany.

- *DU®7500 Spectrophotometer*: purchased from Beckman Coulter, Buckinghamshire, UK.

- *Hotbox Oven with fan size 2 (Baker)*: purchased from Sanyo Gallenkamp plc. Loughborough, UK.

- *RNaseZap®*: purchased from Ambion® (www.ambion.com); catalogue number: 9780. 9782.

- *Diethylpyrocarbonate (DEPC)*: purchased from Sigma Chemical Co (www.sigmaaldrich.com/). P O Box 14508, St. Louis Mo 63178 USA 314-771-5750; D-5758.
- *DEPC-treated water*: purchased from Ambion (www.ambion.com); catalogue number: 9906.

- *Filter Tips 10*: purchased from Greiner Labortechnik GmbH, A-4550 Kremsmünster, Bad Hallerstr, 32 Austria. Item-Nr. 771288.

- *Filter tips 200*: purchased from Greiner Labortechnik GmbH, A-4550 Kremsmünster, Bad Hallerstr, 32 Austria. Item-Nr. 739288.

- *Filter tips 1000*: purchased from Greiner Labortechnik GmbH, A-4550 Kremsmünster, Bad Hallerstr, 32 Austria. Item-Nr. 740288.

- *Filter tips 20*: purchased from Greiner bio-One GmbH, A-4550 Kremsmünster, Bad Hallerstr, 32 Austria. Item-Nr. 774288.

- *Dri-Block DB-1 (Heat block)*: purchased from Tecam® (Cambridge) Limited, Duxford Cambridge England. Model DB; Serial number: 1475 19.

2.1.2.4 Reverse Transcriptase (RT)

- *Omniscript^{TM} RT Kit*: purchased from Qiagen Ltd., West Sussex, RH10 9AX, UK (http://www1.qiagen.com/), catalogue number: 205113 containing:

- Ominiscript Revrse transcriptase
- Buffer RT, 10x
- dNTP Mix, 5mM
- Rnase-free water

- *Oligo dT (16mer)*: purchased from Qiagen Ltd. (http://www1.qiagen.com/), West Sussex, RH10 9AX, UK, catalogue number: SP230

- *RNasin® RNase inhibitor 40u/µl*: purchased from Promega (www.promega.com); catalogue number: N211A.

2.1.2.5 Restriction Enzymes

- *Restriction enzyme Bpu10 I*: purchased from Sibenzyme Co., catalogue number: V0149S. (www.sibenzyme.com).

- *Restriction Enzyme Mnl I*: purchased from New England Biolabs inc., catalogue number: R0163S. (www.neb.com).

2.1.2.6 Destaining with $MgSO_4$

- *Magnesium Sulfate (1.00M)*: purchased from Sigma Chemical Co (www.sigmaaldrich.com/). P O BOX 14508, St. Louis Mo 63178 USA 314-771-5750; D-5758.

2.1.3 Materials Used for TaqMan Assay

- *Human 18S rRNA (20X)*: purchased from ABI Co. (www.appliedbiosystems.com), P. N.: 4319713E, Lot. No. 0309091, 7 Kingsland Grange Woolston, Warrington, Cheshire WA1 4SR, U.K.
- *Assays- by - Design™ Product*: GX V- Scale, product number: 4331348, S.O. No. 1450612, Plate I. D. 163139 (www.appliedbiosystems.com).

- *384- well PCR plate*: Thermo – Fast® 384, catalogue number: TF – 0384/BC128, purchased from abgene Co., ABgene House, Blenheim Rd., Epsom, Surrey KT19 9AP, UK. (www.abgene.com).

- *Optical Adhesive Covers*: product number: 4311971, purchased from ABI Co., Foster City, CA 94404.

- *ABI PRISM*: 7900 HT Sequence Detection System, purchased from ABI Co. (www.appliedbiosystems.com).

- *qPCR™ Mastermix*: RT – QP2X – 03 – 175^{+}, purchased from EGT group (www.eurogentec.com).

2.1.3.1 Software Used for TaqMan Assays

- *SDS Software*: used for analysis purchased from ABI Co. (www.appliedbiosystems.com).

- *Probability Calculator*: http://www.ncss.com/download.html.

- *File builder*: downloaded from ABI Co. website (www.appliedbiosystems.com).

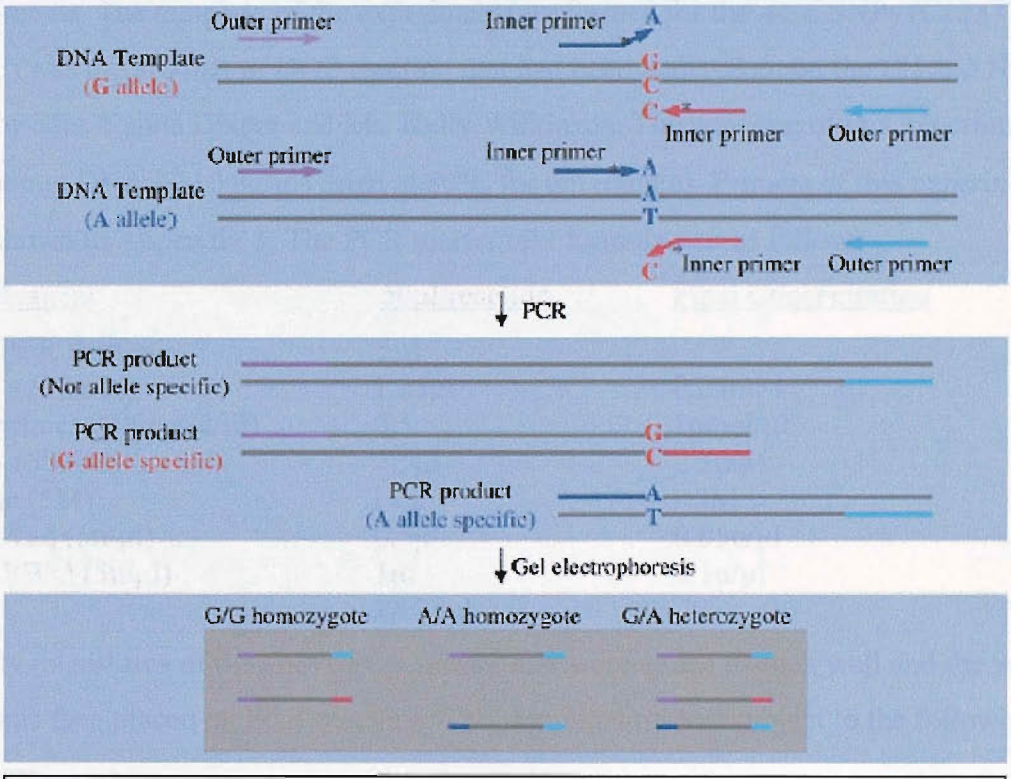
2.2 Methods

2.2.1 Methods Used for PCR and Genotyping

2.2.1.1 Amplification Refractory Mutation system (ARMS)

This is a rapid and valuable method for detection of point mutations or small deletions. Because of its high accuracy, it is generally used in clinical diagnosis. Its specificity and sensitivity has also made it as a useful method and ideal for routine applications. The basic idea behind this method is that primers with a mismatch at 3′ end can not amplify. Under normal condition, primers must be completely matched at the 3′ end to allow amplification. The rate of extension from matches vs. mismatched templates differs significantly: about 10^3 to $>10^6$; and a deliberate mismatch at -2 position of the ARMS primer increases the specificity of the reaction. In this condition, the amplification of the test DNA occurs if the target sequence is present in the test sample and does not occur when the target sequence is not present [127]. In the case of tetra primer ARMS [148], four primers (two outer primers and two allele specific ones) are included in one reaction (Figure 2.1), while in a three primer ARMS, there would be two reactions: two outer primers with one allele specific primers were included in each reaction.

Figure 2.1: Schematic Representation of Tetra Primer ARMS



Primers design in a tetra primer ARMS assay: Two outer primers and two allele specific ones (each for one allele of the SNP). On the gel, apart from a PCR product band (from two outer primers), there are two allele specific bands, depending on the genotype of the subject, with different sizes. Adopted from Ye S et al. (2001) [148]. The stars show the positions of mismatches in primers.

2.2.1.2 DOP (whole genome amplification)

Because of the importance of the NH and EH DNA banks (most individuals not now available for re-bleeding), it is of crucial importance to use methods to save the stock DNA. DOP is one such method in which, the genomic DNA is amplified randomly using specially designed primers and PCR formulae. In this way, we can keep the reaction products for future experiments. The template of the experiments performed for the *ACE* SNPs (C1237T and A-5466C) was the product of DOP reaction that had been undertaken on the EH and NH DNA bank by Mrs. Sylvia Diaper and Ms. Kelly Wilkinson. The template of this experiment was 2µl of genomic DNA (7-10 ng/µl) dried at 80°C for ten minutes. Primers of this experiment have been shown in Appendix 1. The PCR master mix formula was as follows:

PCR Reagent	50µl/reaction	Final Concentration
Long PCR Buffer	5µl	
dNTP's (10mM)	1.25µl	0.25mM
DOP primer (100pmol/µl)	0.5	1pmol/µl
$MgCl_2$ (50mM)	2.5µl	2.5mM
Betaine (5M)	13µl	1.3M
Gibco Taq (5u/µl)	0.5µl	0.05u/µl
1/250 PWO (5u/µl)	1µl	0.1u/µl
H_2O	26.25µl	

Fifty microlitres of the above PCR master mix were added to each well and the 96 well plate was then placed on PCR machine. This combination was subject to the following cycling program:

94°C	5mins	
94°C	1min	× 7
30°C	1min	
72°C	3mins	
94°C	1min	× 27
60°C	1min	
72°C	3mins	

2.2.1.3 Genotyping Markers of the RAS

It was aimed to type a panel of markers in the RAS (Table 2.1) and study their associations with anthropometric and cardiovascular phenotypes in the NH and EH populations. In addition to this, the data was used for LD and haplotype analysis (for both *ACE* and *AGTR1*). In the case of *ACE* (in collaboration with colleagues in the department) haplotype analyses has been performed within the *ACE* gene and with markers in the *GH* gene. Haplotype phenotype study for the *AGTR1* haplotypes was also carried out.

Table 2.1: Markers typed in this project on NH and EH populations

Gene	Marker Typed	Code in the Server	Method	Template
AGT	M235T	AGTV001	Three primer ARMS assay & Restriction digestion	Genomic DNA
AGTR1	C-521T A-153G L191L A1166C	AT1RV003 AT1RV004 AT1RV002 AT1RV001	Three primer ARMS assay " " " " " " " " " " Tetra primer ARMS assay	1/100 dilution of long PCR
ACE	C1237T A-5466C	ACEV003 ACEV002	Three primer ARMS assay	DOP

2.2.1.3.1 Genotyping of SNPs in the *AGTR1* Gene

Four SNPs of the *AGTR1* were typed in this project: C-521T, A-153G, L191L and A1166C. Apart from A1166C, which was performed using tetra-primer ARMS assay, the other three were studied using three primer ARMS assays. To increase the specificity of allele specific primers in ARMS assays, it is recommended to perform the experiment on the products of long PCR. To achieve this, two long PCRs were performed: one on exon 5 and 3′ UTR (= 3kb) for L191L and A1166C, and the other (= 1.8kb) on 5′ UTR for C-521T and A-153G. Sequence used for these experiments was downloaded from GenBank, accession number: AF245699.

2.2.1.3.1.1 Long PCRs

There were two long PCRs:

I) This experiment amplified 3kb consisting exon 5 of the *AGTR1* gene, which was then used as the template for the ARMS reactions. The sequence was downloaded from Genbank (http://www.ncbi.nlm.nih.gov), accession number: AF245699. Templates were 3µl (6-7ng/µl) of genomic DNA. They were dried at 80°C for ten minutes before loading. Then 20µl of the PCR mix was aliquotted into each well of 96 well plates. Optimisation was performed with different $MgCl_2$ concentrations (1.5mM, 2.0mM and 2.5mM) and annealing temperatures (ranging from 60°C to 72°C). The experiment was performed using 96-well plate. This experiment was performed in a 20µl volume PCR reaction using the protocol that was developed by Ms. Lesley Hinks (Human Genetic Division, University of Southampton):

Long PCR Reagents	20µl × 1 reaction	Final Concentration
10 × long PCR Buffer	2µl	
10mM dNTP	0.5µl	0.25mM
100pmol/µl primers	0.08+0.08	0.4pmol
50mM $MgCl_2$	0.8µl	2mM
5m Betaine	5.2µl	1.3mM
Gibco Taq (5u/µl)	0.2µl	0.05u/µl
1/250 PWO (5u/µl)	0.4µl	0.1u/µl
H_2O	10.74	

The thermocycler programme was as follows: 94°C for 2 minutes as the initial denaturing temperature step; 94°C for 20sec, 65°C for 30sec, 68°C for 3 minutes, last three steps were repeated for 35 cycles followed by a final extension time at 68°C for 20 minutes. PCR products were mixed with MADGE dye (2µl with 5µl PCR product) and then loaded on the gel. Electrophoresis was performed using a 0.7% agarose gel in a submerged tank at 100V for 30mins consisting Ethidium Bromide (400ml of 1× TBE containing 12µl of ethidium bromide 10mg/ml).

II) This experiment amplified 1863bp of 5′ UTR of the *AGTR1* gene. Primers were designed using Primer3 software and their sequences and positions are provided in Appendix 1. Template optimisation (annealing temperatures ranging from 50°C to 64°C) and reaction steps including electrophoresis were the same as the long PCR on the 3′ UTR. Apart from $MgCl_2$ concentration, which was 2.5mM with adjusted water of 9.5µl, the rest of the PCR formula was the same as for the long PCR on the 3′ UTR.

The thermocycler programme was as follows: 94°C for 2 minutes as the initial denaturing temperature step; 94°C for 20sec, 63°C for 30sec, 70°C for 2 minutes, last three steps were repeated for 35 cycles with a final extension time at 68°C for 20 minutes.

2.2.1.3.1.2 Genotyping of the *AGTR1* A1166C

This SNP is located in 3′ UTR of the *AGTR1* gene. 2µl of 1/100 dilution of long PCR products were taken as templates for *AGTR1* tetra-primer ARMS reaction [148] and were dried at 80°C for ten minutes before loading the PCR mixture. Optimisation of the reaction was performed using different concentrations of magnesium (1.5mM, 2mM and 2.5mM), and a range of temperatures (60°C to 72°C) for the annealing temperature and different concentrations of four primers. The sequence of primers are listed in Appendix 1. 10µl of PCR master mix was loaded to each well containing dried template.

The oligos formula was: 2.5µl of allele A specific primer, 10µl of allele C specific primer and 0.1µl of each forward and reverse primers. The PCR cycling program began with an initial denaturing temperature step at 94°C for 2 minutes followed by 25 cycles of 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute with a final extension at 72°C for 2 minutes. PCR formula was as follows:

PCR Reagents	10µl × reaction	Final Concentration
10 × PCR Buffer	1µl	1 × PCR Buffer
1% W1	0.5µl	
50mM $MgCl_2$	0.4µl	2 mM
5mM dNTP	0.4µl	0.2 mM
Oligos	0.22µl	2.2 pmol/µl
Gibco Taq (5 u/µl)	0.1µl	0.05 u/µl
Water	7.38µl	

5µl of PCR product was mixed with 2µl of MADGE dye and then loaded on 5% polyacrylamide gel, pre-stained with ethidium bromide. Electrophoresis was performed at 150V for 30 minutes in dry boxes.

2.2.1.3.1.3 Genotyping of *AGTR1* L191L (T ⟶ C)

This SNP is located in coding region (exon 5) of the *AGTR1*. This SNP does not change any amino acid because in either case the amino acid Leucine is coded (i.e. CTC/T). The experiment was performed using three primer ARMS method. Templates were 3µl of 1/100 dilution of long PCR product of 3′ end (consisting exon 5 and 3′ UTR of the gene) of the *AGTR1*, dried at 80°C for 10 minutes before loading the PCR mixture. Optimization of the reaction was undertaken using different concentrations of magnesium (1.5mM, 2.0mM, 2.5mM) and a range of temperature (50°C to 62°C) for the annealing temperature with different concentrations of primers. The optimization was also performed first on pooled control DNA, then pooled LPCR product and finally on individual LPCR products. The oligo formula was a mixture consisting of 10µl of each primers. PCR formula as follows:

PCR Reagent	10µl per Reaction	Final Concentration
PCR Buffer	1µl	
Betain (5M)	2.6µl	1.3M
$MgCl_2$ (25mM)	0.8µl	2mM
dNTPs (5mM)	0.4µl	0.2mM
Oligos (100pM)	0.2µl	2pM
Taq Polymerase (5U/µl)	0.1µl	0.05U/µl
Water	4.9µl	

Primer sequences are presented in Appendix 1. It should also be mentioned that the experiment was optimised for the 384 well MADGE format plate. 10μl of PCR master mix was loaded to each well containing dried template and sealed with microseal^{TM} `A` film. The thermocycler program was: initial denaturing step at 92°C for 2 minutes followed by 35 cycles: 92°C for 30 Sec, 51°C for 30 Sec, 70°C for 30 Sec; with a final extension step at 70°C for 2 minutes. The electrophoresis steps are the same as genotyping the *AGTR1* A1166C.

2.2.1.3.1.4 Genotyping *AGTR1* C-521T

This SNP is located in 5′ UTR of the *AGTR1*. Allele specific primers were designed using Tixis software and up and down control primers were downloaded from Inserm website (http://genecanvas.idf.inserm.fr/;) their sequences are presented in Appendix 1. The template of this experiment was 3μl of 1/100 dilution of long PCR of the 5′ UTR, this was dried at 80°C for ten minutes before loading the PCR. Optimization was done using different $MgCl_2$ concentrations (1.5-2.5mM) and annealing temperatures (ranging from 45°C to 60°C) for 384 well plate. The PCR formula was as follows:

PCR Reagent	10μl per Reaction
PCR Buffer	1μl
Betaine (5M)	2.6μl (1.3M)
$MgCl_2$ (25mM)	0.8μl (2.0mM)
dNTPs (5mM)	0.4μl (0.2mM)
Oligos(100pM)	0.2μl (2.0pM)
Gibco Taq (5u/μl)	0.1μl (0.05u/μl)
Water	4.9μl

An oligo mix of 16μl of allele specific primer, 0.4μl of up-control, and 10μl of down-control primer was prepared and eventually 0.2μl of it was used in each reaction. 10μl of PCR mixture was loaded in each well containing the dried DNA, and after sealing the plate with microseal^{TM} `A` film, it was placed on PCR machine. The thermocycler programme using 384-plate was: 92°C for 2 minutes as initial denaturing temperature step; 92°C for 30sec, 46°C for 30sec, 70°C for 30sec, last three steps were repeated for 25 cycles with a final extension time

at 70°C for 2 minutes. The electrophoresis steps are the same as genotyping the *AGTR1* A1166C.

2.2.1.3.1.5 Genotyping *AGTR1* A-153G

Apart from the primers (presented in Appendix 1), other steps were the same as genotyping *AGTR1* C-521T.

2.2.1.3.2 Genotyping of SNPs in the *ACE* gene

Two SNPs were genotyped in the *ACE* gene: C1237T and A-5466C. Both experiments were performed using three primer ARMS assay. The templates of these assays were products of DOP. *ACE* sequence was downloaded from GenBank; accession number: AF118569.

2.2.1.3.2.1 Genotyping of the *ACE* C1237T

This SNP located in exon 8 of the *ACE* gene. The experiment was performed using three-primer ARMS method. 2μl of DOP product (15-20ng DNA) were aliquotted to each well and dried at 80°C for ten minutes. Primers were designed using TIXIS software. Optimisation of the reaction was undertaken with different magnesium concentrations (1.5mM, 2.0mM and 2.5mM), annealing temperature ranging from 60°C to 72°C and different primer concentrations. It is worth mentioning that reaction optimisation was performed on pooled control DNAs, individual DNAs, pooled DOPs and individual DOPs respectively. Oligos formulae are 10μl of each of allele specific and down control primers and 0.75μl of up control one. Primers sequences are shown in Appendix 1.

It should also be mentioned that the experiment was optimised for the 384 well MADGE format plates. 10μl of the above PCR mix was aliquotted to each well containing dried template sealed by microseal ™ `A` film before placing on the thermal cycler (DNA engine Tetrad, MJ Research). The cycling programme was: initial denaturing step at 92°C for 2

minutes followed by 35 cycles of 92°C for 30Sec, 65°C for 30Sec and 70°C for 30Sec; with final extension time at 70°C for 2 minutes. The PCR formula was as follows:

PCR Reagent	10µl/reaction	Final Concentration
PCR Buffer	1µl	
Betaine (5M)	2.6µl	1.3mM
$MgCl_2$ (50mM)	0.3µl	1.5mM
dNTPs (5mM)	0.4µl	0.2mM
Oligos (100pmol/µl)	0.2µl	2pmol/µl
Gibco Taq (5u/µl)	0.1µl	0.05u/µl
H_2O	5.4µl	

2µl of the PCR products were then mixed with 5µl of MADGE dye and were loaded on to a 384 well MADGE format polyacrylamide gel using 96-pin passive replicator. Electrophoresis was performed in 11 minutes at 150v in dry box (MADGE technique).

2.2.1.3.2.2 Genotyping of the *ACE* A-5466C

This SNP is located in 5′ region of the *ACE* gene. All steps of this experiment are the same as genotyping of the *ACE* T1237C except for $MgCl_2$ concentration, which was 2.0mM (0.4µl, and adjusted water of 5.3µl). Oligos formulae was: 10µl of each of allele specific and down control primers and 3µl of up control one. Primer sequences are available in Appendix 1. The thermocycler program was: initial denaturing step at 92°C for 2 minutes followed by 35 cycles: 92°C for 30Sec, 59°C for 30Sec and 70°C for 30Sec; with a final extension step at 70°C for 2minutes.

2.2.1.3.3 Genotyping of the *AGT* M235T

This SNP, M235T (SNP ID: rs699); C (Thr) to T (Met), is located in exon 2 of the *AGT* gene. Genotyping was performed using two methods: three primer ARMS assay and restriction enzyme digest.

For the ARMS assay, the sequence was downloaded from GenBank, accession number: X15324. Control primers were downloaded from GenCanvas websit: http://genecanvas.idf.inserm.fr/. Allele specific primers were designed using Tixis software. PCR formula was as follows (10µl per reaction): PCR Buffer 1µl, Betaine (5M) 2.6µl (1.3M), $MgCl_2$ (50mM) 0.5µl (2.5mM), dNTPs (5mM) 0.4µl (0.2mM), Oligos 0.2µl (of a mixture of primers UP/CO 10µl + DO/CO 10µl + T/F or C/F 0.6µl), Taq DNA Polymerase 0.1µl, water 5.2µl. The tetrad programme was: 92°C for 2 minutes, 92°C for 30 seconds, 60.5°C for 30 seconds, 70°C for 30 seconds (last three steps were repeated for 33 times) and 70°C for 2 minutes.

For restriction enzyme, the sequence was downloaded from GenBank, accession number: NM_000029. A digest mutagenesis (highlighted in primer sequence) was induced 3 (Figure 2.2) nucleotides after SNP position to create a cut point for enzyme Tth111I which cuts the C allele in the new sequence. Frossard P M et al. (1998) [56] have studied M235T using this enzyme, but we used a longer primer to obtain a better resolution of bands on the gel.

Figure 2.2: The Position of the Primers for Genotyping *AGT* M235T Using *Tth111I*

TGGACCACACAGCTGACAGGCTACAGGCAATCCTGGGTGTTCCTTGGAAGGACAAGAACTGCACCTCCCG
GCTGGATGCGCACAAGGTCCTGTCTGCCCTGCAGGCTGTACAGGGCCTGCT*AGT*GGCCCAGGGCAGGGCT
GATAGCCAGGCCCAGCTGCTGCTGTCCAYGGTGGTGGGCGTGTTCACAGCCCCAGGCCTGCACCTGA
AGCAGCCGTTTGTGCAGGGCCTGGCTCTCTATACCCCTGTGGTCCTCCCACGCTCTCTGGACTTCACAGA
ACTGGATGTTGCTGCTGAGAAGATTGACAGGTTCATGCAGGCTGTGACAGGATGGAAGACTGGCTGCTCC
CTGAYGGGAGCC*AGT*GTGGACAGCACCCTGGCTTTCAACACCTACGTCCACTTCCAAGGGAAGATGA
AGGGCTTCTCCCTGCTGGCCGAGCCCCAGG*AGT*TCTGGGTGGACAACAGCACCTC*AGT*GTCTGTTCCCAT

Shown is a part of the sequence for *AGT*; accession number: NM_000029. The positions of two SNPs have been represented with red Y: first for T174M (rs4762) and second Y for M235T (rs699). Primers used for genotyping *AGT* M235T using *Tth111I* have been highlighted in yellow with the two nucleotide mutagenesis (AC in stead of GA), in blue, to induce a restriction site for *Tth111I*.

Optimization was undertaken using different concentration of Magnesium and annealing temperatures. The PCR formula for 10µl per reaction was: PCR Buffer 1µl, Betaine (5M) 2.6µl (1.3mM), $MgCl_2$ (25mM) 0.8µl (2mM), dNTPs (5mM) 0.4µl (0.2mM), Oligos 0.1µl of each primers, Taq DNA Polymerase 0.1µl and water 4.9µl. The tetrad programme was: 92°C for 2 minutes, 92°C for 30 seconds, 69°C for 30 seconds, 70°C for 30 seconds, last three steps were repeated for 35 times with 70°C for 2 minutes as final step.

Electrophoresis was performed on 5% polyacrylamide gel at 150V for 25 minutes according to the MADGE method. Gel images were transferred to Phoretix software and genotyping was done manually. Genotyping results were transferred to Excel software for assessing HW compatibility and further statistical analysis.

2.2.1.4 Statistical Analysis

Genotype phenotype association studies were performed using both analysis of variance (ANOVA) and linear regression models. The purpose of these analyses was to explore the relationship between genotyped SNPs (*AGTR1* A1166C, L191L, C-521T and A-153G and also *AGT* M235T), and resolved haplotypes in the *AGTR1* with various anthropometric and cardiovascular phenotypes. Two datasets were used:

1 - Toplevel.dta, which contains data on cardiovascular phenotypes e.g. blood pressure, pulse rate, glucose and insulin levels for the EH cohort.
2 - Avan3.dta, which contains data on ageing phenotypes for the NH cohort.

The NH and the EH data files contain data on early life and adult anthropometry. Therefore, both files were combined for an analysis of genotype in relation to anthropometric phenotypes on men and women separately. A separate analysis was carried out using the toplevel.dta (EH) database to investigate the effect of SNPs genotypes on different cardiovascular phenotypes.

2.2.1.5 LD and Haplotype Analysis

The 2LD software (http://linkage.rockefeller.edu) was used for pairwise LD study among the *ACE* SNPs and the *GH* microsattelite and SNPs. *GH* Microsatellite and SNPs were obtained from Human Genetics Division (genotyped by: Holloway, Voropanov, Patel and King).

Genotyped SNPs data in the *AGTR1* gene and *ACE* gene were subjected to LD and haplotype analyses (using either Arlequin or Phase programmes). In the case of *ACE*, these analyses were performed with markers in the *GH* gene. In the case of *AGTR1*, data were also analysed for LD unit map (to resolve the presence of haplotype block) and haplotype phenotype association.

2.2.1.6 RNA Structure

RNA does not only have a linear structure, but also has a spatial structure which is of crucial importance for its functions, and it is likely to be affected by mutations. To evaluate the effect of *AGTR1* A1166C and L191L, and their haplotypes on its RNA structure, one transcript (ENST00000326871) was chosen from ensemble (www.ensembl.org) and after inducing mutations, RNA structural changes were studied.

2.2.1.7 LD Unit Map

To test the idea of the haplotype block [97] in the *AGTR1* gene, the data was submitted to the LD unit map programme (http://cedar.genetics.soton.ac.uk/public_html/).

2.2.1.8 GC Content Assessment

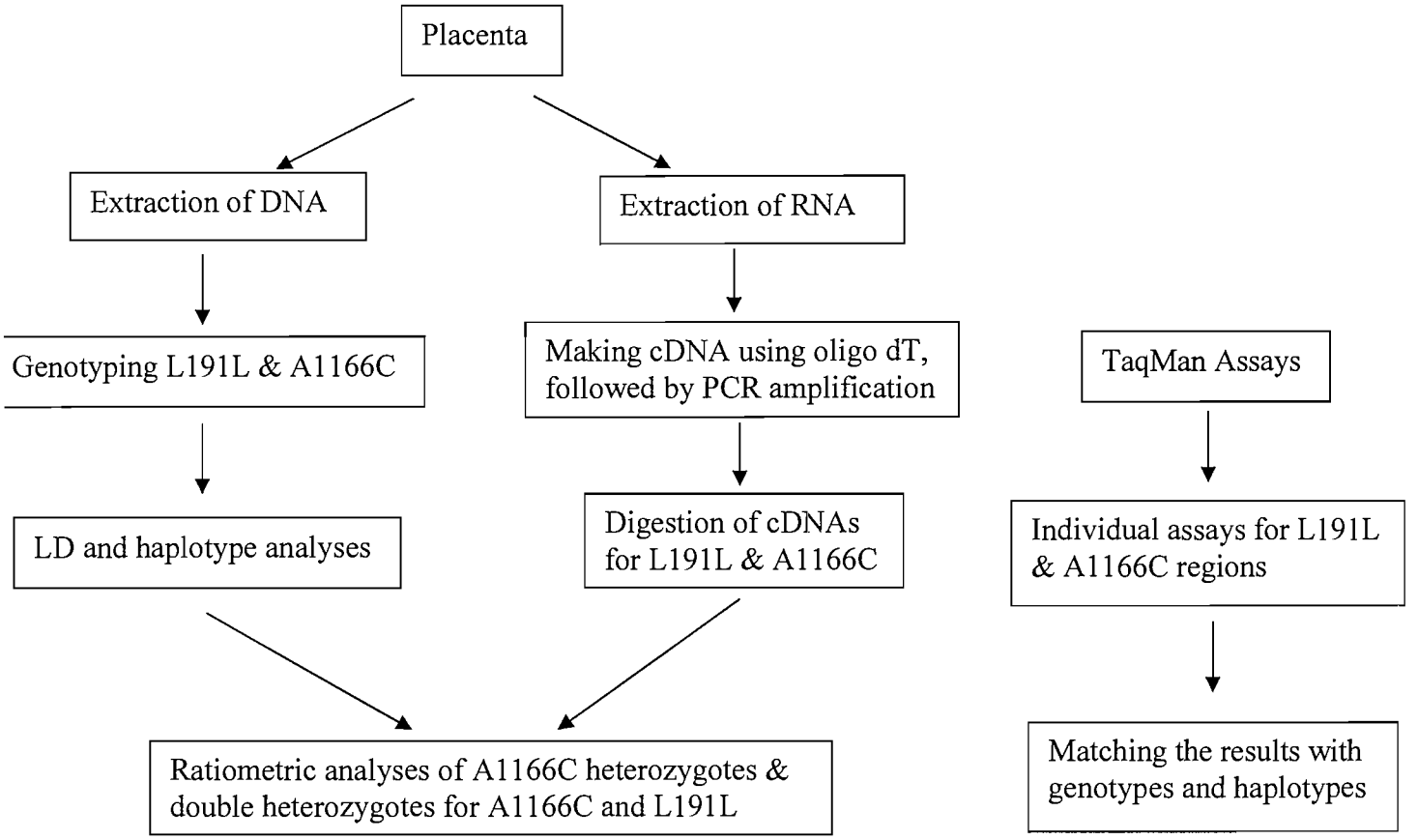
It has been suggested that the gap between two haplotype blocks are GC rich regions [47]. To test this idea, the *AGTR1* sequence (accession number: AF245699) was analysed using relevant

programme (http://bioweb.pasteur.fr/seqanal/interfaces/isochore.html#_data) to calculate the GC percentage in the *AGTR1* gene.

2.2.2 Ratiometric Analysis

Figure 2.3 Shows the plans for ratiometric and TaqMan assay.

Figure 2.3: The Workflow of the Expression Studies on *AGTR1*



2.2.2.1 DNA Extraction

30mg of placenta was taken and extraction was performed according to the manufacturer's instruction. DNA samples were quantified by spectrophotometry and then stored at –20°C.

2.2.2.2 RNA Extraction

80mg of placenta was taken and extraction was carried out according to the manufacturer's instructions. All RNA samples were treated with DNA-free™ according to manufacturer's instructions and after quantification by spectrophotometry were diluted in RNA storage solution (50ng/µl) and stored at -70°C. Contamination of RNA with DNA was checked with primers spanning exon-intron boundary.

2.2.2.3 Genotyping A1166C Using *Bpu10 I*

30ng of extracted DNA from placenta samples was used as template for PCR. Primer sequences are presented in Table 1 in Appendix 6. Templates were put in a 96 well plate and dried at 80°C before loading the PCR master mix. PCR formula was as follows:

PCR Reagent	20µl per reaction	Final Concentration
PCR Buffer	2µl	
Betaine (5M)	5.2µl	1.3M
$MgCl_2$ (25mM)	1.6µl	2mM
dNTPs (5mM)	0.8µl	0.2mM
Oligos (100pM/µl)	0.2µl+0.2µl	1pM
Taq DNA polymerase (5U/µl)	0.2µl	0.05U
Water	9.8µl	

After loading the PCR master mix in each well containing dried template, the plate was sealed with a rubber mat and placed on thermal cycler.

PCR thermal cycling program: First denaturising step at 92°C followed by 92°C for 30 seconds, 61°C for 30 seconds, 70°C for 30 seconds, repeated for 35 cycles with a final step of 70°C for 2 minutes.

10µl of PCR products was subjected to digestion with 1U of *Bpu10 I* (5U/µl) at 37°C for 16 hours. Digestion products were then loaded on a 5% polyacrylamide gel pre-stained with ethidium bromide and electrophoresis was performed at 150V for 12 minutes in a dry box (direct electrode contacts onto gel). Gels were scanned using a fluoroimager FI595 (Molecular Dynamics, Sunvyvale, CA) and the pictures were transferred to Phoretix software for further analysis.

2.2.2.4 Genotyping L191L (C to T) Using *Mnl I*

Apart from the primers, (Table 1 in Appendix 6), restriction enzyme (which was 1U of *Mnl I*) and electrophoresis time (which was 14 minutes), the formula and steps for genotyping of L191L were the same as for genotyping A1166C using *Bpu10 I.*

2.2.2.5 Haplotype and LD Study of A1166C and L191L in Placenta Samples

Genotypes of L191L and A1166C were subjected to 2LD and Arlequin Ver1.1 software to resolve the level of LD, structure and frequency of haplotypes.

2.2.2.6 Destaining with $MgSO_4$

To minimise background fluorescence of ethidium bromide, gels were equilibrated with 500ml of 1mM $MgSO_4$ for 20 minutes the destaining agent.

2.2.2.7 cDNA Synthesis

500ng (50ng/µl) of RNA was used as template and cDNAs were synthesised using oligo (dT), according to the manufacturer's instruction.

2.2.2.8 Standard Curve for A1166C

To perform the ratiometric analysis of A1166C on cDNA, a standard curve was necessary. To make the standard curve, a series of dilutions of AA and CC (30ng as whole for each template) genotypes of the above SNP were prepared from genomic DNA. DNA templates were provided from Laboratory stocks (kind gift from Patricia Briggs in Professor Day's Laboratory) with concentrations of 10ng/µl. DNA was then arranged in a PCR plate as shown in Table 2.2. PCR and digestion formula were the same as for the genotyping experiments for A1166C using *Bpu10 I* (2.2.2.3). Along with the above dilutions, nine heterozygotes (AC) were amplified simultaneously.

Table 2.2: AA and CC dilution series of A1166C using control DNAs for making standard curve

	1	2	3	4	5	6	7	8	9	10	11
AA	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0
CC	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

This experiment was performed in duplicate and stained with ethidium bromide followed by loading in three different conditions: first load on the gel, second load on the same gel and third load on different gel (Figure 3 and 4 in Appendix 8). In the next step, gels were subjected to two steps of destaining with $MgSO_4$ (1.3.6) and one step re-staining with Vistra Green™. Figures 3 and 4 in Appendix 6 show these steps.

After the gels were scanned in the fluoroimager, the pictures were transferred to Phoretix software for further analysis. There were 23 (1 gel was damaged and thus left out of the analysis) images including different staining and loadings. In Phoretix, bands were detected and background removed, and volume (or pixels) of each band was then considered for

analysis (Figure 6 in Appendix 6). The proportions of bands C/(C+A) were plotted on the Y axis against the proportion of C/A, which is the product of CC/AA in each individual column of Table 2.2, on X axis. Finally, means of each reading were calculated and then matched against C/A proportion.

2.2.2.9 Ratiometric Analysis of A1166C on cDNA

The templates for this experiment were 3µl of each of the 27 cDNAs of placenta samples that were heterozygous for A1166C. These were selected along with 3 AA, 3 CC and 3 water templates. The PCR and digestion formula are the same as genotyping of A1166C using *Bpu10 I* (2.2.2.3) except that the template was 3µl of cDNA and water volume was 6.8µl. This experiment was performed in duplicate. The PCR products were stained first with ethidium bromide followed by two steps of destaining with $MgSO_4$ and one step of re-staining with Vistra Green™. Figures 7 and 8 in Appendix 6 show the gels in different steps of this analysis.

There were 16 gels including different staining and loadings. After scanning in the fluoroimager, gels pictures were transferred to Phoretix where bands were detected and backgrounds removed. The volume of each band was then considered for analysis (Figure 6 in Appendix 6). The mean proportions of bands C/(C+A) was found on Y axis of standard curve calculated with genomic DNA and matched against the proportion of C/A on X axis of the same curve to find the proportion of C/A in the cDNA.

2.2.2.10 Standard Curve for L191L

To make a standard curve, a series of dilutions of TT and CC (30ng as whole for each template) genotypes of L191L were prepared from genomic DNA. DNA templates were provided from Laboratory stocks (kind gift from Patricia Briggs in Professor Day's Laboratory) with concentrations of 10ng/µl. The samples were then arranged on PCR plate as outlined in Table 2.3. The digestion formula was the same as for genotyping L191L using *MnI 1* (see 2.2.2.4). Along with the above dilutions, five heterozygotes (TC) were amplified and analysed simultaneously.

Table 2.3: TT and CC dilution series of L191L using control DNAs for making standard curve

	1	2	3	4	5	6	7	8	9	10	11
TT	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0
CC	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

The experiment was performed in duplicate. Three steps of loadings on different gels were followed by two steps of destaining with $MgSO_4$ and one step of re-staining with Vistra Green™ (Figure 5 in Appendix 6).

After the gels were scanned in the fluoroimager, pictures were transferred to Phoretix software for further analysis. There were 24 gels which included different stainings and loadings. In Phoretix, bands were detected and backgrounds removed, the volume of each band was then quantified for analysis (Figure 6 in Appendix 6). Two proportions of bands C1/(C1+T) and (C1+C2)/(C1+C2+T) were plotted on the Y axis against the proportion of C/T, which is the product of CC/TT in each individual column of Table 2.3 on the X axis. The reason that two proportions were studied was to investigate the possible interference of band C2 with standard curve. Finally, means of each reading were calculated and plotted against C/T proportion and then regressed.

2.2.2.11 Ratiometric Analysis of L191L on cDNAs

16 (unselected) cDNAs identified as heterozygous for both A1166C and L191L along with 3 TT, 3 CC and 5 water samples. These samples were then subjected to digestion with *Mnl 1* (see 2.2.2.4). 3µl of cDNAs were used as template for PCR with the same formula as described for the L191L standard curve. The experiment was performed in duplicate with two loadings on two different gels followed by two steps of destaining with MgSO4 and one re-staining step with Vistra Green™ (Figures 9 and 10 in Appendix 6); 16 gels in total. After the gels were scanned in the fluoroimager, they were transferred to Phoretix software where bands were detected and backgrounds removed (Figure 6 in Appendix 6). Bands volumes were then taken and proportions of bands C1/(C1+T) and (C1+C2)/(C1+C2+T) were calculated. Means of the

products of these proportions were found on the Y axis of the standard curve made with genomic DNA and matched with appropriate value on the X axis.

2.2.2.12 Statistical Analysis

These analyses consist of calculating descriptive statistics such as mean, standard deviation (sd), coefficient of variance (C.V) and t test. Analyses of variability were also performed as follow:

1. To check the variability between experiments undertaken for the standard curve (using control DNA) and those undertaken on cDNA templates, a t test analysis was achieved using two sets of data with similar experimental conditions:
A) Second loading of heterozygote (for A1166C) control DNA samples, stained with ethidium bromide.
B) Second loading of heterozygote (for A1166C) cDNA samples, stained with ethidium bromide.

2. To check the variability between two different loadings of one PCR, a comparison was performed between first and second loadings of the same digested heterozygote cDNA after the first destaining step, and the values of band ratios C/(C+A) were then subtracted from each other, and the variance of these differences was calculated.

3. To evaluate the variability of two different PCRs, a comparison was performed between first loading and first destaining steps of two PCRs. PCRs were digestion of 27 heterozygote cDNA for A1166C. The values of C/(C+A) of two PCRs were subtracted from each other, and the variance of these differences was calculated.

2.2.3 TaqMan Assay

2.2.3.1 Experimental Design

To study the effect of two SNPs, *AGTR1* L191L and A1166C, on the expression of the *AGTR1* gene, two assays were designed (Figure 2.3). The gene sequence of *AGTR1* was downloaded from Genbank, Acc. No. AF245699. 300bp around each SNP along with suggestive locations for the probes were selected, and modified (i.e. file format compatibility) according to file builder software format. Assays were then ordered to Applied Biosystems (ABI). The submitted sequence was arranged in a way that each SNP was in an amplified segment without overlapping either the primers or probe (Figure 2.4). The company then sent back the working assays in which the probes were FAM labelled along with experimental protocols.

To find the right concentration of the cDNA template, the experiment was carried out with different amounts of cDNA: 1.5, 2.0 and 3.0μl. 2.0μl was chosen for subsequent explained assay. The experiment was undertaken in one plate for both A1166C and L191L and 18S [labelled with VIC was used for endogenous control as described by Miller B C et al. (2003) [103]]. Three NTCs (Non-template control) were also considered to verify the lack of contamination in the experiment. Failed samples were repeated in duplicate.

After loading templates and PCR reagents, the plate was sealed with an optical adhesive cover and centrifuged to prevent any bubble formation, and placed in ABI Prism 7900 HT machine. The PCR formula was: cDNA 2μl, qPCR™ Mastermix 10μl, 20 × assay mix 1μl and 7μl of RNase-free water; 20μl in total. Thermal cycler conditions were: 2 minutes at 50°C followed by 10 minutes at 95°C, 15 seconds at 95°C and 1 minute at 60°C, repeated for forty times.

Figure 2.4: Design of TaqMan Assays Showing Sequence of the *AGTR1*, SNPs, Primers and Probes

```
49261 cattgatcgatacctggctattgttcacccaatgaagtcccgccttcgacgcacaatgct
49321 tgtagccaaagtcacctgcatcatcatttggctgctggcaggcttggccagtttgccagc
49381 tataatccatcgaaatgtatttttcattgagaacaccaatattacagtttgtgctttcca
49441 ttatgagtcccaaaattcaaccctYccgatagggctgggcctgaccaaaaatatactggg
49501 tttcctgtttccttttctgatcattcttacaagttatactcttatttggaaggccctaaa
49561 gaaggcttatgaaattcagaagaacaaaccaagaaatgatgatatttttaagataattat
49621 ggcaattgtgcttttcttttttcttttcctggattccccaccaaatattcacttttctgga
49681 tgtattgattcaactaggcatcatacgtgactgtagaattgcagatattgtggacacggc
49741 catgcctatcaccatttgtatagcttattttaacaattgcctgaatcctctttttatgg
49801 ctttctggggaaaaaatttaaaagatattttctccagcttctaaaatatattcccccaaa
49861 agccaaatcccactcaaacctttcaacaaaaatgagcacgctttcctaccgcccctcaga
49921 taatgtaagctcatccaccaagaagcctgcaccatgttttgaggttgagtgacatgttcg
49981 aaacctgtccataaagtaatttgtgaaagaaggagcaagagaacattcctctgcagcac
50041ttcactaccaaatgagccMttagctacttttcagaattgaaggagaaaatgcattatgtgg
50101 actgaaccgacttttctaaagctctgaacaaaagcttttctttccttttgcaacaagaca
50161 aagcaaagccacattttgcattagacagatgacggctgctcgaagaacaatgtcagaaac
50221 tcgatgaatgtgttgatttgagaaattttactgacagaaatgcaatctccctagcctgct
50281 tttgtcctgttatttttttatttccacataaaggtatttagaatatattaaatcgttagag
```

The sequence of primers and probes for L191L (Y) and A1166C (M) TaqMan Assays (Genbank accession number: AF245699). Y(C/T): L191L, M(C/A): A1166C. ■ = Primers and ■ = Probes

2.2.3.2 Analysis

2.2.3.2.1 TaqMan Gene Expression Analysis

PCR products were analysed using SDS software. Each individual experimental product was finally represented as Ct (Point at which sample will fluoresce through and above threshold level, and threshold level means the cycle at which the amplification of PCR product is detected). Data (Ct values) were then exported to Excel where Ct values were arranged according to genotypes. Relative expression of the *AGTR1* was calculated using normalisation to endogenous control (18S) as described in TaqMan cytokine gene expression plate I protocol (www.appliedbiosystems.com):

$$\Delta Ct\ [sample] = Ct\ [sample] - Ct\ [18S]$$

Lowest expressed (highest ΔCt) genotype was selected as calibrator and then:

$$\Delta\Delta Ct\ [sample] = \Delta Ct\ [sample] - \Delta Ct\ [calibrator]$$

In this work, the mean of ΔCt of each genotype group was subtracted from the mean of the calibrator (highest expressed genotype group i.e. lowest ΔCt). Normalisation of samples was then carried out:

$$2^{-\Delta\Delta Ct\ [sample]}$$

In this analysis, the mean of ΔCt and ΔΔCt of each genotype group were used rather than samples' ΔΔCt.

2.2.3.2.2 Phase Analysis

Genotypes were subjected to Phase analysis [133] to calculate the structure and frequencies of haplotypes.

2.2.3.2.3 ANOVA and Regression

In this analysis, the possible association and correlation between genotypes and ΔCts were studies. ANOVA was also performed for Phase results i.e. haplotypes and ΔCts.

2.2.3.2.4 Haplotype Trend Regression (HTR)

In this analysis, ΔCt of the both SNPs were separately analysed with individual's haplotype to study the possible association of haplotypes with ΔCt [152].

3 Results of Genotyping, Genotype Phenotype and Haplotype Studies of *AGTR1*

AGTR1 A1166C, L191L, C-521T and A-153G genotype data were analysed in conjunction with the MRC Environmental Epidemiology Unit at the Southampton General Hospital, which holds phenotypes for genotype-phenotype analysis.

3.1 Results of Genotyping of the SNPs in the *AGTR1* Gene

3.1.1 Result of Genotyping of *AGTR1* A1166C

Based on the designed primers (Appendix 1) a control band (420bp) and two other allele specific ones were expected: A (251bp) and C (224bp); a schematic representation of bands has been provided in Appendix 3 (Figure 1). Gel images (Appendix 3, Figure 3) were examined using Phoretix software 1D version 4.0, Phoretix Intl. Newcastle UK, genotypes assigned manually, and genotype counts were exported to Excel for further analysis. Genotypes and Hardy-Weinberg equilibrium (HW eq.) were then tested using standard χ^2 test for total population sample (Table 3.1). Table 3.2 shows the overall gene frequency distribution in men and women of the NH and the EH populations (where 11, 12 and 22 represent AA, AC and CC genotypes respectively). The results indicate Hardy-Weinberg equilibrium (Appendix 2); and not significant at 1df, $\chi^2 = 0.0$ and p value = 0.992; the call rate was 93.3% (Table 3.1).

Table 3.1: Genotype distribution of the *AGTR1* A1166C in NH and EH populations

Genotype / Counts	11	12	22	χ^2
982	514	393	75	0.0

Table 3.2: Genotype distribution of A1166C in EH and NH, according to gender

*AGTR1*V001	Men	Women	Total
11	321	193	514
	54.59%	48.98%	52.34%
12	222	171	393
	37.76%	43.40%	40.02%
22	45	30	75
	7.65%	7.61%	7.64%
Total	588	394	982
	0.598%	0.401%	

The frequency of allele A = 0.72 and allele C = 0.28. EH population dataset was separately studied vs cardiovascular phenotypes and also subjected to a combined analysis. The distribution of A1166C in EH population is presented in Table 3.3. This dataset was completely in Hardy-Weinberg equilibrium: not significant at 1df, $\chi^2 = 3.1$ and p value = 0.08. Frequency of allele A = 0.71 and allele C = 0.29.

Table 3.3: Genotype distribution of A1166C in EH, according to gender

*AGTR1*V001	Men	Women	Total
11	122	61	183
	50.83%	44.2%	48.41%
12	101	69	170
	42.8%	50.00%	44.97%
22	17	8	25
	7.08%	5.80%	6.61%
Total	240	138	378
	0.634%	0.365%	

3.1.2 Result of Genotyping of *AGTR1* L191L

According to the primers design, two bands were expected: a 151bp for control band and a 103bp for allele specific band (Figures 2 and 4 in Appendix 3). Electrophoresis was performed using 5% polyacrylamide gel previously stained with ethidium bromide and on 384 well MADGE gel format in dry box for ten minutes at 150v. A picture of the gel is presented in Appendix 3 (Figure 4). Gels were analysed using Phoretix software and genotypes were called manually and results were then transferred to Excel for further statistical analysis. Final results are presented in Table 3.4. Results were in Hardy-Weinberg equilibrium; non significant at one degree of freedom; p = 0.854; Call rate was 96.1%. The frequencies of alleles are: C = 0.55 and T = 0.45.

Table 3.4: Genotype distribution of the *AGTR1* L191L in NH and EH populations

Genotype / Counts	11(CC)	12(TC)	22(TT)	χ^2
1013	**295**	**506**	**212**	**0.0**

3.1.3 Result of Genotyping of *AGTR1* C-521T

Based on the primers design, two bands were expected, the control band of 270bp in size and the allele specific band of 100bp (Figures 5 in Appendix 3). Electrophoresis was performed using 5% polyacrylamide gel previously stained with ethidium bromide and in 384 well MADGE format in dry box for nine minutes at 150v. A picture of the gel is presented in Appendix 3 (Figure 5). After scanning gels using fluoroimager, pictures were transmitted to Phoretix software for manual typing, and calls were copied to Excel for further statistical analysis and Hardy-Weinberg calculation. The final data was in Hardy-Weinberg equilibrium, $\chi^2 = 0.1$ and p = 0.75 (Table 3.5). The frequencies of alleles were: C = 0.63 and T = 0.37.

Table 3.5: Genotype distribution of the *AGTR1* C-521T in NH and EH population

Genotype / Counts	11 (CC)	12 (CT)	22 (TT)	χ^2
949	**379**	**445**	**125**	**0.1**

3.1.4 Result of Genotyping of *AGTR1* A-153G

According to the primer design, two bands were expected: one control band of 301bp, and one allele specific band of 170bp in size. Electrophoresis was performed using 5% polyacrylamide gel previously stained with ethidium bromide and in 384 well MADGE format in a dry box for nine minutes at 150v. An image of the gel is presented in Appendix 3 (Figure 6). Data was tested for Hardy-Weinberg equilibrium, p = 0.529 (Table 3.6). The frequencies of alleles were: A = 0.79, G = 0.21.

Table 3.6: Genotype distribution of the *AGTR1* A-153G in the NH and EH population

Genotype / Counts	11 (CC)	12 (CT)	22 (TT)	χ^2
925	**574**	**306**	**46**	**0.4**

3.2 Results of Genotype Phenotype Studies of *AGTR1*

Details of analyses have been provided in Appendix 4.

3.2.1 Result of Genotype Phenotype Study of the *AGTR1* A1166C

EH Population

Many significant associations and trends of associations of the *AGTR1* A1166C with anthropometric and metabolic traits have been found in this study. These findings are more prominent in the EH which are summarised in following and in Table 3.7.

CC genotype, in males, was associated with lower BMI (p = 0.03), waist-hip-ratio (p = 0.01), waist circumference (p = 0.00), glucose at 30 minute (p = 0.01), insulin at 0 (p = 0.04) and trends of associations with lower adult weight (p = 0.06), fasting glucose (p = 0.08) and glucose at 120 minute (p = 0.06) and height (p = 0.07). The same genotype (CC), in women, was significantly associated with lower fasting triglyceride (p = 0.04) and fibrinogen (p =

0.01), and also with trends of associations with lower waist circumference (p = 0.09), adult weight (p = 0.07) and fasting cholesterol (p = 0.07).

In the cases of BMI (p = 0.01), waist-hip-ratio (p = 0.004), waist circumference (p = 0.001), adult weight (p = 0.008), glucose at 30 minutes and fasting fibrinogen, the associations remained significant in combined analysis adjusted for gender.

Additionally, significant associations of C allele with lower waist-hip-ratio (p = 0.01), waist circumference (p = 0.02), glucose at 0 (p = 0.03), 30 (p = 0.008) and 120 minutes (p = 0.02), and also insulin at 0 (p = 0.05) in men were observed. The same effect was seen with fasting fibrinogen (p = 0.002) in women.

C allele, in combined analysis, was significantly associated with waist-hip-ratio (p = 0.01), waist circumference (p = 0.02), insulin at 0 (p = 0.03), glucose at 30 (p = 0.01) and 120 minutes (p = 0.03), and also fasting fibrinogen (p = 0.01).

EH and NH Populations

Men:

- The significant association of CC genotype with lower weight (p = 0.03 with 2df) and lower waist circumference (p = 0.10 on 2df) were observed.

Women:

- Regardless of some suggestive evidence of the association of the CC genotype with lower height (p = 0.09 on 2df), no significant association was found between *AGTR1* genotype and anthropometric phenotypes.

Table 3.7: The results of ANOVA and regression (Reg.) analyses of anthropometric and metabolic traits for AGTR1 A1166C polymorphism in 240 men and 138 women (EH). In each cell, mean and SD of each genotype groups is presented. Geometric means and SDs were used for glucose, insulin, cholesterol, triglyceride and fibrinogen values. p values are on 2df from ANOVA and 1df from regression on allele unadjusted unless mentioned

	Men			*p* value		Women			*p* value		Combined Analysis			*p* value	
	AA	AC	CC	ANOVA	Reg.	AA	AC	CC	ANOVA	Reg.	AA	AC	CC	ANOVA	Reg.
BMI	26.87	27.43	25.19	0.03	0.61	26.33	27.32	25.46	0.25	0.56	26.69	27.39	25.28	0.01	0.99
(kg/m^2)	3.29	3.39	2.98			3.57	4.69	1.90			3.39	3.96	2.65		
Waist to Hip	0.94	0.94	0.90	0.01	0.01	0.80	0.79	0.77	0.48	0.40	0.89	0.88	0.86	0.004	0.013
Ratio	0.05	0.05	0.05			0.06	0.05	0.04			0.09	0.09	0.08		
Waist Circumference	99.23	98.41	91.45	0.008	0.02	82.57	83.86	76.5	0.09	0.61	93.68	92.51	86.67	0.001	0.02
(cm)	10.18	9.01	8.17			8.99	9.65	2.93			12.55	11.70	9.88		
Adult Weight	80.40	80.33	73.62	0.06	0.12	67.67	70.51	62.13	0.07	0.86	76.16	76.35	69.94	0.008	0.24
(kg)	11.46	10.81	11.01			9.82	12.01	5.32			12.46	12.27	10.91		
Fasting Glucose	6.11	5.92	5.57	0.08	0.03	5.66	5.65	5.79	0.89	0.82	5.96	5.81	5.64	0.18	0.07
(mmol/l)	1.21	1.16	1.08			1.11	1.16	1.15			1.19	1.16	1.11		
Glucose at 30	9.68	9.34	8.23	0.01	0.008	8.76	8.74	8.09	0.58	0.50	9.37	9.09	8.19	0.01	0.01
(mmol/l)	1.25	1.21	1.20			1.22	1.23	1.23			1.25	1.22	1.21		
Glucose at 120	6.82	6.31	5.73	0.06	0.02	7.07	7.12	6.56	0.73	0.73	6.91	6.63	6.01	0.08	0.03
(mmol/l)	1.38	1.42	1.24			1.29	1.33	1.39			1.35	1.39	1.30		
Insulin at 0	42.67	40.77	27.44	0.04	0.05	48.41	42.34	50.06	0.34	0.41	44.56	41.41	33.53	0.08	0.04
(pm/l)	1.98	1.81	2.02			1.73	1.80	1.25			1.90	1.80	1.91		
Insulin at 30	271.28	266.74	238.23	0.74	0.53	248.29	249.86	352.96	0.24	0.30	263.35	259.63	271.58	0.95	0.96
(pm/l)	1.89	1.87	1.59			1.69	1.84	1.57			1.83	1.85	1.63		
Insulin at 120	161.90	135.65	121.45	0.21	0.08	231.01	238.11	223.79	0.94	0.92	183.13	172.64	151.68	0.35	0.15
(pm/l)	2.28	2.43	1.77			1.89	1.93	1.70			2.18	2.32	1.86		
Systolic BP	165.03	162.88	156.88	0.30	0.15	156.38	155.99	161.25	0.82	0.78	162.15	160.08	158.28	0.61	0.39
(mmHg)	21.83	20.85	16.82			21.20	23.55	17.14			21.95	22.18	16.69		
Pulse Pressure	74.76	72.68	69.24	0.29	0.12	75.13	72.84	76.88	0.61	0.70	74.89	72.75	71.68	0.32	0.14
(mmHg)	16.05	14.56	13.44			14.59	15.77	17.57			15.54	15.02	14.96		
Fasting Chol.	6.65	6.54	6.21	0.32	0.16	6.98	7.21	6.18	0.07	0.72	6.76	6.80	6.20	0.08	0.18
(mmol/l)	1.17	1.21	1.21			1.23	1.17	1.19			1.20	1.20	1.20		
Fasting TG	1.48	1.39	1.26	0.41	0.19	1.25	1.40	1.01	0.04	0.72	1.40	1.39	1.16	0.19	0.28
(mmol/l)	1.68	1.74	1.54			1.56	1.46	1.40			1.65	1.63	1.51		
Fasting Fibrinogen	309.54	295.67	302.92	0.28	0.22	302.92	283.80	267.12	0.01	0.002	307.38	290.88	290.48	0.03	0.01
(g/l)	1.20	1.28	1.21			1.16	1.15	1.13			1.19	1.23	1.20		

Generally, in both men and women, the CC genotype seems to be a strong correlate of a range of cardiovascular risk phenotypes.

3.2.2 Result of Genotype Phenotype Study of *AGTR1* L191L

The associations found from genotype phenotype study of the *AGTR1* L191L with anthropometric and cardiovascular phenotypes are presented in Table 3.8 and Table 3.9.

EH and NH Populations

Men: There are trends of associations between TT genotype and higher weight (p = 0.09), higher waist circumference (p = 0.15) and waist to hip ratio (p = 0.11). There are also significant association of T allele with higher height (p = 0.04), higher waist to hip ratio (p =0.04), and trends of association with higher waist circumference (p = 0.06).

Women: There are trends of association of TT genotype with lower height (p = 0.08) and lower birth weight (p = 0.17). There are also trends of association between T allele and higher BMI (p = 0.08) and lower birth weight (p = 0.07).

EH Population

Men: There are trends of associations of TT genotype with higher glucose features (p = 0.15, 0.06 and 0.14 relative to glucose at 0, 30 and 120 minutes respectively). There are significant associations of T allele with higher fasting glucose (p = 0.05), glucose at 120 minute (p = 0.05), and trends of association with higher glucose at 30 minutes (p = 0.19) and higher fasting cholesterol (p = 0.07).

Women: There are significant association of TT genotype with higher fasting cholesterol (p = 0.01), higher LDL (p = 0.01), higher apolipoprotein A1 (p = 0.05) and higher apolipoprotein B (p = 0.03). There are also significant association of T allele with

higher fasting cholesterol (p = 0.006), higher LDL (p = 0.006), higher apolipoprotein A (p = 0.02) and higher apolipoprotein B (p = 0.009)

Table 3.8: Results of the ANOVA and regression (Reg.) analyses of the *AGTR1* L191L with cardiovascular phenotypes in the EH population (388 individuals). In each cell, mean and SD of each genotype groups is presented. Geometric means and SDs were used for glucose, cholesterol, and lipoprotein values. p values are on 2df from ANOVA and 1df from regression on allele unadjusted unless mentioned.

	Men (245)			p value		Women (143)			p value	
	CC	CT	TT	ANOV	Reg.	CC	CT	TT	ANOVA	Reg.
Fasting Cholesterol	6.45	6356	6.85	0.17	0.07	6.58	7.22	7.37	0.01	0.006
(mmol/l)	1.19	1.21	1.17			1.24	1.18	1.79		
Glucose 0	5.84	6.03	6.23	0.15	0.05	5.64	5.68	5.62	0.93	0.98
(mmol/l)	1.11	1.19	1.28			1.09	1.17	1.08		
Glucose 30	8.1	9.46	9.89	0.07	0.19	8.59	8.68	8.89	0.81	0.54
(mmol/l)	1.23	1.21	1.30			1.19	1.25	1.2		
Glucose 120	6.12	6.12	6.94	0.14	0.05	6.79	7.36	6.75	0.19	0.76
(mmol/l)	1.32	1.40	1.50			1.30	1.33	1.25		
Low Density	4.43	4.76	4.75	0.2	0.16	4.48	5.10	5.21	0.01	0.006
Lipoprotein (mmol/l)	1.30	1.33	1.27			1.33	1.26	1.22		
Apolipoprotein A1	1.36	1.32	1.34	0.69	0.72	1.29	1.38	1.41	0.05	0.02
(g/l)	1.25	1.25	1.27			1.16	1.18	1.16		
Apolipoprotein B	1.07	1.10	1.16	0.24	0.09	1.03	1.06	1.12	0.03	0.009
(g/l)	1.29	1.31	1.29			1.25	1.25	1.23		

Table 3.9: Results of the ANOVA and regression (Reg.) of the *AGTR1* L191L with anthropometric phenotypes in the EH and NH populations. In each cell, mean and SD of each genotype groups is presented. p values are on 2df from ANOVA and 1df from regression on allele unadjusted unless mentioned.

	Men			p value		Women			p value	
	CC	CT	TT	ANOVA	Reg.	CC	CT	TT	ANOVA	Reg.
Weight	78.14	80.23	81.04	0.09	0.36	67.53	68.74	69.16	0.54	0.29
(kg)	11.08	12.48	12.55			12.38	11.07	11.65		
Height	1.71	1.72	1.73	0.13	0.05	1.60	1.60	1.58	0.09	0.18
(m)	0.07	0.06	0.07			0.06	0.06	0.06		
Waist Circumference	97.26	98.77	99.42	0.15	0.06	83.63	83.84	85.09	0.54	0.33
(cm)	9.57	10.32	9.93			10.88	8.96	9.83		
Waist to Hip Ratio	0.93	0.94	0.94	0.11	0.04	26.47	26.84	27.57	0.66	0.96
	0.05	0.05	0.05			4.45	4.17	4.41		
BMI	26.55	27.09	27.06	0.27	0.19	26.47	26.84	27.57	0.20	0.08
(kg/m^2)	3.15	3.64	3.72			4.45	4.17	4.41		
Birth Weight	125.16	124.33	25.77	0.74	0.82	122.44	121.19	117.7	0.17	0.07
(Ounces)	17.81	19.73	16.72			20.16	17.18	14.79		

3.2.3 Result of Genotype Phenotype Study of *AGTR1* C-521T

The associations found from genotype phenotype study of the *AGTR1* C-521T with cardiovascular and anthropometric phenotypes are summarised in Table 3.10 and Table 3.11.

EH and NH Populations

Men: There are significant associations of TT allele with higher waist circumference ($p = 0.05$), and trends of association with higher weight ($p = 0.07$), BMI ($p = 0.09$) and higher waist to hip ratio ($p = 0.07$). There are also significant associations of T allele with higher weight ($p = 0.02$), higher BMI ($p = 0.03$), higher waist circumference ($p = 0.02$), and higher waist to hip ratio ($p = 0.03$).

Women: TT is significantly associated with higher height ($p = 0.02$), and allele T is significantly associated with higher height ($p = 0.006$) and also with trends of association with higher weight ($p = 0.09$).

EH Population

Men: Genotype TT is significantly associated with lower HDL ($p = 0.03$) and higher lipoprotein A ($p = 0.05$). Allele T is significantly associated with lower HDL ($p = 0.01$), and with trends of higher lipoprotein A ($p = 0.1$).

Women: Genotype TT is significantly associated with lower lipoprotein A ($p = 0.04$) and lower glucose at 30 minute ($p = 0.02$). Allele T is significantly associated with lower lipoprotein A ($p = 0.02$), and with trends of association with lower glucose at 30 minute ($p = 0.09$).

Table 3.10: Results of the ANOVA and regression (Reg.) analyses of the *AGTR1* C-521T with anthropometric phenotypes in the whole population (NH & EH: 949). In each cell, mean and SD of each genotype groups is presented. p values are on 2df from ANOVA and 1df from regression on allele unadjusted unless mentioned.

	Men (543)			p value		Women (406)			p value	
	CC	CT	TT	ANOVA	Reg.	CC	CT	TT	ANOVA	Reg.
Weight	78.34	80.21	81.78	0.07	0.02	67.67	68.74	70.79	0.23	0.09
(kg)	12.60	12.15	10.50			11.83	12.05	10.15		
BMI	26.49	27.08	27.36	0.09	0.03	26.86	26.85	27.40	0.7	0.54
(kg/m^2)	3.33	3.64	3.26			4.48	4.25	4.41		
Waist Circumference	97.28	98.87	100.27	0.05	0.02	84.22	83.91	85.25	0.68	0.69
(cm)	9.79	10.23	8.68			10.53	9.71	9.23		
Waist to Hip Ratio	0.93	0.93	0.95	0.07	0.03	0.80	0.79	0.80	0.87	0.75
	0.05	0.05	0.41			0.05	0.05	0.06		
Height (m)	1.71	1.72	1.72	0.38	0.2	1.59	1.60	1.61	0.02	0.006
	0.07	0.06	0.06			0.06	0.6	0.06		

Table 3.11: Results of the ANOVA and regression (Reg.) analyses of the *AGTR1* C-521T with cardiovascular phenotypes in the EH population (344). In each cell, mean and SD of each genotype groups is presented. Geometric means and SDs were used for glucose and lipoproteins values. p values are on 2df from ANOVA and 1df from regression on allele unadjusted unless mentioned.

	Men			p value		Women			p value	
	CC	CT	TT	ANOVA	Reg.	CC	CT	TT	ANOVA	Reg.
High Density	1.26	1.17	1.07	0.03	0.01	1.43	1.50	1.42	0.49	0.72
Lipoprotein (mmol/l)	1.28	1.29	1.44			1.32	1.22	1.23		
Lp (a) lipoprotein	7.31	11.62	8.51	0.05	0.1	15.71	12.69	5.92	0.04	0.02
(mg/l)	3.39	3.46	3.83			4.09	3.69	3.77		
Glucose 30	9.37	9.28	10.07	0.27	0.42	9.16	8.27	8.79	0.02	0.09
(mmol/l)	1.21	1.27	1.27			1.24	1.21	1.19		

3.2.4 Result of Genotype Phenotype Study of *AGTR1* A-153G

The associations found in the genotype phenotype study of the *AGTR1* A-153G with cardiovascular and anthropometric phenotypes are presented in Table 3.12 and Table 3.13.

NH and EH Populations

Men: There is significant association of the genotype GG with higher weight at 1 year ($p = 0.04$).

Women: There is significant association of the genotype GG and allele G with higher height: p values = 0.02 and 0.006 respectively.

EH Population

Men: There is significant association of the genotype GG with lower HDL ($p = 0.04$), and trend of association with lower insulin at 120 minute ($p = 0.03$).

Table 3.12: Results of the ANOVA and regression (Reg.) analyses of the association of the *AGTR1* A-153G with anthropometric phenotypes in the whole population (NH & EH: 926). In each cell, mean and SD of each genotype groups is presented. p values are on 2df from ANOVA and 1df from regression on allele unadjusted unless mentioned.

	Men (537)			p value		Women (389)			p value	
	AA	AG	GG	ANOVA	Reg.	AA	AG	GG	ANOVA	Reg.
Weight at 1 year	362.80	360.87	382.55	0.04	0.23	342.01	340.48	336.06	0.78	0.51
(Ounces)	41.98	43.91	41.29			36.70	37.95	26.77		
Height (m)	1.72	1.72	1.73	0.54	0.29	1.59	1.60	1.62	0.02	0.006
	0.07	0.06	0.08			0.06	0.06	0.05		

Table 3.13: Results of the ANOVA and regression (Reg.) of the *AGTR1* A-153G with cardiovascular phenotypes in the EH population (346). In each cell, geometric and SDs means of each genotype groups is presented. p values are on 2df from ANOVA and 1df from regression on allele unadjusted unless mentioned.

	Men			p value		Women			p value	
	AA	AG	GG	ANOVA	Reg.	AA	AG	GG	ANOVA	Reg.
High Density	1.21	1.17	1.01	0.04	0.03	1.47	1.43	1.47	0.8	0.64
Lipoprotein (mmol/l)	1.27	1.3	1.58			1.3	1.2	1.24		
Insulin 120 (pmol/l)	139.57	180.17	112.96	0.06	0.31	233.35	238.55	142.43	0.51	0.6
	2.36	1.95	1.85			1.88	2.03	1.30		

3.3 Result of LD and Haplotype analyses of the *AGTR1* Gene

Table 3.14 shows the frequencies of alleles of the studied SNPs of the *AGTR1* and their location in the gene.

Table 3.14: Characteristics of the studied SNPs in the *AGTR1* gene

	SNP	Accession Number	No. of Nucleotide	Allele 1	Frequency of Allele 1	Allele 2	Frequency of Allele 2
1	A1166C	AF245699	50058	A	0.72	C	0.27
2	L191L	" "	49465	C	0.54	T	0.45
3	C-521T	" "	5245	C	0.63	T	0.36
4	A-153G	" "	5612	A	0.78	G	0.21

The result of haplotype studies of the SNPs, listed in Table 3.14, using Arlequin with comparison with available data on the INSERM website in the Etude Cas- Temoin de l'Infarctus du Myocarde (ECTIM) study [121] is presented in Table 3.15. Total number of individuals was 807 = 1614 haplotype compared with 728 in the ECTIM study. A comparison was made between these two dataset, and they were not significantly different: $\chi^2 = 0.00$ and $p = 1$.

Table 3.15: Represents the frequency and structure of the haplotypes of the *AGTR1* in NH & EH, Belfast and France (ECTIM study)

	Haplotypes	Haplotype Structure	Frequency in NH & EH	Frequency in Belfast	Frequency in France
1	1 1 2 1	CATA	0.29608	0.33	0.34
2	1 1 1 1	CACA	0.15316	0.12	0.12
3	1 1 1 2	CACC	0.14365	0.2	0.18
4	2 1 2 1	TATA	0.08731	0.08	0.09
5	2 2 1 1	TGCA	0.07890	0.06	0.07
6	2 2 1 2	TGCC	0.07495	0.07	0.07
7	2 1 1 1	TACA	0.05393	0.06	0.06
8	2 2 2 1	TGTA	0.05166	0.05	0.04
9	2 1 1 2	TACC	0.02818	0.04	0.04
10	1 1 2 2	CATC	0.02043	0.0	0.0
11	1 2 1 1	CGTA	0.00257	0.0	0.0
12	1 2 1 2	CGCC	0.00247	0.0	0.0
13	2 1 2 2	TATC	0.00227	0.0	0.0
14	2 2 2 2	TGTC	0.00199	0.0	0.0
15	1 2 2 1	CGTA	0.00192	0.0	0.0
16	1 2 2 2	CGTC	0.00054	0.0	0.0

The order of SNPs in the haplotypes in the second column (from left to right) is: C-521T, A-153G, L191L and A1166C.

The values of D′ obtained from LD analysis among SNPs of the *AGTR1* gene are presented in Table 3.16. As it is seen this value is significantly high between SNPs in the 5′ UTR and 3′ UTR, while it is not very high between these two sets, though mainly more than zero. These values are very similar to those of the ECTIM study [121].

Table 3.16: Represents the value of D′ among SNPs of the *AGTR1*

	C-521T	A-153G	L191L	A1166C
C-521T	-	-	-	-
A-153G	0.94	-	-	-
L191L	0.16	0.43	-	-
A1166C	0.00	0.12	0.80	-

The data was also subjected to Phase programme [133] to calculate the structure and frequency of haplotypes. Table 3.17 shows the Phase results and comparison with those obtained from Arlequin.

Table 3.17: Represents the frequencies and sequence of haplotypes of *AGTR1*, yielded from Phase and Arlequin programmes

	Haplotypes	Haplotype Frequency by Arlequin	Haplotype Frequency by Phase
1	1 1 2 1	0.29608	0.32156
2	1 1 1 1	0.15316	0.12701
3	1 1 1 2	0.14365	0.14931
4	2 1 2 1	0.08731	0.09170
5	2 2 1 1	0.07890	0.08426
6	2 2 1 2	0.07495	0.09293
7	2 1 1 1	0.05393	0.06691
8	2 2 2 1	0.05166	0.03097
9	2 1 1 2	0.02818	0.01177
10	1 1 2 2	0.02043	0.01610
11	1 2 1 1	0.00257	0.00185
12	1 2 1 2	0.00247	0.00309
13	2 1 2 2	0.00227	0.0006
14	2 2 2 2	0.00199	0.0006
15	1 2 2 1	0.00192	0.00123
	1 2 2 2*	0.00054	

* found only by Arlequin

To compare these two results (haplotypes obtained from Phase and Arlequin), seven common haplotypes (highlighted in Table 3.17) consisting 93.37% of individuals were chosen and compared. There was no significant difference between them $\chi^2 = 9.97$, p = 0.12 (6 degree of freedom) (Table 3.18).

Table 3.18: Value of D′ based on Arlequin and Phase programmes

SNPs	D′*	p value	D′**	p value
2and1	0.800153	0.000817	0.863244	0.000000
3and1	0.007432	0.001003	0.152822	0.000001
3and2	0.162601	0.001168	0.430803	0.000000
3and4	0.943735	0.000247	0.953713	0.000000
4and1	0.120730	0.000968	0.293044	0.000000
4and2	0.433892	0.002203	0.669493	0.000000

Numbers 1 to 4 in the first column refer to the number of the SNPs in Table 3.14.

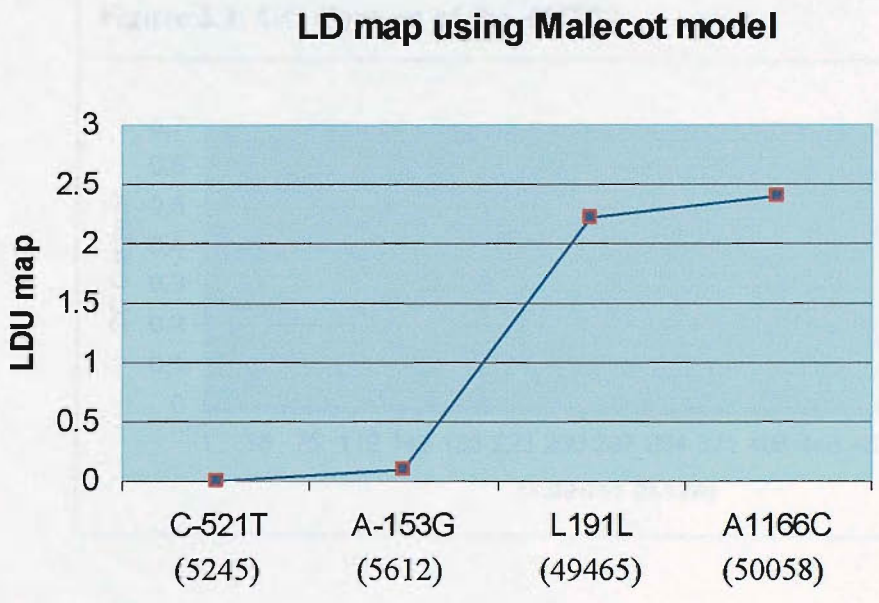
* Based on haplotypes frequencies yielded from Arlequin.

** Based on haplotypes frequencies yielded from Phase.

3.4 Result of LD Unit map Analysis

The result of the LD unit map analysis shows that there are two haplotype blocks spanning 19.9 kb with a gap of 44 kb between them (Figure 3.1).

Figure 3.1: The Structure of the Haplotype Blocks in the *AGTR1*

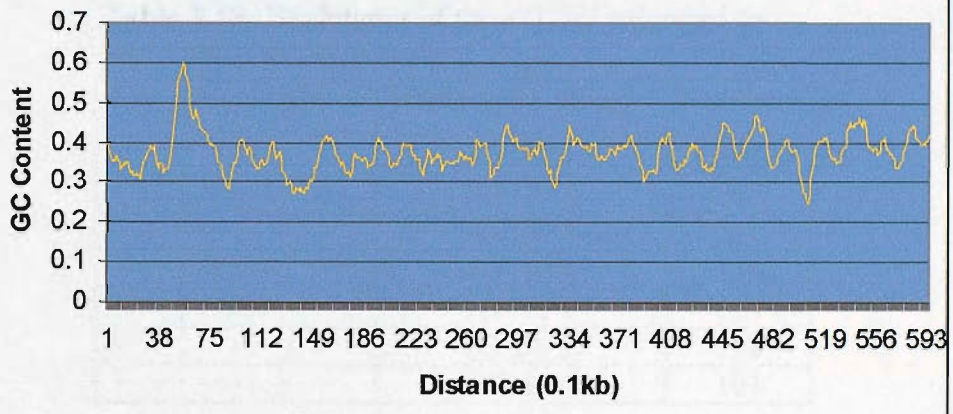


On the X axis each marker has been shown along with its physical location (in base pair) in the *AGTR1* sequence (accession number: AF245699). LDU: LD unit.

3.5 Result of GC content study

Figure 3.2 shows the content of GC throughout the *AGTR1* gene. The average GC percentage is approximately 40%, apart from a peak in the beginning of the gene, and there does not seem to be a GC rich region in the middle of the gene where LD analyses predict a recombination hot spot between two blocks.

Figure 3.2: GC Content of the *AGTR1*



3.6 Result of Haplotype Phenotype Study in the *AGTR1* Gene

Details of analyses are presented in Appendix 4.

To study the possible association between haplotypes of the four studied SNPs in *AGTR1* with phenotypes in EH and NH populations, first seven common haplotypes found by Phase analysis consisting 93.37% of individuals were selected and named from AT1R*1 to AT1R*7 according to the haplotype nomenclature [114], and were then subjected to regression against phenotypes. The number and structure of each haplotype is represented in Table 3.19. The number of individuals was 708 and of haplotypes 1416.

Table 3.19: Haplotypes of the *AGTR1* subjected to haplotype phenotype study

No.	Haplotype	Haplotype Calling	Number
1	1121	AT1R*1	481
2	1111	AT1R*2	205
3	1112	AT1R*3	231
4	2212	AT1R*4	138
5	2121	AT1R*5	129
6	2211	AT1R*6	128
7	2111	AT1R*7	104

Table 3.20: Haplotypes carrying allele C of the *AGTR1* A1166C

	Haplotype (Phase)+ number	Haplotype Frequency	Haplotype (Arlequin)	Frequencies
1	1 1 1 2 (241)	0.14931	2 2 1 2	0.07495
2	2 2 1 2 (150)	0.09293	2 1 1 1	0.05393

Allele 2 (C) of the *AGTR1* A1166C has only appeared in two haplotypes out of seven (Table 3.20). A unique structure is seen in these two haplotypes, because in the first one wild type alleles of SNPs in 5′ UTR are presented and mutant ones in the second one. Besides, the structure of haplotypes belonged to SNPs in exon 5 and 3′ UTR is unchanged in these two. This situation implies that if there is any significant difference in the level of *AGTR1* expression between these two haplotypes, it should come from the effect of 5′ UTR.

In males, haplotype 1112 (CATC) is associated with:

Lower fasting glucose ($p = 0.01$).

Lower 30′ glucose ($p = 0.04$).

Lower BMI ($p = 0.05$).

In females, haplotype 1111(CATA) is associated with:

Lower BMI ($p = 0.03$)

Trends of association with higher level of fasting glucose ($p = 0.07$)

3.7 Summary of Results

In the case of *AGTR1* A1166C, it can be briefly summarised as:

A) The CC genotype of *AGTR1* A1166C is a strong determinant of anthropometric measures. Its association was found with lower weight, lower height, lower waist circumference, lower BMI and lower waist-to-hip ratio.

B) In terms of cardiovascular phenotypes CC genotype was also associated with lower insulin at 0 and 120 minutes, lower glucose at 0, 30 and 120 minutes, lower fasting proinsulin and lower fasting split proinsulin.

In women's group, in spite of some significant associations between the CC genotype and cardiovascular phenotype, no strong relationship was found between the A1166C and anthropometric phenotypes.

In the case of the *AGTR1* L191L, it is seen that it is widely associated with cardiovascular and anthropometric phenotypes, though not as prominent as A1166C:

T allele, in men, is significantly associated with higher fasting glucose, glucose at 120 minutes, height, and waist to hip ratio, and with trends of association with higher fasting cholesterol, apolipoprotein B, and waist circumference. In women, it is significantly associated with higher fasting cholesterol, LDL, apolipoprotein A1 and apolipoprotein B, with trends of association with higher BMI and lower birth weight.

TT genotype, in men, has trends of association with higher glucose at 30 minute and weight. In women, it is significantly associated with higher fasting cholesterol, LDL, apolipoprotein A1, and apolipoprotein B, and with trends of association with lower height.

In the case of the *AGTR1* C-521T, to a limited extent it is associated with cardiovascular and anthropometric:

T allele, in men, is significantly associated with higher weight, BMI, waist circumference, waist to hip ratio and lower HDL, and with trends of association with higher lipoprotein a. In women, it is associated with higher height, lower lipoprotein (a), with trends of association with lower glucose at 30 minute and higher weight.

TT genotype, in men, is significantly associated with lower HDL, higher lipoprotein (a), and waist circumference, and with trends of association with higher waist to hip ratio, waist circumference, BMI, and weight. In women, it is significantly associated with higher height, lower glucose at 30 minutes and lower lipoprotein a.

Lower significant association was observed between *AGTR1* A-153G and the studied phenotypes:

G allele, in men, is significantly associated with lower HDL, and in women with higher height. GG genotype, in men, is significantly associated with higher weight at 1 year and lower HDL, and with trends of association with lower insulin at 120 minutes; in women it is significantly associated with lower height.

The general pattern of associations found from individual SNP phenotype studies shows that more signals are coming up from the coding region of the gene (i.e. the block covering exon 5 and 3′ UTR) compared with that of markers in the 5′ UTR. This is consistent with the general trend found in the literature, in which not much association have been observed from markers in the 5′ UTR. This is a point of crucial importance emphasising the greater role of the coding region of the gene in causing any change in phenotypes.

The presence of two haplotype blocks in the *AGTR1* gene has also been recently by Zhu X et al. (2003) [156], although the extent of these blocks beyond is not clear yet. The

idea of harbouring a GC rich region between haplotype blocks was not confirmed completely in this result, though it does not exclude the presence of a recombination hot spot. The GC content is relatively high (60%) in the 5′ UTR, which is nearly compatible with the GC content in promoter region.

All together, it is seen that the block extending coding region is more related to phenotypic changes rather than the other one extending into the 5′ UTR of the gene.

In the case of A1166C, CC genotype is a strong determinant of anthropometric measures in men and also shows associations with traits of insulin resistance. This effect is also justified for L191L, to a lesser extent, as well. However, in the cases of C-521T and A-153G, the volume of associations is not as much as those coming from L191L and A1166C.

4 Results of Genotyping and Haplotype Studies of the *ACE* and *GH* Genes

4.1 Results of Genotyping of the SNPs in the *ACE* Gene

4.1.1 Result of Genotyping of *ACE* C1237T

This SNP is located in exon 8 of the *ACE* gene, and does not involve an amino acid change (Proline in the presence of both alleles). According to the primer design, one control band with 294bp size and an allele specific band of 170bp would be expected. A schematic representation of proposed bands and gel image are provided in Appendix 3 (Figures 2 and 8). Gel images were examined using Phoretix software in which genotypes were assigned manually and counts were then exported to Excel for further analysis. Genotypes and Hardy-Weinberg equilibrium were tested using standard χ^2 test for each individual array and for the total population sample as well. Results were compatible with Hardy-Weinberg equilibrium, non significant at 1df, p value = 0.12 and the call rate was 91.9% (Table 4.1). The frequency of the allele C was 0.563 and allele T was 0.436.

Table 4.1: Genotype distribution of the *ACE* C1237T in the NH and EH populations

Genotype / Counts	11	12	22	χ^2
819	296	501	173	2.4

4.1.2 Result of Genotyping of *ACE* A-5466C

This SNP is located in the 5′ region of the *ACE* gene. The method of the gel analysis and the pattern of bands are the same as *ACE* C1237T experiment. However, based on the designed primers, one control band with 257bp in size and an allele specific one of

189 bp were expected. An image of a gel is presented in Appendix 3 (Figure 7). Results were slightly deviated from Hardy-Weinberg equilibrium, significant at 1df, $\chi^2 = 4.4$ and p value = 0.04 and the call rate was 92.9% (Table 4.2). The frequency of allele A = 0.61 and allele C = 0.39.

Table 4.2: Genotype distribution of the *ACE* A-5466C in the NH and EH population

Genotype / Counts	11	12	22	χ^2
980	353	496	131	4.4

4.2 Results of Linkage Disequilibrium Analysis

4.2.1 Result of LD Study of the *ACE* Gene

In the first step of the LD analysis of the *ACE* gene, the D′ value was measured (using 2LD software) between three polymorphisms of the *ACE*. Distances between polymorphisms are calculated according to their positions in the *ACE* gene sequence, accession number: AF118569. (See also Figure 1.5 for the *ACE* gene map):

1- *ACE* A-5466C

2- *ACE* C1237T

3- *ACE* I/D (provided by Day I N M et al., unpublished)

Hereafter, these three SNPs will be referred to SNPs 1, 2 and 3 respectively. The result of this analysis is presented in Table 4.3. The number of subjects in this analysis was 904. As was expected all of these three polymorphisms are in significant LD with high intensity.

Table 4.3: Represents the value of D' between polymorphisms in the *ACE* gene

SNP	D'	df	p value	χ^2	Distance
1×2	0.94	1	0.0000	788.82	~ 6kb
1×3	0.84	1	0.0000	669.77	~ 11kb
2×3	0.81	1	0.0000	1116.73	~ 6kb

4.2.2 Result of LD Study of the Polymorphisms of the *ACE* and *GH* Genes

The growth hormone (*GH*) gene is located about 300-500kb downstream of the *ACE* gene, according to different databases (Ensemble and NCBI and HapMap project). Here it is assumed that the real distance is somewhere between these two figures: ~ 400kb. A diagram showing the *ACE* and *GH* genes positions has been provided in Appendix 5 (Figure 1).

1) In the second step of the LD analysis, LD calculations were performed between the discussed above polymorphisms of the *ACE* and two SNPs of the *GH*;

4- A5157G (GH1V001), which is about 21bp upstream to the *GH* gene. Accession number J03071, nucleotide number: 5157.
5- C5187A (GH1V002), which is located inside the exon 1 of the *GH* gene. Accession number J03071, nucleotide number: 5187.

The number of individuals analysed was 728. Hereafter, these SNPs will be referred to as 4 and 5 respectively. Before doing LD analysis, haplotype analysis was performed using Arlequin software. A Table of haplotype frequency of these five SNPs is provided in Appendix 5 (Table 1). The result of LD analysis in this stage is represented in Table 4.4.

Table 4.4: D′ values between the *ACE* SNPs and two SNPs of the *GH*

SNP	D′	df	p value	χ^2
1×2	0.94	1	0.0000	641.85
1×3	0.86	1	0.0000	579.81
2×3	0.84	1	0.0000	956.46
3×5	0.57	1	0.0000	16.60
4×5	0.48	1	0.0000	19.05
2×5	0.43	1	0.0031	8.73
1×5	0.28	1	0.0071	7.24
3×4	0.22	1	0.0000	41.50
2×4	0.17	1	0.0000	24.35
1×4	0.12	1	0.0000	17.53

The numbers in the first column of Table 4.4 refer to the markers in the *ACE* and *GH* genes, as were presented before.

2) In the third part of this analysis, the D′ value was calculated between those three polymorphisms of the *ACE* gene and two SNPs and one microsatellite (*GH*1V004, typed by King T. (2002); Human Genetics Division, University of Southampton) of the *GH* gene, referred to as 6 in this analysis. The sequence of this microsatellite has been described in Figure 4.1; the accession number of the reference sequence in Genbank is J03071. It has 32 alleles ranging from 84 to 140bp (nucleotide number 25766). The result of the LD analysis is presented in Table 4.5.

Figure 4.1: The Sequence of the *GH* microsatellite

$(aaga)_3$-caaa-$(ga)_3$-$(aaga)_7$-$(ga)_{11}$-$(aaga)_{14}$-aaga-$(aaga)_2$-aagg

Table 4.5: D′ values between polymorphisms of the *ACE* and *GH* genes and *GH* microsatellite

	D'	df	p value	χ^2
4×6	0.53	1	0.0000	325.79
5×6	0.44	1	0.0000	17.90
3×6	0.25	1	0.0000	42.99
2×6	0.18	1	0.0000	20.42
1×6	0.12	1	0.0000	18.22

The distribution of the microsatellite alleles with SNP 4 (C5187A) is shown as a histogram in Appendix 5 (Figure 2). To make the analysis more understandable, microsatellite alleles were dichotomised based on their sizes: alleles smaller than 110bp (allele 17) were considered as allele 1 and those greater than 110bp as 2. After removing dropouts the number of subjects was 676. The haplotype structures and frequencies combining SNPs and microsatellite are presented in Appendix 5 (Table 2). As shown in

Table 4.5, the LD analysis was undertaken between the *GH* microsatellite and the other five polymorphisms. The D′ values between the *ACE* polymorphisms and the microsatellite are much lower than those between the growth hormone SNPs and microsatellite.

3) In the fourth step of LD analysis, alleles 17, 18, 19 (110, 120 and 114bp respectively) of the *GH* microsatellite were deleted to get two pure peaks as they are located in the middle part of the histogram, see Appendix 5 (Figure 2). The number of subjects in this stage was 605, and the results are shown in Table 4.6.

Table 4.6: Shows the LD results after deleting alleles 17, 8 and 19 of the GH microsatellite

	D'	df	p Value	χ^2
4 × 6	0.6	1	0.0000	333.96
5 ×6	0.44	1	0.0000	17.90
3 × 6	0.24	1	0.0000	32.55
2 × 6	0.17	1	0.0001	14.98
1 × 6	0.11	1	0.0003	13.07

4) In the last (the fifth) step, alleles 10, 12, 14, 16 of the *GH* microsatellite, which look to be four nucleotide alleles in the middle of the left peak (Figure 2, Appendix 5) holding high frequencies, were deleted. The number of subjects in this part of analysis was 348, and the results are presented in Table 4.7.

Table 4.7: Shows the results of LD between the *ACE* and *GH* polymorphisms, after deleting alleles 10, 12, 14, 16 of the microsatellite

	D'	df	p value	χ^2
5×6	0.75	1	0.0001	14.44
4×6	0.49	1	0.0000	158.80
3×6	0.21	1	0.0000	24.72
1×6	0.17	1	0.0005	12.16
2×6	0.15	1	0.0002	13.83

4.3 Result of the Haplotype Studies of the *ACE* Gene

In the first step of this analysis, haplotype frequencies of the *ACE* gene markers studied in this thesis were calculated using Arlequin and Phase (Table 4.8); and in the next step a comparison was made between obtained results and those of Keavney's [88] (see also Figure 1.7) as a reference for British Caucasians. In this analysis, *ACE* I/D data was provided by the Human Genetics Division at the University of Southampton. The final number of individuals in the NH and EH populations is 904 and 183 in the above paper.

Table 4.8: Represents the structure and frequency of the *ACE* haplotypes obtained from Arlequin and Phase

	A-5466C	C1237T	D/I	Haplotype Structure	Arlequin Frequency	Phase Frequency
1	A	T	I	122	0.3851	0.3899
2	C	C	D	211	0.3528	0.3628
3	A	C	D	111	0.1445	0.1416
4	A	T	D	121	0.0432	0.0404
5	A	C	I	112	0.0401	0.0409
6	C	C	I	212	0.0197	0.016
7	C	T	I	222	0.0081	0.0061
8	C	T	D	221	0.0022	0.0022

By comparing these two results, it is aimed to find out the compatibility of the observed results with those of Keavney's [88]. To understand the comparison process fully, outlined in Table 4.9, it is necessary to bear in mind the cladograms structure (Figure 1.7). It should also be emphasised that haplotypes (of our results) with rare frequency (less than 0.04) have been taken out of this comparison. As it is seen in the Table 4.9, the obtained results show no significant difference between them (χ^2=3.11 with 3 df and p value = 0.375). It could be then concluded that observed results in our study are similar to those found in another part of the UK [88].

Table 4.9: Shows the comparison between our study and Keavney B et al. (1998) [88] paper

	Haplotype†	Clade	A-5466C	T1237C	I/D	Hap. Freq. 1‡	Hap.Freq. 2*
1	1&7	A	A	T	I	0.38	0.38
2	2&6	B	C	C	D	0.35	0.31
3	4&3	C	A	C	D	0.14	0.16
4	5	A	A	C	I	0.04	0.05

†: Haplotype's number in Keavney's paper.

‡: Haplotype frequency in this study.

*: Haplotype frequency in Keavney's paper.

4.4 Result of Haplotype Analysis of the *ACE* and *GH* Genes

The frequency and structure of haplotypes obtained from Arlequin is provided in Table 4.10. The orders of the markers in the haplotypes are the same as have been referred in the above.

Extending the comparison that was performed between the result presented here and cladograms found in Keavney's publication [88] to our final results of the *ACE* and *GH* polymorphisms haplotypes (Table 4.11) reveals similar clades and basic haplotype structures. The purpose of this analysis could be considered as the first step in defining the haplotype block of these two genes. Before performing the above comparison, haplotypes with frequencies less than 0.01 were deleted (as presented in Table 4.11).

Table 4.10: Represents the frequency of haplotypes in 4 SNPs of *ACE* and *GH* genes, *ACE* I/D and *GH* microsatellite

No	Haplotype	Frequency
1	1 2 1 1 2 1 A T I A A 1	0.21916
2	2 1 2 1 2 1 C C D A A 1	0.13697
3	2 1 2 2 2 2 C C D G A 2	0.11033
4	1 2 1 2 2 2 A T I G A 2	0.08755
5	2 1 2 2 2 1 C C D G A 1	0.05917
6	1 2 1 2 2 1 A T I G A 1	0.05075
7	1 1 2 1 2 1 A C D A A 1	0.04710
8	2 1 2 1 2 2 C C D A A 2	0.04663
9	1 1 2 2 2 2 C C D G A 2	0.04397
10	1 1 1 1 2 1 A C I A A 1	0.03112
11	1 1 2 2 2 1 A C D G A 1	0.02616
12	1 2 1 1 2 2 A T I A A 2	0.02504
13	1 2 2 1 2 1 A T D A A 1	0.01500
14	2 1 2 2 1 2 C C D G C 2	0.01371
15	1 1 2 1 2 2 A C D A A 2	0.01322

Table 4.11: Represents the haplotypes and clades in the *ACE* and *GH* genes compared with Keavney's [88] paper

	Hap†	Clade	A-5466C	T1237C	I/D	Hap. Freq. 1‡	Hap.Freq. *	*ACE* & *GH* Hap.	Hap. Fre.♣	Clade Fre.κ
1	1&7	A	**A**	**T**	**I**	0.38	0.38	A T I A A 1 A T I G A 2 A T I G A 1 A T I A A 2	0.21916 0.08755 0.05075 0.02504	0.3852
2	2&6	B	**C**	**C**	**D**	0.35	0.31	C C D A A 1 C C D G A 2 C C D G A 1 C C D A A 2 C C D G A 2 *C C D G C 2*	0.13697 0.11033 0.05917 0.04663 0.04397 0.01371	0.41078
3	4&3	C	**A**	**C**	**D**	0.14	0.16	A C D A A 1 A C D G A 1 A C D A A 2	0.04710 0.02616 0.01322	0.08648
4	5	A	**A**	**C**	**I**	0.04	0.05	A C I A A 1	0.03112	0.03112
										0.91358

†: Haplotypes number in Keavney's paper [88].

‡: Haplotype frequency in our study. The orders of markers in the haplotypes are the same as theirs in the text.

*: Haplotype frequency in Keavney's paper [88].

♣: *ACE* and *GH* haplotypes frequencies.

κ: Frequency of clades in the final *ACE* and *GH* gene haplotypes.

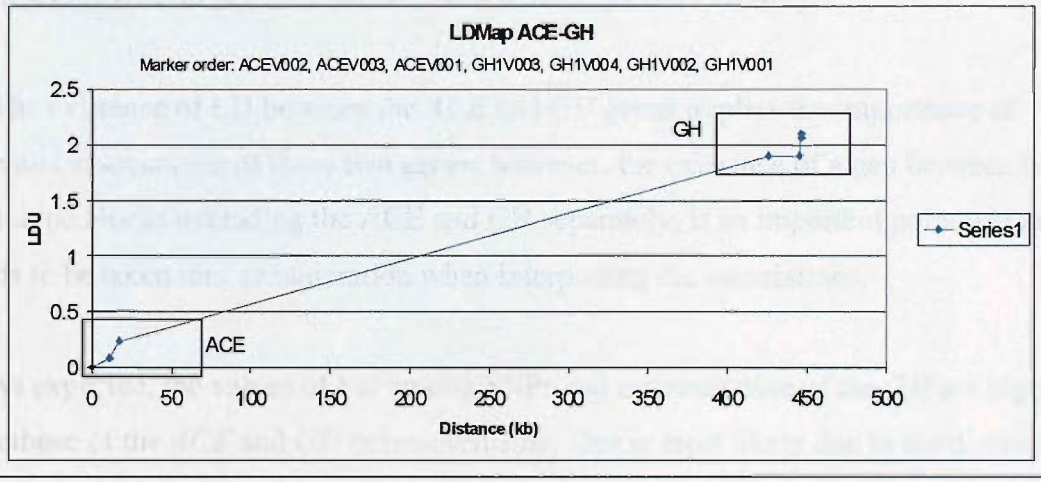
In Table 4.11, in position 5 of the *ACE* and *GH* haplotypes, there has always been allele A, except for high-lighted haplotype, in which there is allele C rather than A; the possible explanation for this event is that allele C might have a low frequency.

4.5 Result of Haplotype Block Analysis of the *ACE* and *GH* Genes

A LD unit map analysis was performed (by Dr. A Collins, Dr. T Gaunt and Dr. S Rodriguez) to resolve any haplotype block extending in these two genes, and it was found that *ACE* and *GH* markers fall into two discrete haplotype blocks with low LD between them. The results also indicated that an intergenic gap of 374kb is more realistic than one of 1.5Mb (Figure 4.2).

Figure 4.2: The LD Unit Map Study of the *ACE* and *GH*

LDMAP ACE/GH	LDU	KB
	0	0
LDMAP of ACE/GH region assuming:	0.087607	10.952
orientation ACE-CSH1-GH1	0.238736	16.918
374 kb distance separating ACE and GH cluster	1.904012	426.114
-2lnL kb: 80.70	1.904012	445.549
-2lnL LDU: 63.62	2.068155	446.693
Using d2/t for GH1V003	2.099938	446.723



4.6 Conclusion

The high levels of D' values found among studied polymorphisms of the *ACE* show that it is very likely that there is one haplotype block in the *ACE* gene. This point was also reported by Zhu X et al. (2003) [156], i.e. one block was found in American whites and blacks.

As is presented in Table 4.11, it could be concluded that the three typed polymorphisms in our study (*ACE* A-5466C, C1237T and I/D) are informative enough to verify almost all haplotypes or clades of the *ACE* (found in this thesis) to those of Keanvey's [88] (see 1.2.2.2). Furthermore, these haplotypes could be extended to the studied *GH* polymorphisms. Therefore, with typing these three polymorphisms it would be possible to put subjects in the proposed clades. However, the presence of a gap in between the *ACE* and *GH* is a point which needs to be considered in association and haplotype studies of these two genes.

The slight difference in the frequencies of the haplotypes obtained from Phase and Arlequin might be due to different algorithms used by these two programmes. These two results, which are statistically non significant, may not affect the robustness of analysis, but it seems wise to perform both of them to have greater certainty.

The existence of LD between the *ACE* and *GH* genes implies the importance of potential associations of these two genes; however, the existence of a gap between two haplotype blocks extending the ACE and GH separately, is an important point which needs to be taken into consideration when interpreting the associations.

As expected, the values of LD among SNPs and microsatellite of the *GH* are higher than those of the *ACE* and *GH* polymorphisms. This is most likely due to the distance between the *ACE* and *GH*. Interestingly, the LD value between the C5187A (number 5 in the analysis) increased after deleting four nucleotide alleles (alleles 17, 18 and 19) of the microsatellite.

5 Result of Genotyping and Genotype Phenotype Study of *AGT* M235T

Based on designed primers (Appendix 1), in the ARMS assay two bands of 354bp for control band and 76bp for allele specific band, and in the restriction digestion assay three bands of 316bp, 285bp and 31bp were expected. Gel images (Figures 9 and 10 in Appendix 3), after being scanned in the fluoroimager, were examined using Phoretix software in which genotypes were assigned manually and counts were then exported to Excel for further analysis. Genotypes were tested for Hardy-Weinberg equilibrium using χ^2 (Table 5.1). Results were slightly deviated from Hardy-Weinberg equilibrium, p = 0.05 Frequency of alleles were: C = 0.43, T = 0.57.

Table 5.1: Genotype distribution of the *AGT* M235T in NH and EH populations

Genotype / Counts	11(TT)	12(TC)	22 (CC)	χ^2
950	299	494	157	3.9

5.1 Result of the Genotype Phenotype Study of *AGT* M235T

Detail of the analysis is provided in Appendix 4. No significant association was found in this analysis.

6 Results of the Predicted Secondary RNA Structures of *AGTR1* RNA

6.1 Secondary RNA Structures of the Haplotypes of *AGTR1*

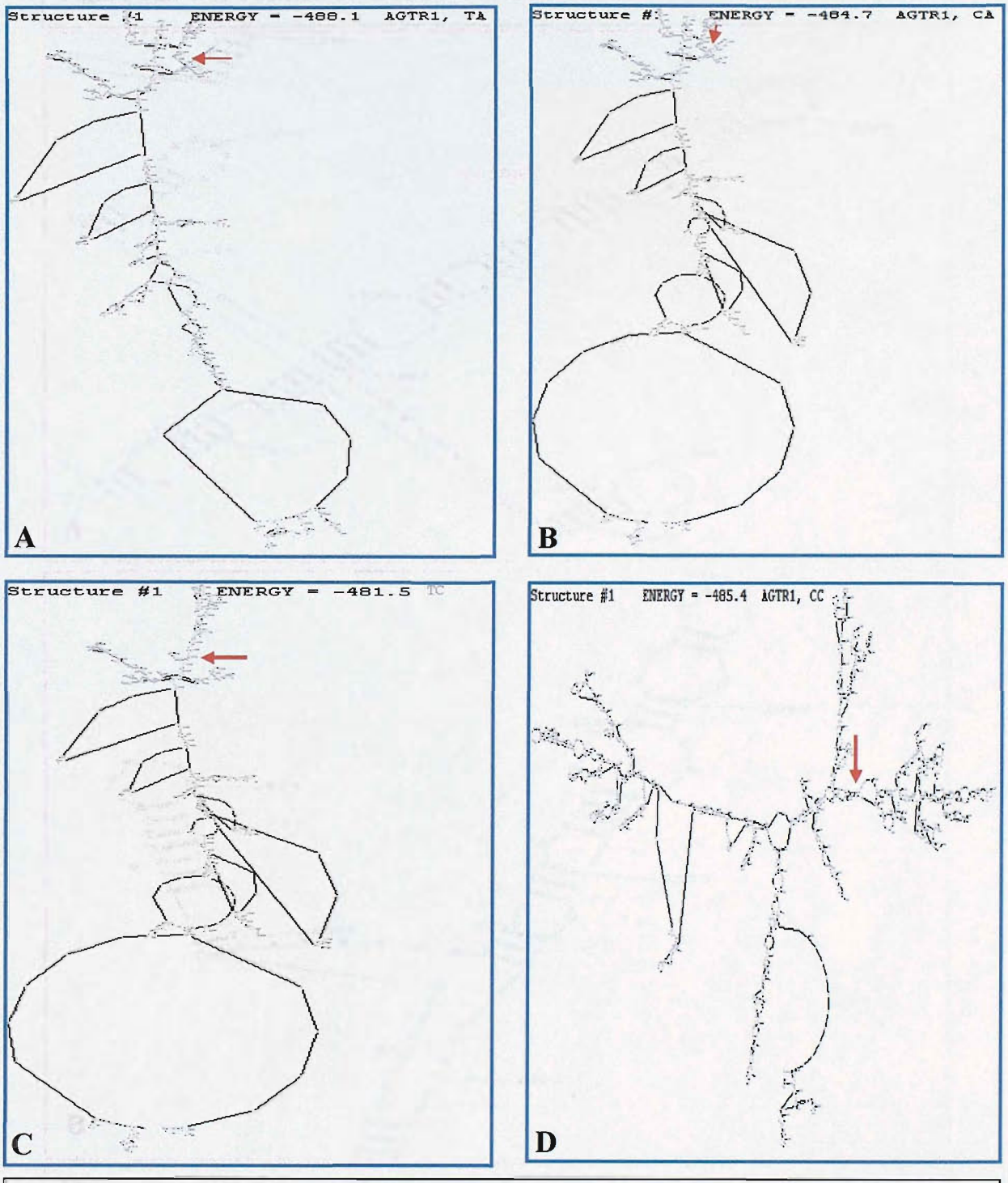
The predicted secondary RNA structures relative to the submitted haplotypes of L191L (T to C) and A1166C are presented in Figure 6.1. The position of A1166C in each structure has also been represented by red arrows. The required amount of energy [Gibbs free energy of formation (ΔG)] for formation of each structure has also been mentioned in Table 6.1 as well as Figures 6.1.

Table 6.1: The value of ΔG for each haplotype of L191L and A1166C

Haplotype L191L-A1166C	ΔG (kcal/mol)
TC	-481.5
TA	-488.1
CC	-485.4
CA	-487.7

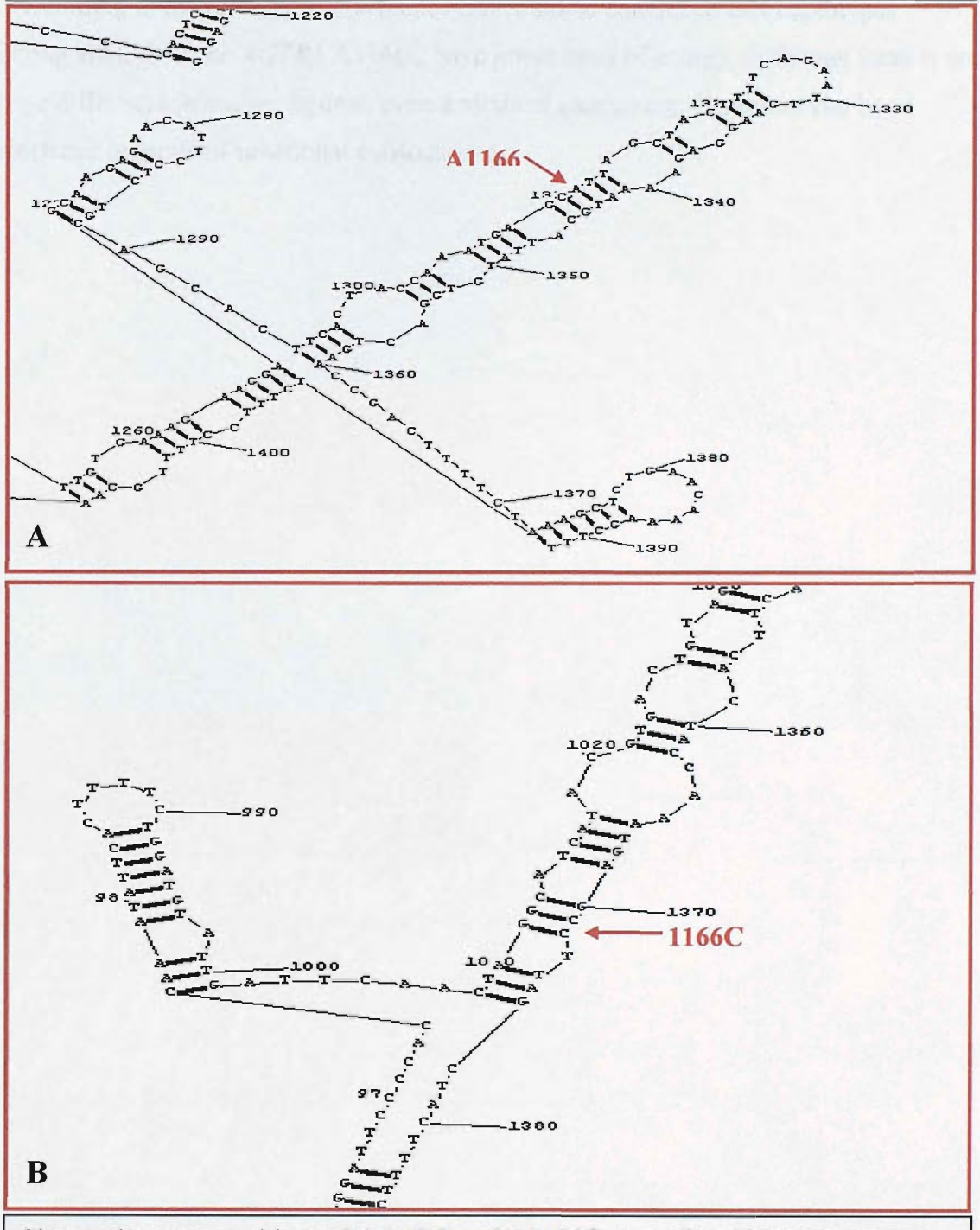
It was also interesting to look at these structures regarding the alleles of the *AGTR1* A1166C. The purpose of this approach was to check if the alleles of this SNP make any essential change in the structure. Figure 6.2 shows the position of alleles A and C of A1166C in the predicted RNA structures.

Figure 6.1: Predicted Secondary Structures for *AGTR1* mRNA



Shown above are predicted structures for *AGTR1* mRNA in respect to haplotypes of L191L and A1166C. Figures A to D refer to haplotypes TA, CA, TC and CC respectively. Red arrows show the position of A1166C in each structure. The amount of energy required for each structure is also shown.

Figure 6.2: The Position of the alleles of A1166C in RNA Structure



Shown above are positions of the alleles of A1166C in predicted RNA structures. A shows the position of allele A1166, and B dose that of allele 1166C.

6.2 Conclusion

According to the results presented above, it could be concluded that haplotypes carrying allele C of the *AGTR1* A1166C have lower level of energy. Although there is not a huge difference in energy figures, even a minimal change e.g. 1kcal/mol can be of importance in terms of functional consequences.

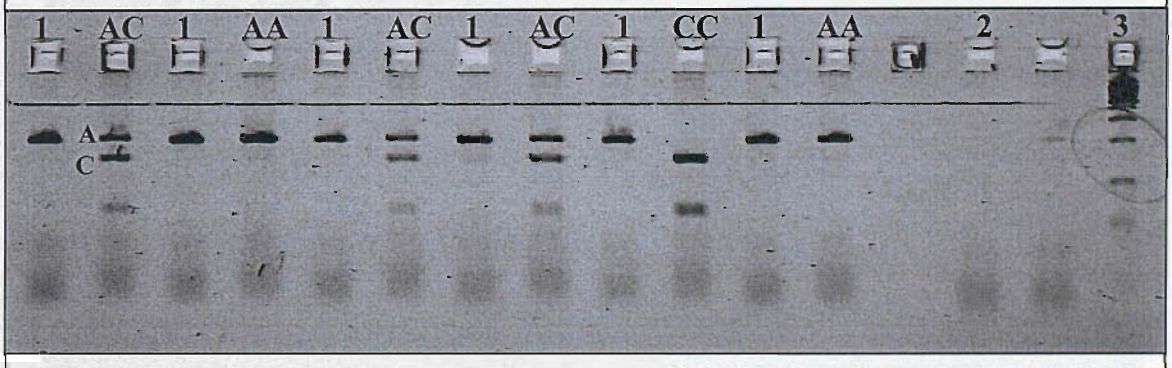
7 Results of the Ratiometric Analyses of *AGTR1* L191L and A1166C

7.1 Results of Genotyping *AGTR1* L191L and A1166C

7.1.1 Result of Genotyping *AGTR1* A1166C

Based on primer design, a PCR product of 198bp was expected. After digestion, three bands were expected: an undigested or A allele 198bp and two other bands in the case of the presence of C allele, 56bp and 142bp (in AC and CC genotypes), (Figure 7.1 in the text & Figure 1 in Appendix 6). Gel images were examined using Phoretix software; genotypes assigned manually, and genotypes counts were exported to Excel for further

Figure 7.1: Gel Showing the Genotyping of A1166C Using *Bpu10 I*



A 5% polyacrylamide gel showing the pattern of different genotypes of A1166C as digested with Bpu10 I. 1: Undigested band, 2: Negative control (PCR without template), 3: 100bp ladder.

analyses (Table 2 in Appendix 6). Genotypes and Hardy-Weinberg equilibrium were then tested using standard χ^2 test for total sample. Table 7.1 shows genotype frequencies.

Table 7.1: Distribution of the genotypes of A1166C in placenta samples

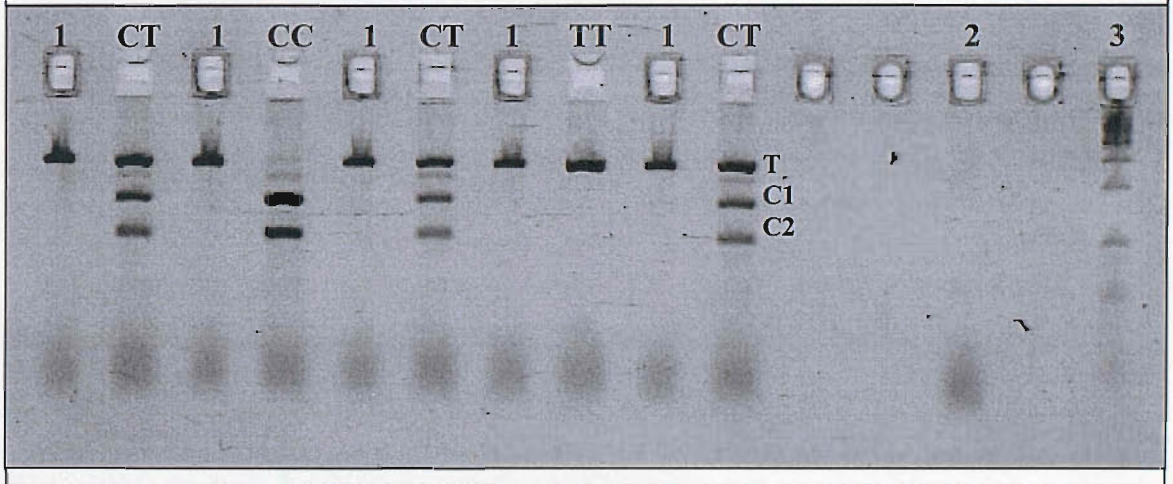
Genotypes / Counts	11(AA)	12(AC)	22(CC)	Dropouts	χ^2
73(Observed)	39 (53.42%)	27 (36.98%)	7 (9.58%)	0	0.5
Expected	37.8	29.5	5.8	0	

The frequencies of alleles: A = 0.72 and C = 0.28, p = 0.471

7.1.2 Result of Genotyping *AGTR1* L191L

Based on primer design, a PCR product of 250bp was expected. After digestion, three bands were expected: an undigested or T allele of 250bp and two other bands in the case of the presence of C allele: 153bp (C1) and 97bp (C2) (in TC and CC genotypes), (Figure 7.2 in the text and Figure 2 in Appendix 6). Gel images were examined using Phoretix software; genotypes assigned manually, and genotypes counts were exported to Excel

Figure 7.2: Gel Showing Genotyping L191L Using *Mnl I*



A 5% polyacrylamide gel showing the patterns of genotypes of the L191L after digestion with Mnl I. 1: Undigested PCR, 2: Negative control (PCR without template), 3: 100bp ladder.

for further analysis (Table 2 in Appendix 6). Genotypes and Hardy-Weinberg equilibrium were then tested using standard χ^2 test for total sample. Table 7.2 shows genotype frequencies.

Table 7.2: Genotypes distribution of L191L in placenta samples

Genotypes / Counts	11(TT)	12(TC)	22(CC)	Dropouts	χ^2
73(observed)	14(19.17%)	39(53.42%)	20(27.39%)	0	0.4
Expected	15.4	36.3	21.4	0	

The frequencies of allele T = 0.46 and C = 0.54, p = 0.517

7.2 Result of Haplotype and LD Analyses

The structure and frequencies of haplotypes are presented in Table 7.3. The two SNPs are in complete LD: D′ = -1, $\chi^2 = 45.67$, df = 1, $p < 0.0001$.

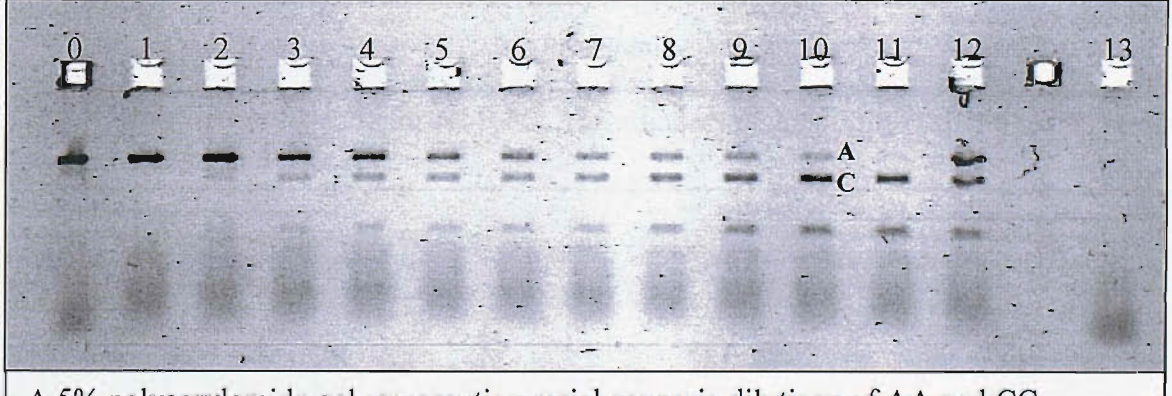
Table 7.3: Structures and frequencies of haplotypes in placenta samples obtained from LD and Arlequin analysis

	Haplotypes (L191L-A1166C)	Haplotype Structures	Haplotype Frequencies
1	21	TA	0.46
2	11	CA	0.26
3	12	CC	0.28

7.3 Standard Curve for A1166C

A gel showing the serial dilutions of AA and CC used for analysis, stained with ethidium bromide is represented in Figure 7.3 (see also Figures 3 and 4 in Appendix 6).

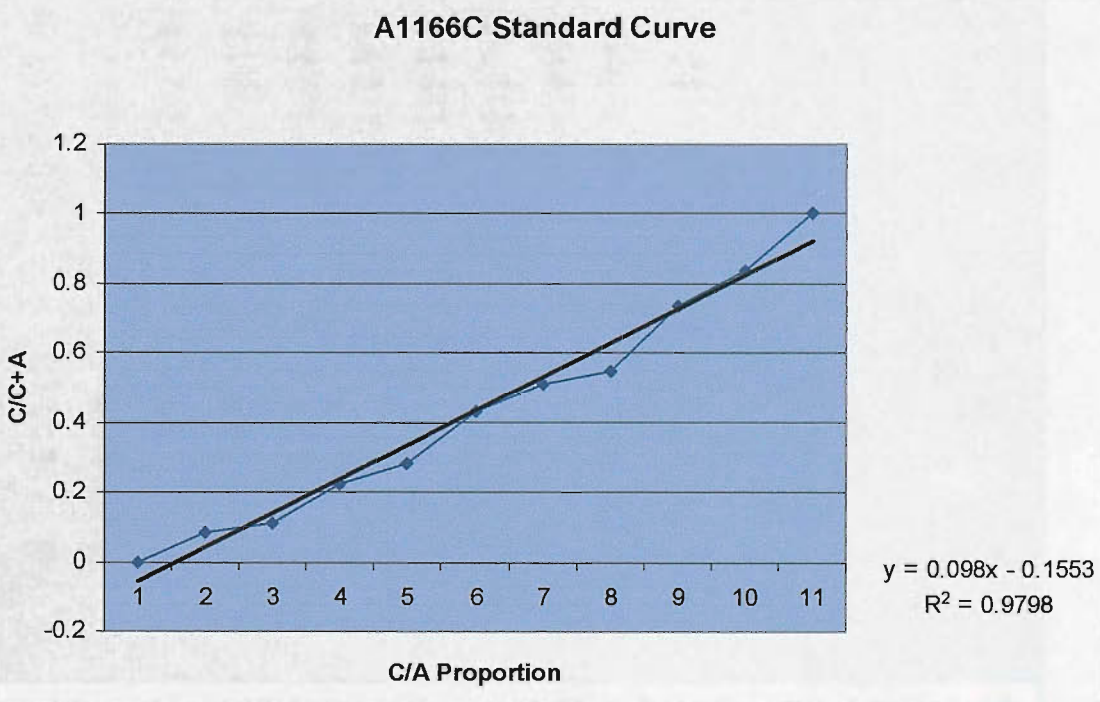
Figure 7.3: The Gel Used for Making Standard Curve for A1166C



A 5% polyacrylamide gel representing serial genomic dilutions of AA and CC genotypes (see Table 1.1) of A1166C. Templates were control DNA to make standard curve.
0: undigested template, 1: AA, 11: CC (digested), 1 to 11: Serial dilutions of AA and CC (see Table 1.1), 12: AC, 13: Negative control (PCR mix without template).

The standard curve for A1166C is presented in Figure 7.4. The means of the proportions of bands C/(C+A) in heterozygote genomic DNA is 0.280, and when it is inserted as Y in regression formula, y = 0.098x-0.1553, the value of x would be 4.44.

Figure 7.4: Standard Curve for A1166C

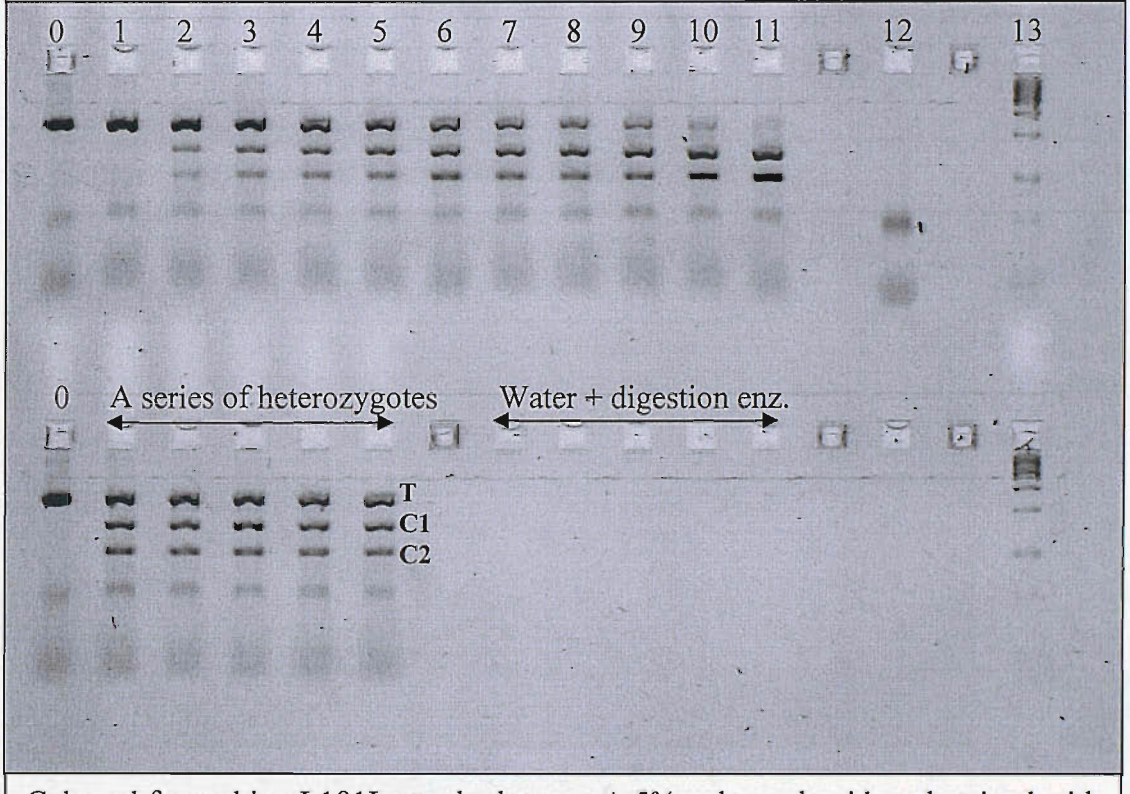


Standard curve made for A1166C. The numbers on axis X refer to the numbers of columns in Table 2.2.

7.4 Standard Curve for L191L

A gel representing the serial dilutions of TT, CC and five heterozygotes (of genomic DNA) is shown in Figure 7.5 (see also Figures 5 in Appendix 6). Two band proportions were measured and regressed against C/T proportions (Table 2.3): C1/(C1+T) and (C1+C2)/(C1+C2+T).

Figure 7.5: The Gel Used for Making Standard Curve for L191L

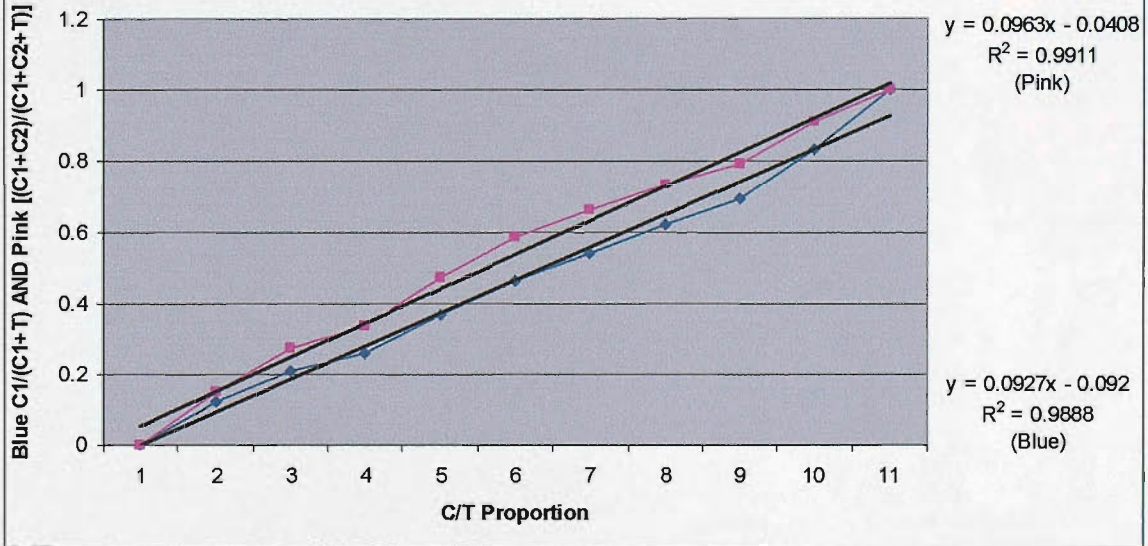


Gel used for making L191L standard curve. A 5% polyacrylamide gel stained with ethidium bromide, loaded with digestion products of L191L with *Mnl 1*, used for making standard curve. 0: Undigested PCR product, 1: TT, 11: CC, 1 to 11: Corresponds to related dilutions of C/T (see Table 1.2). 12: Negative control, 13: 100bp ladder. In bottom row, a series of heterozygous samples are loaded.

Hence, there would be two standard curves (Figure 7.6). The purpose of these two analyses was to test if there would be any significant difference with including the smaller digestion band (97bp = C2).

The means of bands proportions C1/(C1+T) and (C1+C2)/(C1+C2+T) are 0.34 and 0.45 (which are not significantly different p = 0.99) respectively. If these values (as Y) are included in related regression formulae (y = 0.0927x – 0.092 and y = 0.0963x -0.0408 respectively), the values of obtained X(s) would be 4.63 for C1/(C1+T) and 5.11 for (C1+C2)/(C1+C2+T).

Figure 7.6: Standard Curves for L191L



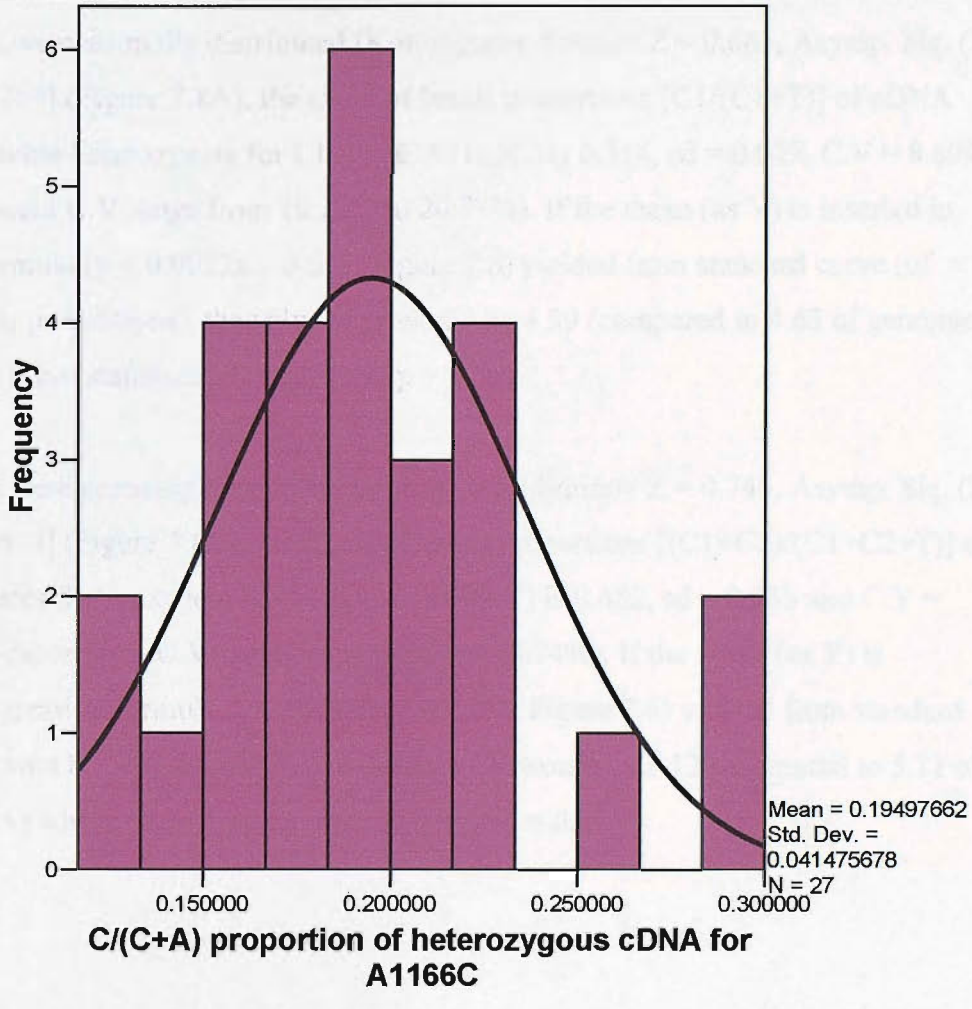
L191L standard curves with related regression formulae. The numbers on axis X refer to the number of columns in Table – 2.3.

7.5 Results of Ratiometric Analysis of A1166C on cDNA

A picture of a gel is presented in Figure 7 in Appendix 6 and gels de-stained with $MgSO_4$ (twice) and restained with Vistra Green™ are presented in Figure 8 in Appendix 6. Contamination of cDNA with genomic DNA was ruled out before this experiment.

The results of bands proportions C/(C+A) are normally distributed [Kolmogorov-Smirnov Z = 0.638 and Asymp. Sig. (2-tailed) = 0.810] (Figure 7.7), the mean of the proportions of bands was 0.195 and sd = 0.041 and C.V = 21.27% (inter-experiments C.V range from 12.64% to 39.89%). If the mean is inserted as Y in the regression formula obtained from the standard curve of genomic DNAs (presented in Figure 7.4), the value of X would be 3.57, which is lower than the X value for genomic DNA (= 0.868) and is significantly different (p = 0.000). This means 18% decrease in the expression of *AGTR1*.

Figure 7.7: The Normal Distribution of the Proportions of Bands C/(C+A) in cDNA of Heterozygous Placenta for A1166C



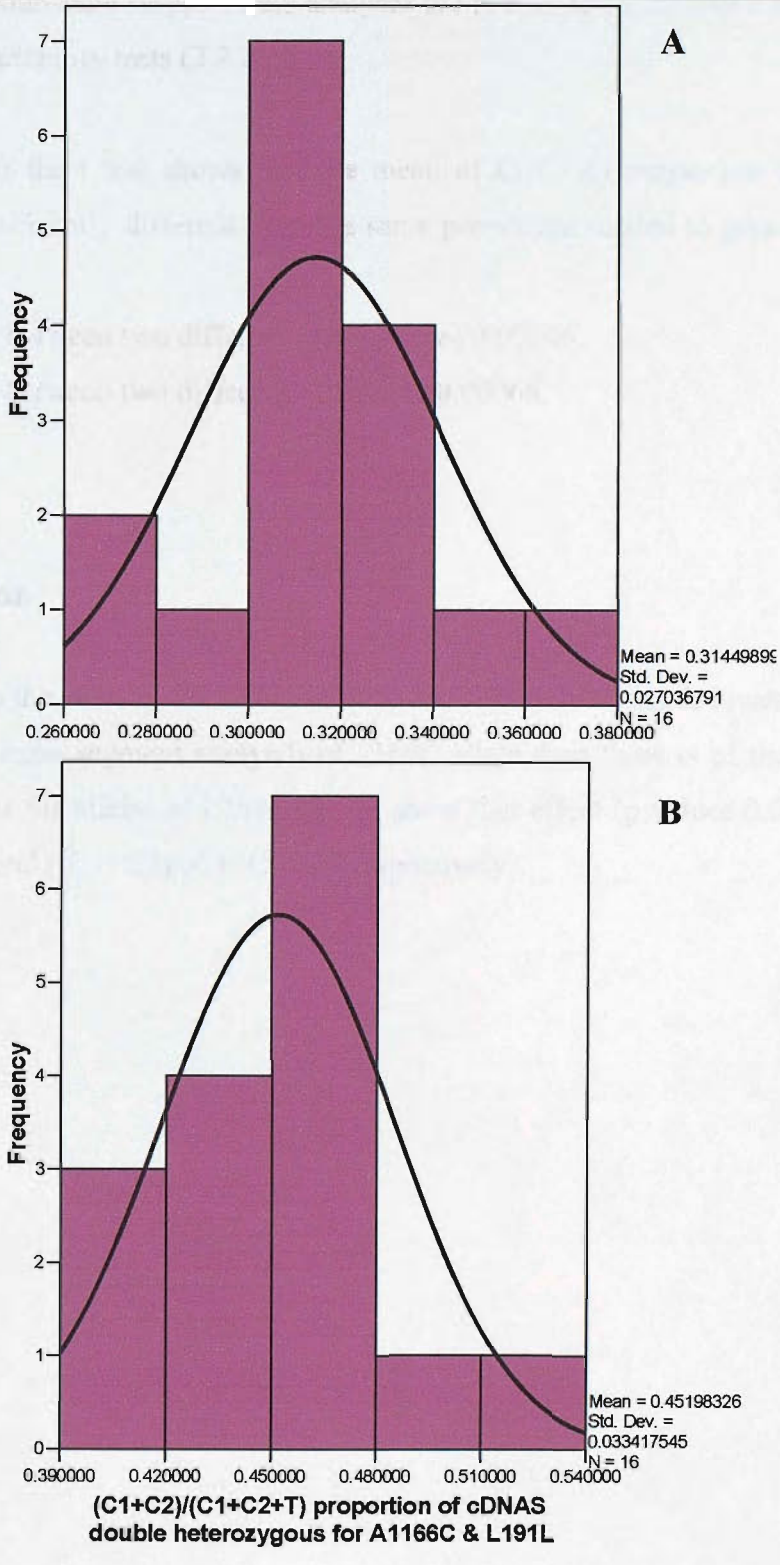
7.6 Results of Ratiometric Analysis of L191L on cDNA

Figure 9, in Appendix 6, represents a gel loaded with digestion products of L191L of double heterozygote (A1166C & L191L) cDNAs, and Figure 10 in Appendix 6 shows two steps of destaining and one step of restaining with Vistra Green™. Contamination of cDNA with genomic DNA was ruled out before this experiment. As previously mentioned, two proportions were calculated: **1**) C1/(C1+T) and **2**) (C1+C2)/(C1+C2+T)

1) Results were normally distributed [Kolmogorov-Sminov Z = 0.665, Asymp. Sig. (2 – tailed) = 0.769] (Figure 7.8A), the mean of bands proportions [C1/(C1+T)] of cDNA templates (double heterozygote for L191L & A1166C) is 0.314, sd = 0.027, C.V = 8.60% (inter-experiment C.V range from 10.29% to 20.71%). If the mean (as Y) is inserted in regression formula (y = 0.0927x – 0.092, Figure 7.6) yielded from standard curve (of relevant bands proportions), the value of X would be 4.39 (compared to 4.63 of genomic DNA) which is not statistically significant (p = 0.06).

2) Results were normally distributed [Kolmogorov-Sminov Z = 0.746, Asymp. Sig. (2 – tailed) = 0.634] (Figure 7.8B), the mean of bands proportions [(C1+C2)/(C1+C2+T)] of cDNA templates (heterozygote for L191L & A1166C) is 0.452, sd = 0.033 and C.V = 7.39% (inter-experiment C.V range from 9.63% to 16.74%). If the mean (as Y) is inserted in regression formula (y = 0.0963x – 0.0408, Figure 7.6) yielded from standard curve (of relevant bands proportions), the value of X would be 5.12 (compared to 5.11 of genomic DNA) which is not significantly different (p = 0.97).

Figure 7.8: The Normal Distribution of Different Proportions: A = C1/(C1+T) & B = (C1+C2)/(C1+C2+T) in L191L Analysis



7.7 Results of the Statistical Analyses

Descriptive statistical results of the analyses are presented in 7.5 and 7.6. Here are the results of the variability tests (2.2.2.12):

1. The result of the t test shows that the mean of C/(C+A) proportion of a group of cDNAs are significantly different from the same proportion related to genomic DNAs ($p < 0.001$).
2. The variance between two different loadings was 0.00046.
3. The variance between two different PCRs was 0.00066.

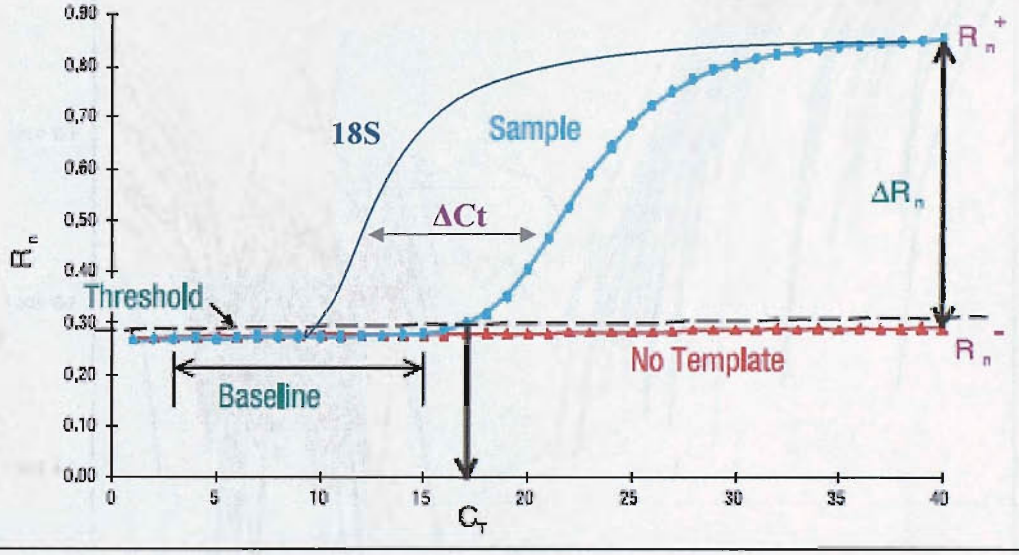
7.8 Conclusion

According to the presented results, it can be concluded that there is lower mRNA level (at least of the exon segment assayed) of 1166C allele than there is of the A1166 ($p = 0.000$). However the alleles at L191L do not show that effect (p values 0.061 and 0.970 for C1/(C1+T) and [(C1+C2)/(C1+C2+T)] respectively).

8 Results of TaqMan Assays of *AGTR1* L191L and A1166C

To place the TaqMan assays results and plots in context, Figure 8.1 is presented to show the schematic amplification of a sample along with general terms used in TaqMan.

Figure 8.1: Model of a Single Amplification Plot in Real-time Quantitative PCR

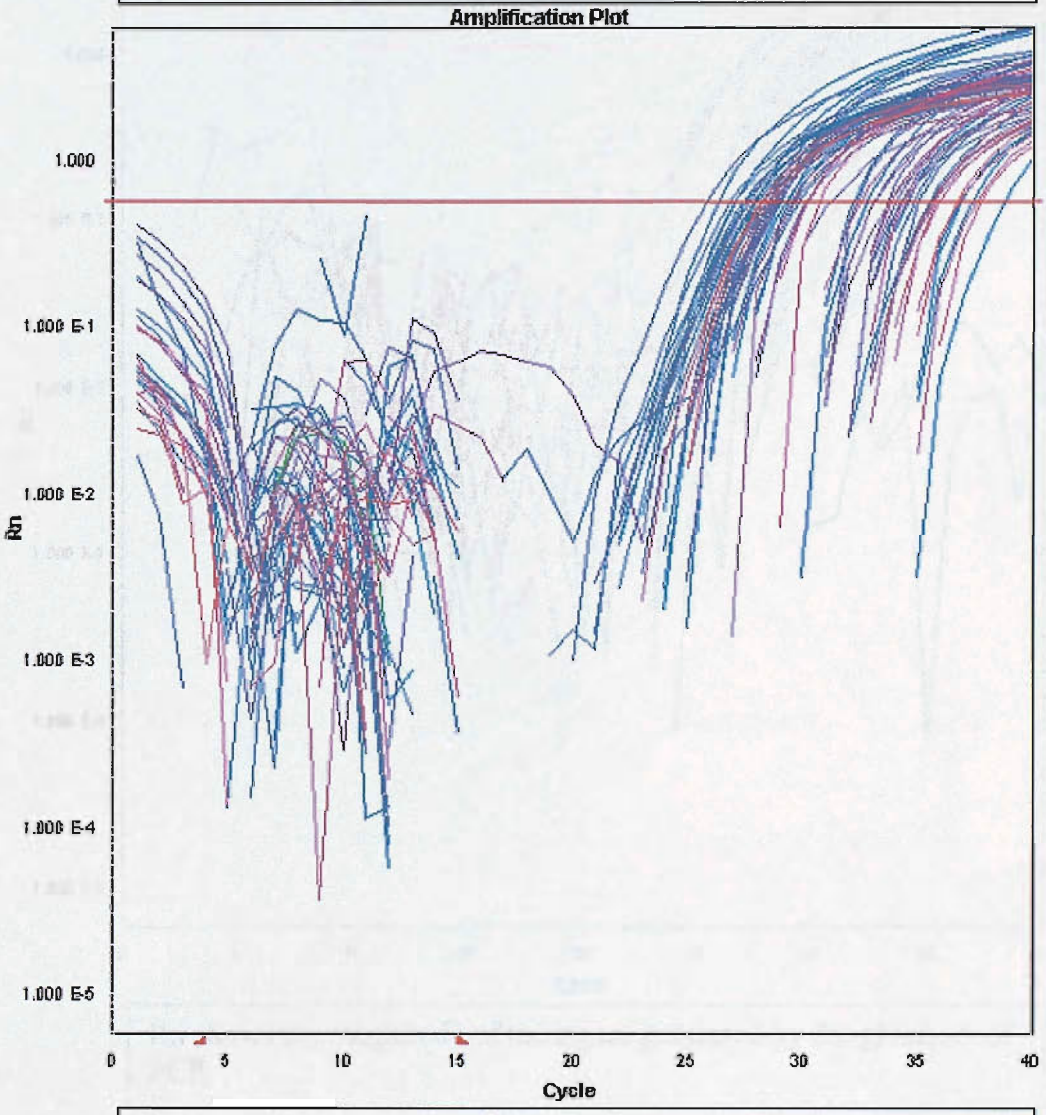


See 2.2.3.2.1 for definition of terms. The above is a schematic representation of linear amplification of a sample in TaqMan assay. ΔR_n is the magnitude of the signal generated by the given set of PCR compared with internal fluorescence (Rox). As shown ΔCt (or C_T) is the Ct difference between the sample and internal control (18S in this case) measured from the threshold set in the geometric phase of amplification.

8.1 Amplification Plots of the Assays

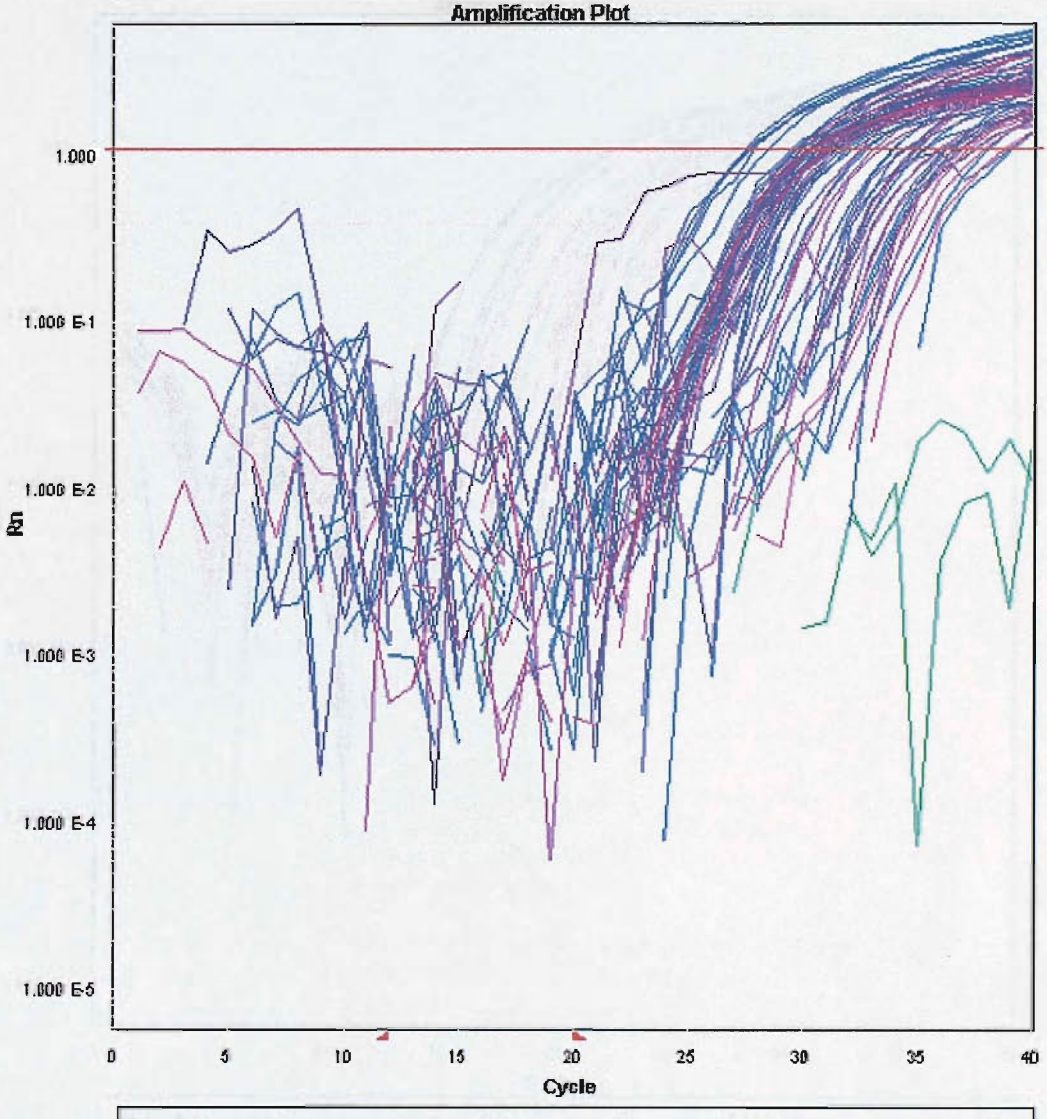
Amplification plots of L191L, A1166C and 18S are presented in Figures 8.2, 8.3 and 8.4 respectively. Failed samples were repeated in duplicate and the total number of samples in the TaqMan experiment after deleting dropouts (four) was 69.

Figure 8.2: Amplification plot of L191L reaction in TaqMan assay



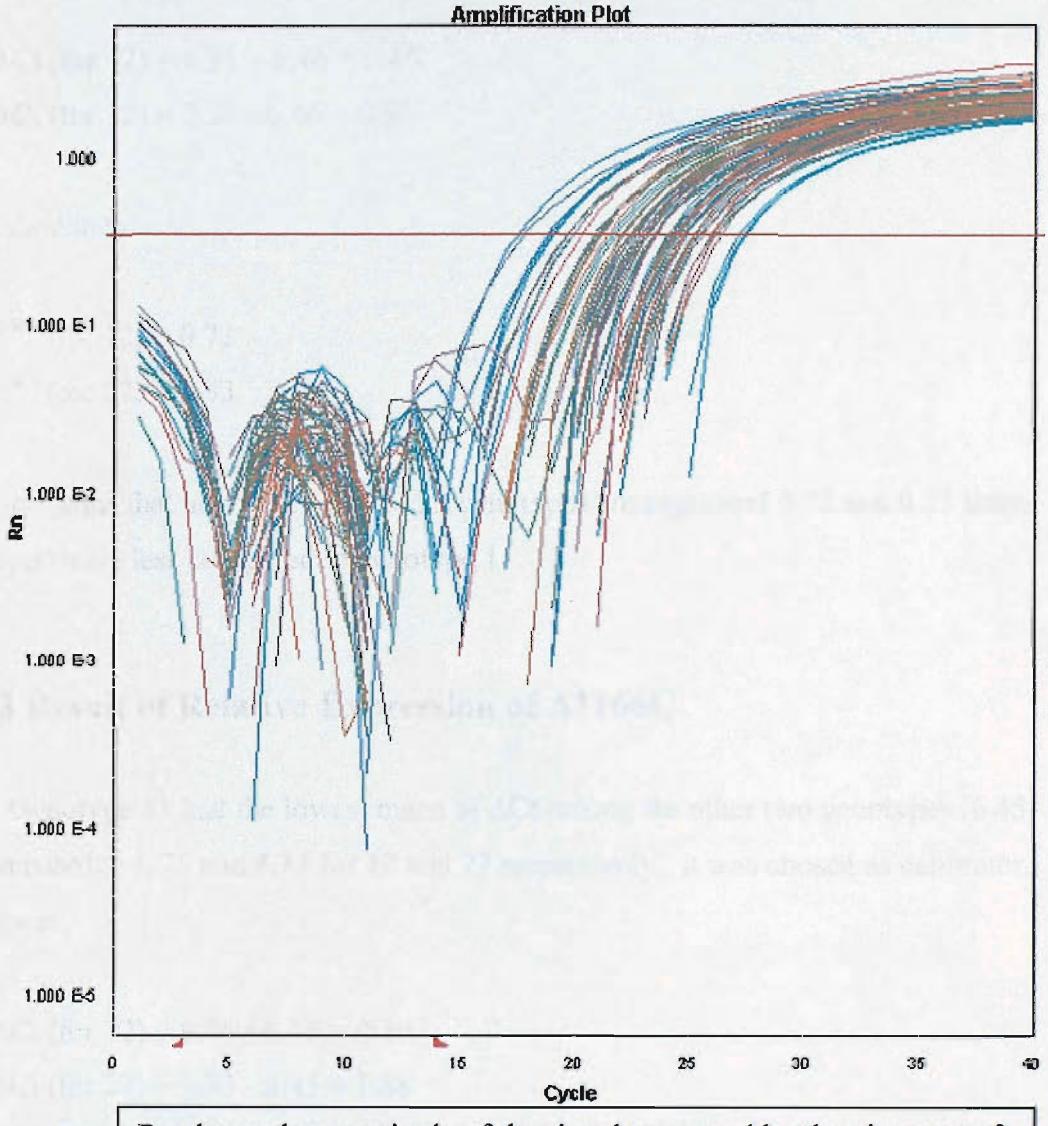
Rn shows the magnitude of the signal generated by the given set of PCR.

Figure 8.3: Amplification plot of A1166C reaction in TaqMan assay



Rn shows the magnitude of the signal generated by the given set of PCR.

Figure 8.4: Amplification plot of 18S reaction in TaqMan assay



Rn shows the magnitude of the signal generated by the given set of PCR.

8.2 Result of Relative Expression of L191L

Genotype 11 had the lowest mean of ΔCt among the other two genotypes (6.46 compared to 6.91 and 7.39 for genotypes12 and 22 respectively), and was chosen as calibrator. Hence:

ΔΔCt (for 12) = 6.91 – 6.46 = 0.45
ΔΔCt (for 22) = 7.39 - 6.46 = 0.93

And then:

$2^{-\Delta\Delta Ct}$ (for 12) = 0.73
$2^{-\Delta\Delta Ct}$ (for 22) = 0.53

It means that genotypes 12 and 22 genotypes are expressed 0.73 and 0.53 times respectively less compared to genotype 11.

8.3 Result of Relative Expression of A1166C

Genotype 11 had the lowest mean of ΔCt among the other two genotypes (6.45 compared to 6.75 and 8.33 for 12 and 22 respectively), it was chosen as calibrator. Hence:

ΔΔCt (for 12) = 6.75 - 6.45 = 0.30
ΔΔCt (for 22) = 8.33 - 6.45 = 1.88

And then:

$2^{-\Delta\Delta Ct}$ (for 12) = 0.8
$2^{-\Delta\Delta Ct}$ (for 22) = 0.27

It means that genotypes 12 and 22 are expressed 0.8 and 0.27 times respectively less compared with genotype 11.

8.4 Results of Phase Analysis

The structure and frequency of haplotypes obtained from Phase analysis are presented in Table 8.1.

Table 8.1: Haplotype structures and frequencies obtained from Phase analysis

Haplotype	Haplotype Structure (L191L & A1166C)	Frequency (Total No. 138)	Percentage of Haplotype
1	21	63	46%
2	11	36	26%
3	12	39	28%

8.5 Results of ANOVA and Regression

There was no significant association between L191L and ΔCt (p values: 0.52 and 0.33 for ANOVA and regression respectively). In the case of A1166C, there was no significant association between 22 genotype and ΔCt ($p = 0.24$), while there was a trend of association between allele 2 of A1166C and higher ΔCt ($p = 0.09$). Details of analyses are presented in the Appendix 6.

These analyses were repeated between ΔCts of the SNPs and haplotypes. Since there was only one haplotype 2.2, it was deleted from this analysis. In the analysis for L191L, no significant association was found between haplotypes and L191L ΔCt, p values 0.28 and 0.80 for ANOVA and regression respectively. Similarly, in the analysis for A1166C, no significant association or correlation was found between haplotypes and A1166C ΔCt,

p values 0.23 and 0.49 for ANOVA and regression respectively. Details of analyses are presented in the Appendix 6.

8.6 Results of Haplotype Trend Regression (HTR) Analysis

The results of HTR are presented in Table 8.2. Details of the analyses are provided in Appendix 6. As shown in Table 8.2, there are significant associations between haplotype 1_1 and lower ΔCt of both L191L and A1166C regions, p values 0.032 and 0.046 respectively. In both SNPs, the trends of association between higher ΔCt and the presence of their own allele 2 are seen.

Table 8.2: Results of HTR analysis

Haplotype Structure	Estimated Haplotype Frequencies	L191L			A1166C		
		Mean	2^-(ΔΔct)	p value	Mean	2^-(ΔΔct)	p value
1_1	0.26	6.29		0.0319	6.12		0.046
1_2	0.28	7.04	0.59	0.6015	7.23	0.46	0.1423
2_1	0.46	7.11	0.57	0.2545	6.76	0.64	0.8769
	Total No. = 138	Overall p value		0.1	Overall p value		0.1

Haplotype 1_1 is considered as calibrator in both SNPs.

8.7 Conclusion

In the case of L191L, *AGTR1* mRNA abundance was 0.73 and 0.53 times less in genotypes 12 and 22, compared with genotype 11, respectively. A similar scenario was seen for A1166C i.e. the *AGTR1* was expressed 0.8 and 0.27 times less in genotypes 12 and 22, compared with genotype 11, respectively. In HTR analysis, it is seen that haplotype 1_1, in both SNPs, is significantly associated with lower ΔCt i.e. higher expression; furthermore, carrying allele 2 of each SNP in haplotypes, though non significant, causes higher ΔCt, i.e. lower expression.

9 Discussion

9.1 *AGTR1* Studies

As an initial comment regarding the technique chose, the tetra-primer ARMS assay developed for *AGTR1* A1166C gave reasonable discrimination but required significant time to achieve optimisation. It might have been more efficient to establish two separate allele specific assays requiring twice as many PCR reactions that probably have been set up more readily. Therefore, three-primer ARMS assays were designed for the other SNPs.

9.1.1 Genotype and Haplotype Phenotype Studies

(see chapter 3)

Anthropometric traits and the principal traits of metabolic syndrome were examined in relation to *AGTR1* A1166C. These have been extensively studied regarding hypertension and coronary artery disease. Our analyses suggest that *AGTR1* A1166C affects BMI and weight, waist or waist-hip-ratio. Baseline glucose and insulin values and 30 and 120 minutes glucose were also significantly affected in men with comparable trends in women (see 3.2.1 and Table 3.7).

It was demonstrated that CC, of *AGTR1* A1166C, genotype associates with lower values for these variables. Given the known gender differences for anthropometric and metabolic traits, males were examined separately from females. The lower level of significance in women may reflect the smaller number studied (138 vs 240 in the EH). Furthermore, similar magnitude differences are seen for CC genotype women for BMI and glucose values at OGTT (oral glucose tolerance test) time-points and a post-hoc combined analysis is also shown in Table 3.7. CC genotype seems to associate with lower BMI by approximately 2 units, lower waist by about 7cm, lower glucose level at all points in OGTT by about 0.5 mmol/l, and (non-significant) triglyceride and cholesterol by about 0.2-0.3mM each. However, the pattern for insulin levels in OGTT differs between males and females. These findings add to the observations of metabolic

associations for the *ACE* I/D polymorphism [27,108,109] may implicate diversity of the RAS pathway more generally in influence on anthropometric and metabolic traits.

It is possible but uninvestigated that *AGTR1* A1166C in the 3′ UTR might affect mRNA stability or polyadenylation. Alternatively, it may be in linkage disequilibrium (LD) with some other functional marker(s) located elsewhere in the *AGTR1* gene or within a nearby gene that could explain the observed associations of this SNP with cardiovascular and metabolic phenotypes. This was a reason we studied other SNPs in both exon 5 (L191L) and the 5′ region of the gene (C-521T and A-153G) to resolve haplotypes and LD between them. In this part of the study, it was observed that the value of D′ between SNPs in 5′ UTR was much higher than that of between SNPs in 5′ UTR and SNPs in exon 5. Similarly, the D′ value between L191L and A1166C was significantly high. This finding is in general agreement with the literature, in which no LD has been detected between A1166C and SNPs in the 5′ UTR and promoter region (G-2228A, C-1424G, T-810A, T-713G, C-521T, A-214C, G-213C and A–153G) and T55C in exon 4 of the *AGTR1* gene [121,121]. However, Lajemi et. al. (2001) [91] found an additive effect of 1166C and -153G on aortic stiffness.

Moczulski D K et al. (1998) [106] achieved a linkage study in 66 Caucasian siblings with type 1 diabetes. They found linkage between 20cM region on chromosome 3 containing *AGTR1* and diabetic nephropathy. This point underscores the importance of the *AGTR1* and its polymorphisms in terms of conceivable associations with metabolic traits.

The interaction of A1166C with a nearby microsateelite is potentially an explanation for associations seen with this SNP. Doria A et al. (1997) [44] found higher frequencies of 1166C and a 140 bp allele of an adjacent CA repeat microsatellite in nephropathy cases of the IDDM compared to normoalbuminuric control subjects.

Another important issue, especially in the context of multifactorial diseases, is the possible role of environmental factors. As investigated by Schmid C et al. (1997) [130] in Sprague-Dawley rats, low salt diet induces a temporary low expression of the *AGTR1* in

examined organs: kidney, liver and lung, but not adrenal gland; while this effect was not reported in rats fed with high salt diet. In another study [129], it was seen that the expression of *AGTR1* in rats kidneys exposed to low protein diet during intra-uterine life increased by 24%.

Effects of the 3′ UTR on cell signaling, translation and cell proliferation have been reported. Studies on Chinese hamster ovary (CHO-K1) have revealed the effect of *AGTR1* 3′ UTR on the angiotensin II receptor-mediated cell signaling pathway and have shown the presence of a 55 kDa RNA-binding protein which interacts with *AGTR1* 3′ UTR and influences specific receptor function [137]. They also showed that normally unstable *AGTR1* becomes more stable after removing the 3′ UTR, emphasizing that either 3′ UTR harbors a destabilizing element or a destabilizing reaction is happening as a result of reaction of a trans-acing element with a marker in 3′ UTR of the *AGTR1* gene.

While the mechanism of *AGTR1* A1166C genotype-phenotype associations remain uncertain, this study suggests that in addition to effects on vascular function, it can influence anthropometric and metabolic traits, providing further evidence of the integral effects of this gene and genotype on cardiovascular risk traits.

Angiotensin II has widespread effects on different organs of the body. The expression of *AGTR1* and *AGTR2* in different tissues such as adrenal cortex, kidney and rat uterus has been reported. The former is the predominant form in vascular smooth muscle and human uterus, whereas the latter is expressed in the adrenal medulla and brain more predominantly [37]. Giacchetti G et al. (2002) [61] reported the expression of angiotensin, *ACE* and *AGTR1* genes in visceral and subcutaneous adipose.

Our study suggests that, like *ACE* I/D genotype, *AGTR1* genotype may also influence metabolic as well as vascular phenotypes and invites investigation of both *AGTR1* and the RAS pathway with respect to metabolic traits.

Our findings showing the presence of two haplotypes in the *AGTR1* gene was confirmed by another report [156] showing that there are two main haplotype blocks in American whites and blacks. One of these haplotypes spans the 5′ UTR and the other one spans exon 5. However, the extent of these blocks outside *AGTR1* remains unknown. Nevertheless, the lack of significant LD between SNPs in the promoter region and A1166C is suggestive of a functional effect arising from the 3′ block.

9.1.2 Expression Studies

To place expression studies reported here in context, it would perhaps be better to address in brief the role of 3′ UTR in regulation of gene.

9.1.2.1 Regulatory Effects of 3′ UTR

There is an increasing number of papers showing the role of 3′ UTR on gene expression. This is a growing field and the number of genes shown to be involved is increasing. Moreover, such a concept could have a great impact on medicine. Sequences in 3′ UTR regulate transcript cleavage (e.g. as examplified in *IGF2* mRNA degradation [102]), polyadenylation, nuclear export, transcript stability, level of translation and mRNA targeting. mRNA degradation can occur through endonucleolytic cleavage, arrest of translation at a premature stop codon [nonsense-mediated decay, (NMD)] or poly (A) shortening. Binding of trans-acting elements (RNA binding protein = RNAbp) affects both of the last two steps. mRNA translatability is the most important factor determining the expression pattern, which is influenced by: translation repression/activation, sequestering mRNAs into messenger ribonucleoprotein (mRNP) particles, which makes them translationally inaccessible and poly (A) shortening with subsequent, regulated cytoplasmic polyadenylation. List of regulatory motifs (cis-acting elements) at 3′ UTR can be found in the UTR database [64]: http://bighost.area.ba.cnr.it/BIG/UTRHome/ and http://igs-server.cnrs-mrs.fr/~gauthere/UTR/3utr.html/.

9.1.2.1.1 Cis-acting elements

Adenylate/uridylate-rich elements (AREs) have been found in many mRNAs, such as: proto-oncoproteins, growth factors and their receptors, inflammatory mediators and cytokines. AU-rich elements range in size from 50 to 150 nucleotides containing one or several copies of AUUUA pentamer or UUAUUUA(U/A) nanomer. Their function is to mark mRNAs for degradation. However, this process by itself is influenced by stress conditions, cell stimulation, or oncogenic transformation [64]. Some of these elements are destabilizing, such as: AUUUA in mononucleocyte colony stimulating factor (GM-CSF), *c-myc* and *c-fos* genes, as well as non AUUUA elements e.g. GUUUG in 3′ UTR of *c-jun* mRNA or the iron response element in 3′ UTR of the transferrin receptor (CAGUGU/C). On the other hand, there are some stabilizing cis-acting determinants in the 3′ UTR, such as: GC-rich sequence in *β-globin* (very stable mRNA), UGGGGGGAGGGAGGGAGGGA in elastin and $PuU_{2-5}Pu_{1-2}U_{2-5}Pu$ (Pu = purine) in *HuC*. There might be more than one cis-acting element and they may act independently as for *GM-CSF* or in interaction with each other as *c-jun*. These elements in 3′ UTR may also react with elements in coding region e.g. *c-myc* or with elements in 5′ UTR e.g. *v-lc-fos* [104].

9.1.2.1.2 Trans-acting elements

There are specific RNAbp, such as: AUF1 (hnRNP D, which bind to A+U rich elements), four members of the ELAV family (HuR [human]/HuA [murine], HuB/Hel-N1, HuC, HuD), 3-oxoacyl-CpA thiolase, glycealdehyde-3-phosphate-dehydrogenase (GAPDH), hnRNPA1 and hnRNPC, AUH [detailed list is presented at Gouble A et. al. (2000) [63]]. Members of the AUF1 family increase the rate of degradation while HuR, which shuttles between nucleus and cytoplasm, stabilizes the messenger RNA. Binding of proteins to cis-acting elements in 3′ UTR can be either sequence specific or changed by structural elements (stem loops) formed in mRNA, in which nucleotide sequence inside the loop is crucial. The latter case is studied very well in the iron-dependent interaction of the iron-responsive element (IRE) with iron regulatory proteins (IRPs) as

IRE forms a stem-loop structure with which IRPs react [125]. In this model, both 5′ UTR and 3′ UTR are involved. Another example is a 106-nucleotide GC-rich region in the 3′ UTR of Glucose tranporter (*GLUT1*). It contributes to rapid mRNA turn over as well as its stabilization in response to TNF treatment [64]. The AUF1 family reacts with mRNAs of *c-myc*, *c-fos*, *β-adrenergic receptors*, *leutinizing hormone receptor*, *interleukin-1β*, *GROα*, *GM-CSF* and *H4 histone*. While Hu proteins stabilize mRNAs of cytokines, proto-oncogenes and lymphokines by reacting to AU-rich motifs in 3′ UTR.

The involvement of 3′ UTR in pathogenesis of different diseases has been mentioned in conditions such as: myotonic dystrophy (MD), acute myelogeneous leukaemia (AML), α- Thalassemia and Alzheimer's disease [35,64]. In cardiovascular genetics, some genes whose 3′ UTR is involved in the process of mRNA decay are: β1- and β2-adrenergic receptors, nitric oxide syntheses, cyclooxygenase 2, angiotensin II receptors, endothelial growth factor, globin, elastin and cytokines such as tumour necrosis factor-α (TNF-α) [104].

9.1.2.1.3 The importance of the 3′ UTR in *AGTR1*

Many studies have been performed on the *AGTR1* to investigate the role of 3′ UTR on mRNA stability. Thekkumkara T J et al. (1998) [137] performed experiments on Chinese hamster ovary (CHO-K1). They studied the *AGTR1* expression in the above cells with and without 3′ UTR. Both cell lines expressed similar levels of cell-surface receptors and high affinity receptors, both coupled to heterotrimeric G-protein, but significant differences were observed for receptor-mediated cell signalling pathways. In cells with 3′ UTR, after stimulation by angiotensin II, no increase in intracellular cAMP was observed; however, a sustained level of intracellular calcium was seen. These effects were not reported in cells without 3′ UTR. Decay analysis revealed that normally unstable *AGTR1* receptor mRNA became highly stable by removing its 3′ UTR. Finally, they identified a major cellular protein of 55 kDa, which specifically interacts with the 3′ UTR of *AGTR1* mRNA.

Later on it was shown that post-transcriptional modification of the *AGTR1* receptor is critical for regulating tissue-specific receptor functions [136]. It was shown that wild type

AGTR1 couples with G-protein alpha i, whereas in cells expressing the 3′ UTR decoy, it would couple with G-protein alpha q.

So far, the following points have been demonstrated:

1 – The 3′ UTR of the *AGTR1* is important and functional in terms of mRNA stability.

2 – There are AU rich motifs in 3′ UTR of the *AGTR1*.

3 – Wild type *AGTR1* is less stable than *AGTR1* without 3′ UTR.

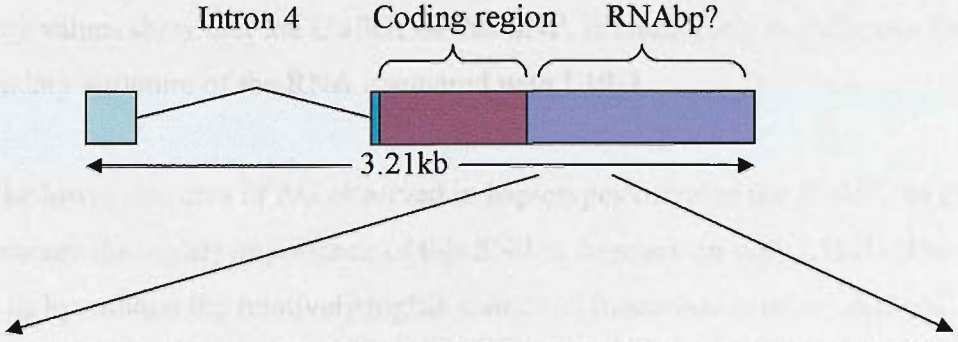
4 – There is an RNAbp reacting with 3′ UTR of the *AGTR1*.

5 – According to Thekkumkara T J et al. (1998) [137], it might be suggested that the RNAbp has a stabilizing effect.

Considering the associations found between A1166C and different phenotypes, it is very important to find the possible effect of A1166C on the reaction of cis- and trans-acting elements at the 3′ UTR of the *AGTR1*. In fact, this idea was tested by Pende A et. al. (1999) [119], and no difference appeared from alleles A and C in interaction of AUF1 with 3′ UTR of the *AGTR1*. However, this finding does not rule out the interaction of other RNAbps with the 3′ UTR of *AGTR1* with a possible influence of A1166C. Indeed, it would perhaps be logical to hypothesise the interaction of stabiliser RNAbps (e.g. Hu - family) with the 3′ UTR of *AGTR1*.

The functional role of 3′ UTR is also supported by the identification of mRNA localisation motifs (zipcodes) in the 3′ UTR of β-actin mRNA [89]. Zipcodes are evolutionary conserved sequences of 40 to 50 nucleotides flanked by GGACT and AATGC, which in case of β-actin are responsible for specific localisation of mRNA. Deleting them causes disordering of the actin filaments and altered cell function. These flanking motifs, GGACT and AATGC, are also present in 3′ UTR of the *AGTR1*, although separated by about 155 nucleotides (Figure 9.1).

Figure 9.1: The Structure of the 3′ UTR of the *AGTR1*



AAAGAAGGAGCAAGAGAACAUUCCUCUGCAGCACUUCACUACCAAAUGAGCMUUAGCUAC
UUUUCAGAAUUGAAGGAGAAAAUGCAUUAUGUGGACUGAACCGACUUUUCUAAAGCUCUG
AACAAAAGCUUUUCUUUCCUUUUGCAACAAGACAAAGCAAAGCCACAUUUUGCAUUAGAC
AGAUGACGGCUGCUCGAAGAACAAUGUCAGAAACUCGAUGAAUGUGUUGAUUUGAGAAAU
UUUACUGACAGAAAUGCAAUCUCCCUAGCCUGCUUUUGUCCUGUUAUUUUUUAUUUCCAC
AUAAAGGUAUUUAGAAUAUAUUAAAUCGUUAGAGGAGCAACAGGAGAUGAGAGUUCCA

AGTR1 transcript sequence represented in GenBank (ensemble gene browser: ENSt00000326871). The A/C polymorphism is indicated by an M, and two putative zip code sequences [89] responsible for localization of mRNA in *β*-actin are highlighted, and A+U motifs, potential sequences capable of reacting with some trans-acting elements, are highlighted in blue. According to the report of Thekkumkara et al (1998) [137], there is an RNA binding protein (RNAbp) interacting with the 3′ UTR of the *AGTR1*. The exact location (s) of interaction is not yet known.

9.1.2.1.4 Predicted Secondary RNA Structures of *AGTR1*

(see chapter 6)

It is generally believed that there is more to RNA than linear structure. They make spatial structures which are important in terms of their functions. Thus it is logical to hypothesis that mutations would affect the secondary structure of RNA and its function consequently.

It seems to be very difficult or perhaps impossible to judge based on the overall shape of the secondary RNA structures, while energy figures of each structure might be a better variable to think and discuss about.

The positions of the alleles of the *AGTR1* A1166C in the structures, do not represent a pivotal location to make a dramatic change in the structures, e.g. stem loop; though the energy values show that the C allele of this SNP, is more likely to influence the secondary structure of the RNA compared with L191L.

The lower amounts of ΔG observed in haplotypes carrying the allele C of the A1166C underscore the higher importance of this SNP in comparison with L191L. This point can help us hypothesis the relatively higher chance of functionality of the A1166C.

To my knowledge, there is no reference for the magnitude of the effects of the energy changes on the function or behaviour of the mRNA. However, it seems logic to assume that even minimal energy change would have significant influence on the characteristics of mRNA. In addition to this, whether or not these SNPs induce any change in localization of the RNA is a point which needs to be determined.

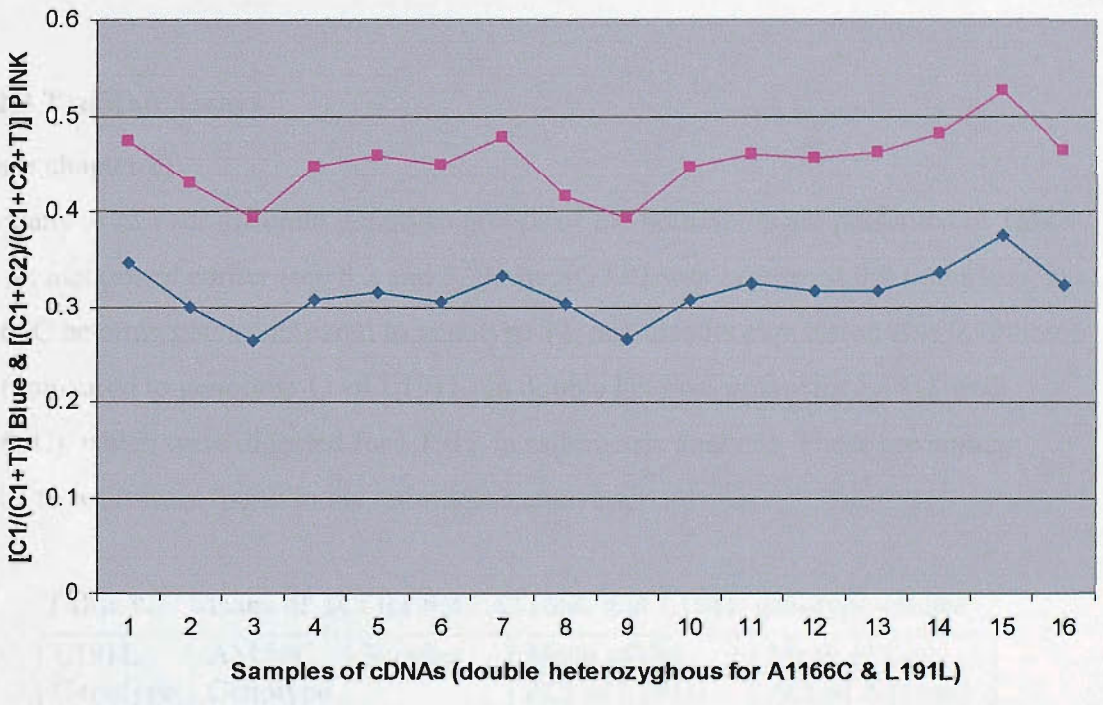
9.1.2.2 Ratiometric Analysis

(see chapter 7)

The presence of the two SNPs (L191L & A1166C) in the transcript of the *AGTR1* gene provided a good opportunity to study their relationship to mRNA expression. This enabled us to look at expression of the gene with respect to haplotypes.

Results of the analyses of variability show that the variances were low: the variance between two loadings was lower than that of two PCRs (0.00046 compared to 0.00066), and the t test result was also significant, i.e. the data are reliable.

Figure 9.2: Distributions of Means of the Two Bands Proportions of L191L



Distributions of means of the two bands proportions C1/(C1+T) (Blue) and (C1+C2)/(C1+C2+T) (Pink) of L191L performed on cDNA of double heterozygous (A1166C & L191L) samples of placenta.

In the case of heterozygosity of each SNP, there would be three bands after digestion. In A1166C analysis, the third band was too small to resolve clearly in electrophoresis. In L191L analysis, C2 band was clear enough to be included in the analysis. Hence, two analyses were undertaken on L191L. In Figure 9.2, it is shown that the mean of two proportions C1/(C1+T) and (C1+C2)/(C1+C2+T) change accordingly, and as mentioned before, no significant difference turned up between them, i.e. either of these two analyses is appropriate for our study. This can also explain the lack of possible interference of heterodouplexes of L191L digestion on the analyses performed.

The effect of allele C of the A1166C on mRNA, represented here, would be an interpretation for associations found either in this thesis or literature. Technically, our method was not expensive, compared with traditional gene expression technology "TaqMan", but needed significant time and labour. However, the main purpose of performing TaqMan assays was confirming what were found in ratio metric analyses.

9.1.2.3 TaqMan Assays

(see chapter 8)

Means of ΔCt for different genotype groups of the both SNPs are presented in Table 9.1. As mentioned earlier (see 8.2 and 8.3), the *AGTR1* was expressed 0.8 times less, in A1166C heterozygotes compared to genotype 11, and also its expression was 0.49 times less (compared to genotype 11 of L191L) in double heterozygotes (for L191L and A1166C), which were digested for L191L in ratiometric analysis. These are similar results to what were found in the ratiometric analysis.

Table 9.1: Means of ΔCt for both A1166C and L191L genotype groups

L191L Genotype	A1166C Genotype	Number	Mean of the ΔCt of L191L	Mean of the ΔCt of A1166C
22	11	13	7.39	6.80
12	11	22	6.53	6.26
12	12	15	7.48	7.43
11	12	12	5.90	5.90
11	22	6	7.65	8.33

The lower level of *AGTR1* expression evident from A1166C analyses suggests differential allelic breakdown of mRNA, implying that either mRNAs carrying allele C would degrade faster or mRNAs carrying allele A would last longer. Whichever is true, the final effect is that individuals with C allele of A1166C would have lower level of *AGTR1* expression or higher/faster mRNA degradation. An allele specific difference in mRNA expression has also been reported in *apolipoprotein C-III* gene by Esterbauer H et al. (1999) [49]. They showed that mRNAs encoded by allele S2, of SstI polymorphism located in 3' UTR of *apolipoprotein C-III* gene, were greater (14%-29%) in three out of five liver biopsies taken from obese patients.

The different expression levels of the *AGTR1* in genotypes show the influence of the mutant alleles in reducing the expression of the *AGTR1*. This is more prominent in the case of A1166C, compared to L191L, which is comparable with our findings in association studies of the two SNPs. Considering the point that there is a haplotype spanning exon five and 3' UTR of the *AGTR1*, it could perhaps be concluded that A1166C might be potentially capable of being referred as the `SNP tag haplotype`.

The results can be interpreted with respect to haplotypes:

1) There are three transcribed haplotypes: 1_1, 2_1 and 1_2 (Table 8.2).
2) Message level by haplotype shows 1_1 to be about 2 fold greater than 2_1 or 1_2.
3) The most profound effects are:

   A - In haplotype analysis at A1166C region, haplotype 1_2 level is 0.46 of haplotype 1_1.

   B - In genotype analysis for 1166 AA vs AC vs CC, CC is at 0.27 the level of AA. CC must represent (1_2, 1_2).
4) Effect 3B (one 1_2 haplotype interacting with the other to get multiplicative effect) suggests interdependence of alleles in some feed back or regression loop.
5) Evidence points to 1_1, 2_1 and 1-2 each behaving differently. Most prominent effects (mRNA and clinical phenotype) are for 1_2.

6) Recombination hotspot in the middle of the *AGTR1* and 3′ UTR breakdown data suggests (but do not prove) that the effect is at breakdown not transcript level.
7) Our original interpretation of within heterozygotes analyses of L191L and A1166C mRNA region levels was confounded by the presence of three different transcribed haplotypes not two, while we were premising our interpretation.

The mRNA destabilisation role of 3′ UTR has also been studied in other genes. Tholanikunnel B G et. al. (1995) [138] showed the presence of the AUUUA destabilisation pentamer in the 3′ UTR of β- adrenergic receptor, which is also a member of G -protein-coupled receptors, reacting with a 35 kDa RNA binding protein. Many members of G -protein-coupled receptors have this pentamer in their 3′ UTR, so does *AGTR1* (Figure - 9.1).

The idea of the effect of haplotypes harbouring polymorphisms of 3′ UTR has been investigated. Frittitta L et. al. (2001) [55] in a study on Caucasians from Sicily, Italy, showed that a haplotype consisting of 3 mutant alleles of three SNPs in the 3′ UTR of *Glycoprotein PC-1* modulates the expression of *PC-1* and carries increased risk for insulin resistance. These individuals had greater risk for insulin resistance and higher levels of plasma glucose and insulin during an oral glucose tolerance test and higher levels of cholesterol, HDL cholesterol and systolic blood pressure. And also J-C Lambert J-C et al. (2003) [92] showed an association of the 3′ UTR +1073 C/T polymorphism of the *OLR1* (oxidised LDL receptor I) on chromosome 12 with Alzheimer's disease (AD) in French (589 cases and 663 controls) and Americans (230 affected sibs and 143 unaffected sibs). The *OLR1* expression was significantly lower in AD patients carrying CC and CT genotypes compared with controls with the same genotypes. They found another polymorphism 2 nucleotides upstream of 1073 C/T, 1071 T/A which was not associated with the disease. However, they showed that polymorphisms in 3′ UTR affect the binding of regulatory proteins: haplotype +1071 A and +1073 C alleles bind with higher affinity compared with the +1071 T and +1073 T respectively. Finally, it was suggested that the +1073 C allele may be associated with a specific decrease of *OLR1* expression in lymphocytes from AD cases. Similarily, The effect of polymorphisms (allelic variation) in 3′ UTR of TNFRSF1B (tumour necrosis factor receptor superfamily

member 1B) gene on bone mass has been reported by Albagha O M et al. (2002) [10]. They showed that a haplotype ATC (consisting homozygous alleles for G593A, T598G AND T620C) is significantly associated with a 5.7% lower femoral neck bone mineral density (BMD) compared with other haplotypes.

There are increasing number of evidence showing the implication of 3′ UTR in the regulation of gene expression e.g. myotonic dystrophy, neuroblastoma and tumor necrosis factor (TNF) [35].

Another possible explanation would be that A1166C is a proxy marker for another unknown functional marker. This idea is explained in more details in the discussion of the *AGTR1* (see 9.1).

The exact effect of these polymorphisms on the protein, receptor or intracellular signalling remains obscure.

9.2 *ACE* Studies

(see chapter 4)

The final goal of defining haplotypes is to find blocks of markers associated with special phenotypes. The real advantage of this approach is resolving causal markers hiding in haplotypes that are associated with diseases. Potentially, a great amount of time and money could then be saved; as rather than typing many markers a few disease markers found in haplotype blocks could be studied. These haplotypes could be used as reference for matching susceptibility of different populations to some disorders or environmental factors. This is the real purpose of haplotype map project in the Human Genome Centre (http://www.hapmap.org/).

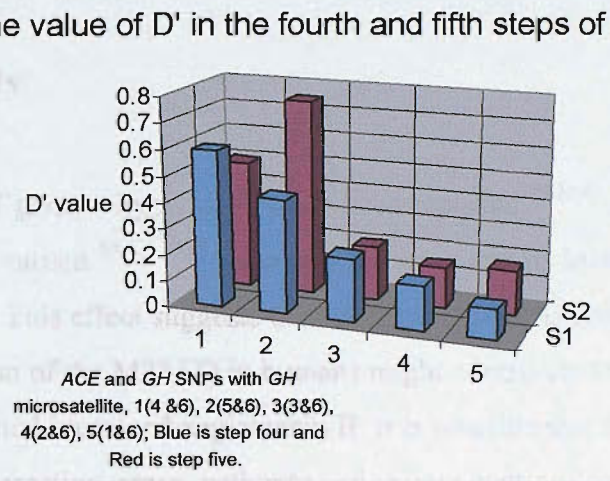
Another advantage of haplotype blocks is in studying human evolution as the characteristics of haplotype blocks, such as the extent of a block, are different among populations and with comparison of these structures, important historical events such as recombination could be resolved.

I showed that the structure of haplotypes in the *ACE* gene is compatible with previous literature performed for British Caucasians [88]. The crucial issue in the *ACE* gene, presently, is to find the functional marker with which the *ACE* I/D is in LD. If it happens, it would be a real breakthrough in cardiovascular and metabolic genetics.

The observed values of D′ between the *GH* polymorphisms are consistent with those of King's (Data not shown). According to the reasonable high LD between the *ACE* and *GH*, it could be suggested that *GH* might be considered as a proxy marker.

The value of D′ between polymorphisms numbered 5 & 6 increased after deleting alleles 17, 18 and 19 of the *GH* microsatellite; this event has been presented in Figure - 9.3. The rise in the D′ should be considered as a signal for possible functional relationship, and warrants further investigation.

Figure 9.3: Comparison of Two Steps of LD Analysis of the *ACE* and *GH* Genes



	1	2	3	4	5
Series1	0.598671	0.437318	0.241902	0.169972	0.112106
Series2	0.494529	0.748516	0.206427	0.153979	0.172521

The above comparison was performed between steps 5th and 4th of the ACE and GH genes. The graph highlights the increase in the value of LD between markers 5 and 6 in this part of analysis.

In a study (unpublished) performed by Dr. T Gaunt and Dr. S Rodriguez in Human Genetics Division at the University of Southampton, it was found that *ACE* and *GH* markers fall into two discrete haplotype blocks with low LD between blocks. The results also indicated that an intergenic gap of 374kb is more realistic than one of 1.5Mb.

The existence of LD between the *ACE* and *GH* gene polymorphisms together with other physiological and phenotypic relationship are supported by evidence (see 1.3). However, genetic interactions between these two genes still demand more accurate scrutiny. Furthermore, as different elements of the RAS pathway have similar effects on metabolic and cardiovascular phenotypes, further genetic analysis of other components of this pathway is highly recommended to find the possible gene-gene interactions; and forms the main part of this thesis. Meanwhile, because there are some parallel pathways involving counter regulatory hormones and the autonomic system with known effects on

metabolic and cardiovascular traits, a comprehensive approach investigating all elements of these pathways is warranted.

9.3 *AGT* M235T Study

(see chapter 5)

The linkage of the *AGT* gene to hypertension has been reported [29,30,82], as well as association of the M235T variant [80,131,132,139]. Indeed this gene and its locus are known to be linked to hypertension. This effect suggests that increase in plasma angiotensinogen level (due to the association of the M235T) in humans might contribute to elevated blood pressure through the eventual increased angiotensin II. It is possible that this effect is modulated by different interacting genes, pathophysiological situations (e.g. obesity, gender and oestrogen) and perhaps some unknown environmental factors [1]. However, there are many other studies which have not found association between *AGT* M235T and hypertension, either in British families [22,29] or other Caucasians [23].

9.4 General Discussion

As planned, it was aimed to study a panel of markers of the RAS in the NH and EH populations with cardiovascular and anthropometric phenotypes. It was shown, in this thesis, that well known polymorphisms of this pathway, i.e. *AGTR1* A1166C and *ACE* I/D (in collaboration with other colleagues in the department) are significantly associated with cardiovascular and metabolic traits. *AGT* M235T was also studied in the NH and EH populations; however, no significant association was observed.

This thesis has presented evidence to show that SNPs in the coding region of the *AGTR1* are more associated with the studied phenotypes than those in the 5′ UTR. This observation along with little signal arising from the 5′ UTR in the literature guided us to think more seriously about the coding region and its SNPs.

The effect of the *AGTR1* L191L and A1166C SNPs and their haplotypes on the expression of the gene was demonstrated. This result suggests that the A1166C SNP may have a role in causality of the observed phenotypical changes. Though the exact mechanism still needs to be determined, but mRNA breakdown effect would be worthy of investigation. Nevertheless, this finding does not exclude the possible role of other functional markers either inside or nearby the gene. To the writer's knowledge no gene nearby has as yet been found at the moment to explain such a proximity effect; however, it is expected that with fast growing genetic databases and with complete sequencing of the human genome other possible affecting genes or markers, if any, will be found in near future.

The results of quantitation of haplotypes of the *AGTR1* in conjunction with the association of its haplotypes (1112) with metabolic traits emphasise that haplotypes which span the coding region of the gene is more likely to carry functional markers causing observed phenotypical changes. This would narrow the field of searching for the causal marker to the 3′ UTR. This is another example representing the implication of 3′ UTR in modification of gene expression.

Furthermore, these findings highlight the importance and implication of SNPs in the human genome, emphasising that even a tiny change in nucleotide sequence could potentially be able to induce some changes.

Our findings, showing the simultaneous effects of different polymorphisms on a special category of phenotypes especially those in one physiological pathway, support the importance of expression studies of groups of genes. As most phenotypical changes happen in chain with other(s) elements especially in the case of complex diseases, it seems wiser and closer to physiology to study the expression of different genes simultaneously. Nowadays, with availability of advanced technologies such as microarray, this demand can be met, and is becoming ever more popular.

One of the implications of our findings is their potential usefulness for pharmacogenetic purposes, i.e. the studied markers reveal the importance of these genes in the development of some diseases, e.g. hypertension and metabolic syndrome. This would strengthen the significance of *ACE* inhibitors and *AGTR1* antagonists in the management of associated diseases. The final goal would be to predict which specific therapy is appropriate for which patient with respect to their individual genotype.

The significance levels of LD between markers of the *ACE* and *GH* suggest that these two genes might work or cross talk with each other in affecting phenotypes. This, if proved, is of major importance in explaining complexity of phenotypical changes. However, the gap between these two genes needs to be considered in interpretation of any result.

With the unravelling of the human genome sequence and identification of new mutations (e.g. SNPs), a new window is being opened to medicine. This new era, although in its early stage, would mainly impact on the fields of preventive and diagnostic medicine. Nowadays, screening people for the suspected mutations in genes could allow preventive measures to be taken, to stop or modify the risk of a dangerous

outcome. Well known examples are mutations in genes coding for ion channel proteins (e.g. *KVLQT1*, *HERG*, *SCN5A*, *minK*, *MiRP1*, and *RyR2*) which are associated with long QT syndrome, and those in *CYP2A6* associated with smoking [42]. The same principle is also of major importance in cancer genetics and dyslipidemias.

In an ideal situation, the final objective of this kind of research is to unravel the genetic pathology for disease, i.e. a mutation which makes people more or less susceptible to a disease or which result in a person having an altered response to a drug. These tests could work at population level or individualise patient risk assessment and mangement. Apart from traditionally roles of laboratory tests i.e. diagnosis, prognosis, screening and monitoring, it seems that genetic tests would provide potential advantages in counselling and even "right to know" [40].

Many genetic tests are presently available which help predict the susceptibility (+/or their response) of patients to a drug, e.g. CardiaRisk™ developed by Myriad Genetic Laboratories, Inc (www.myriad.com) based on *AGT* M235T, and there is also another test available for diagnosis of the mutation "R3500Q" in *APOB* causing familial defective apolipoprotein B (FDB) [40]. The work presented here did not test any drug response, but the associations of the *AGTR1* A1166C and *ACE* I/D with broader range of metabolic traits were presented in this thesis.

Considering the fast growing advances in genetics and molecular pathology, the role of genetic tests will be more prominent in future. In the next years or decade, genotyping individuals for known causative SNPs or mutations will become an important part of preventive medicine with many advantages in choosing the best drug based on patient's genotype. This may surpass chemical tests. However, as clinicians need clear guidelines for their diagnostic purposes, new tools or standards may need to be developed.

The results of this thesis either at the level of genotype (or haplotype) phenotype or from expression studies underscore the importance of the RAS and its polymorphisms in relation to metabolic and cardiovascular phenotypes.

Overall, I have identified relationship of genetic diversity in the RAS with metabolic and anthropometric traits in another group of Caucasians extending previous literature for *ACE* and making new discovery concerning *AGTR1*. Moreover, a functional explanation for the observed effects of the *AGTR1* A1166C was determined; however, details of these mechanisms remain to be discovered.

9.5 Future Work

Considering the significant associations found in this thesis and in the recent literature between the polymorphisms of the RAS and cardiovascular and metabolic phenotypes, it is necessary to resolve the mechanisms of these associations.

It was already known that there is a RNAbp reacting with 3′ UTR of the *AGTR1*, but appropriate studies are required to determine the nature of the reacting trans and cis acting elements and possible interference of the A1166C. Specific methods may be of great value. A luciferase assay may be able to show any effect (cis and trans acting reactions) from 3′ UTR or even a short sequence of it (where the proposed SNP is located) [62,147]. Then other assays such as a UV cross-linking experiment [119] and the electro mobility shift assay (EMSA) could determine the trans acting factor [94], especially as the antibody for *AGTR1* is available. In the case of *AGTR1*, perhaps it would be more logical to test a stabiliser RNAbp because the 3′ UTR of this gene is naturally unstable; particularly as AUF1 (which is a destabiliser RNAbp) has already been tested. The important point in these experiments is that the examined sequence must have known genotype for A1166C.

Regarding the reported synergistic effects of the polymorphisms of the RAS, it would also be desirable to study the expression of different elements of the RAS simultaneously as well as physiological pathways which have either agonistic or antagonistic effects.

Appendices

Appendix 1

Long PCR (for coding region of the *AGTR1*) primers

AGTR1 F	5´- TCCTCAAAGTCGAGCCCTACCTCCTACG - 3´
AGTR1 R	5´- TGATTTTTGACCGGGGAAGCTAAACATGA - 3´

Primers for the *AGTR1* A1166C experiment

Allele Specific A	5´- TCTGCAGCACTTCACTACCAAATGAACA - 3´
Allele Specific C	5´- TCTCCTTCAATTCTGAAAAGTAGCTGAG - 3´
Forward	5´- GCCAAATCCCACTCAAACCTTTCAACAA - 3´
Reverse	5´- AAGCAGGCTAGGGAGATTGCATTTCTGT - 3´

AGTR1 L191L primer sequences

AGTR1, L191L, T/F	5´ - AGTCCCAAAATTCAACCATT - 3´
AGTR1, L191L, C/F	5´ - AGTCCCAAAATTCAACCATC - 3´
AGTR1, L191L, UP/C	5´ - TATTTTTCATTGAGAACACCAATATTA - 3´
AGTR1, L191L, CO/C	5´ - CAAATAAGAGTATAACTTGTAAGAATGA - 3´

Primers for Long PCR on 5' UTR of *AGTR1*

AGTR1,L5'-UTR,Up/Control	5´ - CTTGTCCAATTGCCCTCACT - 3´
AGTR1,L5'-UTR,Do/Control	5´ - CGTGGCAAACAAACCTACCT - 3´

AGTR1 C-521T primer sequences

AGTR1, C-521T, C/F	5´ - ATTATTTCTTCTTTAAAGAC - 3´
AGTR1, C-521T, T/G	5´ - ATTATTTCTTCTTTAAAGAT - 3´
AGTR1, C-521T, UP/C	5´ - GCCTACATTTTGTTGAGAAT - 3´
AGTR1, C-521T, DO/C	5´ - ATTCAAGGGTGGACACTCAT - 3´

AGTR1 A-153G primer sequences

AGTR1, A-153G, A/F	5´ - ATCATCCTTGCTGCCGTAAA - 3´
AGTR1, A-153G, G/F	5´ - ATCATCCTTGCTGCCGTAAG - 3´
AGTR1, A-153G, UP/C	5´ - TGAAGAACACGAATCTCCGC - 3´
AGTR1, A-153G, DO/C	5´ - CTGGAATCATTGGCGAGGAG - 3´

Primsrs of the DOP reaction

5´ - CCGACTCGACNNNNNNATGTGG - 3´	

Primers of the *ACE* C1237T genotyping

Forward/C	5´- TACTACCTGCAGTACAAGGATCTGACC - 3´
Forward/T	5´- TACTACCTGCAGTACAAGGATCTGACT - 3´
Up/Control	5´- CCTGCCCTGTTCTGTCCATCC - 3´
Down/Control	5´- TCTCAGCCCTCCCATACCCG - 3´

Primers of the *ACE* A-5466C genotyping

Forward/A	5´- CATGCCATGTCACATATATTATAGTATGTA - 3´
Forward/C	5´- CATGCCATGTCACATATATTATAGTATGTC - 3´
Up/Control	5´- CACTACTAATGTCAGAACATTTCACT - 3´
Down/Control	5´- CCAAACCCTTTCTCTCCA - 3´

Primers for ARMS reaction of the *AGT* M235T

M235T, T/F	5´ - AAGACTGGCTGCTCCCTTAT - 3´
M235T, C/F	5´ - AAGACTGGCTGCTCCCTTAC - 3´
M235T, UP/C	5´ - GATGCGCACAAGGTCCTGTC - 3´
M235T, DO/C	5´ - GCCAGCAGAGAGGTTTGCCT - 3´

Primers for the restriction digestion of the *AGT* M235T

M235T/RES/UP	5´ - GCTGGATGCGCACAAGGTCCTG - 3´
M235T/RES/DO	5´ - TGAAAGCCAGGGTGCTGTCCACACTGGACCCC - 3´

Appendix 2

Hardy-Weinberg Law (HWL)

It is one of the basic principles of population genetics. Some conditions are presumed in this law:
1- The population size is indefinite or large enough to ignore sampling error.
2- Random mating
3- No genotype has any kind of selective advantage, i.e. all of them have equal chance of living and fertility.
4- Confounding factors like mutation, migration and random genetic drift are absent.
Suppose that in such a population a locus has two alleles M and m. The frequency of dominant allele M is represented as p and that of recessive one, m, as q. Obviously, $p + q = 1$. A punnett square shows the possible combinations of gametes:

M(p)	m(q)	
MM p^2	Mm pq	M(p)
mM qp	Mm q^2	M(q)

We see that the probability of the occurrence of MM is $p \times p = p^2$. It is the same as other genotypes:

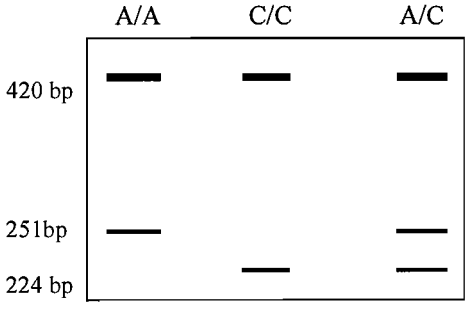
Mm and mM = $pq \times qp = 2pq$
mm $q \times q = q^2$
Above explanation implies that genotype frequencies are determined by allele frequencies. The distribution of genotypes in the next generation will be:

$p^2 + 2pq + q^2 = 1$

Another concept of HWL is that the frequencies of alleles remain constant from one generation to the next. A population in such a condition is said to be in a state of genetic equilibrium.

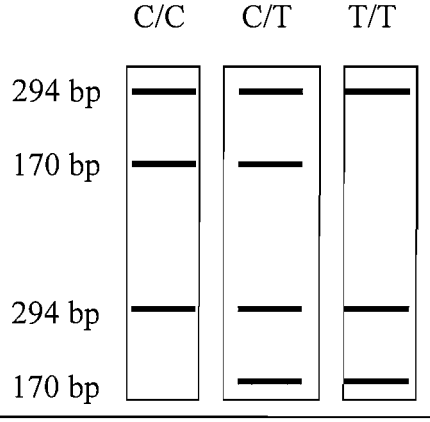
Appendix 3

Figure 1: Schematic Representation of Bands Patterns in a Tetra Primer ARMS Assay



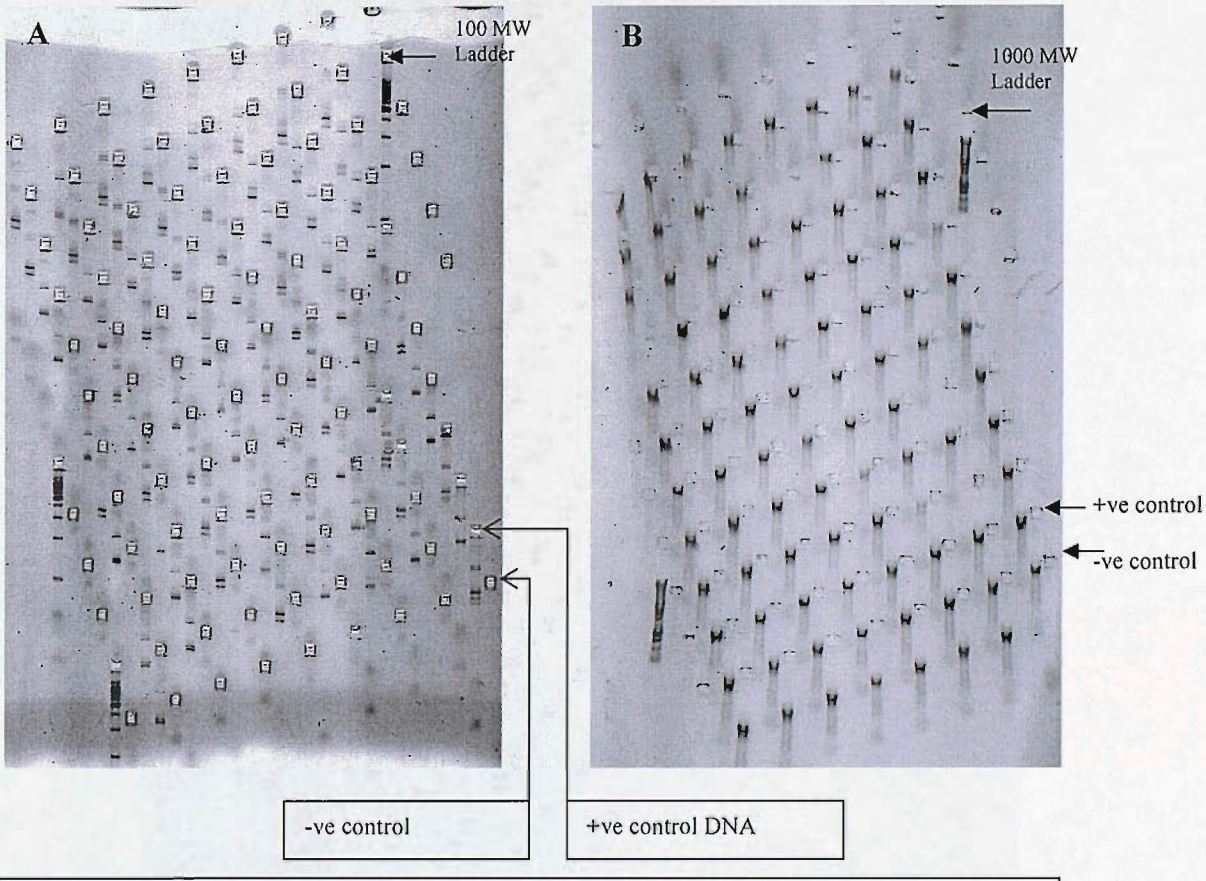
The above figure shows the pattern of bands in genotyping *AGTR1* A1166C assay. The above style can be applied to all tetra primer ARMS assay, regardless of bands sizes.

Figure 2: Schematic Representation of Bands Patterns in a Three Primer ARMS Assay



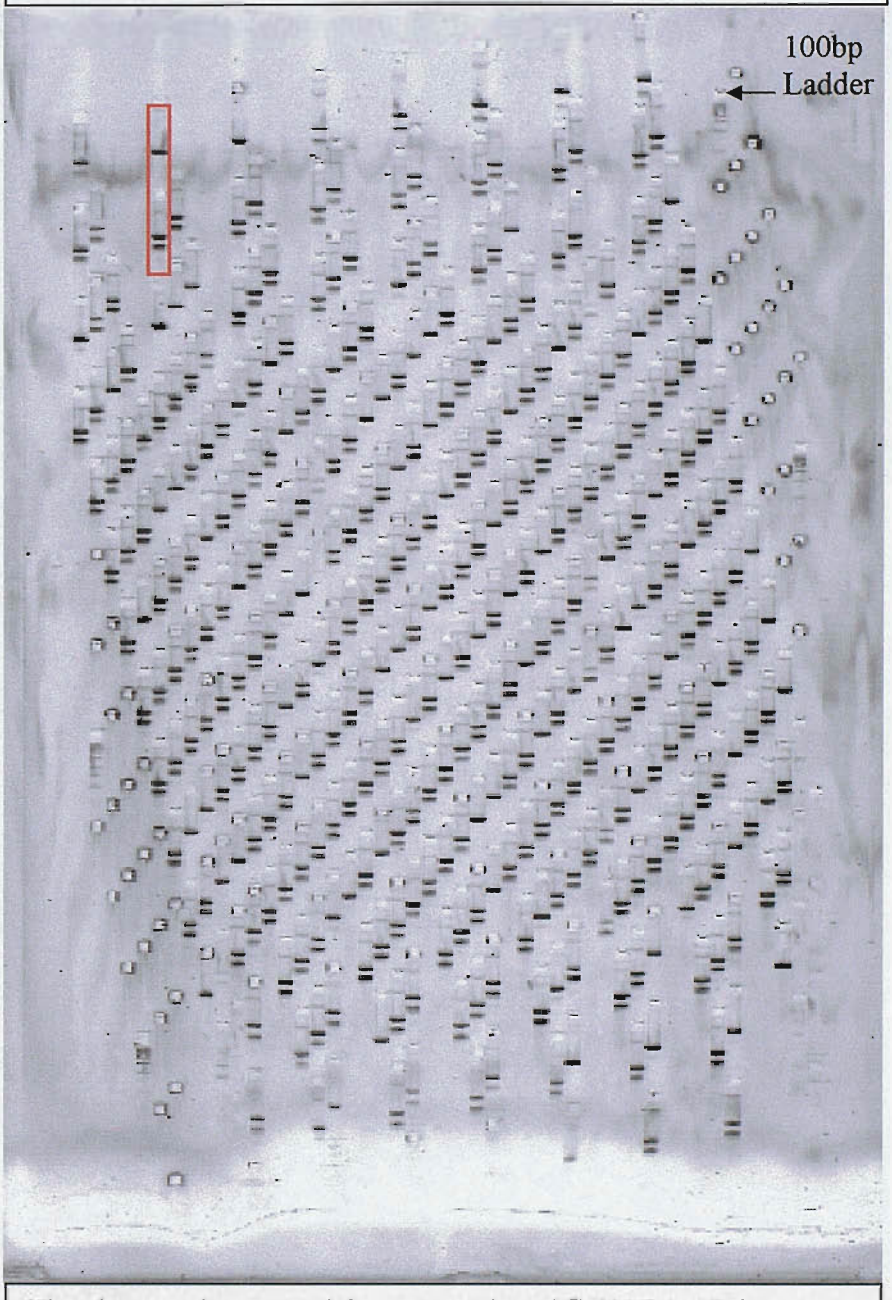
The above figure shows the pattern of bands in genotyping *ACE* C1237T assay. The above style can be applied to all three primer ARMS assay, regardless of bands sizes.

Figure 3: Gels Used for Genotyping *AGTR1* A1166C



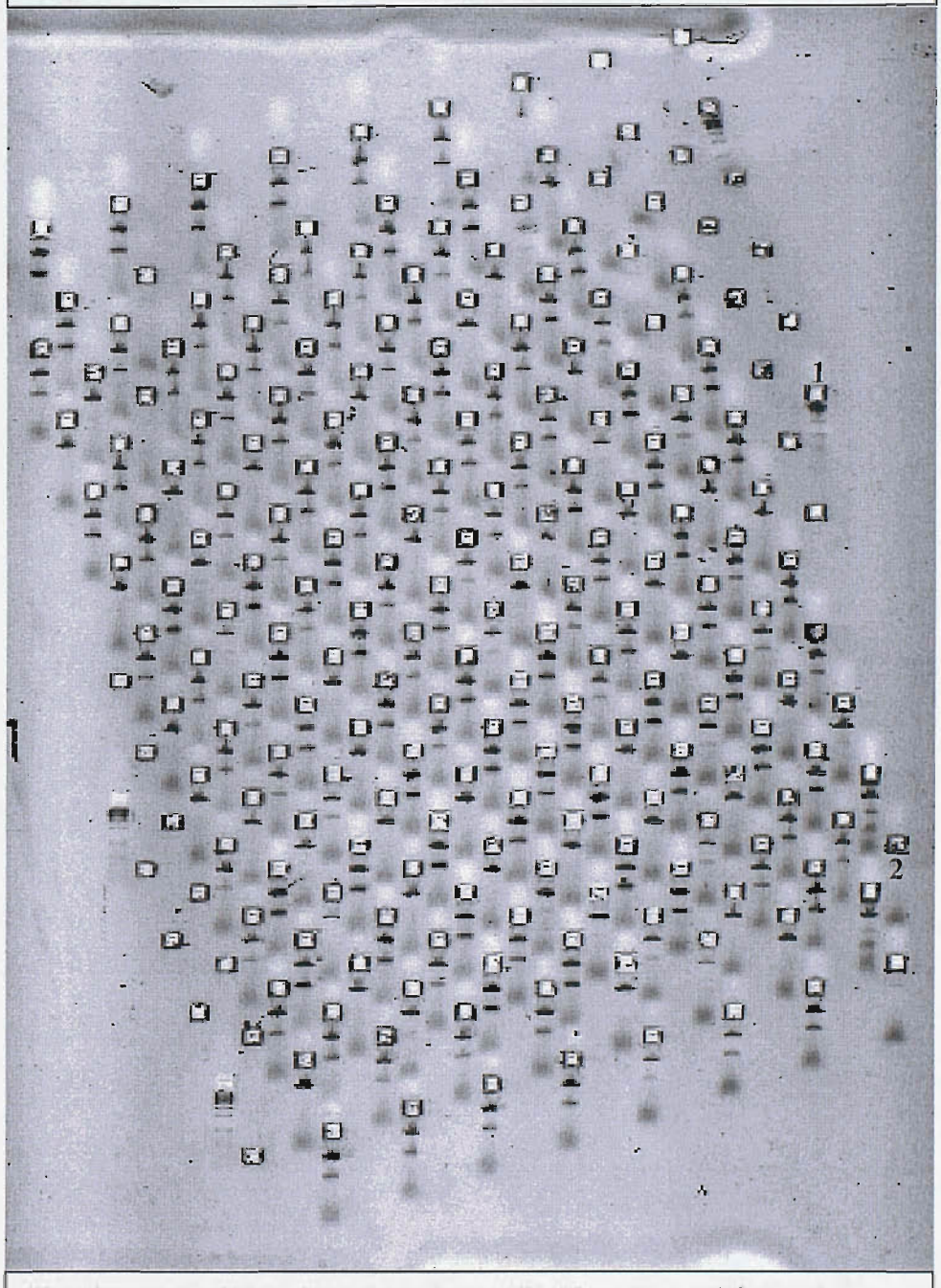
A: shows *AGTR1* A1166C assay in a 96 well MADGE format.
B: shows long PCR reaction in 96 well MADGE format, used as template for *AGTR1* A1166C assay.
+ve control: A freshly prepared DNA as template with PCR mix.
-ve control: PCR mix without any template.

Figure 4: A Gel Showing *AGTR1* L191L Assay



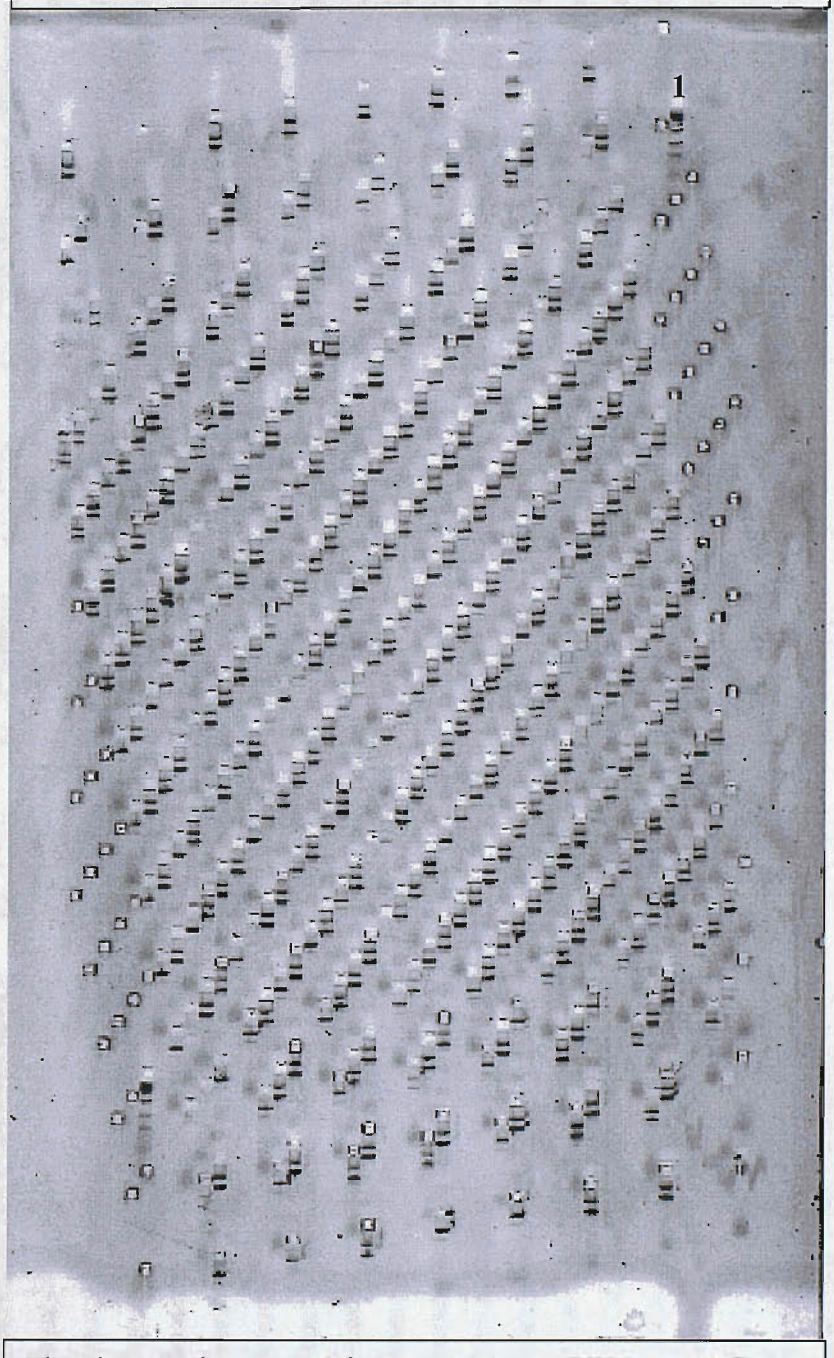
The above gel was used for genotyping *AGTR1* L191L in arrays 6 and 7 of the NH. The red column shows the genotype of an individual (TT).

Figure 5: A gel showing *AGTR1* C-521T Assay



The above gel shows the three primer ARMS assay used for typing *AGTR1* C-521T in array 1 of the NH in a 96 well MADGE format gel. 1: 100bp ladder, 2: negative control, i.e. PCR mix without template.

Figure 6: A Gel Showing *AGTR1* A-153G Assay

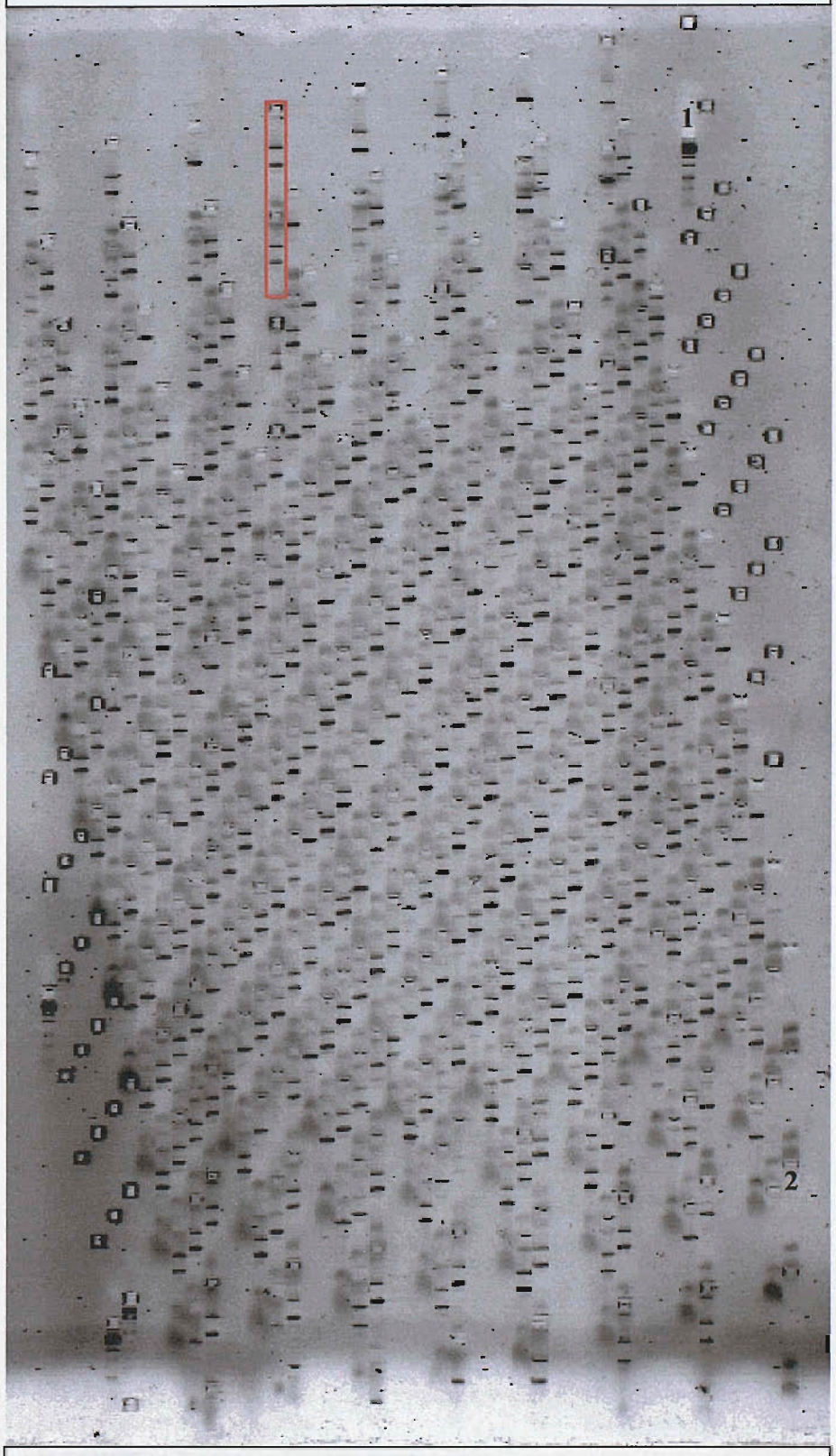


The above gel was used for genotyping *AGTR1* A-153G, which was performed by a three primer ARMS assay.
1: 100bp ladder

Figure 7: A Three Primer ARMS Assay for Genotyping *ACE* A-5466C in the Phoretix Software

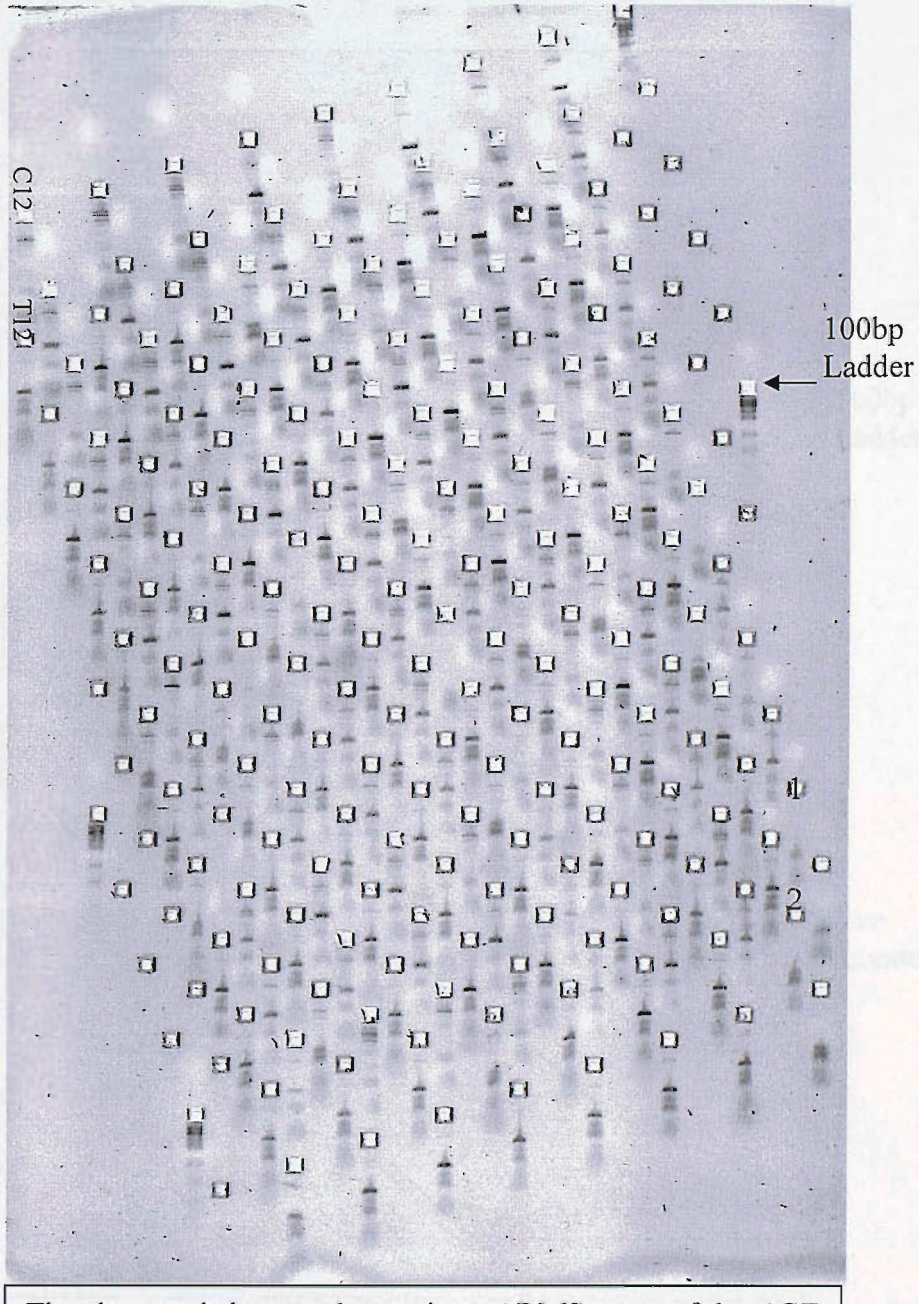
The above image shows the mode of genotyping of a three primer ARMS assay in the Phoretix software. Wild type allele reaction is loaded on the upper row, and mutant allele on the lower row. Green column is a homozygote for wild type and pink column is for mutant allele, and yellow column is a heterozygotes.

Figure 8: A Gel Showing the *ACE* C1237T Assay



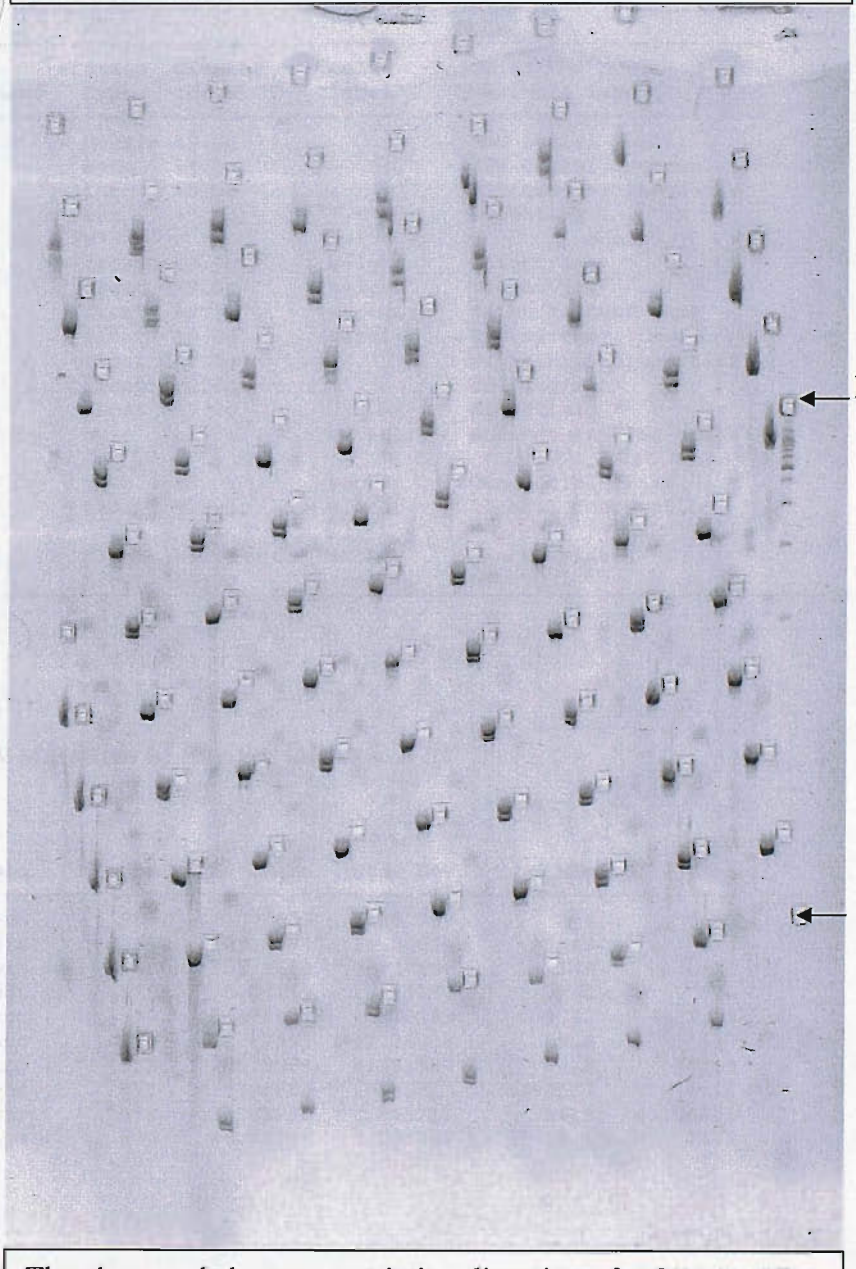
The above image shows the gel used for genotyping *ACE* C1237T in arrays 6 and 7 of the NH. 1: 100bp ladder, 2: negative control, i.e. PCR mix without template. The red column shows a guide how to type an individuals's genotype.

Figure 9: A Gel Showing *AGT* M235T Assay



The above gel shows a three primer ARMS assay of the *AGT* M235T in 192 MADGE gel format.
1 & 2, i.e –ve control: PCR reagents without template.
C12 & T12: allele C and T reactions respectively.

Figure 10: A Gel Showing the Restriction Digestion of the *AGT* M235T



The above gel shows a restriction digestion of *AGT* M235T with Tth111I in a 96 well MADGE format gel.
-ve control: PCR reaction without template.

Appendix 4

Variables in the dataset

Anthropometric phenotypes

variable name	storage type	display format	value label	variable label
serno	double	%9.0g		MRC serial number
sex	byte	%4.0g		Sex 0=men, 1=women
bwt_ozs	int	%8.0g		birthweight in ounces
wt2_ozs	int	%8.0g		weight at 1 yr in ounces
hwgt	float	%9.0g	weight	Weight (kg)
hhgt	float	%9.0g	height	Height (m)
hwast	float	%9.0g	wastcirc	Waist circumf (cm)
hhip	float	%9.0g	hipcirc	Hip circumf (cm)
hwhr	float	%9.0g		Waist:Hip (proportion)
hbmi	float	%9.0g		BMI (kg/m2)
hage	float	%9.0g		Age clinic
category	byte	%8.0g	category	whether east or north-west hert
soccat	float	%9.0g	soccat	Own social class
alccat	float	%23.0g	alccat	Alcohol Consumption Group
smoker1	float	%8.0g	smoker1	Smoker status
at1rv001	float	%9.0g	ace	AT1RV001

Summary statistics of key variables

Variable	Obs	Mean	Std. Dev.	Min	Max
serno	982	4100.828	2238.789	4	6651
bwt_ozs	982	123.3574	18.21615	48	184
wt2_ozs	982	353.9613	41.24609	176	480
hwgt	979	75.24259	13.23176	40	138
hhgt	979	1.670827	.0870349	1.42	1.93
hwast	978	92.67955	12.26088	59.9	137
hhip	979	105.3064	8.381967	84.3	148.5
hwhr	978	.8792963	.0865711	.6394557	1.12
hbmi	979	26.88986	3.868211	16.9	43.06485
hage	976	66.25369	3.008802	59.1	73.7

AT1RV001 vs cardiovascular phenotypes in East Herts
Variables in the dataset

variable name	storage type	display format	value label	variable label
serno	double	%9.0g		MRC serial number
sex	byte	%4.0g		Sex 0=men, 1=women
bwt_ozs	int	%8.0g		birthweight in ounces
wt2_ozs	int	%8.0g		weight at 1 yr in ounces
sysbpa1	int	%8.0g		EH1 Systolic BP (mmHg)
diabpa1	int	%8.0g		EH1 Diastolic BP (mmHg)
pulsea1	int	%8.0g		EH1 Pulse rate (bpm)
pulspres	float	%9.0g		Pulse pressure mmHg (SBP-DBP)
echolvm	float	%9.0g		EH2 echo left ventricular mass (g)
lecholvm	float	%9.0g		Ln(EH2 echo LVM (g))
fastfib	int	%8.0g		fasting fibrinogen
lfastfib	float	%9.0g		Ln(fasting fibrinogen g/l)
fastchol	float	%9.0g		fasting cholesterol
lfstchol	float	%9.0g		Ln(fasting cholesterol mmol/l)
trig	float	%9.0g		triglycerides
ltrig	float	%9.0g		Ln(fasting triglycerides mmol/l)
hdl	float	%9.0g		high density lipoproteins
lhdl	float	%9.0g		Ln(high density lipoproteins mmol/l)
ldl	float	%9.0g		low density lipoproteins
lldl	float	%9.0g		Ln(low density lipoproteins mmol/l)
lldlhdl	float	%9.0g		Ln(LDL:HDL ratio)
fastapa1	float	%9.0g		fasting apo a1
lapa1	float	%9.0g		Ln(Apolopoprotein A1 g/l)
fastapb	float	%9.0g		fasting apo b
lapb	float	%9.0g		Ln(Apolopoprotein B g/l)
fstlpa	float	%9.0g		fasting lp(a)
lfstlpa	float	%9.0g		Ln(Lp(a) lipoprotein mg/l)
fastvii	int	%8.0g		fasting factor vii
lfastvii	float	%9.0g		Ln(fasting factor VII %ofstd)
weight	float	%9.0g	weight	EH1 weight (kg)
height	float	%9.0g	height	EH1 height (m)
wastcirc	float	%9.0g	wastcirc	EH1 Waist circumf (cm)
hipcirc	float	%9.0g	hipcirc	EH1 Hip circumf (cm)
waisthip	float	%9.0g		EH1 Waist:Hip (proportion)
bmi1	float	%9.0g		EH1 BMI (kg/m2)
weight2	float	%9.0g		EH2 weight (kg)

height2	float	%9.0g		EH2 height (m)
bmi2	float	%9.0g		EH2 BMI (kg/m2)
fev1	float	%9.0g		EH1 forced expiratory volume in 1second (l)
fvc	float	%9.0g		EH1 forced ventilatory capacity (l)
fevratio	float	%9.0g		EH1 FEV1 observed:predicted
insul0	float	%9.0g		insulin at 0
insul30	int	%8.0g		insulin at 30
insul120	float	%9.0g		insulin at 120
linsul0	float	%9.0g		Ln(Insulin0 pmol/l)
linsul30	float	%9.0g		Ln(Insulin30 pmol/l)
lins120	float	%9.0g		Ln(Insulin120 pmol/l)
gluc0	float	%9.0g		glucose at 0
gluc30	float	%9.0g		glucose at 30
gluc120	float	%9.0g		glucose at 120
lgluc0	float	%9.0g		Ln(Glucose0 mmol/l)
lgluc30	float	%9.0g		Ln(Glucose30 mmol/l)
lgluc120	float	%9.0g		Ln(Glucose120 mmol/l)
proinsul	float	%9.0g		proinsulin
lproi	float	%9.0g		Ln(Fasting Proinsulin pmol/l)
proi3233	float	%9.0g		proinsulin split 32-33
lsplit	float	%9.0g		Ln(Fast. 32-33 Split Proinsulin pmol/l)
lauigtt	float	%9.0g		Ln(Insulin AUC during OGTT)
ageclif1	float	%9.0g		EH1 age fasting clinic (OGTT)
ageclin2	float	%9.0g		EH2 age fasting clinic (ECG,Rose,ECHO,Bone)
category	byte	%8.0g	category	whether east or north-west hert
soccat	float	%9.0g	soccat	Own social class
fathsoc	byte	%4.0g	fathsoc	father - social class
fsoccat	float	%13.0g	fsoccat	Father's social class
alccat	float	%23.0g	alccat	EH1 Alcohol Consumption Group
alccat2	float	%23.0g	alccat2	EH2 Alcohol Consumption Group
smoker1	byte	%8.0g	smoker1	EH1 smoker status
smoker2	float	%9.0g	smoker2	EH2 smoker status
at1rv001	float	%9.0g	ace	AT1RV001

Summary statistics of key variables

Variable	Obs	Mean	Std. Dev.	Min	Max
bwt_ozs	378	125.2963	19.19817	48	184
wt2_ozs	378	354.8889	42.06315	224	480
sysbpa1	378	160.963	21.73163	110	232

diabpa1	378	87.25132	11.53166	53	127
pulsea1	378	69.11376	10.91905	42	105
pulspres	378	73.71164	15.27394	31	126
echolvm	272	187.037	56.73643	82.89	376.81
lecholvm	272	5.186479	.3007024	4.417514	5.931741
fastfib	363	304.314	60.02983	144	582
lfastfib	363	5.69958	.1915614	4.969813	6.36647
fastchol	376	6.853989	1.241219	3.1	11.4
lfstchol	376	1.908402	.1828432	1.131402	2.433613
trig	376	1.567287	.8855396	.5	7.7
ltrig	376	.3220138	.4914435	-.6931472	2.04122
hdl	370	1.336784	.3512667	.3	3
lhdl	370	.2547563	.2741732	-1.203973	1.098612
ldl	376	4.913032	1.316182	1.7	9.9
lldl	376	1.557425	.2643934	.5306283	2.292535
lldlhdl	370	1.290748	.3872066	-.5108256	3.091042
fastapa1	370	1.370919	.2924668	.7	2.6
lapa1	370	.2940641	.2057763	-.356675	.9555114
fastapb	370	1.113892	.2819742	.39	2.79
lapb	370	.0768514	.2504967	-.9416085	1.026042
fstlpa	368	22.30326	29.26819	.9	194
lfstlpa	368	2.319221	1.332809	-.1053605	5.267858
fastvii	353	122.5722	35.32288	59	258
lfastvii	353	4.768586	.2838958	4.077538	5.552959
weight	378	75.83201	12.35044	46	138
height	378	1.677201	.0827987	1.437	1.927
wastcirc	378	92.6873	12.10414	60.5	137
hipcirc	378	104.5942	7.394946	89	139
waisthip	378	.8850529	.0870265	.65	1.12
bmi1	378	26.91111	3.647016	16.9	39.6
weight2	373	75.89973	12.56781	45.5	145
height2	372	1.681414	.0828457	1.449	1.926
bmi2	372	26.79594	3.688571	17.46	39.59
fev1	374	2.339759	.614048	.07	3.78
fvc	374	2.882406	.6683677	.07	4.69
fevratio	374	.9249465	.1918902	.02	1.42
insul0	370	51.58622	41.1795	5	560
insul30	359	312.2897	200.1168	23	1660
insul120	359	235.1373	197.4117	5.1	1448
linsul0	370	3.745039	.6217091	1.609438	6.327937
linsul30	359	5.569115	.6002852	3.135494	7.414573
lins120	359	5.172191	.7999964	1.629241	7.277938
gluc0	376	5.960106	1.28663	3.4	17.3
gluc30	367	9.381199	2.218662	5.1	22.3
gluc120	367	7.093733	2.75412	3	26.4
lgluc0	376	1.770064	.1588762	1.223776	2.850706
lgluc30	367	2.215067	.2120164	1.629241	3.104587
lgluc120	367	1.9056	.3114181	1.098612	3.273364
proinsul	372	3.903495	3.055138	1	25
lproi	372	1.149453	.6359984	0	3.218876
lsplit	371	1.501754	.8331091	0	3.89182
lauigtt	342	10.16802	.5523814	8.066051	11.73615
ageclif1	375	64.35627	3.016879	59.1	71.5
ageclin2	375	66.46133	3.044197	61.9	73.3

AGTR1 A1166C Analyses

Overall gene frequency distribution in men and women

AT1RV001	Sex 0=men, 1=women 0	1	Total
11	321	193	514
	54.59	48.98	52.34
12	222	171	393
	37.76	43.40	40.02
22	45	30	75
	7.65	7.61	7.64
Total	588	394	982
	100.00	100.00	100.00

Pearson chi2(2) = 3.2966 Pr = 0.192

Comments: No significant difference in AT1RV001 distribution in men and women.

Analyses in Men

AT1RV001	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	125.10592	18.950297	321
12	125.29279	18.540401	222
22	122.84444	14.132418	45
Total	125.0034	18.45405	588

P= 0.71 on 2 df for bwt_ozs vs at1r, N= 588
Beta= -0.55, p= 0.65 for trend in bwt_ozs vs at1r

AT1RV001	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	363.7757	39.893681	321
12	363.05405	44.180284	222
22	351.57778	48.266537	45
Total	362.56973	42.275807	588

P= 0.19 on 2 df for wt2_ozs vs at1r, N= 588
Beta= -3.73, p= 0.17 for trend in wt2_ozs vs at1r

AT1RV001	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.7203762	.06639303	319
12	1.7231982	.06136566	222
22	1.7042222	.07590332	45
Total	1.7202048	.0653848	586

P= 0.21 on 2 df for hhgt vs at1r, N= 586
Beta= -0.00, p= 0.44 for trend in hhgt vs at1r

AT1RV001	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	79.804075	12.1546	319
12	80.645496	12.104988	222
22	75.4	11.968519	45
Total	79.784642	12.173627	586

P= 0.03 on 2 df for hwgt vs at1r, N= 586
Beta= -0.86, p= 0.28 for trend in hwgt vs at1r

AT1RV001	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	98.921069	10.052212	318
12	98.542342	9.9805289	222
22	95.533333	9.993725	45
Total	98.516752	10.042125	585

P= 0.10 on 2 df for hwast vs at1r, N= 585
Beta= -1.11, p= 0.09 for trend hwast in vs at1r

AT1RV001	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	105.33009	7.7222604	319
12	105.11937	7.1991849	222
22	103.12444	7.5570001	45
Total	105.08089	7.5246132	586

P= 0.18 on 2 df for hhip vs at1r, N= 586
Beta= -0.71, p= 0.15 for trend in hhip vs at1r

AT1RV001	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.929054	3.4945241	319
12	27.126465	3.5162759	222
22	25.933864	3.69483	45
Total	26.927419	3.5251728	586

P= 0.12 on 2 df for hbmi vs at1r, N= 586
Beta= -0.19, p= 0.40 for trend in hbmi vs at1r

AT1RV001	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.93786664	.05095201	318
12	.93602446	.05204319	222
22	.92569361	.05631281	45
Total	.93623117	.05179864	585

P= 0.34 on 2 df for hwhr vs at1r, N= 585
Beta= -0.00, p= 0.21 for trend in hwhr vs at1r

Analyses in Women

AT1RV001	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	120.58031	16.383268	193
12	120.97661	18.611493	171
22	122.53333	19.588408	30
Total	120.90102	17.593743	394

P= 0.85 on 2 df for bwt_ozs vs at1r, N= 394
Beta= 0.71, p= 0.61 for trend in bwt_ozs vs at1r

AT1RV001	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	339.62176	33.4201	193
12	343.48538	39.84667	171
22	337.2	29.353259	30
Total	341.11421	36.066163	394

P= 0.49 on 2 df for wt2_ozs vs at1r, N= 394
Beta= 1.10, p= 0.70 for trend in wt2_ozs vs at1r

AT1RV001	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.5953886	.05992164	193
12	1.6025146	.05741586	171
22	1.577931	.05420723	29
Total	1.597201	.05864806	393

P= 0.09 on 2 df for hhgt vs at1r, N= 393
Beta= -0.00, p= 0.77 for trend in hhgt vs at1r

AT1RV001	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	67.888601	11.622727	193
12	69.085965	11.436867	171
22	68.706897	14.774204	29
Total	68.469975	11.782108	393

P= 0.62 on 2 df for hwgt vs at1r, N= 393
Beta= 0.77, p= 0.41 for trend in hwgt vs at1r

Summary of Waist circumf (cm)			
AT1RV001	Mean	Std. Dev.	Freq.
11	83.748187	9.7661141	193
12	84.183626	9.6364915	171
22	84.465517	12.323041	29
Total	83.990585	9.8942333	393

P= 0.88 on 2 df for hwast vs at1r, N= 393
Beta= 0.39, p= 0.62 for trend in hwast vs at1r

Summary of Hip circumf (cm)			
AT1RV001	Mean	Std. Dev.	Freq.
11	105.4772	9.1895149	193
12	105.7538	9.1535418	171
22	106.08965	13.421686	29
Total	105.64275	9.5183579	393

P= 0.93 on 2 df for hhip vs at1r, N= 393
Beta= 0.29, p= 0.70 for trend in hhip vs at1r

Summary of BMI (kg/m2)			
AT1RV001	Mean	Std. Dev.	Freq.
11	26.641574	4.1263598	193
12	26.927766	4.372413	171
22	27.559854	5.4082143	29
Total	26.833862	4.3336923	393

P= 0.53 on 2 df for hbmi vs at1r, N= 393
Beta= 0.38, p= 0.28 for trend in hbmi vs at1r

Summary of Waist:Hip (proportion)			
AT1RV001	Mean	Std. Dev.	Freq.
11	.793505	.05255337	193
12	.79539679	.05031937	171
22	.79645548	.05175261	29
Total	.79454587	.05141257	393

P= 0.92 on 2 df for hwhr vs at1r, N= 393
Beta= 0.00, p= 0.69 for trend in hwhr vs at1r

AGTR1 A1166C In Relation to Cardiovascular Phenotypes

Sex 0=men, 1=women			
AT1RV001	0	1	Total
11	122	61	183
	50.83	44.20	48.41
12	101	69	170
	42.08	50.00	44.97
22	17	8	25
	7.08	5.80	6.61
Total	240	138	378
	100.00	100.00	100.00

Pearson chi2(2) = 2.2359 Pr = 0.327

Comment: No significant difference in at1r in men and women.

AGTR1 A1166C in relation to cardiovascular phenotypes in men

Summary of EH1 Systolic BP (mmHg)			
AT1RV001	Mean	Std. Dev.	Freq.
11	165.03279	21.827246	122
12	162.88119	20.84768	101
22	156.88235	16.822167	17
Total	163.55	21.129943	240

P= 0.30 on 2 df for sysbpa1 vs at1r, N= 240
P= 0.39 on 2 df adjusted for ageclif1 and bmi1, N= 237
P= 0.37 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -3.16, p= 0.15 for trend in sysbpa1 vs at1r
Beta= -2.97, p= 0.17 for trend adjusted for ageclif1 and bmi1
Beta= -3.20, p= 0.14 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

Summary of EH1 Diastolic BP (mmHg)			
AT1RV001	Mean	Std. Dev.	Freq.
11	90.270492	10.668589	122
12	90.19802	11.140036	101
22	87.647059	9.5390066	17
Total	90.054167	10.774271	240

P= 0.63 on 2 df for diabpa1 vs at1r, N= 240
P= 0.80 on 2 df adjusted for ageclif1 and bmi1, N= 237
P= 0.73 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -0.72, p= 0.52 for trend in diabpa1 vs at1r
Beta= -0.68, p= 0.55 for trend adjusted for ageclif1 and bmi1
Beta= -1.02, p= 0.37 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

Summary of EH1 Pulse rate (bpm)			
AT1RV001	Mean	Std. Dev.	Freq.
11	69.868852	10.921925	122
12	68.633663	11.81501	101
22	66.882353	10.647079	17
Total	69.1375	11.275267	240

P= 0.50 on 2 df for pulsea1 vs at1r, N= 240
P= 0.42 on 2 df adjusted for ageclif1 and bmi1, N= 237
P= 0.39 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -1.37, p= 0.24 for trend in pulsea1 vs at1r
Beta= -1.56, p= 0.19 for trend adjusted for ageclif1 and bmi1
Beta= -1.55, p= 0.20 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

Summary of Pulse pressure mmHg (SBP-DBP)			
AT1RV001	Mean	Std. Dev.	Freq.
11	74.762295	16.051597	122
12	72.683168	14.558112	101
22	69.235294	13.442142	17
Total	73.495833	15.284197	240

P= 0.29 on 2 df for pulspres vs at1r, N= 240
P= 0.32 on 2 df adjusted for ageclif1 and bmi1, N= 237
P= 0.38 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -2.44, p= 0.12 for trend in pulspres vs at1r
Beta= -2.29, p= 0.14 for trend adjusted for ageclif1 and bmi1
Beta= -2.17, p= 0.16 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

Summary of Ln(EH2 echo LVM (g))			
AT1RV001	Mean	Std. Dev.	Freq.
11	5.314981	.22538486	84
12	5.3176305	.26784979	70
22	5.2458187	.18729475	12
Total	5.3110986	.24130739	166

P= 0.62 on 2 df for lecholvm and at1r, N= 166
P= 0.88 on 2 df adjusted for ageclin2 and bmi2, N= 166
P= 0.92 on 2 df adjusted for ageclin2 bmi2 father and current social class, smoking and alcohol, N= 164
Beta= -0.02, p= 0.57 for trend in lecholvm vs at1r
Beta= -0.01, p= 0.65 for trend adjusted for ageclin2 and bmi2
Beta= -0.00, p= 0.93 for trend adjusted for ageclin2 bmi2 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting fibrinogen g/l) Mean	Std. Dev.	Freq.
11	5.7350994	.18020243	119
12	5.6892577	.24698778	98
22	5.7134792	.19100015	16
Total	5.7143337	.21181946	233

P= 0.28 on 2 df for lfastfib vs at1r, N= 233
P= 0.22 on 2 df adjusted for ageclif1 and bmi1, N= 233
P= 0.29 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 230
Beta= -0.03, p= 0.22 for trend in lfastfib vs at1r
Beta= -0.02, p= 0.25 for trend adjusted for ageclif1 and bmi1
Beta= -0.02, p= 0.31 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting cholesterol mmol/l) Mean	Std. Dev.	Freq.
11	1.8953159	.15928901	121
12	1.8773983	.19389007	101
22	1.8267666	.19354468	16
Total	1.8831038	.17715643	238

P= 0.32 on 2 df for lfstchol vs at1r, N= 238
P= 0.41 on 2 df adjusted for ageclif1 and bmi1, N= 237
P= 0.48 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -0.03, p= 0.16 for trend in lfstchol vs at1r
Beta= -0.02, p= 0.19 for trend adjusted for ageclif1 and bmi1
Beta= -0.02, p= 0.18 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting triglycerides mmol/l) Mean	Std. Dev.	Freq.
11	.39352468	.52089444	121
12	.32864728	.5529506	101
22	.23028244	.42916219	16
Total	.35501841	.52929559	238

P= 0.41 on 2 df for ltrig vs at1r, N= 238
P= 0.39 on 2 df adjusted for ageclif1 and bmi1, N= 237
P= 0.52 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -0.07, p= 0.19 for trend in ltrig vs at1r
Beta= -0.07, p= 0.21 for trend adjusted for ageclif1 and bmi1
Beta= -0.06, p= 0.24 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(high density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	.18030821	.22600768	120
12	.19428805	.28028953	97
22	.11671184	.4340314	16
Total	.18176101	.26692176	233

P= 0.56 on 2 df for lhdl vs at1r, N= 233
P= 0.49 on 2 df adjusted for ageclif1 and bmi1, N= 232
P= 0.66 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 229
Beta= -0.01, p= 0.73 for trend in lhdl vs at1r
Beta= -0.01, p= 0.77 for trend adjusted for ageclif1 and bmi1
Beta= -0.02, p= 0.52 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(low density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	1.5445276	.2219327	121
12	1.5408567	.31469533	101
22	1.4631966	.28857314	16
Total	1.5375021	.26906916	238

P= 0.52 on 2 df for lldl vs at1r, N= 238
P= 0.60 on 2 df adjusted for ageclif1 and bmi1, N= 237
P= 0.70 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -0.02, p= 0.43 for trend in lldl vs ar1r
Beta= -0.02, p= 0.46 for trend adjusted for ageclif1 and bmi1
Beta= -0.02, p= 0.44 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(LDL:HDL ratio) Mean	Std. Dev.	Freq.
11	1.357986	.32894444	120
12	1.3155716	.4392927	97
22	1.3464847	.60217906	16
Total	1.3395387	.39902276	233

P= 0.74 on 2 df for lldlhdl vs at1r, N= 233
P= 0.68 on 2 df adjusted for ageclif1 and bmi1, N= 232
P= 0.92 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 229
Beta= -0.02, p= 0.58 for trend in lldlhdl vs at1r
Beta= -0.02, p= 0.57 for trend adjusted for ageclif1 and bmi1
Beta= -0.01, p= 0.75 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Apolopoprotein A1 g/l) Mean	Std. Dev.	Freq.
11	.27690912	.23967989	119
12	.29759796	.21381749	98
22	.26472277	.23346151	15
Total	.28486046	.22797704	232

P= 0.75 on 2 df for lapal vs at1r, N= 232
P= 0.71 on 2 df adjusted for ageclif1 and bmi1, N= 231
P= 0.94 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 229
Beta= 0.01, p= 0.76 for trend in lapal vs at1r
Beta= 0.01, p= 0.70 for trend adjusted for ageclif1 and bmi1
Beta= -0.00, p= 0.96 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Apolopoprotein B g/l) Mean	Std. Dev.	Freq.
11	.11309018	.23124891	119
12	.09832754	.28285461	98
22	.00345465	.33234439	15
Total	.09976573	.26134545	232

P= 0.31 on 2 df for lapb with at1r, N= 232
P= 0.46 on 2 df adjusted for ageclif1 and bmi1, N= 231
P= 0.68 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 229
Beta= -0.03, p= 0.22 for trend in lapb vs at1r
Beta= -0.03, p= 0.23 for trend adjusted for ageclif1 and bmi1
Beta= -0.029, p= 0.31 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Lp(a) lipoprotein mg/l) Mean	Std. Dev.	Freq.
11	2.1442383	1.2161964	117
12	2.0834396	1.3771028	97
22	2.7610587	.95686496	16
Total	2.1615064	1.277529	230

P= 0.14 on 2 df for lfstlpa with at1r, N= 230
P= 0.30 on 2 df adjusted for ageclif1 and bmi1, N= 229
P= 0.27 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 26
Beta= 0.13, p= 0.33 for trend in lfstlpa vs at1r
Beta= 0.13, p= 0.34 for trend adjusted for ageclif1 and bmi1
Beta= 0.14, p= 0.30 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting factor VII %ofstd) Mean	Std. Dev.	Freq.
11	4.7231247	.28711911	117
12	4.6828473	.3087071	96
22	4.6401912	.22864546	16
Total	4.7004454	.29281171	229

P= 0.42 on 2 df for lfastvii with atlr, N= 229
P= 0.44 on 2 df adjusted for ageclif1 and bmil, N= 229
P= 0.30 on 2 df adjusted for ageclif1 bmil father and current social class, smoking and alcohol, N= 226
Beta= -0.04, p= 0.19 for trend in lfastvii vs atlr
Beta= -0.04, p= 0.20 for trend adjusted for ageclif1 and bmil
Beta= -0.04, p= 0.15 for trend adjusted for ageclif1 bmil father and current social class, smoking and alcohol

AT1RV001	Summary of EH1 weight (kg) Mean	Std. Dev.	Freq.
11	80.401639	11.45953	122
12	80.334653	10.810286	101
22	73.617647	11.012109	17
Total	79.892917	11.24788	240

P= 0.06 on 2 df for weight with atlr, N= 240
Beta= -1.81, p= 0.12 for trend in weight vs atlr

AT1RV001	Summary of EH1 height (m) Mean	Std. Dev.	Freq.
11	1.7290574	.06597135	122
12	1.7110594	.05470171	101
22	1.7074706	.07343119	17
Total	1.7199542	.06247579	240

P= 0.07 on 2 df for height with atlr, N= 240
Beta= -0.001, p= 0.03 for trend in height vs atlr

AT1RV001	Summary of EH1 Waist circumf (cm) Mean	Std. Dev.	Freq.
11	99.233607	10.176578	122
12	98.411881	9.0084436	101
22	91.452942	8.1674754	17
Total	98.336667	9.7280806	240

P= 0.008 on 2 df for wastcirc with atlr, N= 240
Beta= -2.43, p= 0.02 for trend in wastcirc vs atlr

AT1RV001	Summary of EH1 Hip circumf (cm) Mean	Std. Dev.	Freq.
11	105.07459	6.9417359	122
12	104.76931	7.1686783	101
22	101.47059	5.6453264	17
Total	104.69083	6.989083	240

P= 0.14 on 2 df for hipcirc with atlr, N= 240
Beta= -1.09, p= 0.13 for trend in hipcirc vs atlr

AT1RV001	Summary of EH1 Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.94278688	.05315286	122
12	.93841584	.04753383	101
22	.90058824	.04762754	17
Total	.93795833	.05137032	240

P= 0.01 on 2 df for waisthip with atlr, N= 240
Beta= -0.01, p= 0.01 for trend in waisthip vs atlr

AT1RV001	Summary of EH1 BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.87377	3.2910549	122
12	27.429703	3.3874339	101
22	25.194118	2.9846422	17
Total	26.98875	3.3464899	240

P= 0.03 on 2 df for bmil with atlr, N= 240
Beta= -0.18, p= 0.61 for trend in bmil vs atlr

AT1RV001	Summary of EH1 forced expiratory volume in 1second (1) Mean	Std. Dev.	Freq.
11	2.5831967	.60899493	122
12	2.5839604	.59536724	101
22	2.4088235	.42041471	17
Total	2.5711667	.59154529	240

P= 0.50 on 2 df for fev1 with atlr, N= 240
P= 0.47 on 2 df adjusted for ageclif1 and height, N= 237
P= 0.54 on 2 df adjusted for ageclif1 height father and current social class, smoking and alcohol, N= 234
Beta= -0.04, p= 0.46 for trend in fev1 vs atlr
Beta= -0.02, p= 0.69 for trend adjusted for ageclif1 and height
Beta= -0.03, p= 0.56 for trend adjusted for ageclif1 height father and current social class, smoking and alcohol

AT1RV001	Summary of EH1 forced ventilatory capacity (1) Mean	Std. Dev.	Freq.
11	3.1533606	.714641	122
12	3.0579208	.6001405	101
22	2.8776471	.46220299	17
Total	3.0936667	.6551635	240

P= 0.21 on 2 df for fvc with atlr, N= 240
P= 0.26 on 2 df adjusted for ageclif1 and height, N= 237
P= 0.19 on 2 df adjusted for ageclif1 height father and current social class, smoking and alcohol, N= 234
Beta= -0.12, p= 0.08 for trend in fvc vs atlr
Beta= -0.10, p= 0.12 for trend adjusted for ageclif1 height
Beta= -0.10, p= 0.09 for trend adjusted for ageclif1 height father and current social class, smoking and alcohol

AT1RV001	Summary of EH1 FEV1 observed:predicted Mean	Std. Dev.	Freq.
11	.9004918	.20786141	122
12	.91742575	.19346139	101
22	.85705883	.1320873	17
Total	.90454167	.19733527	240

P= 0.48 on 2 df for fevratio with atlr, N= 240
P= 0.52 on 2 df adjusted for ageclif1 height, N= 237
P= 0.58 on 2 df adjusted for ageclif1 height father and current social class, smoking and alcohol, N= 234
Beta= -0.00, p= 0.87 for trend in fevratio vs atlr
Beta= -0.01, p= 0.61 for trend adjusted for ageclif1 height
Beta= -0.01, p= 0.49 for trend adjusted for ageclif1 height father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Insulin0 pmol/l) Mean	Std. Dev.	Freq.
11	3.7535156	.68376618	117
12	3.7080323	.59530594	99
22	3.3119714	.70102199	16
Total	3.7036555	.65492883	232

P= 0.04 on 2 df for linsul0 with atlr, N= 232
P= 0.14 on 2 df adjusted for ageclif1 bmil, N= 231
P= 0.15 on 2 df adjusted for ageclif1 bmil father and current social class, smoking and alcohol, N= 228
Beta= -0.13, p= 0.05 for trend in linsul0 vs atlr
Beta= -0.12, p= 0.06 for trend adjusted for ageclif1 bmil
Beta= -0.12, p= 0.06 for trend adjusted for ageclif1 bmil father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Insulin30 pmol/l) Mean	Std. Dev.	Freq.
11	5.6031663	.6380916	115
12	5.586279	.62388217	95
22	5.4732294	.46647843	16
Total	5.5868686	.62005411	226

P= 0.74 on 2 df for linsul30 with at1r, N= 226
P= 0.89 on 2 df adjusted for ageclif1 bmi1, N= 226
P= 0.93 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 223
Beta= -0.04, p= 0.53 for trend in linsu130 vs at1r
Beta= -0.03, p= 0.66 for trend adjusted for ageclif1 bmi1
Beta= -0.03, p= 0.68 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Insulin120 pmol/l) Mean	Std. Dev.	Freq.
11	5.0869874	.8240401	115
12	4.9100861	.88613905	92
22	4.799466	.56978169	14
Total	4.9951312	.84007585	221

P= 0.21 on 2 df for lins120 with at1r, N= 221
P= 0.15 on 2 df adjusted for ageclif1 bmi1, N= 221
P= 0.32 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 218
Beta= -0.16, p= 0.08 for trend in lins120 vs at1r
Beta= -0.14, p= 0.10 for trend adjusted for ageclif1 bmi1
Beta= -0.13, p= 0.16 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Glucose0 mmol/l) Mean	Std. Dev.	Freq.
11	1.8105367	.19471525	121
12	1.778755	.14559367	101
22	1.7175105	.07983665	16
Total	1.7907956	.17078588	238

P= 0.08 on 2 df for lgluc0 with at1r, N= 238
P= 0.10 on 2 df adjusted for ageclif1 bmi1, N= 237
P= 0.20 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 234
Beta= -0.04, p= 0.03 for trend in lgluc0 vs at1r
Beta= -0.04, p= 0.03 for trend adjusted for ageclif1 bmi1
Beta= -0.03, p= 0.05 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Glucose30 mmol/l) Mean	Std. Dev.	Freq.
11	2.2702091	.22692827	119
12	2.2341888	.18769109	99
22	2.1083464	.18321257	16
Total	2.2439022	.21145442	234

P= 0.01 on 2 df for lgluc30 with at1r, N= 234
P= 0.03 on 2 df adjusted for ageclif1 bmi1, N= 234
P= 0.04 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 231
Beta= -0.06, p= 0.008 for trend in lgluc30 vs at1r
Beta= -0.05, p= 0.01 for trend adjusted for ageclif1 bmi1
Beta= -0.05, p= 0.02 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Glucose120 mmol/l) Mean	Std. Dev.	Freq.
11	1.9204219	.31845973	117
12	1.8422514	.35144061	97
22	1.7465837	.21554227	15
Total	1.8759236	.33023341	229

P= 0.06 on 2 df for lgluc120 with at1r, N= 229
P= 0.07 on 2 df adjusted for ageclif1 bmi1, N= 229
P= 0.17 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 226
Beta= -0.08, p= 0.02 for trend in lglucl20 vs at1r
Beta= -0.08, p= 0.02 for trend adjusted for ageclif1 bmi1
Beta= -0.07, p= 0.05 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Fasting Proinsulin pmol/l) Mean	Std. Dev.	Freq.
11	1.1534676	.72518684	119
12	1.0459034	.63278439	100
22	.778729	.50367721	16
Total	1.0821815	.67845962	235

P= 0.09 on 2 df for lproi with at1r, N= 235
P= 0.16 on 2 df adjusted for ageclif1 bmi1, N= 234
P= 0.24 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 231
Beta= -0.15, p= 0.04 for trend in lproi vs at1r
Beta= -0.13, p= 0.06 for trend adjusted for ageclif1 bmi1
Beta= -0.11, p= 0.09 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Fast. 32-33 Split Proinsulin pmol/l) Mean	Std. Dev.	Freq.
11	1.1782872	.75339776	118
12	1.0767534	.70095575	101
22	.78199496	.63879021	15
Total	1.1090594	.72813563	234

P= 0.12 on 2 df for lsplit with at1r, N= 234
P= 0.18 on 2 df adjusted for ageclif1 bmi1, N= 233
P= 0.20 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 230
Beta= -0.15, p= 0.05 for trend in lsplit vs at1r
Beta= -0.13, p= 0.06 for trend adjusted for ageclif1 bmi1
Beta= -0.13, p= 0.07 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Insulin AUC during OGTT) Mean	Std. Dev.	Freq.
11	10.1598	.62073109	108
12	10.13295	.4990918	87
22	9.9525122	.4062041	14
Total	10.134738	.56042501	209

P= 0.43 on 2 df for lauigtt with at1r, N= 209
P= 0.69 on 2 df adjusted for ageclif1 bmi1, N= 209
P= 0.78 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 206
Beta= -0.06, p= 0.29 for trend in lauigtt vs at1r
Beta= -0.05, p= 0.40 for trend adjusted for ageclif1 bmi1
Beta= -0.05, p= 0.43 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AGTR1 A1166C in relation to cardiovascular phenotypes in women

AT1RV001	Summary of EH1 Systolic BP (mmHg) Mean	Std. Dev.	Freq.
11	156.37705	21.19604	61
12	155.98551	23.553751	69
22	161.25	17.136011	8
Total	156.46377	22.103988	138

P= 0.82 on 2 df for sysbpa1 with at1r, N= 138
P= 0.76 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.81 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= 0.89, p= 0.78 for trend in sysbpa1 vs at1r
Beta= 0.90, p= 0.78 for trend adjusted for ageclif1 bmi1

Beta= 0.49, p= 0.89 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of EH1 Diastolic BP (mmHg) Mean	Std. Dev.	Freq.
11	81.245902	10.482773	61
12	83.144928	12.224945	69
22	84.375	6.7387472	8
Total	82.376812	11.212742	138

P= 0.55 on 2 df for diabpa1 with at1r, N= 138
P= 0.55 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.57 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= 1.75, p= 0.28 for trend in diabpa1 vs at1r
Beta= 1.75, p= 0,28 for trend adjusted for ageclif1 bmi1
Beta= 1.47, p= 0.37 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of EH1 Pulse rate (bpm) Mean	Std. Dev.	Freq.
11	69.032787	10.44185	61
12	69.449275	10.133718	69
22	66.125	11.703937	8
Total	69.072464	10.310251	138

P= 0.69 on 2 df for pulsea1 with at1r, N= 138
P= 0.58 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.81 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= -0.43, p= 0.77 for trend in pulsea1 vs at1r
Beta= -0.44, p= 0.77 for trend adjusted for ageclif1 bmi1
Beta= -0.32, p= 0.84 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Pulse pressure mmHg (SBP-DBP) Mean	Std. Dev.	Freq.
11	75.131148	14.59392	61
12	72.84058	15.770993	69
22	76.875	17.569759	8
Total	74.086957	15.304442	138

P= 0.61 on 2 df for pulspres with at1r, N= 138
P= 0.48 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.53 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= -0.85, p= 0.70 for trend in pulspres vs at1r
Beta= -0.85, p= 0.70 for trend adjusted for ageclif1 bmi1
Beta= -0.98, p= 0.65 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(EH2 echo LVM (g)) Mean	Std. Dev.	Freq.
11	5.0140687	.2943676	49
12	4.9876827	.27602257	49
22	4.8742528	.2168854	8
Total	4.9913192	.28083675	106

P= 0.43 on 2 df for lecholvm with at1r, N= 106
P= 0.28 on 2 df adjusted for ageclin2 bmi2, N= 105
P= 0.37 on 2 df adjusted for ageclin2 bmi2 father and current social class, smoking and alcohol, N= 103
Beta= -0.05, p= 0.26 for trend in lecholvm vs at1r
Beta= -0.06, p= 0.11 for trend adjusted for agecclin2 bmi2
Beta= -0.06, p= 0.12 for trend adjusted for ageclin2 bmi2 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting fibrinogen g/l) Mean	Std. Dev.	Freq.
11	5.7134774	.14786958	57
12	5.6482783	.13754549	65
22	5.5876948	.12329245	8
Total	5.6731374	.14555427	130

P= 0.01 on 2 df for lfastfib with at1r, N= 130
P= 0.005 on 2 df adjusted for ageclif1 bmi1, N= 130
P= 0.009 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 128
Beta= -0.06, p= 0.002 for trend in lfastfib vs at1r
Beta= -0.07, p= 0.001 for trend adjusted for ageclif1 bmi1
Beta= -0.06, p= 0.002 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting cholesterol mmol/l) Mean	Std. Dev.	Freq.
11	1.9427208	.20902848	61
12	1.9753386	.15715679	69
22	1.8220365	.17101317	8
Total	1.9520335	.18489955	138

P= 0.07 on 2 df for lfstchol for at1r, N= 138
P= 0.03 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.05 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= -0.01, p= 0.72 for trend in lfstchol vs at1r
Beta= -0.01, p= 0.75 for trend adjusted for ageclif1 bmi1
Beta= -0.01, p= 0.71 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting triglycerides mmol/l) Mean	Std. Dev.	Freq.
11	.22239225	.44465259	61
12	.33527405	.37832128	69
22	-.01462652	.33664953	8
Total	.26509293	.41386501	138

P= 0.04 on 2 df for ltrig with at1r, N= 138
P= 0.11 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.24 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= -0.01, p= 0.72 for trend in ltrig vs at1r
Beta= -0.00, p= 0.99 for trend adjusted for ageclif1 bmi1
Beta= 0.01, p= 0.90 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(high density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	.40119131	.23022974	60
12	.35448033	.25403511	69
22	.42236404	.19050736	8
Total	.3789017	.24033928	137

P= 0.48 on 2 df for lhdl with at1r, N= 137
P= 0.70 on 2 df adjusted for ageclif1 bmi1, N= 137
P= 0.56 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, n= 135
Beta= -0.01, p= 0.72 for trend in lhdl vs at1r
Beta= -0.02, p= 0.49 for trend adjusted for ageclif1 bmi1
Beta= -0.03, p= 0.39 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(low density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	1.5882332	.28191526	61
12	1.6146132	.22518036	69
22	1.4219591	.21184598	8
Total	1.5917841	.2534175	138

P= 0.12 on 2 df for ldl with at1r, N= 138
P= 0.09 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.08 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= -0.02, p= 0.52 for trend in ldl vs at1r
Beta= -0.02, p= 0.55 for trend adjusted for ageclif1 bmi1
Beta= -0.02, p= 0.53 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(LDL:HDL ratio) Mean	Std. Dev.	Freq.
11	1.1753036	.3325147	60
12	1.2601328	.370611	69
22	.99959508	.24623547	8
Total	1.2077674	.35237552	137

P= 0.09 on 2 df for lldlhdl with atlr, N= 137
P= 0.14 on 2 df adjusted for ageclif1 bmi, N= 137
P= 0.11 on 2 df adjusted for ageclif1 bmi father and current social class, smoking and alcohol, N= 135
Beta= 0.00, p= 0.91 for trend in lldlhdl vs atlr
Beta= 0.01, p= 0.83 fir trend adjusted for ageclif1 bmi
Beta= 0.01, p= 0.78 for trend adjusted for ageclif1 bmi father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Apolopoprotein A1 g/l) Mean	Std. Dev.	Freq.
11	.31935398	.15361463	61
12	.30485403	.17277478	69
22	.2750729	.12170987	8
Total	.30953699	.1613427	138

P= 0.72 on 2 df for lapa1 with atlr, N= 138
P= 0.54 on 2 df adjusted for ageclif1 bmi, N= 138
P= 0.47 on 2 df adjusted for ageclif1 bmi father and current social class, smoking and alcohol, N= 136
Beta= -0.02, p= 0.44 for trend in lapa1 vs atlr
Beta= -0.02, p= 0.37 for trend adjusted for ageclif1 bmi
Beta= -0.02, p= 0.30 for trend adjusted for ageclif1 bmi father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Apolopoprotein B g/l) Mean	Std. Dev.	Freq.
11	.03162965	.26206146	61
12	.06537601	.1858886	69
22	-.14387159	.1930648	8
Total	.03832885	.22684751	138

P= 0.04 on 2 df for lapb with atlr, N= 138
P= 0.07 on 2 df adjusted for ageclif1 bmi, N= 138
P= 0.12 on 2 df adjusted for ageclif1 bmi father and current social class, smoking and alcohol, N= 136
Beta= -0.02, p= 0.51 for trend in lapb vs atlr
Beta= -0.02, p= 0.48 for trend adjusted for ageclif1 bmi
Beta= -0.02, p= 0.49 for trend adjusted for ageclif1 bmi father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Lp(a) lipoprotein mg/l) Mean	Std. Dev.	Freq.
11	2.7881107	1.4529105	61
12	2.3987006	1.362418	69
22	2.5927327	.85695792	8
Total	2.5820794	1.3853472	138

P= 0.28 on 2 df for lfstlpa with atlr, N= 138
P= 0.28 on 2 df adjusted for ageclif1 bmi, N= 138
P= 0.38 on 2 df adjusted for ageclif1 bmi father and current social class, smoking and alcohol, N= 136
Beta= -0.26, p= 0.20 for trend in lfslpa vs atlr
Beta= -0.27, p= 0.18 for trend adjusted for ageclif1 bmi
Beta= -0.24, p= 0.24 for trend adjusted for ageclif1 bmi father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(fasting factor VII %ofstd) Mean	Std. Dev.	Freq.
11	4.9248211	.23078967	55
12	4.8708651	.20229629	61
22	4.8651177	.23037732	8
Total	4.8944264	.21705229	124

P= 0.38 on 2 df for lfastvii with atlr, N= 124
P= 0.32 on 2 df adjusted for ageclif1 bmi, N= 124
P= 0.35 on 2 df adjusted for ageclif1 bmi father and current social class, smoking and alcohol, N= 122
Beta= -0.04, p= 0.19 for trend in lfastvii vs atlr
Beta= -0.04, p= 0.17 for trend adjusted for ageclif1 bmi
Beta= -0.04, p= 0.19 for trend adjusted for ageclif1 bmi father and current social class, smoking and alcohol

AT1RV001	Summary of EH1 weight (kg) Mean	Std. Dev.	Freq.
11	67.672131	9.8233927	61
12	70.510145	12.006728	69
22	62.125	5.3234656	8
Total	68.769565	10.950123	138

P= 0.07 on 2 df for weight with atlr, N= 138
Beta= 0.28, p= 0.86 for trend in weight vs atlr

AT1RV001	Summary of EH1 height (m) Mean	Std. Dev.	Freq.
11	1.6028033	.05961204	61
12	1.6075652	.0538231	69
22	1.5625	.06015931	8
Total	1.6028478	.05731842	138

P= 0.11 on 2 df for height with atlr, N= 138
Beta= -0.01, p= 0.42 for trend in height vs atlr

AT1RV001	Summary of EH1 Waist circumf (cm) Mean	Std. Dev.	Freq.
11	82.567213	8.9886728	61
12	83.86087	9.6481515	69
22	76.5	2.9277002	8
Total	82.862319	9.2158321	138

P= 0.09 on 2 df for wastcirc with atlr, N= 138
Beta= -0.68, p= 0.61 for trend in wastcirc vs atlr

AT1RV001	Summary of EH1 Hip circumf (cm) Mean	Std. Dev.	Freq.
11	103.81639	7.4487402	61
12	105.54348	8.6636745	69
22	99.4375	5.274179	8
Total	104.42609	8.0761369	138

P= 0.09 on 2 df for hipcirc with atlr, N= 138
Beta= -0.05, p= 0.96 for trend in hipcirc vs atlr

AT1RV001	Summary of EH1 Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.7952459	.05696802	61
12	.79362319	.05101819	69
22	.77125	.03522884	8
Total	.79304348	.05297856	138

P= 0.48 on 2 df for waisthip with atlr, N= 138
Beta= -0.01, p= 0.40 for trend in waisthip vs atlr

AT1RV001	Summary of EH1 BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.327869	3.5725405	61
12	27.324638	4.6902716	69
22	25.4625	1.9025828	8
Total	26.776087	4.1273974	138

P= 0.25 on 2 df for bmi1 with atlr, N= 138
Beta= 0.35, p= 0.56 for trend in bmi1 vs atlr

AT1RV001	Summary of EH1 forced expiratory volume in 1second (l) Mean	Std. Dev.	Freq.
11	1.827069	.39479067	58
12	1.9995588	.38698001	68
22	2.00625	.41996385	8
Total	1.9252985	.3986739	134

P= 0.04 on 2 df for fev1 with atlr, N= 134

P= 0.02 on 2 df adjusted for ageclif1 height, N= 134
P= 0.05 on 2 df adjusted for ageclif1 height father and current social class, smoking and alcohol, N= 132
Beta= 0.13, p= 0.02 for trend in fev1 vs at1r
Beta= 0.14, p= 0.006 for trend adjusted for ageclif1 height
Beta= 0.13, p= 0.01 for trend adjusted for ageclif1 height father and current social class, smoking and alcohol

| Summary of EH1 forced ventilatory capacity (l)

AT1RV001	Mean	Std. Dev.	Freq.
11	2.4301724	.47604914	58
12	2.5854412	.52390165	68
22	2.3475	.51690701	8
Total	2.5040299	.50665217	134

P= 0.15 on 2 df for fvc with at1r, N= 134
P= 0.26 on 2 df adjusted for ageclif1 height, N= 134
P= 0.23 on 2 df adjusted for ageclif1 height father and current social class, smoking and alcohol, N= 132
Beta= 0.06, p= 0.39 for trend in fvc vs at1r
Beta= 0.08, p= 0.18 for trend adjusted for ageclif1 height
Beta= 0.06, p= 0.30 for trend adjusted for ageclif1 height father and current social class, smoking and alcohol

| Summary of EH1 FEV1 observed:predicted

AT1RV001	Mean	Std. Dev.	Freq.
11	.9163793	.18190703	58
12	.98911765	.1652744	68
22	1.05375	.16664227	8
Total	.96149254	.17661609	134

P= 0.02 on 2 df for fevratio with at1r, N= 134
P= 0.02 on 2 df adjusted for ageclif1 height, N=134
P= 0.05 on 2 df adjusted for ageclif1 height father and current social class, smoking and alcohol, N= 132
Beta= 0.07, p= 0.005 for trend in fevratio vs at1r
Beta= 0.07, p= 0.006 for trend adjusted for ageclif1 height
Beta= -0.06, p= 0.01 for trend adjusted for ageclif1 height father and current social class, smoking and alcohol

| Summary of Ln(Insulin0 pmol/l)

AT1RV001	Mean	Std. Dev.	Freq.
11	3.879682	.54814909	61
12	3.7456457	.58720325	69
22	3.9132577	.22003863	8
Total	3.8146103	.55683019	138

P= 0.34 on 2 df for linsul0 with at1r, N= 138
P= 0.05 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.04 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, n= 136
Beta= -0.06, p= 0.41 for trend in linsul0 vs at1r
Beta= -0.09, p= 0.22 for trend adjusted for ageclif1 bmi1
Beta= -0.07, p= 0.29 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

| Summary of Ln(Insulin30 pmol/l)

AT1RV001	Mean	Std. Dev.	Freq.
11	5.5146136	.52329386	58
12	5.5209182	.60744952	67
22	5.8663664	.45183522	8
Total	5.5389477	.56612169	133

P= 0.24 on 2 df for linsul30 with at1r, N= 133
P= 0.04 on 2 df adjusted for ageclif1 bmi1, N= 133
P= 0.03 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, n= 131
Beta= 0.08, p= 0.30 for trend in linsul30 with at1r
Beta= 0.06, p= 0.44 for trend adjusted for ageclif1 bimi
Beta= 0.05, p= 0.49 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

| Summary of Ln(Insulin120 pmol/l)

AT1RV001	Mean	Std. Dev.	Freq.
11	5.4424425	.63496501	61
12	5.4727233	.66006857	69
22	5.4107237	.53039984	8
Total	5.4557441	.63839418	138

P= 0.94 on 2 df for lins120 with at1r, N= 138
P= 0.96 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.88 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= 0.01, p= 0.92 for trend in lins120 vs at1r
Beta= -0.01, p= 0.92 for trend adjusted for ageclif1 bmi1
Beta= -0.01, p= 0.91 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

| Summary of Ln(Glucose0 mmol/l)

AT1RV001	Mean	Std. Dev.	Freq.
11	1.7337632	.10022718	61
12	1.7323308	.15031906	69
22	1.7555173	.13679485	8
Total	1.7343081	.12883876	138

P= 0.89 on 2 df for lgluc0 with at1r, N= 138
P= 0.85 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.84 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= 0.00, p= 0.82 for trend in lgluc0 vs at1r
Beta= 0.00, p= 0.89 for trend adjusted for ageclif1 bmi1
Beta= 0.00, p= 0.84 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

| Summary of Ln(Glucose30 mmol/l)

AT1RV001	Mean	Std. Dev.	Freq.
11	2.1704057	.20092309	58
12	2.1678412	.2079858	67
22	2.0909481	.20524124	8
Total	2.1643344	.20406927	133

P= 0.58 on 2 df for lgluc30 with at1r, N= 133
P= 0.64 on 2 df adjusted for ageclif1 bmi1, N= 133
P= 0.75 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 131
Beta= -0.02, p= 0.50 for trend in lgluc30 vs at1r
Beta= -0.02, p= 0.48 for trend adjusted for ageclif1 bmi1
Beta= -0.02, p= 0.56 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

| Summary of Ln(Glucose120 mmol/l)

AT1RV001	Mean	Std. Dev.	Freq.
11	1.955754	.25291378	61
12	1.962559	.28347875	69
22	1.881392	.32617151	8
Total	1.9548456	.27144451	138

P= 0.73 on 2 df for lgluc120 with at1r, N= 138
P= 0.79 on 2 df adjusted for ageclif1 bmi1, N= 138
P= 0.70 on 2 df adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol, N= 136
Beta= -0.01, p= 0.73 for trend in lgluc120 vs at1r
Beta= -0.01, p= 0.69 for trend adjusted for ageclif1 bmi1
Beta= -0.01, p= 0.69 for trend adjusted for ageclif1 bmi1 father and current social class, smoking and alcohol

| Summary of Ln(Fasting Proinsulin pmol/l)

AT1RV001	Mean	Std. Dev.	Freq.
11	1.21896	.54685632	61
12	1.2877946	.53522116	68
22	1.4196718	.53124018	8
Total	1.2648465	.53870469	137

P= 0.54 on 2 df for lproi with at1r, N= 137
P= 0.44 on 2 df adjusted for ageclif1 bmi1, N= 137

P= 0.66 on 2 df adjusted for ageclifl bmil father and current social class, smoking and alcohol, N= 135
Beta= 0.08, p= 0.28 for trend in lproi vs atlr
Beta= 0.07, p= 0.33 for trend adjusted for ageclifl bmil
Beta= 0.05, p= 0.49 for trend adjusted for ageclifl bmil father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Fast. 32-33 Split Proinsulin pmol/l) Mean	Std. Dev.	Freq.
11	2.1494339	.48620904	61
12	2.196872	.54221099	68
22	2.1409955	.47701006	8
Total	2.1724871	.51130618	137

P= 0.86 on 2 df for lsplit with atlr, N= 137
P= 0.91 on 2 df adjusted for ageclifl bmil, N= 137
P= 0.88 on 2 df adjusted for ageclifl bmil father and current social class, smoking and alcohol, N= 135
Beta= 0.02, p= 0.75 for trend in lsplit vs atlr
Beta= 0.00, p= 0.96 for trend adjusted for ageclifl bmil
Beta= -0.01, p= 0.85 for trend adjusted for ageclifl bmil father and current social class, smoking and alcohol

AT1RV001	Summary of Ln(Insulin AUC during OGTT) Mean	Std. Dev.	Freq.
11	10.190484	.52665921	58
12	10.221315	.56805431	67
22	10.428468	.29731101	8
Total	10.22033	.5374209	133

P= 0.50 on 2 df for lauigtt with atlr, N= 0.50
P= 0.15 on 2 df adjusted for ageclifl bmil, N= 133
P= 0.11 on 2 df adjusted for ageclifl bmil father and current social class, smoking and alcohol, N= 131
Beta= 0.07, p= 0.36 for trend in lauigtt vs atlr
Beta= 0.04, p= 0.55 for trend adjusted for ageclifl bmil
Beta= 0.04, p= 0.57 for trend adjusted for ageclifl bmil father and current social class, smoking and alcohol

AGTR1 L191L Analyses

Overall gene frequency distributions in men and women

AT1RV002	Sex 0=men, 1=women 0	1	Total
11	165	128	293
	27.27	31.60	29.01
12	308	197	505
	50.91	48.64	50.00
22	132	80	212
	21.82	19.75	20.99
Total	605	405	1010
	100.00	100.00	100.00

Pearson chi2(2) = 2.3118 Pr = 0.315

Analyses for men

AT1RV002	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	78.140244	11.08245	164
12	80.230519	12.477097	308
22	81.046154	12.552285	130
Total	79.837209	12.158688	602

p= 0.090 on 2 df for hwgt vs at1rv002, N=602
beta= 1.49, p= 0.036 for trend in hwgt with at1rv002

AT1RV002	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.7142683	.06648088	164
12	1.7201299	.06407433	308
22	1.7298461	.06720309	130
Total	1.7206312	.06553454	602

p= 0.127 on 2 df for hhgt vs at1rv002, N=602
beta= 0.01, p= 0.045 for trend in hhgt with at1rv002

AT1RV002	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.552473	3.1499185	164
12	27.086377	3.6416604	308
22	27.056559	3.7240719	130
Total	26.934489	3.5351016	602

p= 0.268 on 2 df for hbmi vs at1rv002, N=602
beta= 0.27, p= 0.194 for trend in hbmi with at1rv002

AT1RV002	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	97.264024	9.5723456	164
12	98.772078	10.3241	308
22	99.423256	9.9291987	129
Total	98.500333	10.054896	601

p= 0.150 on 2 df for hwast vs at1rv002, N=601
beta= 1.11, p= 0.061 for trend in hwast with at1rv002

AT1RV002	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	104.35549	6.7346301	164
12	105.43377	7.5952343	308
22	105.26	8.199344	130
Total	105.10249	7.5108469	602

p= 0.321 on 2 df for hhip vs at1rv002, N=602
beta= 0.49, p= 0.266 for trend in hhip with at1rv002

AT1RV002	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.93075782	.05334787	164
12	.93545443	.0509803	308
22	.94355669	.05352144	129
Total	.93591191	.05228781	601

p= 0.112 on 2 df for hwhr vs at1rv002, N=601
beta= 0.01, p= 0.040 for trend in hwhr with at1rv002

AT1RV002	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	125.15758	17.814064	165
12	124.32792	19.732567	308
22	125.77273	16.721605	132
Total	124.86942	18.576078	605

p= 0.736 on 2 df for bwt_ozs vs at1rv002, N=605
beta= 0.24, p= 0.822 for trend in bwt_ozs with at1rv002

AT1RV002	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	361.61212	45.41658	165
12	361.23377	40.757692	308
22	364.24242	41.870415	132
Total	361.99339	42.262033	605

p= 0.785 on 2 df for wt2_ozs vs at1rv002, N=605
beta= 1.22, p= 0.621 for trend in wt2_ozs with at1rv002

Analyses for women

AT1RV002	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	67.532812	12.379753	128
12	68.739796	11.070689	196
22	69.15625	11.652564	80
Total	68.439851	11.602796	404

p= 0.545 on 2 df for hwgt vs at1rv002, N=404
beta= 0.86, p= 0.294 for trend in hwgt with at1rv002

AT1RV002	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.5969531	.06059493	128
12	1.6007143	.0553636	196
22	1.583625	.06100931	80
Total	1.5961386	.05840681	404

p= 0.086 on 2 df for hhgt vs at1rv002, N=404
beta=-0.01, p= 0.184 for trend in hhgt with at1rv002

AT1RV002	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.466681	4.454955	128
12	26.841173	4.1713962	196
22	27.568821	4.4070158	80
Total	26.866611	4.316279	404

p= 0.200 on 2 df for hbmi vs at1rv002, N=404
beta= 0.53, p= 0.080 for trend in hbmi with at1rv002

AT1RV002	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	83.632812	10.886697	128
12	83.836225	8.9659963	196
22	85.085	9.8326255	80
Total	84.019059	9.7718696	404

p= 0.544 on 2 df for hwast vs at1rv002, N=404
beta= 0.67, p= 0.333 for trend in hwast with at1rv002

AT1RV002	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	104.81562	9.9230144	128
12	105.73061	9.1491953	196
22	106.5375	9.4783612	80
Total	105.6005	9.4616819	404

p= 0.428 on 2 df for hhip vs at1rv002, N=404
beta= 0.87, p= 0.193 for trend in hhip with at1rv002

AT1RV002	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.79715568	.05899817	128
12	.79281974	.04945309	196
22	.79786621	.04197059	80
Total	.7951928	.05129303	404

p= 0.663 on 2 df for hwhr vs at1rv002, N=404
beta=-0.00, p= 0.959 for trend in hwhr with at1rv002

AT1RV002	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	122.44531	20.155228	128
12	121.18782	17.181809	197
22	117.7	14.790007	80
Total	120.8963	17.791118	405

p= 0.165 on 2 df for bwt_ozs vs at1rv002, N=405
beta=-2.24, p= 0.073 for trend in bwt_ozs with at1rv002

AT1RV002	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	342.19531	33.887042	128
12	341.4467	37.577857	197
22	338.2375	36.685887	80
Total	341.04938	36.211802	405

p= 0.729 on 2 df for wt2_ozs vs at1rv002, N=405
beta=-1.84, p= 0.471 for trend in wt2_ozs with at1rv002

AGTR1 A1166C In Relation to Cardiovascular Phenotypes

Overall SNP frequency distributions in men and women

AT1RV002	Sex 0=men, 1=women 0	1	Total
11	60	43	103
	24.49	30.07	26.55
12	128	76	204
	52.24	53.15	52.58
22	57	24	81
	23.27	16.78	20.88
Total	245	143	388
	100.00	100.00	100.00

Pearson chi2(2) = 2.8905 Pr = 0.236

Analyses for men

AT1RV002	Summary of EH1 Systolic BP (mmHg) Mean	Std. Dev.	Freq.
11	160.75	17.233811	60
12	165.35156	22.834078	128
22	161.91228	21.773412	57
Total	163.42449	21.359899	245

p= 0.323 on 2 df for sysbpal vs at1rv002, N=245
p= 0.685 on 2 df adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat, N=239
beta= 0.64, p= 0.749 for trend in sysbpal with at1rv002
beta= 0.71, p= 0.720 for trend adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat

AT1RV002	Summary of EH1 Diastolic BP (mmHg) Mean	Std. Dev.	Freq.
11	88.583333	9.4748403	60
12	90.914063	10.841033	128
22	89.473684	11.952443	57
Total	90.008163	10.801105	245

p= 0.354 on 2 df for diabpal vs at1rv002, N=245
p= 0.563 on 2 df adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat, N=239
beta= 0.47, p= 0.639 for trend in diabpal with at1rv002
beta= 0.42, p= 0.683 for trend adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Pulse pressure mmHg (SBP-DBP) Mean	Std. Dev.	Freq.
11	72.166667	13.8309	60
12	74.4375	15.972787	128
22	72.438596	15.62852	57
Total	73.416327	15.370764	245

p= 0.553 on 2 df for pulspres vs at1rv002, N=245
p= 0.905 on 2 df adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat, N=239
beta= 0.16, p= 0.908 for trend in pulspres with at1rv002
beta= 0.29, p= 0.836 for trend adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(fasting cholesterol mmol/l) Mean	Std. Dev.	Freq.
11	1.8634504	.17732452	59
12	1.881525	.18814024	127
22	1.9239379	.15732106	57
Total	1.8870852	.17934724	243

p= 0.169 on 2 df for lfstchol vs at1rv002, N=243
p= 0.181 on 2 df adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat, N=239
beta= 0.03, p= 0.070 for trend in lfstchol with at1rv002
beta= 0.03, p= 0.108 for trend adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(fasting triglycerides mmol/l) Mean	Std. Dev.	Freq.
11	.36691914	.46290891	59
12	.30670017	.5869945	127
22	.46939494	.44233741	57
Total	.35948421	.52948661	243

p= 0.155 on 2 df for ltrig vs at1rv002, N=243
p= 0.050 on 2 df adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat, N=239
beta= 0.05, p= 0.308 for trend in ltrig with at1rv002
beta= 0.03, p= 0.560 for trend adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(high density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	.1783604	.28849584	59
12	.17699003	.26850403	122
22	.19347102	.2481041	57
Total	.18127687	.26789609	238

p= 0.925 on 2 df for lhdl vs at1rv002, N=238
p= 0.915 on 2 df adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat, N=234
beta= 0.01, p= 0.764 for trend in lhdl with at1rv002
beta= 0.01, p= 0.687 for trend adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(low density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	1.4873569	.26102812	59
12	1.5606867	.28447331	127
22	1.5578574	.24015525	57
Total	1.5422187	.26977981	243

p= 0.200 on 2 df for lldl vs at1rv002, N=243
p= 0.290 on 2 df adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat, N=239
beta= 0.04, p= 0.156 for trend in lldl with at1rv002
beta= 0.03, p= 0.183 for trend adjusted for ageclif1 bmil and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Apolopoprotein A1 g/l) Mean	Std. Dev.	Freq.
11	.30544045	.22344665	58
12	.27446656	.22447418	122
22	.29043395	.24019647	56
Total	.28586766	.22743136	236

p= 0.686 on 2 df for lapal vs at1rv002, N=236
p= 0.653 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=233
beta=-0.01, p= 0.718 for trend in lapal with at1rv002
beta=-0.01, p= 0.702 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Apolopoprotein B g/l) Mean	Std. Dev.	Freq.
11	.06452953	.25400892	58
12	.09877275	.2716086	122
22	.14811578	.25426507	56
Total	.10206556	.26386309	236

p= 0.236 on 2 df for lapb vs at1rv002, N=236
p= 0.351 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=233
beta= 0.04, p= 0.091 for trend in lapb with at1rv002
beta= 0.03, p= 0.188 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Lp(a) lipoprotein mg/l) Mean	Std. Dev.	Freq.
11	2.2072916	1.3126775	57
12	2.1312687	1.2595346	121
22	2.1707538	1.324741	57
Total	2.1592855	1.2833192	235

p= 0.932 on 2 df for lfstlpa vs at1rv002, N=235
p= 0.951 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=231
beta=-0.02, p= 0.880 for trend in lfstlpa with at1rv002
beta= 0.03, p= 0.831 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Insulin0 pmol/l) Mean	Std. Dev.	Freq.
11	3.5833246	.65678003	58
12	3.7674475	.67423883	125
22	3.6935297	.56073371	54
Total	3.7055459	.64760064	237

p= 0.200 on 2 df for linsul0 vs at1rv002, N=237
p= 0.674 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=233
beta= 0.06, p= 0.348 for trend in linsul0 with at1rv002
beta= 0.02, p= 0.736 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Insulin30 pmol/l) Mean	Std. Dev.	Freq.
11	5.596848	.57824164	57
12	5.5772323	.59111037	118
22	5.540913	.67239166	55
Total	5.5734085	.60604877	230

p= 0.884 on 2 df for linsul30 vs at1rv002, N=230
p= 0.534 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=227
beta=-0.03, p= 0.627 for trend in linsul30 with at1rv002
beta=-0.07, p= 0.264 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Insulin120 pmol/l) Mean	Std. Dev.	Freq.
11	4.9014338	.8431575	54
12	5.0190742	.90640695	117
22	4.9865866	.65842885	55
Total	4.9830592	.83507492	226

p= 0.694 on 2 df for lins120 vs at1rv002, N=226
p= 0.851 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=223
beta= 0.04, p= 0.599 for trend in lins120 with at1rv002
beta=-0.02, p= 0.802 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Glucose0 mmol/l) Mean	Std. Dev.	Freq.
11	1.7650225	.10501121	59
12	1.7959241	.17049584	127
22	1.8296181	.24377947	57
Total	1.7963248	.17896931	243

p= 0.151 on 2 df for lgluc0 vs at1rv002, N=243
p= 0.216 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=239
beta= 0.03, p= 0.052 for trend in lgluc0 with at1rv002
beta= 0.03, p= 0.111 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Glucose30 mmol/l) Mean	Std. Dev.	Freq.
11	2.1968494	.20981655	58
12	2.2472784	.19473724	125
22	2.2917478	.26088815	56
Total	2.24546	.2170525	239

p= 0.065 on 2 df for lgluc30 vs at1rv002, N=239
p= 0.127 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=236
beta= 0.05, p= 0.019 for trend in lgluc30 with at1rv002
beta= 0.04, p= 0.049 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Glucose120 mmol/l) Mean	Std. Dev.	Freq.
11	1.811021	.28064734	57
12	1.889895	.3390249	121
22	1.9376022	.39966742	56
Total	1.8820992	.3432768	234

p= 0.137 on 2 df for lgluc120 vs at1rv002, N=234
p= 0.255 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=231
beta= 0.06, p= 0.050 for trend in lgluc120 with at1rv002
beta= 0.05, p= 0.103 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Analyses for women

AT1RV002	Summary of EH1 Systolic BP (mmHg) Mean	Std. Dev.	Freq.
11	158.51163	23.84311	43
12	153.14474	20.556721	76
22	163.70833	21.302131	24
Total	156.53147	21.928759	143

p= 0.093 on 2 df for sysbpa1 vs at1rv002, N=143
p= 0.233 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 1.35, p= 0.623 for trend in sysbpa1 with at1rv002
beta= 0.36, p= 0.900 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of EH1 Diastolic BP (mmHg) Mean	Std. Dev.	Freq.
11	83.395349	12.043848	43
12	80.723684	10.93447	76
22	85.666667	9.471544	24
Total	82.356643	11.144003	143

p= 0.127 on 2 df for diabpa1 vs at1rv002, N=143
p= 0.161 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.54, p= 0.699 for trend in diabpa1 with at1rv002

beta= 0.77, p= 0.605 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Pulse pressure mmHg (SBP-DBP) Mean	Std. Dev.	Freq.
11	75.116279	18.070246	43
12	72.421053	13.977375	76
22	78.041667	13.696966	24
Total	74.174825	15.316526	143

p= 0.262 on 2 df for pulspres vs at1rv002, N=143
p= 0.530 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.81, p= 0.672 for trend in pulspres with at1rv002
beta=-0.41, p= 0.834 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(fasting cholesterol mmol/l) Mean	Std. Dev.	Freq.
11	1.8845306	.21493313	43
12	1.9762784	.16271375	76
22	1.9967896	.15495418	24
Total	1.9521324	.18321957	143

p= 0.013 on 2 df for lfstchol vs at1rv002, N=143
p= 0.017 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.06, p= 0.006 for trend in lfstchol with at1rv002
beta= 0.06, p= 0.010 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(fasting triglycerides mmol/l) Mean	Std. Dev.	Freq.
11	.2233639	.40581674	43
12	.28498422	.40635785	76
22	.30208885	.4382109	24
Total	.26932574	.40985451	143

p= 0.672 on 2 df for ltrig vs at1rv002, N=143
p= 0.962 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.04, p= 0.403 for trend in ltrig with at1rv002
beta= 0.00, p= 0.959 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(high density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	.34480467	.25540027	43
12	.39592753	.21749147	75
22	.34716564	.26824246	24
Total	.37220522	.23796565	142

p= 0.457 on 2 df for lhdl vs at1rv002, N=142
p= 0.340 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=140
beta= 0.01, p= 0.764 for trend in lhdl with at1rv002
beta= 0.03, p= 0.355 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(low density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	1.4992416	.28861277	43
12	1.6291526	.22888516	76
22	1.6512332	.19804555	24
Total	1.5937943	.25018799	143

p= 0.011 on 2 df for lldl vs at1rv002, N=143
p= 0.014 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.08, p= 0.006 for trend in lldl with at1rv002
beta= 0.08, p= 0.010 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Apolopoprotein A1 g/l) Mean	Std. Dev.	Freq.
11	.25797848	.14748519	43
12	.32462613	.16694835	76
22	.34120324	.14675522	24
Total	.3073674	.16042014	143

p= 0.048 on 2 df for lapa1 vs at1rv002, N=143
p= 0.028 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.05, p= 0.022 for trend in lapa1 with at1rv002
beta= 0.05, p= 0.009 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Apolopoprotein B g/l) Mean	Std. Dev.	Freq.
11	-.02829108	.22217709	43
12	.05889544	.2234334	76
22	.10993341	.20443418	24
Total	.04124433	.22397643	143

p= 0.031 on 2 df for lapb vs at1rv002, N=143
p= 0.101 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.07, p= 0.009 for trend in lapb with at1rv002
beta= 0.06, p= 0.041 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Lp(a) lipoprotein mg/l) Mean	Std. Dev.	Freq.
11	2.4769137	1.271206	43
12	2.6049364	1.3972877	76
22	2.6769826	1.4969708	24
Total	2.5785318	1.370135	143

p= 0.825 on 2 df for lfstlpa vs at1rv002, N=143
p= 0.875 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.10, p= 0.542 for trend in lfstlpa with at1rv002
beta= 0.10, p= 0.610 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Insulin0 pmol/l) Mean	Std. Dev.	Freq.
11	3.9159654	.51183496	43
12	3.7649561	.60183804	76
22	3.8348367	.46668562	24
Total	3.8220927	.55543949	143

p= 0.362 on 2 df for linsul0 vs at1rv002, N=143
p= 0.137 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta=-0.06, p= 0.405 for trend in linsul0 with at1rv002
beta=-0.11, p= 0.093 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Insulin30 pmol/l) Mean	Std. Dev.	Freq.
11	5.6418627	.53854307	41
12	5.4893402	.60359507	75
22	5.4600508	.39397583	22
Total	5.5299855	.5574026	138

p= 0.304 on 2 df for linsul30 vs at1rv002, N=138
p= 0.132 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=136
beta=-0.10, p= 0.158 for trend in linsul30 with at1rv002
beta=-0.14, p= 0.046 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Insulin120 pmol/l) Mean	Std. Dev.	Freq.
11	5.4868805	.68950189	43
12	5.4922676	.62954018	76
22	5.2939057	.54832132	24
Total	5.4573562	.6356642	143

p= 0.388 on 2 df for lins120 vs at1rv002, N=143
p= 0.177 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta=-0.08, p= 0.311 for trend in lins120 with at1rv002
beta=-0.12, p= 0.153 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Glucose0 mmol/l) Mean	Std. Dev.	Freq.
11	1.7299858	.08392113	43
12	1.7364086	.15772014	76
22	1.7268533	.07337431	24
Total	1.7328736	.12692113	143

p= 0.935 on 2 df for lgluc0 vs at1rv002, N=143
p= 0.896 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta=-0.00, p= 0.984 for trend in lgluc0 with at1rv002
beta= 0.00, p= 0.986 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Glucose30 mmol/l) Mean	Std. Dev.	Freq.
11	2.1507309	.17522403	41
12	2.160897	.22302512	75
22	2.1854294	.18531315	22
Total	2.1617876	.20303252	138

p= 0.812 on 2 df for lgluc30 vs at1rv002, N=138
p= 0.973 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=136
beta= 0.02, p= 0.539 for trend in lgluc30 with at1rv002
beta= 0.01, p= 0.814 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV002	Summary of Ln(Glucose120 mmol/l) Mean	Std. Dev.	Freq.
11	1.915133	.26474575	43
12	1.9959488	.28502508	76
22	1.9100524	.2260691	24
Total	1.9572313	.2713524	143

p= 0.192 on 2 df for lgluc120 vs at1rv002, N=143
p= 0.135 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=141
beta= 0.01, p= 0.757 for trend in lgluc120 with at1rv002
beta= 0.03, p= 0.437 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AGTR1 C-521T Analyses

Overall frequency distributions in men and women

AT1RV003	Sex 0=men, 1=women 0	1	Total
11	220	159	379
	40.52	39.16	39.94
12	252	193	445
	46.41	47.54	46.89
22	71	54	125
	13.08	13.30	13.17
Total	543	406	949
	100.00	100.00	100.00

Pearson chi2(2) = 0.1785 Pr = 0.915

Analyses for Men

AT1RV003	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	78.342466	12.595109	219
12	80.207171	12.154214	251
22	81.78169	10.50671	71
Total	79.658965	12.172855	541

p= 0.073 on 2 df for hwgt vs at1rv003, N=541
beta= 1.76, p= 0.022 for trend in hwgt with at1rv003

AT1RV003	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.717169	.06894502	219
12	1.7203586	.06132757	251
22	1.7294366	.0642959	71
Total	1.7202588	.06489256	541

p= 0.384 on 2 df for hhgt vs at1rv003, N=541
beta= 0.01, p= 0.195 for trend in hhgt with at1rv003

AT1RV003	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.493082	3.3300497	219
12	27.075538	3.637817	251
22	27.355165	3.2647341	71
Total	26.876454	3.477542	541

p= 0.089 on 2 df for hbmi vs at1rv003, N=541
beta= 0.47, p= 0.032 for trend in hbmi with at1rv003

AT1RV003	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	97.281278	9.786888	219
12	98.871713	10.232225	251
22	100.26761	8.6830672	71
Total	98.41109	9.8986112	541

p= 0.052 on 2 df for hwast vs at1rv003, N=541
beta= 1.52, p= 0.015 for trend in hwast with at1rv003

AT1RV003	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	104.34886	7.4317589	219
12	105.63426	7.8331667	251
22	105.76197	6.8819508	71
Total	105.13068	7.5671182	541

p= 0.139 on 2 df for hhip vs at1rv003, N=541
beta= 0.87, p= 0.071 for trend in hhip with at1rv003

AT1RV003	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.93123559	.04954389	219
12	.9349991	.05323792	251
22	.94699709	.04058676	71
Total	.9350502	.05041131	541

p= 0.073 on 2 df for hwhr vs at1rv003, N=541
beta= 0.01, p= 0.034 for trend in hwhr with at1rv003

AT1RV003	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	125.29091	20.051917	220
12	125.2381	18.019436	252
22	121.09859	17.477459	71
Total	124.71823	18.821745	543

p= 0.221 on 2 df for bwt_ozs vs at1rv003, N=543
beta=-1.53, p= 0.199 for trend in bwt_ozs with at1rv003

AT1RV003	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	363.43182	41.470239	220
12	358.96825	42.303106	252
22	364.85915	42.137969	71
Total	361.54696	41.939566	543

p= 0.399 on 2 df for wt2_ozs vs at1rv003, N=543
beta=-0.72, p= 0.787 for trend in wt2_ozs with at1rv003

Analyses for Women

AT1RV003	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	67.674214	11.828646	159
12	68.742188	12.047026	192
22	70.787037	10.15103	54
Total	68.595556	11.740447	405

p= 0.236 on 2 df for hwgt vs at1rv003, N=405
beta= 1.43, p= 0.098 for trend in hwgt with at1rv003

AT1RV003	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.5872327	.05653398	159
12	1.5992708	.0610166	192
22	1.6107407	.05843402	54
Total	1.5960741	.05934998	405

p= 0.025 on 2 df for hhgt vs at1rv003, N=405
beta= 0.01, p= 0.006 for trend in hhgt with at1rv003

AT1RV003	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.86195	4.4799369	159
12	26.852768	4.2527512	192
22	27.400542	4.4140836	54
Total	26.929409	4.3577449	405

p= 0.696 on 2 df for hbmi vs at1rv003, N=405
beta= 0.19, p= 0.543 for trend in hbmi with at1rv003

AT1RV003	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	84.2239	10.532232	159
12	83.909375	9.7128519	192
22	85.246296	9.2269231	54
Total	84.211111	9.9669055	405

p= 0.685 on 2 df for hwast vs at1rv003, N=405
beta= 0.29, p= 0.692 for trend in hwast with at1rv003

AT1RV003	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	105.45723	9.9743431	159
12	105.48906	9.4923136	192
22	107.01667	8.715585	54
Total	105.68025	9.5785215	405

p= 0.546 on 2 df for hhip vs at1rv003, N=405
beta= 0.58, p= 0.410 for trend in hhip with at1rv003

AT1RV003	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.79777592	.04987467	159
12	.79498153	.04937728	192
22	.79658524	.06149483	54
Total	.79629241	.05121814	405

p= 0.878 on 2 df for hwhr vs at1rv003, N=405
beta=-0.00, p= 0.753 for trend in hwhr with at1rv003

AT1RV003	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	119.38365	17.647677	159
12	121.45596	17.266881	193
22	122.51852	20.470633	54
Total	120.78571	17.862462	406

p= 0.416 on 2 df for bwt_ozs vs at1rv003, N=406
beta= 1.70, p= 0.194 for trend in bwt_ozs with at1rv003

AT1RV003	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	340.67925	37.061584	159
12	341.55959	36.598865	193
22	339.92593	33.96105	54
Total	340.99754	36.361321	406

p= 0.949 on 2 df for wt2_ozs vs at1rv003, N=406
beta=-0.04, p= 0.988 for trend in wt2_ozs with at1rv003

AGTR1 C-521T In Relation to Cardiovascular Phenotypes

Overall SNP frequency distributions in men and women

AT1RV003	Sex 0=men, 1=women 0	1	Total
11	83	55	138
	40.89	39.01	40.12
12	99	69	168
	48.77	48.94	48.84
22	21	17	38
	10.34	12.06	11.05
Total	203	141	344
	100.00	100.00	100.00

Pearson chi2(2) = 0.2945 Pr = 0.863

Analyses for Men

AT1RV003	Summary of EH1 Systolic BP (mmHg) Mean	Std. Dev.	Freq.
11	161.96386	21.831084	83
12	162.88889	21.188603	99
22	164.2381	21.794276	21
Total	162.65025	21.419452	203

p= 0.900 on 2 df for sysbpa1 vs at1rv003, N=203
p= 0.858 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=199
beta= 1.06, p= 0.649 for trend in sysbpa1 with at1rv003
beta= 0.06, p= 0.982 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of EH1 Diastolic BP (mmHg) Mean	Std. Dev.	Freq.
11	89.746988	12.06976	83
12	89.616162	10.861566	99
22	90.428571	9.6879896	21
Total	89.753695	11.212547	203

p= 0.956 on 2 df for diabpa1 vs at1rv003, N=203
p= 0.878 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=199
beta= 0.17, p= 0.887 for trend in diabpa1 with at1rv003
beta= 0.13, p= 0.917 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Pulse pressure mmHg (SBP-DBP) Mean	Std. Dev.	Freq.
11	72.216867	15.298691	83
12	73.272727	14.267716	99
22	73.809524	17.063467	21
Total	72.896552	14.931333	203

p= 0.856 on 2 df for pulspres vs at1rv003, N=203
p= 0.917 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=199
beta= 0.89, p= 0.584 for trend in pulspres with at1rv003
beta=-0.08, p= 0.963 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Ln(fasting cholesterol mmol/1) Mean	Std. Dev.	Freq.
11	1.8652648	.19732118	83
12	1.9133058	.16648198	99
22	1.8867899	.15871613	19
Total	1.8909615	.17981817	201

p= 0.199 on 2 df for lfstchol vs at1rv003, N=201
p= 0.217 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=199
beta= 0.03, p= 0.207 for trend in lfstchol with at1rv003
beta= 0.02, p= 0.241 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Ln(fasting triglycerides mmol/1) Mean	Std. Dev.	Freq.
11	.31267653	.51354303	83
12	.33046184	.52538013	99
22	.39832782	.47723062	19
Total	.32953285	.51424227	201

p= 0.808 on 2 df for ltrig vs at1rv003, N=201
p= 0.836 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=199
beta= 0.03, p= 0.562 for trend in ltrig with at1rv003
beta= 0.03, p= 0.624 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Ln(high density lipoproteins mmol/1) Mean	Std. Dev.	Freq.
11	.2286445	.2466544	81
12	.16040712	.25215459	98
22	.07026058	.37067028	19
Total	.17967198	.26625563	198

p= 0.039 on 2 df for lhdl vs at1rv003, N=198
p= 0.054 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=196
beta=-0.08, p= 0.011 for trend in lhdl with at1rv003
beta=-0.07, p= 0.017 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Ln(low density lipoproteins mmol/1) Mean	Std. Dev.	Freq.
11	1.5156021	.28521702	83
12	1.5780822	.2376075	99
22	1.5430866	.23836362	19
Total	1.5489739	.25887353	201

p= 0.268 on 2 df for lldl vs at1rv003, N=201
p= 0.218 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=199
beta= 0.03, p= 0.257 for trend in lldl with at1rv003
beta= 0.04, p= 0.228 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Ln(Apolopoprotein A1 g/1) Mean	Std. Dev.	Freq.
11	.28292844	.25010305	80
12	.28994546	.22493533	96
22	.27827214	.19311196	19
Total	.28592928	.23178849	195

p= 0.969 on 2 df for lapa1 vs at1rv003, N=195
p= 0.957 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=193
beta= 0.00, p= 0.964 for trend in lapa1 with at1rv003
beta=-0.00, p= 0.962 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Apolopoprotein B g/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	.08201321	.27782207	80
12	.12615381	.26358291	96
22	.15739904	.14654945	19
Total	.11108925	.26099124	195

p= 0.387 on 2 df for lapb vs at1rv003, N=195
p= 0.441 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=193
beta= 0.04, p= 0.170 for trend in lapb with at1rv003
beta= 0.04, p= 0.206 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Lp(a) lipoprotein mg/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	1.9893358	1.221769	81
12	2.452923	1.2415783	96
22	2.1417664	1.3418085	17
Total	2.2320971	1.2555834	194

p= 0.047 on 2 df for lfstlpa vs at1rv003, N=194
p= 0.083 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=192
beta= 0.24, p= 0.100 for trend in lfstlpa with at1rv003
beta= 0.22, p= 0.148 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin0 pmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	3.6604923	.63768165	80
12	3.7129407	.65657652	97
22	3.6847231	.56721498	19
Total	3.6887978	.6381923	196

p= 0.863 on 2 df for linsul0 vs at1rv003, N=196
p= 0.975 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=194
beta= 0.03, p= 0.703 for trend in linsul0 with at1rv003
beta=-0.00, p= 0.994 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin30 pmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	5.5487542	.64044379	81
12	5.5777484	.58867733	94
22	5.6970729	.5272364	19
Total	5.577329	.60389053	194

p= 0.631 on 2 df for linsul30 vs at1rv003, N=194
p= 0.849 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=193
beta= 0.06, p= 0.397 for trend in linsul30 with at1rv003
beta= 0.03, p= 0.669 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin120 pmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	4.92891	.90363196	75
12	4.9772757	.83353373	93
22	4.9793448	.51594339	17
Total	4.9578581	.83636538	185

p= 0.928 on 2 df for linsl20 vs at1rv003, N=185
p= 1.000 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=184
beta= 0.03, p= 0.725 for trend in linsl20 with at1rv003
beta=-0.00, p= 0.984 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose0 mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	1.7901296	.1315481	83
12	1.8001778	.16340959	99
22	1.7797704	.1787488	19
Total	1.7940995	.15198443	201

p= 0.827 on 2 df for lgluc0 vs at1rv003, N=201
p= 0.879 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=199
beta= 0.00, p= 0.967 for trend in lgluc0 with at1rv003
beta= 0.00, p= 0.997 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose30 mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	2.237933	.1945473	81
12	2.2276553	.20239067	98
22	2.3096566	.24090014	19
Total	2.2397286	.2034381	198

p= 0.274 on 2 df for lgluc30 vs at1rv003, N=198
p= 0.380 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=197
beta= 0.02, p= 0.421 for trend in lgluc30 with at1rv003
beta= 0.01, p= 0.693 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose120 mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	1.878016	.33588062	79
12	1.8808757	.32268166	96
22	1.7869328	.31153534	18
Total	1.8709436	.32661255	193

p= 0.520 on 2 df for lgluc120 vs at1rv003, N=193
p= 0.511 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=192
beta=-0.03, p= 0.473 for trend in lgluc120 with at1rv003
beta=-0.03, p= 0.369 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Analyses for Women

Summary of EH1 Systolic BP (mmHg)			
AT1RV003	Mean	Std. Dev.	Freq.
11	155.98182	23.061505	55
12	155.28986	19.46951	69
22	158.76471	26.775757	17
Total	155.97872	21.733933	141

p= 0.842 on 2 df for sysbpa1 vs at1rv003, N=141
p= 0.934 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta= 0.76, p= 0.783 for trend in sysbpa1 with at1rv003
beta= 0.19, p= 0.946 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of EH1 Diastolic BP (mmHg)			
AT1RV003	Mean	Std. Dev.	Freq.
11	82.454545	12.331559	55
12	81.362319	8.9211761	69
22	86.588235	14.062623	17
Total	82.41844	11.071299	141

p= 0.220 on 2 df for diabpa1 vs at1rv003, N=141
p= 0.292 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta= 1.12, p= 0.430 for trend in diabpa1 with at1rv003
beta= 0.90, p= 0.543 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Pulse pressure mmHg (SBP-DBP)			
AT1RV003	Mean	Std. Dev.	Freq.
11	73.527273	15.114201	55
12	73.927536	15.182535	69
22	72.176471	15.899038	17
Total	73.560284	15.141414	141

p= 0.914 on 2 df for pulspres vs at1rv003, N=141
p= 0.793 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta=-0.35, p= 0.856 for trend in pulspres with at1rv003
beta=-0.70, p= 0.713 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(fasting cholesterol mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	1.9522967	.19826042	55
12	1.9332185	.18014323	69
22	2.0148356	.16084157	17
Total	1.9505007	.18582567	141

p= 0.269 on 2 df for lfstchol vs at1rv003, N=141
p= 0.358 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta= 0.02, p= 0.498 for trend in lfstchol with at1rv003
beta= 0.01, p= 0.582 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(fasting triglycerides mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	.27879067	.42558793	55
12	.20245366	.40764246	69
22	.27334848	.32337499	17
Total	.24077811	.40490272	141

p= 0.549 on 2 df for ltrig vs at1rv003, N=141
p= 0.367 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta=-0.02, p= 0.631 for trend in ltrig with at1rv003
beta=-0.05, p= 0.352 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(high density lipoproteins mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	.36091692	.27725967	54
12	.40592232	.19550356	69
22	.35303614	.20865495	17
Total	.3821412	.2314744	140

p= 0.487 on 2 df for lhdl vs at1rv003, N=140
p= 0.447 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=138
beta= 0.01, p= 0.721 for trend in lhdl with at1rv003
beta= 0.03, p= 0.348 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(low density lipoproteins mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	1.6080428	.27128965	55
12	1.5575733	.24304419	69
22	1.6847145	.22255117	17
Total	1.5925891	.2539062	141

p= 0.153 on 2 df for lldl vs at1rv003, N=141
p= 0.223 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta= 0.01, p= 0.721 for trend in lldl with at1rv003
beta= 0.00, p= 0.886 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Apolopoprotein A1 g/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	.30962558	.17080542	55
12	.31549515	.15248374	69
22	.30902869	.12842237	17
Total	.31242596	.15633446	141

p= 0.975 on 2 df for lapa1 vs at1rv003, N=141
p= 0.861 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta= 0.00, p= 0.938 for trend in lapa1 with at1rv003
beta= 0.01, p= 0.624 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Apolopoprotein B g/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	.03747472	.23619944	55
12	.0030163	.22782728	69
22	.13781229	.12769077	17
Total	.03270953	.22445146	141

p= 0.083 on 2 df for lapb vs at1rv003, N=141
p= 0.146 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta= 0.02, p= 0.389 for trend in lapb with at1rv003
beta= 0.02, p= 0.486 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Lp(a) lipoprotein mg/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	2.7543848	1.4079216	55
12	2.5405634	1.3054006	69
22	1.7789967	1.3260979	17
Total	2.5321488	1.3717008	141

p= 0.036 on 2 df for lfstlpa vs at1rv003, N=141
p= 0.064 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta=-0.41, p= 0.020 for trend in lfstlpa with at1rv003
beta=-0.38, p= 0.041 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin0 pmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	3.8048472	.60576643	55
12	3.8288392	.54842401	69
22	3.801457	.45008545	17
Total	3.8161792	.55761471	141

p= 0.966 on 2 df for linsul0 vs at1rv003, N=141
p= 0.578 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta= 0.01, p= 0.933 for trend in linsul0 with at1rv003
beta=-0.04, p= 0.509 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin30 pmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	5.5243538	.57402342	52
12	5.5476058	.60084041	67
22	5.567621	.39088243	17
Total	5.5412173	.56506475	136

p= 0.956 on 2 df for linsul30 vs at1rv003, N=136
p= 0.826 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=134
beta= 0.02, p= 0.763 for trend in linsul30 with at1rv003
beta=-0.03, p= 0.711 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin120 pmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	5.4411572	.63554134	55
12	5.4928156	.65887647	69
22	5.3314965	.61603528	17
Total	5.4532154	.64238053	141

p= 0.643 on 2 df for linsl20 vs at1rv003, N=141
p= 0.273 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139
beta=-0.02, p= 0.782 for trend in linsl20 with at1rv003
beta=-0.08, p= 0.301 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose0 mmol/l)			
AT1RV003	Mean	Std. Dev.	Freq.
11	1.7436443	.15857241	55
12	1.7295653	.10753582	69
22	1.692861	.08361416	17
Total	1.7306318	.12788654	141

p= 0.360 on 2 df for lgluc0 vs at1rv003, N=141
p= 0.285 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=139

beta=-0.02, p= 0.178 for trend in lgluc0 with at1rv003
beta=-0.03, p= 0.131 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Ln(Glucose30 mmol/l) Mean	Std. Dev.	Freq.
11	2.2145174	.21828656	52
12	2.1120391	.19149371	67
22	2.173457	.16981839	17
Total	2.1588992	.20409337	136

p= 0.023 on 2 df for lgluc30 vs at1rv003, N=136
p= 0.034 on 2 df adjusted for ageclif1 bmi1 and alccat
smoker1 soccat fsoccat, N=134
beta=-0.04, p= 0.094 for trend in lgluc30 with
at1rv003
beta=-0.05, p= 0.101 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat fsoccat

AT1RV003	Summary of Ln(Glucose120 mmol/l) Mean	Std. Dev.	Freq.
11	1.9783686	.29352455	55
12	1.938402	.26829596	69
22	1.8983975	.21328189	17
Total	1.9491685	.27221087	141

p= 0.517 on 2 df for lgluc120 vs at1rv003, N=141
p= 0.326 on 2 df adjusted for ageclif1 bmi1 and alccat
smoker1 soccat fsoccat, N=139
beta=-0.04, p= 0.250 for trend in lgluc120 with
at1rv003
beta=-0.05, p= 0.147 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat fsoccat

AGTR1 A-153G Analyses

Overall SNP frequency distributions in men and women

AT1RV004	Sex 0=men, 1=women 0	1	Total
11	338	236	574
	62.94	60.67	61.99
12	170	136	306
	31.66	34.96	33.05
22	29	17	46
	5.40	4.37	4.97
Total	537	389	926
	100.00	100.00	100.00

Pearson chi2(2) = 1.4154 Pr = 0.493

Analyses for Men

AT1RV004	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	79.897321	12.11188	336
12	80.932353	11.882679	170
22	79.034483	11.708461	29
Total	80.179439	12.008562	535

p= 0.573 on 2 df for hwgt vs at1rv004, N=535
beta= 0.33, p= 0.709 for trend in hwgt with at1rv004

AT1RV004	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.7202976	.06672219	336
12	1.7237647	.05880419	170
22	1.7334483	.07816082	29
Total	1.7221122	.06494182	535

p= 0.535 on 2 df for hhgt vs at1rv004, N=535
beta= 0.00, p= 0.293 for trend in hhgt with at1rv004

AT1RV004	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.953323	3.3954785	336
12	27.220311	3.559975	170
22	26.28799	3.357339	29
Total	27.002095	3.446598	535

p= 0.370 on 2 df for hbmi vs at1rv004, N=535
beta=-0.02, p= 0.927 for trend in hbmi with at1rv004

AT1RV004	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	98.56875	9.9672181	336
12	99.502941	9.8610547	170
22	97.765517	7.9583417	29
Total	98.822056	9.8319664	535

p= 0.504 on 2 df for hwast vs at1rv004, N=535
beta= 0.29, p= 0.687 for trend in hwast with at1rv004

AT1RV004	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	105.09256	7.5869312	336
12	105.77529	7.2571107	170
22	104.04483	7.2749854	29
Total	105.25271	7.4656357	535

p= 0.418 on 2 df for hhip vs at1rv004, N=535
beta= 0.10, p= 0.855 for trend in hhip with at1rv004

AT1RV004	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.93692114	.05153079	336
12	.93977407	.05513357	170
22	.93935314	.03434062	29
Total	.93795951	.05187993	535

p= 0.834 on 2 df for hwhr vs at1rv004, N=535
beta= 0.00, p= 0.585 for trend in hwhr with at1rv004

AT1RV004	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	125.37278	19.192684	338
12	122.87059	17.753932	170
22	128.62069	20.654321	29
Total	124.75605	18.853176	537

p= 0.194 on 2 df for bwt_ozs vs at1rv004, N=537
beta=-0.51, p= 0.711 for trend in bwt_ozs with at1rv004

AT1RV004	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	362.79586	41.984698	338
12	360.87059	43.912309	170
22	382.55172	41.289558	29
Total	363.25326	42.748125	537

p= 0.039 on 2 df for wt2_ozs vs at1rv004, N=537
beta= 3.78, p= 0.225 for trend in wt2_ozs with at1rv004

Analyses for Women

AT1RV004	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	68.265957	11.63588	235
12	68.963235	11.568897	136
22	70.970588	13.463458	17
Total	68.628866	11.679401	388

p= 0.601 on 2 df for hwgt vs at1rv004, N=388
beta= 0.97, p= 0.347 for trend in hwgt with at1rv004

AT1RV004	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.5902979	.05861212	235
12	1.60375	.06137845	136
22	1.6211765	.04741866	17
Total	1.596366	.05960629	388

p= 0.023 on 2 df for hhgt vs at1rv004, N=388
beta= 0.01, p= 0.006 for trend in hhgt with at1rv004

AT1RV004	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.997697	4.3725919	235
12	26.800978	4.1413518	136
22	27.041333	5.2179933	17
Total	26.930656	4.3218467	388

p= 0.910 on 2 df for hbmi vs at1rv004, N=388
beta=-0.11, p= 0.778 for trend in hbmi with at1rv004

AT1RV004	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	84.411915	10.110853	235
12	84.024999	9.6579941	136
22	84.394117	9.9990918	17
Total	84.275515	9.9256794	388

p= 0.936 on 2 df for hwast vs at1rv004, N=388
beta=-0.23, p= 0.791 for trend in hwast with at1rv004

AT1RV004	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	105.77362	9.7302584	235
12	105.46544	9.3226956	136
22	107.22941	10.745394	17
Total	105.72938	9.6158038	388

p= 0.772 on 2 df for hhip vs at1rv004, N=388
beta= 0.12, p= 0.890 for trend in hhip with at1rv004

AT1RV004	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.79732548	.04871423	235
12	.79666364	.05517687	136
22	.78619017	.04720553	17
Total	.79660561	.05093254	388

p= 0.686 on 2 df for hwhr vs at1rv004, N=388
beta=-0.00, p= 0.551 for trend in hwhr with at1rv004

AT1RV004	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	120.16949	17.578665	236
12	121.90441	17.807979	136
22	120.70588	18.22006	17
Total	120.79949	17.659462	389

p= 0.660 on 2 df for bwt_ozs vs at1rv004, N=389
beta= 1.13, p= 0.465 for trend in bwt_ozs with at1rv004

AT1RV004	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	342.00847	36.695178	236
12	340.47794	37.952205	136
22	336.05882	26.770951	17
Total	341.21337	36.715235	389

p= 0.780 on 2 df for wt2_ozs vs at1rv004, N=389
beta=-2.12, p= 0.511 for trend in wt2_ozs with at1rv004

AGTR1 A-153G In Relation to Cardiovascular Phenotypes

AT1RV004	Sex 0=men, 1=women 0	1	Total
11	134	80	214
	62.62	60.61	61.85
12	68	46	114
	31.78	34.85	32.95
22	12	6	18
	5.61	4.55	5.20
Total	214	132	346
	100.00	100.00	100.00

Pearson chi2(2) = 0.4643 Pr = 0.793

Analyses for Men

AT1RV004	Summary of EH1 Systolic BP (mmHg) Mean	Std. Dev.	Freq.
11	162.39552	21.168859	134
12	164.01471	22.537195	68
22	160.41667	18.549483	12
Total	162.79907	21.406677	214

p= 0.814 on 2 df for sysbpa1 vs at1rv004, N=214
p= 0.809 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=210
beta= 0.33, p= 0.892 for trend in sysbpa1 with at1rv004
beta= 1.62, p= 0.515 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV004	Summary of EH1 Diastolic BP (mmHg) Mean	Std. Dev.	Freq.
11	90.291045	11.225525	134
12	89.941176	10.822699	68
22	91.583333	9.3755808	12
Total	90.252336	10.963528	214

p= 0.891 on 2 df for diabpa1 vs at1rv004, N=214
p= 0.543 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=210
beta= 0.14, p= 0.911 for trend in diabpa1 with at1rv004
beta= 0.82, p= 0.532 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV004	Summary of Pulse pressure mmHg (SBP-DBP) Mean	Std. Dev.	Freq.
11	72.104478	15.311438	134
12	74.073529	14.761099	68
22	68.833333	13.940349	12
Total	72.546729	15.053431	214

p= 0.464 on 2 df for pulspres vs at1rv004, N=214
p= 0.706 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=210
beta= 0.19, p= 0.911 for trend in pulspres with at1rv004
beta= 0.80, p= 0.642 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV004	Summary of Ln(fasting cholesterol mmol/l) Mean	Std. Dev.	Freq.
11	1.8868234	.19709118	134
12	1.9058143	.13975605	68
22	1.8901278	.18558914	11
Total	1.8930568	.17956716	213

p= 0.777 on 2 df for lfstchol vs at1rv004, N=213
p= 0.801 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=210
beta= 0.01, p= 0.604 for trend in lfstchol with at1rv004
beta= 0.01, p= 0.534 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV004	Summary of Ln(fasting triglycerides mmol/l) Mean	Std. Dev.	Freq.
11	.34812136	.52729702	134
12	.36192217	.55445984	68
22	.50840286	.54873577	11
Total	.3608047	.5357481	213

p= 0.636 on 2 df for ltrig vs at1rv004, N=213
p= 0.359 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=210
beta= 0.04, p= 0.472 for trend in ltrig with at1rv004
beta= 0.06, p= 0.301 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV004	Summary of Ln(high density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	.19157949	.23810573	132
12	.15403325	.26352805	66
22	-.00652726	.45618109	11
Total	.16929611	.26350995	209

p= 0.048 on 2 df for lhdl vs at1rv004, N=209
p= 0.083 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=206
beta=-0.07, p= 0.030 for trend in lhdl with at1rv004
beta=-0.06, p= 0.070 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV004	Summary of Ln(low density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	1.5380839	.28283225	134
12	1.584366	.2097425	68
22	1.5247319	.30281252	11
Total	1.5521699	.2625077	213

p= 0.468 on 2 df for lldl vs at1rv004, N=213
p= 0.499 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=210
beta= 0.02, p= 0.484 for trend in lldl with at1rv004
beta= 0.02, p= 0.487 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

AT1RV004	Summary of Ln(Apolopoprotein A1 g/l) Mean	Std. Dev.	Freq.
11	.26942421	.24397076	131
12	.29159287	.20555427	66
22	.28321769	.19862841	11
Total	.27718796	.22947919	208

p= 0.813 on 2 df for lapa1 vs at1rv004, N=208
p= 0.525 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=205
beta= 0.01, p= 0.581 for trend in lapa1 with at1rv004
beta= 0.03, p= 0.277 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Apolopoprotein B g/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	.09270931	.28572067	131
12	.13797655	.2220167	66
22	.19589745	.17826247	11
Total	.11253002	.26288155	208

p= 0.292 on 2 df for lapb vs at1rv004, N=208
p= 0.194 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=205
beta= 0.05, p= 0.117 for trend in lapb with at1rv004
beta= 0.05, p= 0.080 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Lp(a) lipoprotein mg/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	2.1581957	1.2937001	129
12	2.255499	1.2791891	66
22	2.696605	1.1590575	11
Total	2.2181206	1.2823333	206

p= 0.395 on 2 df for lfstlpa vs at1rv004, N=206
p= 0.672 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=203
beta= 0.18, p= 0.235 for trend in lfstlpa with at1rv004
beta= 0.09, p= 0.558 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin0 pmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	3.711538	.65927994	129
12	3.7460168	.60013413	67
22	3.5381704	.61351349	11
Total	3.713485	.63696527	207

p= 0.606 on 2 df for linsul0 vs at1rv004, N=207
p= 0.877 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=204
beta=-0.02, p= 0.758 for trend in linsul0 with at1rv004
beta= 0.01, p= 0.928 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin30 pmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	5.5807441	.60521262	127
12	5.6388277	.61211847	65
22	5.3693406	.63513787	10
Total	5.5889688	.60845948	202

p= 0.416 on 2 df for linsul30 vs at1rv004, N=202
p= 0.569 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=200
beta=-0.02, p= 0.821 for trend in linsul30 with at1rv004
beta=-0.02, p= 0.795 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin120 pmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	4.9385872	.85842365	126
12	5.193885	.6692482	64
22	4.7270336	.61713873	9
Total	5.0111253	.8005761	199

p= 0.063 on 2 df for linsl20 vs at1rv004, N=199
p= 0.134 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=197
beta= 0.10, p= 0.313 for trend in linsl20 with at1rv004
beta= 0.12, p= 0.209 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose0 mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	1.7879551	.16057069	134
12	1.8034395	.15410413	68
22	1.7864939	.21740712	11
Total	1.792823	.16113091	213

p= 0.806 on 2 df for lgluc0 vs at1rv004, N=213
p= 0.577 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=210
beta= 0.01, p= 0.675 for trend in lgluc0 with at1rv004
beta= 0.02, p= 0.303 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose30 mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	2.2321511	.19492522	132
12	2.2598151	.21368572	68
22	2.3016409	.24847285	10
Total	2.244418	.2035705	210

p= 0.438 on 2 df for lgluc30 vs at1rv004, N=210
p= 0.203 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=208
beta= 0.03, p= 0.203 for trend in lgluc30 with at1rv004
beta= 0.04, p= 0.087 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose120 mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	1.8803654	.33115938	131
12	1.9162677	.31366874	65
22	1.7247887	.35502349	10
Total	1.8841415	.32767286	206

p= 0.223 on 2 df for lgluc120 vs at1rv004, N=206
p= 0.509 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=204
beta=-0.02, p= 0.689 for trend in lgluc120 with at1rv004
beta=-0.00, p= 0.947 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Analyses for Women

Summary of EH1 Systolic BP (mmHg)			
AT1RV004	Mean	Std. Dev.	Freq.
11	155.6875	23.463731	80
12	157.71739	18.216797	46
22	157.5	34.133561	6
Total	156.47727	22.168496	132

p= 0.880 on 2 df for sysbpa1 vs at1rv004, N=132
p= 0.661 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta= 1.56, p= 0.641 for trend in sysbpa1 with at1rv004
beta= 3.00, p= 0.399 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of EH1 Diastolic BP (mmHg)			
AT1RV004	Mean	Std. Dev.	Freq.
11	82.175	11.688764	80
12	82.086957	8.3074962	46
22	89.333333	21.077634	6
Total	82.469697	11.194628	132

p= 0.309 on 2 df for diabpa1 vs at1rv004, N=132
p= 0.477 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta= 1.45, p= 0.388 for trend in diabpa1 with at1rv004
beta= 1.73, p= 0.341 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Pulse pressure mmHg (SBP-DBP)			
AT1RV004	Mean	Std. Dev.	Freq.
11	73.5125	16.086785	80
12	75.630435	13.992631	46
22	68.166667	15.867157	6
Total	74.007576	15.348121	132

p= 0.484 on 2 df for pulspres vs at1rv004, N=132
p= 0.477 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta= 0.10, p= 0.965 for trend in pulspres with at1rv004
beta= 1.27, p= 0.593 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(fasting cholesterol mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	1.9610003	.20104985	80
12	1.9359907	.17356103	46
22	2.0504372	.11888593	6
Total	1.9563502	.18928144	132

p= 0.359 on 2 df for lfstchol vs at1rv004, N=132
p= 0.398 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta= 0.00, p= 0.880 for trend in lfstchol with at1rv004
beta=-0.01, p= 0.638 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(fasting triglycerides mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	.21451252	.44220534	80
12	.24913547	.37795703	46
22	.24757618	.18265187	6
Total	.22808099	.41055377	132

p= 0.896 on 2 df for ltrig vs at1rv004, N=132
p= 0.675 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta= 0.03, p= 0.662 for trend in ltrig with at1rv004
beta=-0.02, p= 0.696 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(high density lipoproteins mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	.38364589	.26383929	79
12	.35458221	.18642275	46
22	.38525878	.21742259	6
Total	.3735142	.23624244	131

p= 0.799 on 2 df for lhdl vs at1rv004, N=131
p= 0.943 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=129
beta=-0.02, p= 0.644 for trend in lhdl with at1rv004
beta= 0.01, p= 0.753 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(low density lipoproteins mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	1.613923	.26948523	80
12	1.5743341	.23056498	46
22	1.737781	.14473403	6
Total	1.6057568	.25306438	132

p= 0.300 on 2 df for lldl vs at1rv004, N=132
p= 0.317 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta= 0.00, p= 0.935 for trend in lldl with at1rv004
beta=-0.02, p= 0.565 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Apolopoprotein A1 g/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	.31294931	.16899097	80
12	.29849054	.15037954	46
22	.30734523	.12118158	6
Total	.30765592	.15999139	132

p= 0.889 on 2 df for lapa1 vs at1rv004, N=132
p= 0.869 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta=-0.01, p= 0.692 for trend in lapa1 with at1rv004
beta= 0.01, p= 0.834 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Apolopoprotein B g/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	.03748498	.24366518	80
12	.03262562	.21458411	46
22	.1568705	.06146742	6
Total	.04121818	.22894023	132

p= 0.449 on 2 df for lapb vs at1rv004, N=132
p= 0.429 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta= 0.02, p= 0.518 for trend in lapb with at1rv004
beta= 0.00, p= 0.967 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Lp(a) lipoprotein mg/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	2.7393047	1.3538858	80
12	2.4003207	1.4249048	46
22	1.9793513	1.1948044	6
Total	2.5866306	1.3786415	132

p= 0.226 on 2 df for lfstlpa vs at1rv004, N=132
p= 0.316 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta=-0.36, p= 0.085 for trend in lfstlpa with at1rv004
beta=-0.35, p= 0.128 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin0 pmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	3.8308096	.58733599	80
12	3.8088141	.55458458	46
22	3.6662187	.40946758	6
Total	3.8156631	.56679988	132

p= 0.789 on 2 df for linsul0 vs at1rv004, N=132
p= 0.381 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta=-0.05, p= 0.579 for trend in linsul0 with at1rv004
beta=-0.10, p= 0.195 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin30 pmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	5.5208292	.58297112	79
12	5.5680443	.5522674	45
22	5.5574823	.3035769	6
Total	5.5388645	.5600081	130

p= 0.901 on 2 df for linsul30 vs at1rv004, N=130
p= 0.949 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=128
beta= 0.03, p= 0.681 for trend in linsul30 with at1rv004
beta=-0.01, p= 0.891 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Insulin120 pmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	5.4525182	.62972382	80
12	5.4745837	.71168935	46
22	5.1499672	.26015715	6
Total	5.4464553	.64810568	132

p= 0.513 on 2 df for lins120 vs at1rv004, N=132
p= 0.216 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130
beta=-0.05, p= 0.602 for trend in lins120 with at1rv004
beta=-0.12, p= 0.217 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose0 mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	1.7176782	.09160136	80
12	1.7446249	.10321288	46
22	1.671559	.07647439	6
Total	1.7249724	.09613272	132

p= 0.120 on 2 df for lgluc0 vs at1rv004, N=132
p= 0.286 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat fsoccat, N=130

beta= 0.01, p= 0.683 for trend in lgluc0 with atlrv004
beta= 0.00, p= 0.750 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose30 mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	2.1645393	.19658969	79
12	2.1323002	.1842651	45
22	2.1275049	.12886231	6
Total	2.1516703	.18934738	130

p= 0.631 on 2 df for lgluc30 vs atlrv004, N=130
p= 0.591 on 2 df adjusted for ageclif1 bmi1 and alccat
smoker1 soccat fsoccat, N=128
beta=-0.03, p= 0.357 for trend in lgluc30 with
atlrv004
beta=-0.03, p= 0.381 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat fsoccat

Summary of Ln(Glucose120 mmol/l)			
AT1RV004	Mean	Std. Dev.	Freq.
11	1.9432389	.24559059	80
12	1.9390927	.26724223	46
22	1.9031378	.2621726	6
Total	1.9399712	.2521873	132

p= 0.932 on 2 df for lgluc120 vs atlrv004, N=132
p= 0.697 on 2 df adjusted for ageclif1 bmi1 and alccat
smoker1 soccat fsoccat, N=130
beta=-0.01, p= 0.776 for trend in lgluc120 with
atlrv004
beta=-0.02, p= 0.661 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat fsoccat

Haplotype Phenotype Analyses of the *AGTR1* Gene

Cardiovascular phenotypes in East Herts

variable name	storage type	display format	value label	variable label
gluc0	float	%9.0g		glucose at 0
gluc30	float	%9.0g		glucose at 30
lgluc0	float	%9.0g		Ln(Glucose0 mmol/l)
lgluc30	float	%9.0g		Ln(Glucose30 mmol/l)
at1rc1	float	%9.0g		AT1R 4 SNP Haplotype 1 Count
at1rc2	float	%9.0g		AT1R 4 SNP Haplotype 2 Count
at1rc3	float	%9.0g		AT1R 4 SNP Haplotype 3 Count
at1rc4	float	%9.0g		AT1R 4 SNP Haplotype 4 Count
at1rc5	float	%9.0g		AT1R 4 SNP Haplotype 5 Count
at1rc6	float	%9.0g		AT1R 4 SNP Haplotype 6 Count
at1rc7	float	%9.0g		AT1R 4 SNP Haplotype 7 Count

Summary statistics of key variables

Variable	Obs	Mean	Std. Dev.	Min	Max
lgluc0	261	1.761856	.1289131	1.435084	2.772589
lgluc30	257	2.19652	.1955848	1.629241	3.039749
at1rc1	262	.6450382	.6127276	0	2
at1rc2	262	.2977099	.5133416	0	2
at1rc3	262	.3396947	.5132989	0	2
at1rc4	262	.240458	.4542315	0	2
at1rc5	262	.1793893	.4132328	0	2
at1rc6	262	.1450382	.3739022	0	2
at1rc7	262	.1526718	.3810307	0	2

AGTR1 haplotypes vs BMI and WHR in East & North Herts combined

variable name	storage type	display format	value label	variable label
hwhr	float	%9.0g		Waist:Hip (proportion)
hbmi	float	%9.0g		BMI (kg/m2)
category	byte	%8.0g	category	whether east or north-west hert
at1rc1	float	%9.0g		AT1R 4 SNP Haplotype 1 Count
at1rc2	float	%9.0g		AT1R 4 SNP Haplotype 2 Count
at1rc3	float	%9.0g		AT1R 4 SNP Haplotype 3 Count
at1rc4	float	%9.0g		AT1R 4 SNP Haplotype 4 Count
at1rc5	float	%9.0g		AT1R 4 SNP Haplotype 5 Count
at1rc6	float	%9.0g		AT1R 4 SNP Haplotype 6 Count
at1rc7	float	%9.0g		AT1R 4 SNP Haplotype 7 Count

Summary statistics of key variables

Variable	Obs	Mean	Std. Dev.	Min	Max
hwhr	707	.8752807	.0850659	.6394557	1.104348
hbmi	707	26.80163	3.837368	16.9	42.70998

AGTR1 haplotype in relation to $\log_e$ fasting glucose in men

AT1R count	Unadjusted associations Beta	P	Adjusted associations Beta	P
AT1RC2	0.004	0.88	0.004	0.88
AT1RC3	-0.06	0.01	-0.06	0.01
AT1RC4	-0.04	0.17	-0.04	0.16
AT1RC5	-0.01	0.69	-0.02	0.61
AT1RC6	-0.03	0.35	-0.03	0.39
AT1RC7	-0.04	0.19	-0.05	0.17

AGTR1 haplotype in relation to $\log_e$ fasting glucose in women

AT1R count	Unadjusted associations Beta	P	Adjusted associations Beta	P
AT1RC2	0.04	0.07	0.04	0.07
AT1RC3	-0.00	0.93	0.00	0.98
AT1RC4	-0.00	0.93	-0.00	0.84
AT1RC5	-0.02	0.31	-0.03	0.16
AT1RC6	0.03	0.28	0.03	0.31
AT1RC7	-0.03	0.18	-0.04	0.16

AGTR1 haplotype in relation to $\log_e$ glucose at 30' in men

AT1R count	Unadjusted associations Beta	P	Adjusted associations Beta	P
AT1RC2	-0.02	0.58	-0.01	0.73
AT1RC3	-0.07	0.04	-0.06	0.10
AT1RC4	-0.04	0.29	-0.05	0.25
AT1RC5	-0.04	0.36	-0.04	0.34
AT1RC6	-0.03	0.50	-0.02	0.64
AT1RC7	-0.04	0.39	0.04	0.39

AGTR1 haplotype in relation to $\log_e$ glucose at 30' in women

AT1R count	Unadjusted associations Beta	P	Adjusted associations Beta	P
AT1RC2	0.06	0.19	0.06	0.17
AT1RC3	-0.00	0.97	-0.01	0.86
AT1RC4	-0.03	0.51	-0.03	0.62
AT1RC5	0.03	0.58	0.02	0.70
AT1RC6	-0.01	0.85	-0.01	0.87
AT1RC7	-0.03	0.58	-0.02	0.67

AGTR1 haplotype in relation to BMI in men

AT1R count	Unadjusted associations Beta	P
AT1RC2	-0.45	0.26
AT1RC3	-0.78	0.05
AT1RC4	-0.06	0.89
AT1RC5	-0.05	0.91
AT1RC6	-0.46	0.35
AT1RC7	0.42	0.40

AGTR1 haplotype in relation to waist-hip-ratio (WHR) in men

AT1R count	Unadjusted associations Beta	P
AT1RC2	-0.00	0.59
AT1RC3	-0.01	0.23
AT1RC4	0.00	0.93
AT1RC5	0.00	0.39
AT1RC6	0.00	0.65
AT1RC7	0.00	0.78

AGTR1 hlotype in relation to waist-hip-ratio (WHR) in women

AT1R count	Unadjusted associations Beta	P
AT1RC2	-0.00	0.92
AT1RC3	0.00	0.42
AT1RC4	0.00	0.72
AT1RC5	-0.00	0.95
AT1RC6	0.00	0.61
AT1RC7	0.01	0.42

AGTR1 haplotype in relation to BMI in women

AT1R count	Unadjusted associations Beta	P
AT1RC2	-1.1	0.03
AT1RC3	-0.18	0.72
AT1RC4	-0.41	0.54
AT1RC5	0.46	0.47
AT1RC6	-0.38	0.56
AT1RC7	0.42	0.55

AGT M235T Analyses

AGTV001 genotype distribution by gender

AGTV001	Sex 0=men, 1=women 0	1	Total
11	94	63	157
	16.82	16.11	16.53
12	285	209	494
	50.98	53.45	52.00
22	180	119	299
	32.20	30.43	31.47
Total	559	391	950
	100.00	100.00	100.00

Pearson chi2(2) = 0.5664 Pr = 0.753

Analyses in men

AGTV001	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	78.75	12.690007	94
12	80.612676	12.383863	284
22	79.370787	11.427375	178
Total	79.90018	12.141004	556

p= 0.340 on 2 df for hwgt vs agtv001, N=556
beta= 0.05, p= 0.943 for trend in hwgt with agtv001

AGTV001	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.7251064	.06781925	94
12	1.7183803	.0612893	284
22	1.7242135	.06863231	178
Total	1.7213849	.06479119	556

p= 0.533 on 2 df for hhgt vs agtv001, N=556
beta= 0.00, p= 0.883 for trend in hhgt with agtv001

AGTV001	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	97.979787	10.623969	94
12	99.29894	9.996423	283
22	97.947191	9.1420562	178
Total	98.641982	9.847123	555

p= 0.277 on 2 df for hwast vs agtv001, N=555
beta=-0.24, p= 0.699 for trend in hwast with agtv001

AGTV001	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	104.34574	7.5625657	94
12	105.59507	7.6987385	284
22	104.77809	6.9584824	178
Total	105.1223	7.4500309	556

p= 0.281 on 2 df for hhip vs agtv001, N=556
beta= 0.05, p= 0.922 for trend in hhip with agtv001

AGTV001	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.93755772	.05328604	94
12	.9388973	.04932266	283
22	.93422984	.05157	178
Total	.93717347	.05068511	555

p= 0.628 on 2 df for hwhr vs agtv001, N=555
beta=-0.00, p= 0.493 for trend in hwhr with agtv001

AGTV001	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.40581	3.5504726	94
12	27.270595	3.6203602	284
22	26.657501	3.184014	178
Total	26.928112	3.4869212	556

p= 0.052 on 2 df for hbmi vs agtv001, N=556
beta= 0.00, p= 0.987 for trend in hbmi with agtv001

AGTV001	Summary of birthweight in ounces Mean	Std. Dev.	Freq.
11	122.89362	18.185702	94
12	125.64912	18.146597	285
22	123.72222	20.314581	180
Total	124.5653	18.878662	559

p= 0.362 on 2 df for bwt_ozs vs agtv001, N=559
beta= 0.02, p= 0.986 for trend in bwt_ozs with agtv001

AGTV001	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	363.03191	43.798525	94
12	363.77193	39.964693	285
22	359.20556	44.726193	180
Total	362.1771	42.173508	559

p= 0.513 on 2 df for wt2_ozs vs agtv001, N=559
beta=-2.36, p= 0.367 for trend in wt2_ozs with agtv001

Analyses in women

AGTV001	Summary of Weight (kg) Mean	Std. Dev.	Freq.
11	67.428571	11.516768	63
12	68.072115	11.234204	208
22	69.634454	11.748007	119
Total	68.444872	11.438509	390

p= 0.368 on 2 df for hwgt vs agtv001, N=390
beta= 1.18, p= 0.173 for trend in hwgt with agtv001

AGTV001	Summary of Height (m) Mean	Std. Dev.	Freq.
11	1.5887302	.06284882	63
12	1.5983654	.06163019	208
22	1.594958	.05030204	119
Total	1.5957692	.05856728	390

p= 0.512 on 2 df for hhgt vs agtv001, N=390
beta= 0.00, p= 0.654 for trend in hhgt with agtv001

AGTV001	Summary of Waist circumf (cm) Mean	Std. Dev.	Freq.
11	84.873016	9.9841682	63
12	83.264904	9.6101831	208
22	84.757983	10.577226	119
Total	83.980256	9.9778929	390

p= 0.318 on 2 df for hwast vs agtv001, N=390
beta= 0.21, p= 0.783 for trend in hwast with agtv001

AGTV001	Summary of Hip circumf (cm) Mean	Std. Dev.	Freq.
11	105.30952	9.2684119	63
12	105.08462	9.1453932	208
22	106.79496	9.8408594	119
Total	105.64282	9.3896397	390

p= 0.272 on 2 df for hhip vs agtv001, N=390
beta= 0.91, p= 0.202 for trend in hhip with agtv001

AGTV001	Summary of Waist:Hip (proportion) Mean	Std. Dev.	Freq.
11	.80578762	.05780938	63
12	.79169801	.05012112	208
22	.79245566	.0491283	119
Total	.79420521	.05126012	390

p= 0.146 on 2 df for hwhr vs agtv001, N=390
beta=-0.01, p= 0.166 for trend in hwhr with agtv001

AGTV001	Summary of BMI (kg/m2) Mean	Std. Dev.	Freq.
11	26.696132	4.1357249	63
12	26.662345	4.2264085	208
22	27.385822	4.5982618	119
Total	26.888556	4.3306169	390

p= 0.324 on 2 df for hbmi vs agtv001, N=390
beta= 0.41, p= 0.212 for trend in hbmi with agtv001

AGTV001	Mean	Std. Dev.	Freq.
11	119.52381	15.865216	63
12	120.49282	18.170962	209
22	120.80672	16.717634	119
Total	120.43223	17.345062	391

p= 0.891 on 2 df for bwt_ozs vs agtv001, N=391
beta= 0.59, p= 0.657 for trend in bwt_ozs with agtv001

AGTV001	Summary of weight at 1 yr in ounces Mean	Std. Dev.	Freq.
11	341.50794	32.685142	63
12	342.10048	38.387879	209
22	339.55462	35.917678	119
Total	341.23018	36.706643	391

p= 0.832 on 2 df for wt2_ozs vs agtv001, N=391
beta=-1.25, p= 0.655 for trend in wt2_ozs with agtv001

AGT M235T in relation to cardiovascular phenotypes in the EH cohort

AGTV001 genotype distribution by gender

Sex 0=men, 1=women	AGTV001 11	12	22	Total
0	33	125	61	219
	15.07	57.08	27.85	100.00
1	15	75	40	130
	11.54	57.69	30.77	100.00
Total	48	200	101	349
	13.75	57.31	28.94	100.00

Pearson chi2(2) = 0.9841 Pr = 0.611

Comment: As would be expected, no difference in AGTV001 distribution by gender.

Analyses in men

AGTV001	Summary of EH1 Systolic BP (mmHg) Mean	Std. Dev.	Freq.
11	162.42424	23.494721	33
12	163.352	21.537011	125
22	165.37705	22.252463	61
Total	163.77626	21.960143	219

p= 0.782 on 2 df for sysbpa1 vs agtv001, N=219
p= 0.532 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.500 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta= 1.57, p= 0.497 for trend in sysbpa1 with agtv001
beta= 1.86, p= 0.422 for trend adjusted for ageclif1 bmi1
beta= 1.84, p= 0.439 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of EH1 Diastolic BP (mmHg) Mean	Std. Dev.	Freq.
11	88.181818	11.131191	33
12	90.168	10.758894	125
22	90.704918	11.343639	61
Total	90.018265	10.959041	219

p= 0.554 on 2 df for diabpa1 vs agtv001, N=219
p= 0.706 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.740 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta= 1.13, p= 0.326 for trend in diabpa1 with agtv001
beta= 0.97, p= 0.409 for trend adjusted for ageclif1 bmi1
beta= 0.92, p= 0.443 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of EH1 Pulse rate (bpm) Mean	Std. Dev.	Freq.
11	69.666667	11.915501	33
12	69.672	11.176571	125
22	68.42623	9.9623609	61
Total	69.324201	10.932651	219

p= 0.754 on 2 df for pulsea1 vs agtv001, N=219
p= 0.799 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.977 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta=-0.73, p= 0.526 for trend in pulsea1 with agtv001
beta=-0.69, p= 0.561 for trend adjusted for ageclif1 bmi1
beta=-0.26, p= 0.829 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Pulse pressure mmHg (SBP-DBP) Mean	Std. Dev.	Freq.
11	74.242424	16.809652	33
12	73.184	14.971685	125
22	74.672131	16.485065	61
Total	73.757991	15.627865	219

p= 0.816 on 2 df for pulspres vs agtv001, N=219
p= 0.503 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.434 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta= 0.44, p= 0.790 for trend in pulspres with agtv001
beta= 0.89, p= 0.582 for trend adjusted for ageclif1 bmi1
beta= 0.92, p= 0.584 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(fasting fibrinogen g/l) Mean	Std. Dev.	Freq.
11	5.7213666	.19871598	29
12	5.7271663	.20260815	122
22	5.704085	.20985645	61
Total	5.7197316	.20348692	212

p= 0.771 on 2 df for lfastfib vs agtv001, N=212
p= 0.898 on 2 df adjusted for ageclif1 bmi1, N=212
p= 0.925 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=210
beta=-0.01, p= 0.595 for trend in lfastfib with agtv001
beta=-0.01, p= 0.675 for trend adjusted for ageclif1 bmi1
beta=-0.01, p= 0.728 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(fasting cholesterol mmol/l) Mean	Std. Dev.	Freq.
11	1.9031866	.14187202	31
12	1.889187	.17543757	125
22	1.8607393	.20866516	61
Total	1.8831901	.1810521	217

p= 0.486 on 2 df for lfstchol vs agtv001, N=217
p= 0.599 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.510 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta=-0.02, p= 0.242 for trend in lfstchol with agtv001
beta=-0.02, p= 0.311 for trend adjusted for ageclif1 bmi1
beta=-0.02, p= 0.247 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(fasting triglycerides mmol/l) Mean	Std. Dev.	Freq.
11	.2537467	.59456599	31
12	.37282821	.50971974	125
22	.41421641	.50607728	61
Total	.36745104	.52142472	217

p= 0.374 on 2 df for ltrig vs agtv001, N=217
p= 0.382 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.455 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta= 0.07, p= 0.193 for trend in ltrig with agtv001
beta= 0.08, p= 0.170 for trend adjusted for ageclif1 bmi1
beta= 0.07, p= 0.239 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(high density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	.23129017	.22808714	30
12	.14614539	.28323111	123
22	.19744623	.25922812	59
Total	.1724713	.27032456	212

p= 0.214 on 2 df for lhdl vs agtv001, N=212
p= 0.242 on 2 df adjusted for ageclif1 bmi1, N=211
p= 0.227 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=209
beta=-0.00, p= 0.907 for trend in lhdl with agtv001
beta= 0.00, p= 0.973 for trend adjusted for ageclif1 bmi1
beta= 0.00, p= 0.942 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(low density lipoproteins mmol/l) Mean	Std. Dev.	Freq.
11	1.589563	.21499135	31
12	1.5516902	.25264854	125
22	1.4946406	.33388743	61
Total	1.5410636	.27393786	217

p= 0.234 on 2 df for lldl vs agtv001, N=217
p= 0.275 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.259 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta=-0.05, p= 0.091 for trend in lldl with agtv001
beta=-0.05, p= 0.108 for trend adjusted for ageclif1 bmi1
beta=-0.05, p= 0.103 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(LDL:HDL ratio) Mean	Std. Dev.	Freq.
11	1.3348404	.30331148	30
12	1.3934986	.40014122	123
22	1.2701471	.4511076	59
Total	1.350869	.40506444	212

p= 0.153 on 2 df for lldlhdl vs agtv001, N=212
p= 0.171 on 2 df adjusted for ageclif1 bmi1, N=211
p= 0.159 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=209
beta=-0.05, p= 0.253 for trend in lldlhdl with agtv001
beta=-0.05, p= 0.237 for trend adjusted for ageclif1 bmi1
beta=-0.06, p= 0.199 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Apolopoprotein A1 g/l) Mean	Std. Dev.	Freq.
11	.26756759	.24008367	31
12	.28283499	.22777984	119
22	.28256774	.223175	60
Total	.28050488	.22728041	210

p= 0.943 on 2 df for lapa1 vs agtv001, N=210
p= 0.810 on 2 df adjusted for ageclif1 bmi1, N=209
p= 0.846 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=208
beta= 0.01, p= 0.805 for trend in lapa1 with agtv001
beta= 0.01, p= 0.651 for trend adjusted for ageclif1 bmi1
beta= 0.01, p= 0.572 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Apolopoprotein B g/l) Mean	Std. Dev.	Freq.
11	.10170675	.24126919	31
12	.11681603	.25430695	119
22	.0742442	.29516963	60
Total	.10242223	.26421605	210

p= 0.598 on 2 df for lapb vs agtv001, N=210
p= 0.822 on 2 df adjusted for ageclif1 bmi1, N=209
p= 0.786 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=208
beta=-0.02, p= 0.500 for trend in lapb with agtv001
beta=-0.02, p= 0.545 for trend adjusted for ageclif1 bmi1
beta=-0.02, p= 0.488 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Lp(a) lipoprotein mg/l) Mean	Std. Dev.	Freq.
11	2.4218311	1.2937511	30
12	2.0949284	1.3025056	121
22	2.1034134	1.2744454	60
Total	2.1438203	1.2922052	211

p= 0.447 on 2 df for lfstlpa vs agtv001, N=211
p= 0.606 on 2 df adjusted for ageclif1 bmi1, N=210
p= 0.709 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=208
beta=-0.13, p= 0.370 for trend in lfstlpa with agtv001
beta=-0.12, p= 0.396 for trend adjusted for ageclif1 bmi1
beta=-0.10, p= 0.477 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(fasting factor VII %ofstd) Mean	Std. Dev.	Freq.
11	4.7161908	.23438509	29
12	4.693926	.29208732	122
22	4.7129978	.32277307	60
Total	4.7024093	.29300669	211

p= 0.886 on 2 df for lfastvii vs agtv001, N=211
p= 0.819 on 2 df adjusted for ageclif1 bmi1, N=211
p= 0.734 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=209
beta= 0.00, p= 0.931 for trend in lfastvii with agtv001
beta= 0.00, p= 0.902 for trend adjusted for ageclif1 bmi1
beta=-0.01, p= 0.859 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Insulin0 pmol/l) Mean	Std. Dev.	Freq.
11	3.7099209	.6902092	30
12	3.7507996	.61137859	123
22	3.7295534	.67350537	61
Total	3.7390128	.6379302	214

p= 0.943 on 2 df for linsul0 vs agtv001, N=214
p= 0.646 on 2 df adjusted for ageclif1 bmi1, N=213
p= 0.555 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=211
beta= 0.00, p= 0.960 for trend in linsul0 with agtv001
beta= 0.02, p= 0.814 for trend adjusted for ageclif1 bmi1
beta= 0.01, p= 0.847 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Insulin30 pmol/l) Mean	Std. Dev.	Freq.
11	5.5600292	.72921768	27
12	5.5911909	.56565687	121
22	5.6355077	.55816339	58
Total	5.5995842	.58478932	206

p= 0.834 on 2 df for linsul30 vs agtv001, N=206
p= 0.559 on 2 df adjusted for ageclif1 bmi1, N=206
p= 0.600 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=204
beta= 0.04, p= 0.549 for trend in linsul30 with agtv001

beta= 0.05, p= 0.414 for trend adjusted for ageclif1 bmi1
beta= 0.05, p= 0.477 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Insulin120 pmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	4.99268	.7037713	27
12	4.9279155	.89576926	118
22	5.0954123	.78663934	58
Total	4.9843857	.84174364	203

p= 0.465 on 2 df for lins120 vs agtv001, N=203
p= 0.111 on 2 df adjusted for ageclif1 bmi1, N=203
p= 0.184 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=201
beta= 0.08, p= 0.411 for trend in lins120 with agtv001
beta= 0.10, p= 0.259 for trend adjusted for ageclif1 bmi1
beta= 0.08, p= 0.384 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Glucose0 mmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	1.7730652	.18073574	31
12	1.8029173	.20627867	125
22	1.7831297	.15339158	61
Total	1.7930903	.18878959	217

p= 0.653 on 2 df for lgluc0 vs agtv001, N=217
p= 0.909 on 2 df adjusted for ageclif1 bmi1, N=216
p= 0.899 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=214
beta= 0.00, p= 0.994 for trend in lgluc0 with agtv001
beta= 0.00, p= 0.926 for trend adjusted for ageclif1 bmi1
beta= 0.01, p= 0.668 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Glucose30 mmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	2.2859994	.18760417	30
12	2.2524599	.2373523	123
22	2.2322663	.19238413	61
Total	2.2514055	.21853925	214

p= 0.545 on 2 df for lgluc30 vs agtv001, N=214
p= 0.374 on 2 df adjusted for ageclif1 bmi1, N=214
p= 0.378 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=212
beta=-0.03, p= 0.279 for trend in lgluc30 with agtv001
beta=-0.02, p= 0.366 for trend adjusted for ageclif1 bmi1
beta=-0.01, p= 0.625 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Glucose120 mmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	1.860294	.28216129	30
12	1.8643989	.37342037	121
22	1.8974942	.312183	59
Total	1.8731107	.34408004	210

p= 0.814 on 2 df for lgluc120 vs agtv001, N=210
p= 0.418 on 2 df adjusted for ageclif1 bmi1, N=210
p= 0.235 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=208
beta= 0.02, p= 0.567 for trend in lgluc120 with agtv001
beta= 0.03, p= 0.438 for trend adjusted for ageclif1 bmi1
beta= 0.04, p= 0.225 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Fasting Proinsulin pmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	1.0037214	.67208713	30
12	1.0812525	.66659739	124
22	1.1385805	.80183247	61
Total	1.0866994	.70613198	215

p= 0.689 on 2 df for lproi vs agtv001, N=215
p= 0.341 on 2 df adjusted for ageclif1 bmi1, N=214
p= 0.296 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=212
beta= 0.07, p= 0.391 for trend in lproi with agtv001

Total	82.292308	11.322233	130

beta= 0.09, p= 0.244 for trend adjusted for ageclif1 bmi1
beta= 0.09, p= 0.212 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Fast. 32-33 Split Proinsulin pmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	.91226641	.73831632	30
12	1.20607	.73274324	123
22	1.1533108	.79738366	61
Total	1.1498437	.75539896	214

p= 0.161 on 2 df for lsplit vs agtv001, N=214
p= 0.372 on 2 df adjusted for ageclif1 bmi1, N=213
p= 0.196 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=211
beta= 0.08, p= 0.297 for trend in lsplit with agtv001
beta= 0.10, p= 0.191 for trend adjusted for ageclif1 bmi1
beta= 0.14, p= 0.073 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Insulin AUC during OGTT)			
AGTV001	Mean	Std. Dev.	Freq.
11	10.13624	.67509589	24
12	10.098618	.5503082	114
22	10.203153	.51616472	55
Total	10.133086	.55666355	193

p= 0.522 on 2 df for lauigtt vs agtv001, N=193
p= 0.215 on 2 df adjusted for ageclif1 bmi1, N=193
p= 0.292 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=191
beta= 0.05, p= 0.432 for trend in lauigtt with agtv001
beta= 0.07, p= 0.301 for trend adjusted for ageclif1 bmi1
beta= 0.05, p= 0.462 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(EH2 echo LVM (g))			
AGTV001	Mean	Std. Dev.	Freq.
11	5.2410004	.1805296	23
12	5.3326516	.26790163	88
22	5.3243852	.18705427	41
Total	5.3165535	.23749917	152

p= 0.251 on 2 df for lecholvm vs agtv001, N=152
p= 0.541 on 2 df adjusted for ageclin2 bmi2, N=152
p= 0.624 on 2 df adjusted for ageclin2 bmi2 and alccat2 smoker2 soccat, N=151
beta= 0.03, p= 0.272 for trend in lecholvm with agtv001
beta= 0.02, p= 0.441 for trend adjusted for ageclin2 bmi2
beta= 0.03, p= 0.343 for trend adjusted for ageclin2 bmi2 and alccat2 smoker2 soccat

Analyses in women

Summary of EH1 Systolic BP (mmHg)			
AGTV001	Mean	Std. Dev.	Freq.
11	156.86667	13.963558	15
12	156.97333	21.527464	75
22	155.375	25.758929	40
Total	156.46923	22.093946	130

p= 0.932 on 2 df for sysbpa1 vs agtv001, N=130
p= 0.930 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.985 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.99, p= 0.752 for trend in sysbpa1 with agtv001
beta=-0.84, p= 0.794 for trend adjusted for ageclif1 bmi1
beta=-0.54, p= 0.873 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of EH1 Diastolic BP (mmHg)			
AGTV001	Mean	Std. Dev.	Freq.
11	79.466667	6.5341593	15
12	82.52	11.989139	75
22	82.925	11.505601	40

p= 0.584 on 2 df for diabpa1 vs agtv001, N=130
p= 0.656 on 2 df adjusted for ageclif1 bmi1, N=130

p= 0.855 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta= 1.35, p= 0.401 for trend in diabpa1 with agtv001
beta= 1.15, p= 0.484 for trend adjusted for ageclif1 bmi1
beta= 0.73, p= 0.674 for trend adjusted for ageclif1 bmi1
and alccat smoker1 soccat

Summary of EH1 Pulse rate (bpm)			
AGTV001	Mean	Std. Dev.	Freq.
11	67	11.753419	15
12	68.32	9.8243492	75
22	67.925	9.033804	40
Total	68.046154	9.7574154	130

p= 0.889 on 2 df for pulsea1 vs agtv001, N=130
p= 0.902 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.741 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta= 0.22, p= 0.876 for trend in pulsea1 with agtv001
beta=-0.12, p= 0.931 for trend adjusted for ageclif1 bmi1
beta=-0.04, p= 0.981 for trend adjusted for ageclif1 bmi1
and alccat smoker1 soccat

Summary of Pulse pressure mmHg (SBP-DBP)			
AGTV001	Mean	Std. Dev.	Freq.
11	77.4	12.653627	15
12	74.453333	13.763734	75
22	72.45	18.56375	40
Total	74.176923	15.24501	130

p= 0.550 on 2 df for pulspres vs agtv001, N=130
p= 0.663 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.837 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-2.34, p= 0.279 for trend in pulspres with agtv001
beta=-1.98, p= 0.364 for trend adjusted for ageclif1 bmi1
beta=-1.28, p= 0.571 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(fasting fibrinogen g/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	5.6956623	.13582347	14
12	5.6831821	.15360926	73
22	5.6654193	.12461548	35
Total	5.6795184	.14311455	122

p= 0.757 on 2 df for lfastfib vs agtv001, N=122
p= 0.831 on 2 df adjusted for ageclif1 bmi1, N=122
p= 0.814 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=120
beta=-0.02, p= 0.458 for trend in lfastfib with agtv001
beta=-0.01, p= 0.564 for trend adjusted for ageclif1 bmi1
beta=-0.00, p= 0.972 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(fasting cholesterol mmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	1.8789605	.17190265	15
12	1.9663338	.19531972	75
22	1.932399	.15653425	40
Total	1.9458108	.18253654	130

p= 0.205 on 2 df for lfstchol vs agtv001, N=130
p= 0.253 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.277 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta= 0.01, p= 0.720 for trend in lfstchol with agtv001
beta= 0.00, p= 0.900 for trend adjusted for ageclif1 bmi1
beta= 0.01, p= 0.766 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(fasting triglycerides mmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	.29785409	.26783485	15
12	.23973885	.4291158	75
22	.22142846	.45094394	40
Total	.24081049	.41879556	130

p= 0.836 on 2 df for ltrig vs agtv001, N=130
p= 0.960 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.990 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.03, p= 0.585 for trend in ltrig with agtv001
beta=-0.02, p= 0.778 for trend adjusted for ageclif1 bmi1
beta=-0.00, p= 0.996 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(high density lipoproteins mmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	.29933673	.24721328	15
12	.36217764	.24998606	75
22	.43057279	.22513926	39
Total	.37554816	.24406757	129

p= 0.160 on 2 df for lhdl vs agtv001, N=129
p= 0.411 on 2 df adjusted for ageclif1 bmi1, N=129
p= 0.690 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=127
beta= 0.07, p= 0.055 for trend in lhdl with agtv001
beta= 0.04, p= 0.190 for trend adjusted for ageclif1 bmi1
beta= 0.02, p= 0.481 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(low density lipoproteins mmol/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	1.5032981	.24713249	15
12	1.6112497	.25177319	75
22	1.575944	.24263825	40
Total	1.5879305	.24896551	130

p= 0.291 on 2 df for lldl vs agtv001, N=130
p= 0.337 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.270 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta= 0.02, p= 0.656 for trend in lldl with agtv001
beta= 0.01, p= 0.744 for trend adjusted for ageclif1 bmi1
beta= 0.03, p= 0.482 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(LDL:HDL ratio)			
AGTV001	Mean	Std. Dev.	Freq.
11	1.2039614	.31431775	15
12	1.2490721	.35488177	75
22	1.1269971	.32953888	39
Total	1.2069203	.34473644	129

p= 0.201 on 2 df for lldlhdl vs agtv001, N=129
p= 0.274 on 2 df adjusted for ageclif1 bmi1, N=129
p= 0.299 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=127
beta=-0.06, p= 0.207 for trend in lldlhdl with agtv001
beta=-0.04, p= 0.393 for trend adjusted for ageclif1 bmi1
beta=-0.01, p= 0.868 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

Summary of Ln(Apolopoprotein A1 g/l)			
AGTV001	Mean	Std. Dev.	Freq.
11	.22622294	.16190901	15
12	.32899699	.16884571	75
22	.31091007	.15291301	40
Total	.31157324	.16520201	130

p= 0.088 on 2 df for lapa1 vs agtv001, N=130
p= 0.128 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.291 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta= 0.02, p= 0.286 for trend in lapa1 with agtv001

beta= 0.01, p= 0.613 for trend adjusted for ageclif1 bmi1
beta=-0.00, p= 0.949 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Apolopoprotein B g/l) Mean	Std. Dev.	Freq.
11	-.03148353	.15366091	15
12	.07498261	.24225482	75
22	-.0102224	.22768983	40
Total	.03648113	.2323291	130

p= 0.083 on 2 df for lapb vs agtv001, N=130
p= 0.070 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.128 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.02, p= 0.608 for trend in lapb with agtv001
beta=-0.01, p= 0.745 for trend adjusted for ageclif1 bmi1
beta=-0.01, p= 0.849 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Lp(a) lipoprotein mg/l) Mean	Std. Dev.	Freq.
11	2.0607345	.99321992	15
12	2.7861405	1.5035038	75
22	2.3121012	1.1959572	40
Total	2.5565815	1.3834651	130

p= 0.072 on 2 df for lfst1pa vs agtv001, N=130
p= 0.068 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.073 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.05, p= 0.812 for trend in lfst1pa with agtv001
beta=-0.07, p= 0.721 for trend adjusted for ageclif1 bmi1
beta=-0.04, p= 0.850 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(fasting factor VII %ofstd) Mean	Std. Dev.	Freq.
11	4.8814615	.22396624	14
12	4.8521426	.20234406	70
22	4.929607	.23986634	33
Total	4.8774998	.21681757	117

p= 0.240 on 2 df for lfastvii vs agtv001, N=117
p= 0.251 on 2 df adjusted for ageclif1 bmi1, N=117
p= 0.162 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=115
beta= 0.04, p= 0.248 for trend in lfastvii with agtv001
beta= 0.04, p= 0.226 for trend adjusted for ageclif1 bmi1
beta= 0.06, p= 0.108 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Insulin0 pmol/l) Mean	Std. Dev.	Freq.
11	4.0295536	.64598919	15
12	3.738252	.51810093	75
22	3.7836041	.62480458	40
Total	3.7858182	.57054159	130

p= 0.197 on 2 df for linsul0 vs agtv001, N=130
p= 0.356 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.606 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.07, p= 0.356 for trend in linsul0 with agtv001
beta=-0.05, p= 0.461 for trend adjusted for ageclif1 bmi1
beta=-0.02, p= 0.756 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Insulin30 pmol/l) Mean	Std. Dev.	Freq.
11	5.7255879	.62142863	15
12	5.4917669	.57457037	72
22	5.5353759	.52648494	38
Total	5.5330826	.56640214	125

p= 0.350 on 2 df for linsul30 vs agtv001, N=125
p= 0.594 on 2 df adjusted for ageclif1 bmi1, N=125
p= 0.419 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=123
beta=-0.06, p= 0.481 for trend in linsul30 with agtv001
beta=-0.03, p= 0.730 for trend adjusted for ageclif1 bmi1
beta=-0.03, p= 0.689 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Insulin120 pmol/l) Mean	Std. Dev.	Freq.
11	5.634946	.71327332	15
12	5.4759839	.61947519	75
22	5.3542761	.70125502	40
Total	5.4568771	.65664617	130

p= 0.345 on 2 df for lins120 vs agtv001, N=130
p= 0.425 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.398 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.13, p= 0.146 for trend in lins120 with agtv001
beta=-0.12, p= 0.194 for trend adjusted for ageclif1 bmi1
beta=-0.13, p= 0.175 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Glucose0 mmol/l) Mean	Std. Dev.	Freq.
11	1.7618125	.13305319	15
12	1.7165395	.07649509	75
22	1.7258659	.11256334	40
Total	1.724633	.09647722	130

p= 0.253 on 2 df for lgluc0 vs agtv001, N=130
p= 0.344 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.524 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.01, p= 0.459 for trend in lgluc0 with agtv001
beta=-0.01, p= 0.496 for trend adjusted for ageclif1 bmi1
beta=-0.01, p= 0.631 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Glucose30 mmol/l) Mean	Std. Dev.	Freq.
11	2.2319707	.18548132	15
12	2.161866	.18440856	72
22	2.1514848	.19064151	38
Total	2.1671227	.1865477	125

p= 0.346 on 2 df for lgluc30 vs agtv001, N=125
p= 0.437 on 2 df adjusted for ageclif1 bmi1, N=125
p= 0.526 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=123
beta=-0.03, p= 0.231 for trend in lgluc30 with agtv001
beta=-0.03, p= 0.280 for trend adjusted for ageclif1 bmi1
beta=-0.03, p= 0.337 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Glucose120 mmol/l) Mean	Std. Dev.	Freq.
11	1.966495	.29899108	15
12	1.9560812	.25266956	75
22	1.9271175	.2614177	40
Total	1.9483709	.2592255	130

p= 0.818 on 2 df for lgluc120 vs agtv001, N=130
p= 0.786 on 2 df adjusted for ageclif1 bmi1, N=130
p= 0.715 on 2 df adjusted for ageclif1 bmi1 and alccat smoker1 soccat, N=128
beta=-0.02, p= 0.543 for trend in lgluc120 with agtv001
beta=-0.02, p= 0.571 for trend adjusted for ageclif1 bmi1
beta=-0.02, p= 0.560 for trend adjusted for ageclif1 bmi1 and alccat smoker1 soccat

AGTV001	Summary of Ln(Fasting Proinsulin pmol/l) Mean	Std. Dev.	Freq.
11	1.3302771	.57288897	15
12	1.2489562	.56715072	75
22	1.306432	.52930097	40
Total	1.2760242	.55307434	130

p= 0.803 on 2 df for lproi vs agtv001, N=130
p= 0.828 on 2 df adjusted for ageclif1 bmi, N=130
p= 0.712 on 2 df adjusted for ageclif1 bmi and alccat
smoker1 soccat, N=128
beta= 0.01, p= 0.919 for trend in lproi with agtv001
beta= 0.04, p= 0.644 for trend adjusted for ageclif1
bmi1
beta= 0.01, p= 0.915 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat

| Summary of Ln(Fast. 32-33 Split Proinsulin pmol/l)

AGTV001	Mean	Std. Dev.	Freq.
11	2.200428	.60410903	15
12	2.1702574	.44951238	75
22	2.2444303	.56594401	40
Total	2.1965611	.50345317	130

p= 0.756 on 2 df for lsplit vs agtv001, N=130
p= 0.604 on 2 df adjusted for ageclif1 bmi, N=130
p= 0.715 on 2 df adjusted for ageclif1 bmi and alccat
smoker1 soccat, N=128
beta= 0.04, p= 0.605 for trend in lsplit with agtv001
beta= 0.06, p= 0.314 for trend adjusted for ageclif1
bmi1
beta= 0.05, p= 0.470 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat

| Summary of Ln(Insulin AUC during OGTT)

AGTV001	Mean	Std. Dev.	Freq.
11	10.399396	.60935627	15
12	10.198104	.53444457	72
22	10.191098	.51825839	38
Total	10.22013	.53855474	125

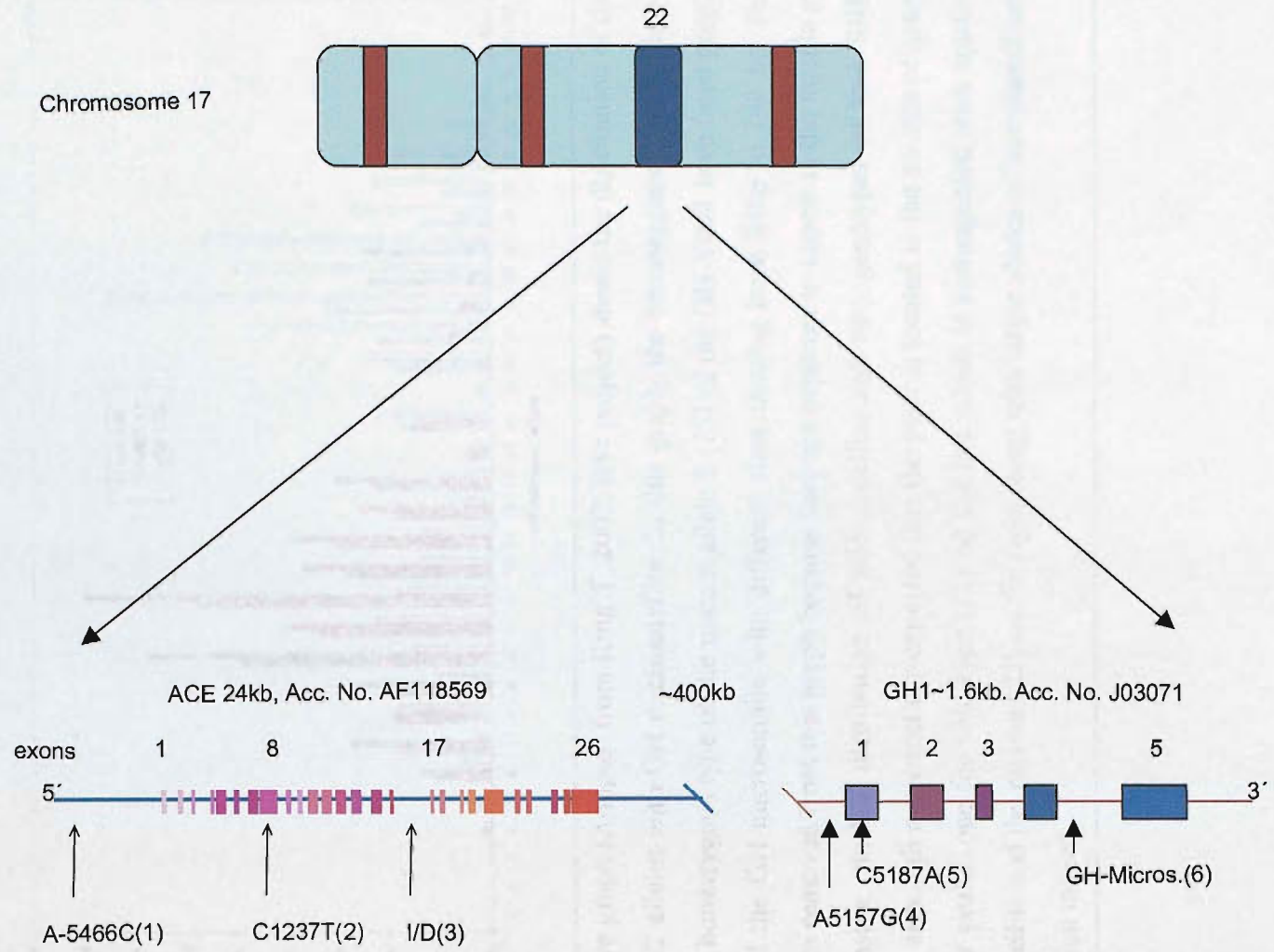
p= 0.391 on 2 df for lauigtt vs agtv001, N=125
p= 0.680 on 2 df adjusted for ageclif1 bmi, N=125
p= 0.595 on 2 df adjusted for ageclif1 bmi and alccat
smoker1 soccat, N=123
beta=-0.08, p= 0.315 for trend in lauigtt with agtv001
beta=-0.05, p= 0.486 for trend adjusted for ageclif1
bmi1
beta=-0.05, p= 0.501 for trend adjusted for ageclif1
bmi1 and alccat smoker1 soccat

| Summary of Ln(EH2 echo LVM (g))

AGTV001	Mean	Std. Dev.	Freq.
11	5.148948	.35278126	12
12	4.9887835	.27231196	54
22	4.9809152	.27103145	33
Total	5.0055746	.28449334	99

p= 0.176 on 2 df for lecholvm vs agtv001, N=99
p= 0.506 on 2 df adjusted for ageclin2 bmi2, N=98
p= 0.643 on 2 df adjusted for ageclin2 bmi2 and
alccat2 smoker2 soccat, N=96
beta=-0.06, p= 0.163 for trend in lecholvm with
agtv001
beta=-0.05, p= 0.251 for trend adjusted for ageclin2
bmi2
beta=-0.03, p= 0.444 for trend adjusted for ageclin2
bmi2 and alccat2 smoker2 soccat

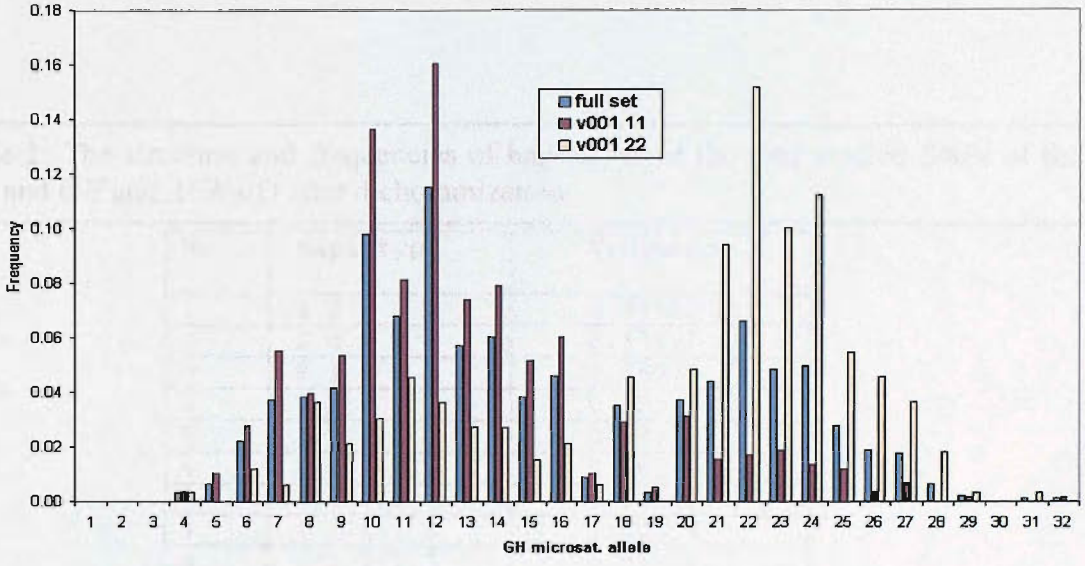
Figure 1: Schematic Representation of the *ACE* and *GH* Genes on Chromosome 17



The diagram shows a schematic representation of the GH and the ACE genes, on Chromosome 17q22-24. Numbers with polymorphisms refer to their number in the LD study.

Appendix 5

Figure 2: *GH* Microsatellite Allele Distribution in GH-V001 11 and 22 Genotype Individuals



The above graph (Adopted from King T. 2002. BSc project) shows the distribution of the GH-V001 11 and 22 alleles with GH microsatellite. In this graph, the homozygous type of the wild allele 1 (11) and homozygous type of the mutant allele 2 (22) of the GH-V001 have been graphed with 32 alleles of the GH microsatelite with different sizes ranging from 84bp to 140 bp. Heterozygous have been removed from this graph because they are expected to appear in the middle part and will be confusing. Such a distribution of microsatellite and two genotypes of that SNP is unique because, although it is generally expected that the peak is located in the middle of graph, there are two clear peaks: one for wild type (11) on the left which is accompanied with smaller alleles of microsatellite and the mutant (22) on the right along with larger alleles of microsatellite; a binomial distribution indeed.

Table 1: The structure and frequencies of haplotypes of the four studied SNPs of the *ACE* and *GH* and *ACE* I/D after dichotomization.

No	Haplotype	Frequency
1	1 2 1 1 2	0.24950
2	2 1 2 1 2	0.17631
3	2 1 2 2 2	0.16970
4	1 2 1 2 2	0.13391
5	1 1 2 2 2	0.07059
6	1 1 2 1 2	0.06347
7	1 1 1 1 2	0.03348
8	1 2 2 1 2	0.02429
9	2 1 2 2 1	0.01744
10	2 1 1 1 2	0.01053
11	1 1 2 1 1	0.00830
12	2 2 1 1 2	0.00799
13	1 2 2 2 2	0.00736
14	1 1 1 2 2	0.00604
15	1 2 1 2 1	0.00537
16	2 1 1 2 2	0.00462
17	1 2 2 2 1	0.00380
18	2 1 2 1 1	0.00357
19	2 2 2 2 2	0.00168
20	2 2 2 2 1	0.00086
21	2 1 1 1 1	0.00084
22	1 1 1 2 1	0.00034

Microsattelite alleles were dichotomised based on their size; alleles smaller than 110 as allele one and those higher than 110 as 2.

Table 2: The structure and frequencies of the haplotypes of the studied polymophisms in the *ACE* and *GH*.

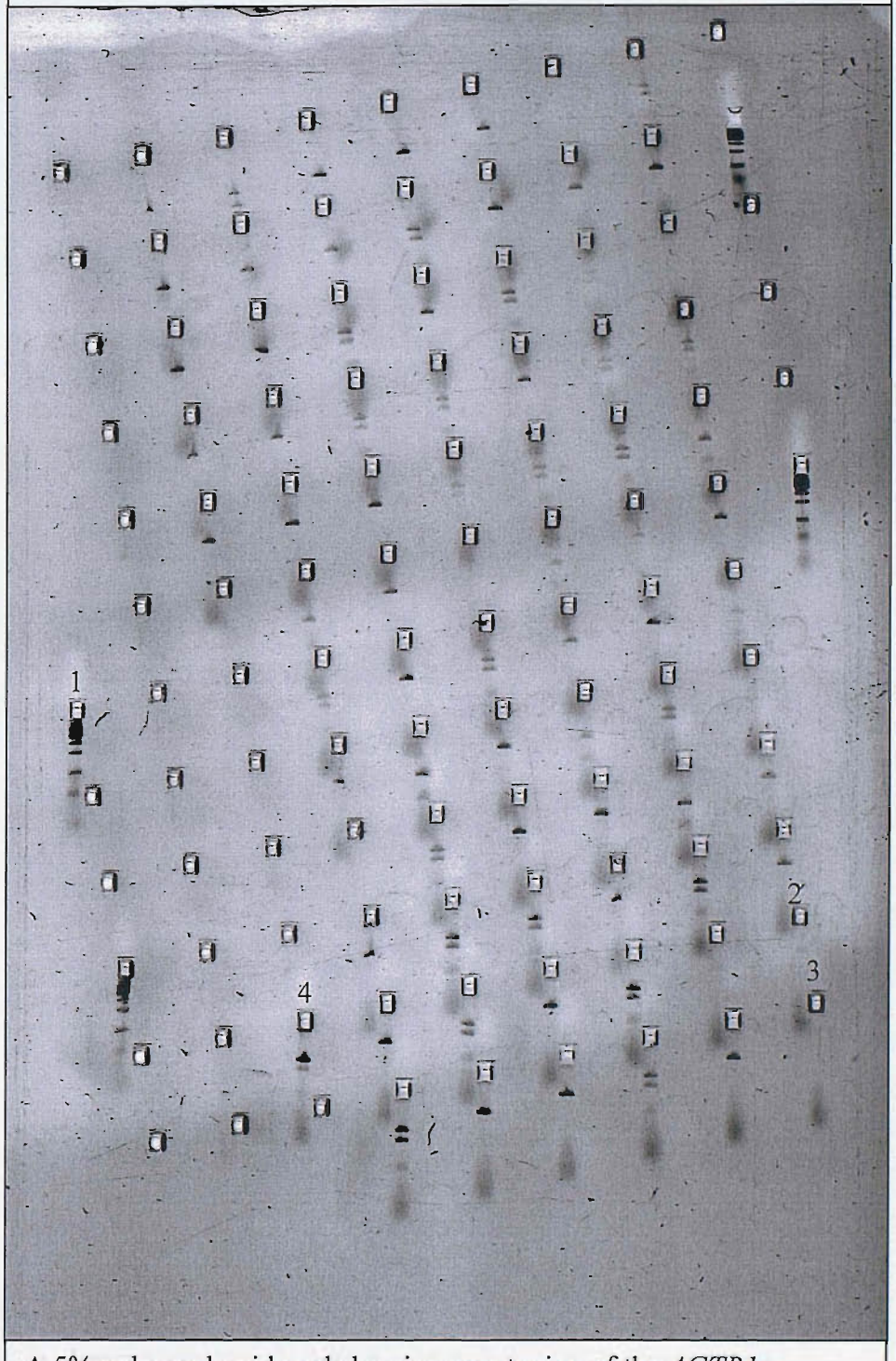
No	Haplotype	Frequency
1	1 2 1 1 2 1	0.21916
2	2 1 2 1 2 1	0.13697
3	2 1 2 2 2 2	0.11033
4	1 2 1 2 2 2	0.08755
5	2 1 2 2 2 1	0.05917
6	1 2 1 2 2 1	0.05075
7	1 1 2 1 2 1	0.04710
8	2 1 2 1 2 2	0.04663
9	1 1 2 2 2 2	0.04397
10	1 1 1 1 2 1	0.03112
11	1 1 2 2 2 1	0.02616
12	1 2 1 1 2 2	0.02504
13	1 2 2 1 2 1	0.01500
14	2 1 2 2 1 2	0.01371
15	1 1 2 1 2 2	0.01322
16	1 2 2 1 2 2	0.00862
17	2 1 1 1 2 1	0.00785
18	1 2 2 2 2 2	0.00618
19	1 1 2 1 1 1	0.00606
20	1 2 1 2 1 2	0.00589
21	2 2 1 1 2 1	0.00554
22	1 1 1 2 2 2	0.00552
23	1 2 2 2 1 2	0.00411
24	2 1 2 1 1 1	0.00397
25	1 1 2 1 1 2	0.00318
26	2 1 1 1 2 2	0.00292
27	2 1 1 2 2 2	0.00286
28	2 2 1 1 2 2	0.00201
29	1 2 2 2 2 1	0.00167
30	2 1 2 2 1 1	0.00149
31	1 1 1 2 2 1	0.00110
32	2 2 2 2 2 1	0.00107
33	2 1 1 2 2 1	0.00097
34	2 2 2 2 1 2	0.00091
35	2 1 1 1 1 1	0.00088
36	2 2 2 2 2 2	0.00066
37	1 1 1 2 1 2	0.00037
38	1 1 1 1 2 2	0.00019
38	1 1 2 2 1 1	0.00012

Appendix 6

Table 1: The sequence of primers used for genotyping *AGTR1* A1166C and L191L

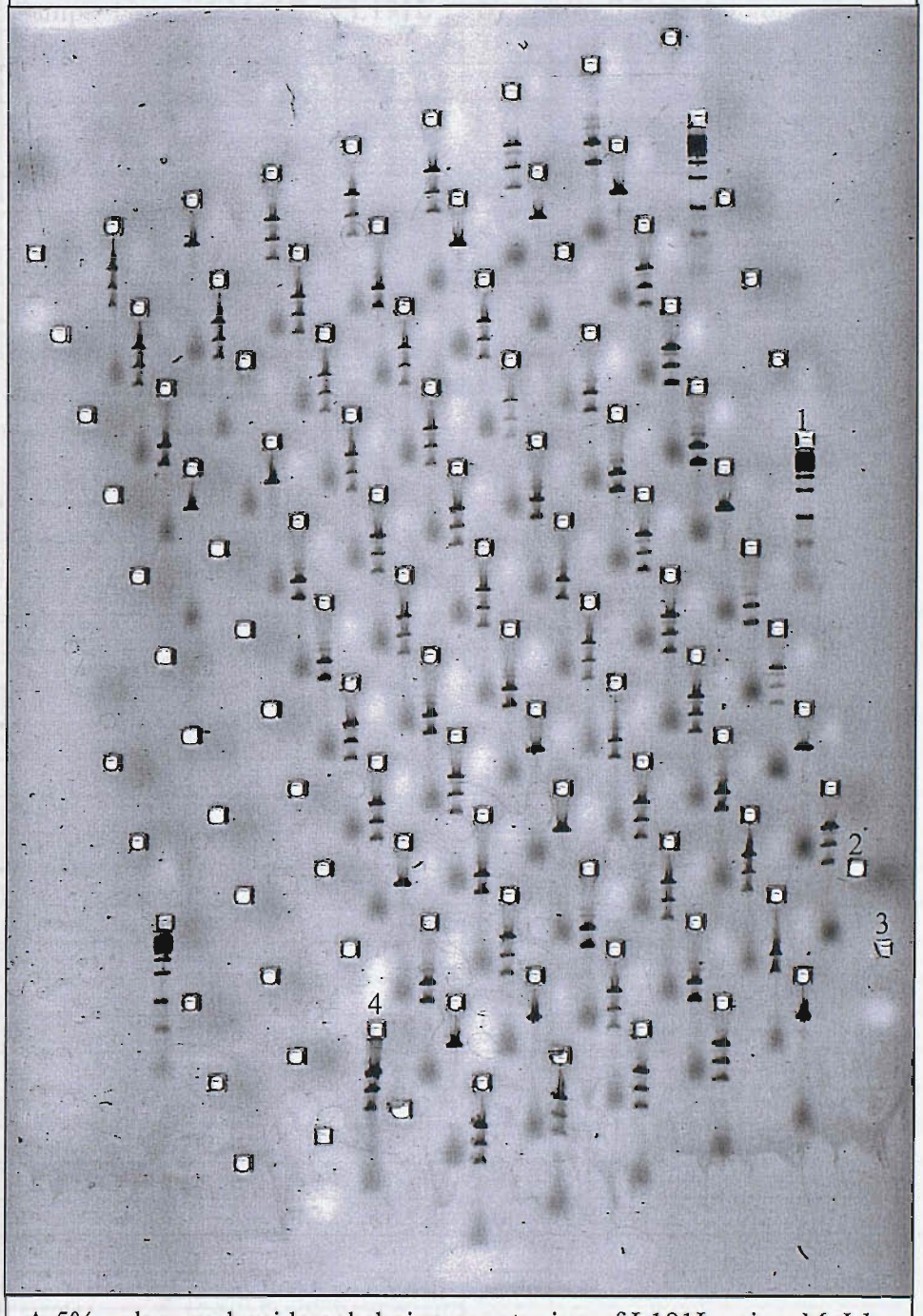
AGTR1 A1166C, Forward:	5′ - TGTGAAAGAAGGAGCAAGAGAA - 3′
AGTR1 A1166C, Reverse:	5′ - GAGCAGCCGTCATCTGTCTA - 3′
AGTR1 L191L, Forward:	5′ - TGTAGCCAAAGTCACCTGCAT - 3′
AGTR1 L191L, Reverse:	5′ - ATAAGCCTTCTTTAGGGCCTTC - 3′

Figure 1: Genotyping of A1166C Using *Bpu10I* in Placenta Samples



A 5% polyacrylamide gel showing genotyping of the *AGTR1* A1166C using *Bpu10I* in placenta samples. 1: 100bp ladder, 2 & 3: PCR mix without template. 4: control DNA.

Figure 2: Genotyping of L191L Using *MnI 1* in Placenta Samples



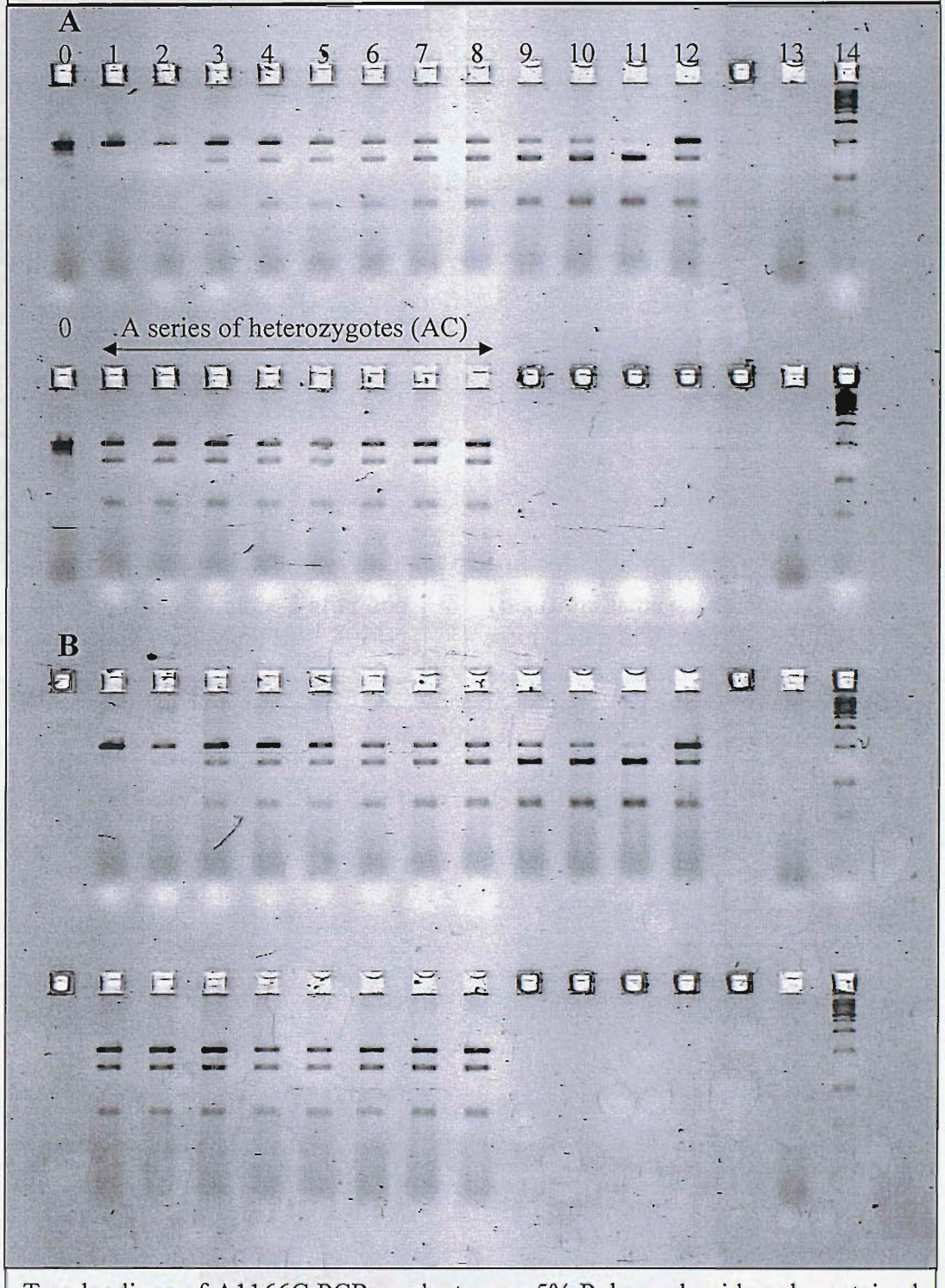
A 5% polyacryolamide gel shoing genotyping of L191L using *MnI 1* in placenta samples. 1: 100bp ladder, 2 & 3: PCR mix without template (-ve con), 4: Control DNA.

Table 2: Represents the distribution of genotypes of A1166C and L191L in placenta samples

No.	Well No.	Sample No.	A1166C	L191L	No.	Well No.	Sample No.	A1166C	L191L
1	A1 (1)	1	12	22	38	D4 (40)	39	12	12
2	A2 (2)	2	11	11	39	D5 (41)	40	12	12
3	A3 (3)	3	12	12	40	D6 (42)	41	12	12
4	A4 (4)	4	12	12	41	D7 (43)	42	12	22
5	A5 (5)	5	22	22	42	D8 (44)	43	11	11
6	A6 (6)	6	11	11	43	D9 (45)	52	11	11
7	A7 (7)	7	22	22	44	D10(46)	59	12	22
8	A8 (8)	8	12	12	45	D11(47)	61	11	12
9	A9 (9)	9	11	11	56	D12(48)	62	11	12
10	A10(10)	10	11	12	47	E1 (49)	64	11	12
11	B1 (13)	11	11	12	48	E2 (50)	65	11	11
12	B2 (14)	12	11	11	49	E3 (51)	77	12	12
13	B3 (15)	13	12	12	50	E4 (52)	78	12	12
14	B4 (16)	14	12	22	51	E5 (53)	79	11	12
15	B5 (17)	16	12	22	52	E6 (54)	80	11	12
16	B6 (18)	17	11	12	53	E7 (55)	87	11	12
17	B7 (19)	18	11	12	54	E8 (56)	91	22	22
18	B8 (20)	19	12	12	55	E9 (57)	92	12	12
19	B9 (21)	20	22	22	56	E10(58)	93	12	22
20	B10(22)	21	12	12	57	E12(60)	103	11	11
21	B11(23)	22	12	22	58	F1 (61)	106	12	12
22	B12(24)	23	11	11	59	F2 (62)	108	11	11
23	C1 (25)	24	11	12	60	F3 (63)	109	11	12
24	C2 (26)	25	11	11	61	F4 (64)	110	11	12
25	C3 (27)	26	12	12	62	F5 (65)	112	11	11
26	C4 (28)	27	11	12	63	F6 (66)	113	22	22
27	C5 (29)	28	12	22	64	F7 (67)	114	12	22
28	C6 (30)	29	22	22	65	F8 (68)	116	11	12
29	C7 (31)	30	11	12	66	F9 (69)	117	11	12
30	C8 (32)	31	22	22	67	F10(70)	119	11	11
31	C9 (33)	32	11	12	68	F11(71)	120	12	22
32	C10(34)	33	11	12	69	F12(72)	125	11	11
33	C11(35)	34	12	22	70	G1 (73)	133	11	12
34	C12(36)	35	12	12	71	G2 (74)	136	11	12
35	D1 (37)	36	11	12	72	G3 (75)	140	11	12
36	D2 (38)	37	12	22	73	G4 (76)	141	11	22
37	D3 (39)	38	11	12					

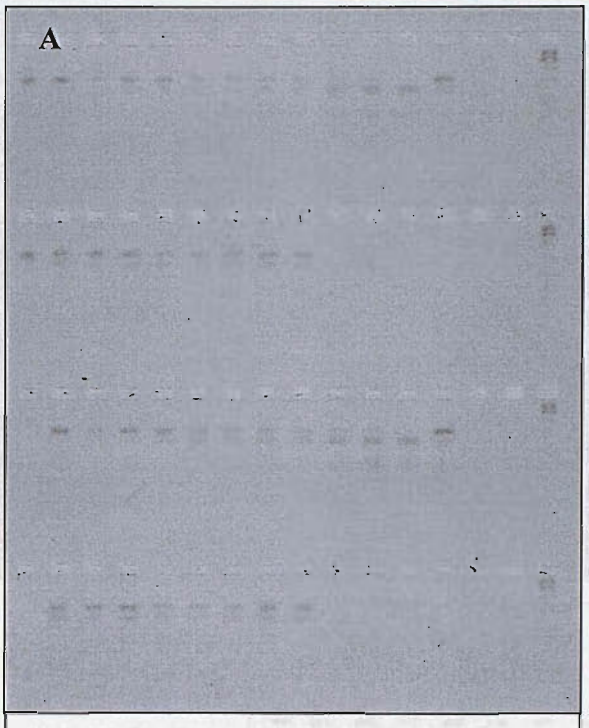
Well No.= Well number: refer to Figures 1 and 2 [in the each given plate, there are 8 rows (A to H) and 12 columns. Thus, well A1 refers to the first loaded well on the top right of the gel]. Sample No. = Sample number: the number of the placenta sample in our bank.

Figure 3: The Gel Used for Making Standard Curve for A1166C

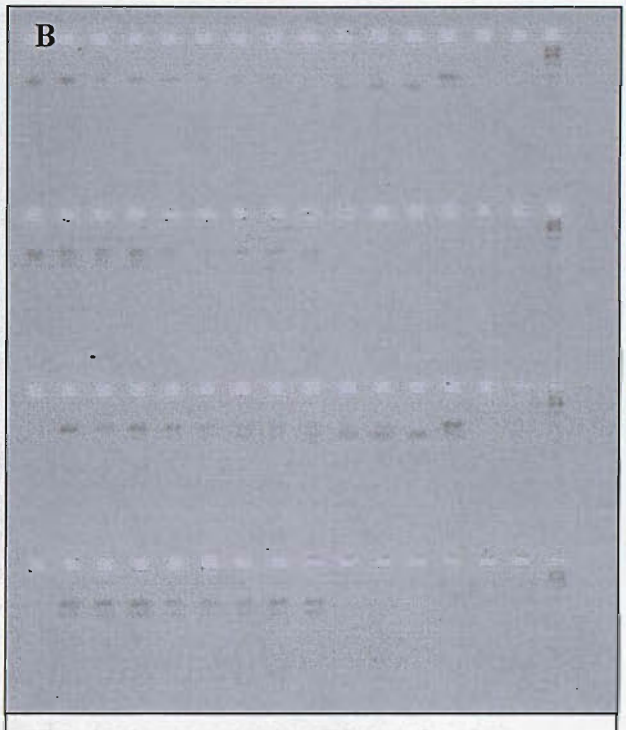


Two loadings of A1166C PCR products on a 5% Polyacrylamide gel prestained with ethidium bromide used for making standard curve. A (top row): 0: undigested, 1 to 11: serial dilutions of AA and CC, 12: AC, 13: Negative control, 14: 100bp ladder. A (lower row): 0: undigested, a series of different heterozygous.
B: 2nd loading of the same experiment as A.

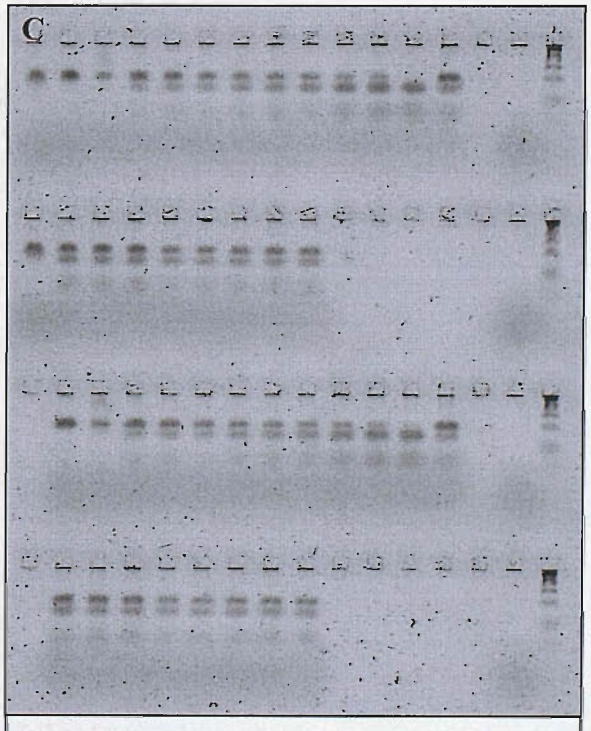
Figures 4: Gels Used to Make Standard Curve for *AGTR1* A1166C



This is the same gel as Figure 3 after one step of destaining with $MgSO_4$.



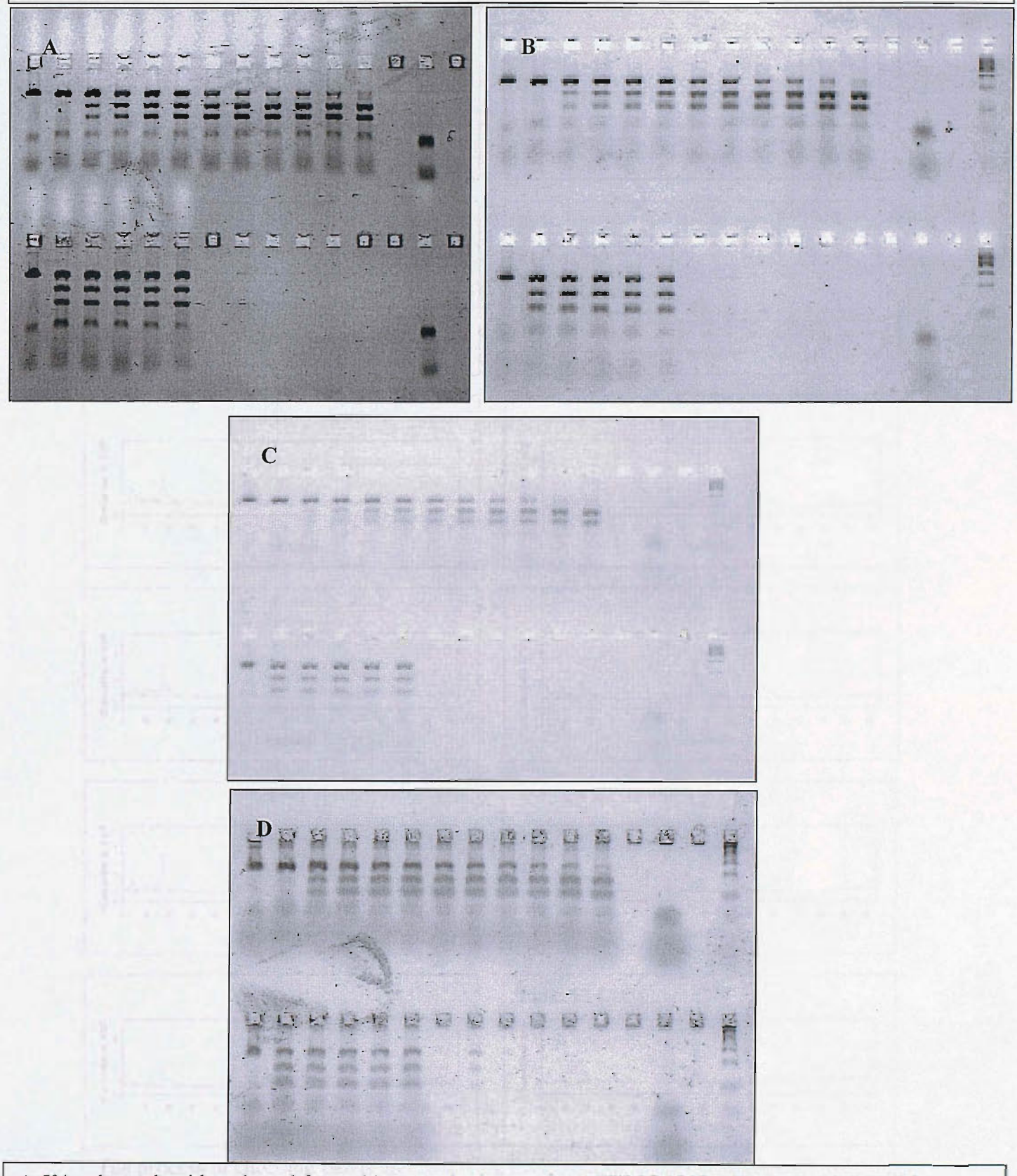
This is the same gel as Figure 3, after second step of destaining with $MgSO_4$.



This is the same gel as Figure 3, after re-staining with Vistra Green™.

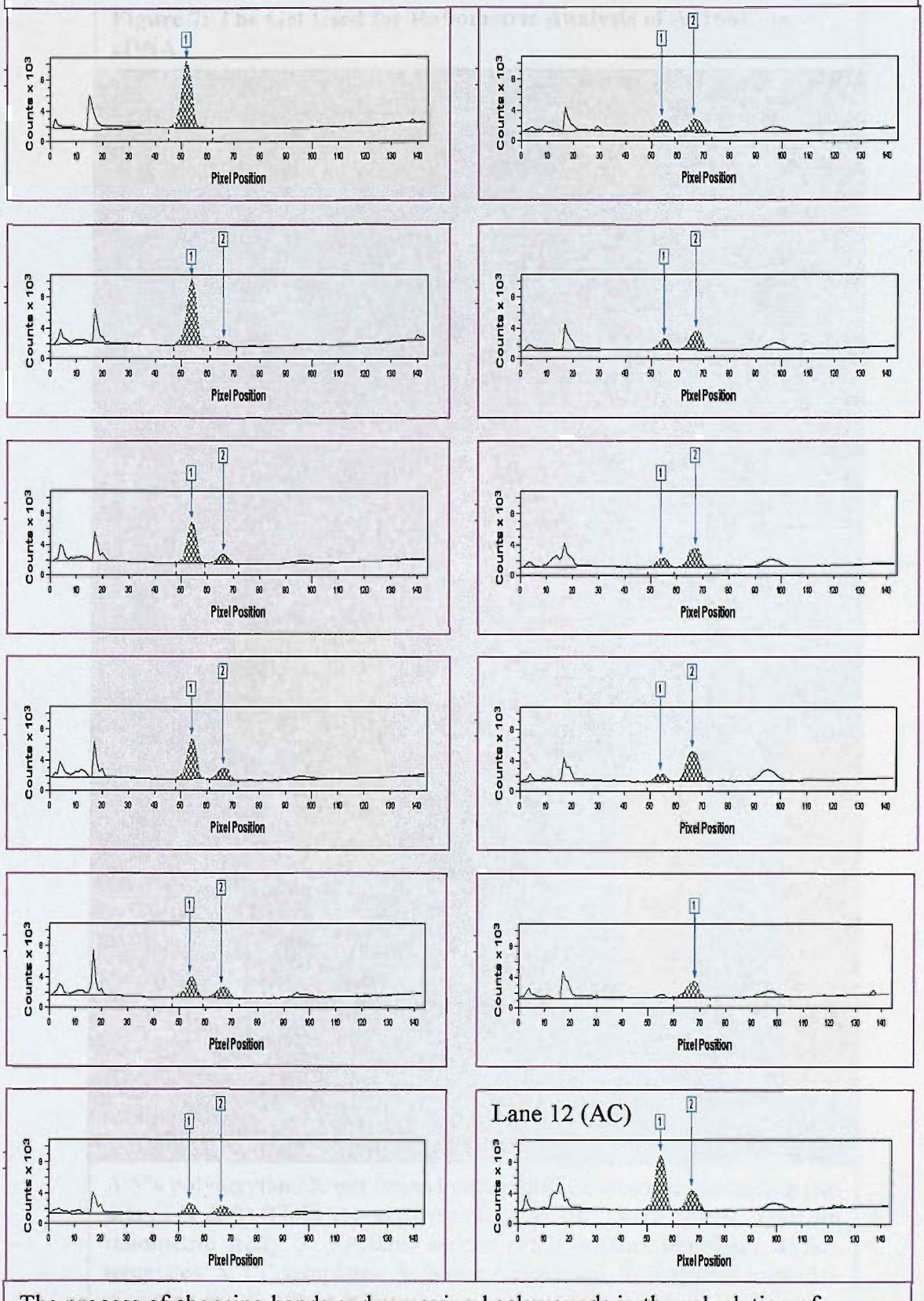
A 5% polyacrylamide gel used for making standard curve for *AGTR1* A1166C; after one (A) and two steps of destaining (B) and restaining with Vistra Green™ (C).

Figure 5: Gels Used to Make Standard Curve for L191L



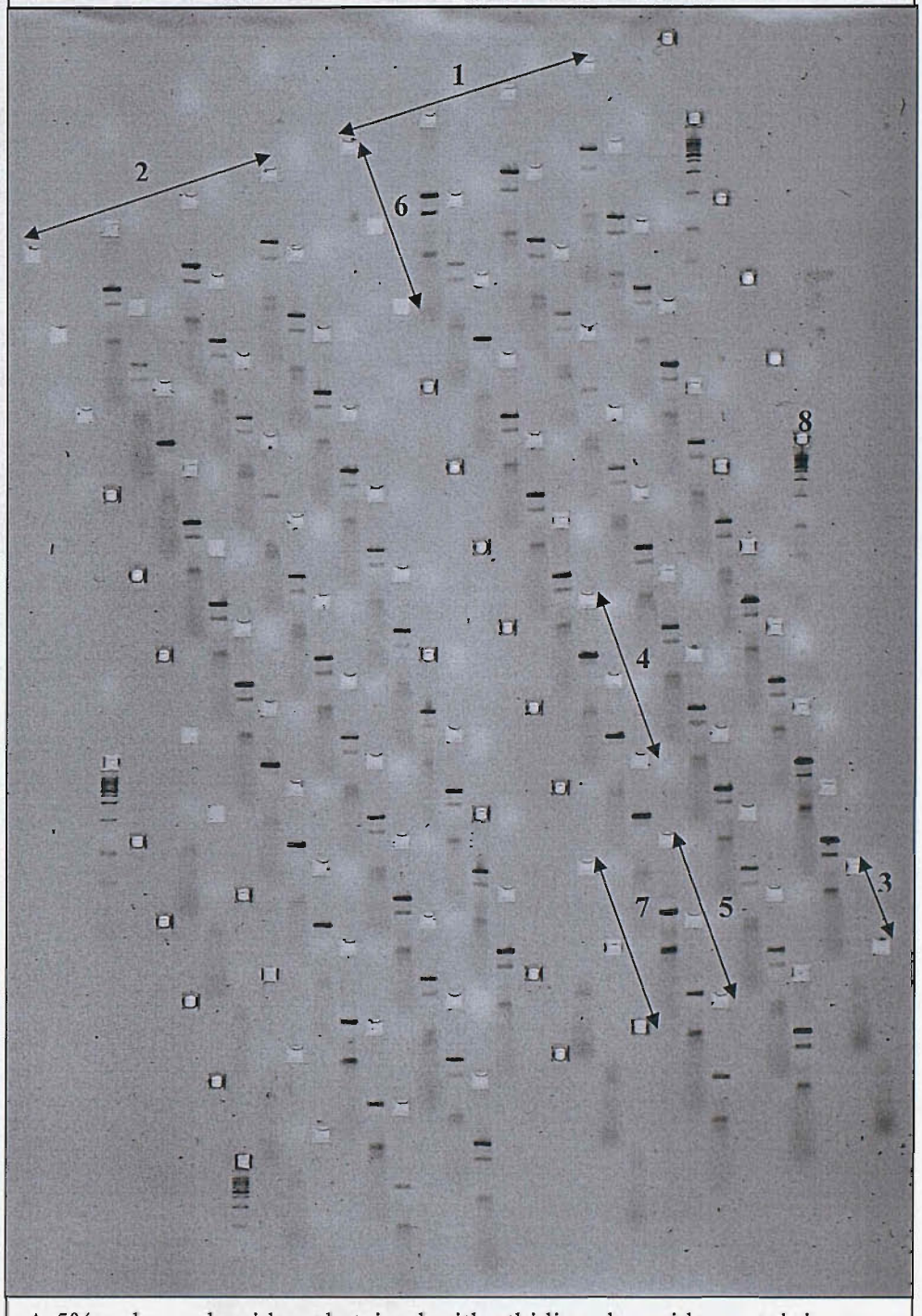
A 5% polyacrylamide gel used for making standard curve for *AGTR1* L191L, showing staining with ethidium bromide (A), two steps of destaining (B&C) and re-staining with Vistra Green™ (D). Each set represents Figure 7.5 in the text.

Figure 6: The Method of Detecting Bands and Removing Background in Phoretix



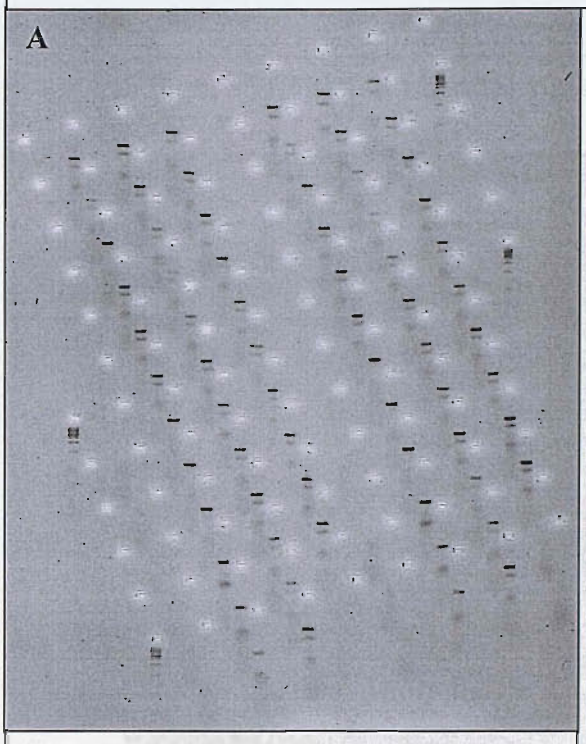
The process of choosing bands and removing backgrounds in the calculation of standard curve and ratiometric analyses in Phoretix software. 1 to 12: represents Figure 7.4 in the text.

Figure 7: The Gel Used for Ratiometric Analysis of A1166C on cDNA

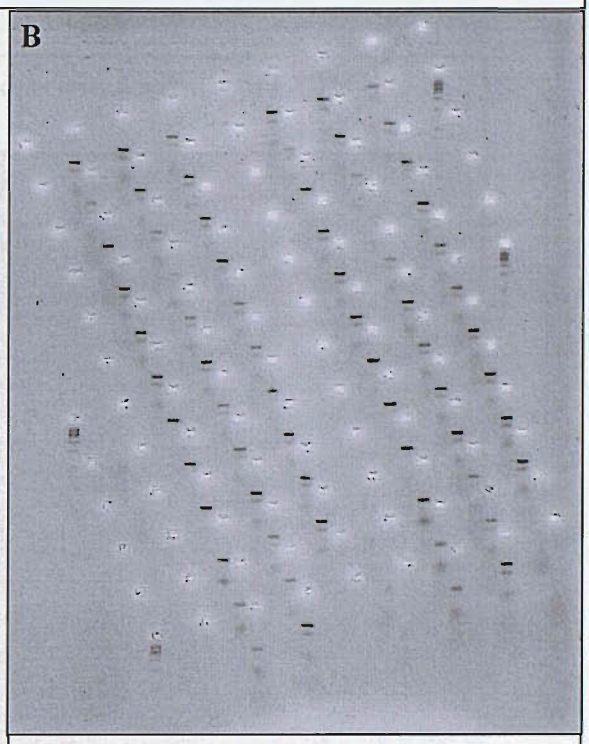


A 5% polyacrylamide gel stained with ethidium bromide containing two sets of (1 & 2) RT-PCR and digestion of A1166C with *Bpu10 I* used for ratiometric study. 3: Negative control (PCR without template), 4: AA templates, 5: CC templates, 6: Water + digestion, 7: Negative control + digestion and 8: 100bp ladder.

Figures 8: Gels Used for Ratiometric Analysis of A1166C on cDNA



This is the same gel as Figure 7 after first destaining step.

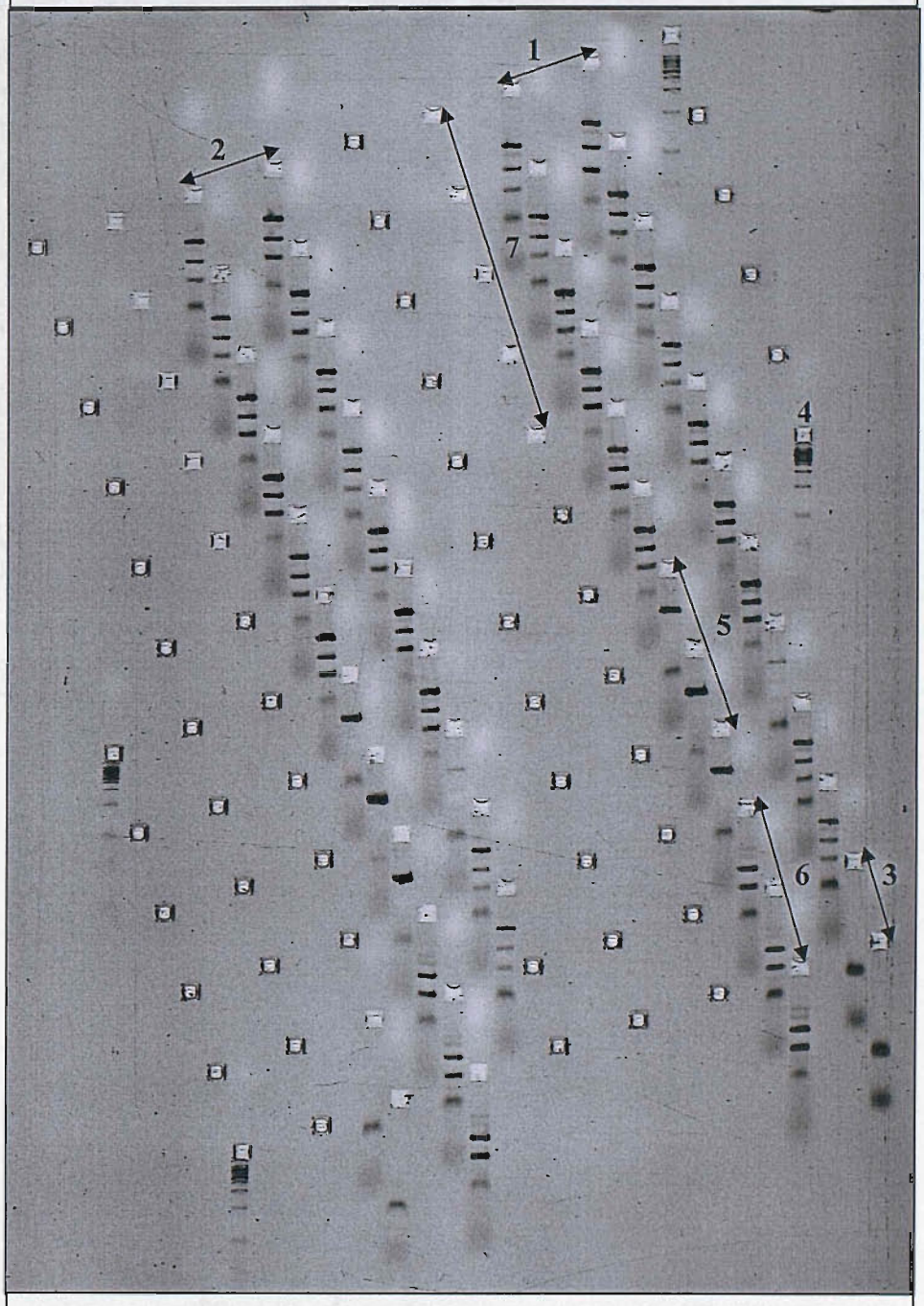


This is the same gel as Figure 7 after second destaining step with $MgSO_4$.

This is the same as Figure 7 after restaining with Vistra Green™.

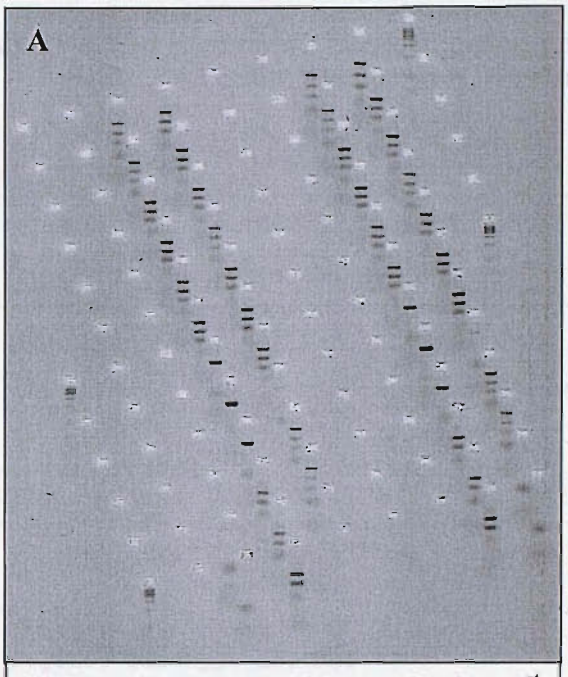
The above gels show the same gel as Figure 7 after one step (A) and second step (B) of destaining and restaining with Vistra Green™ (C).

Figure 9: The Gel Used for Ratiometric Analysis of L191L on cDNA

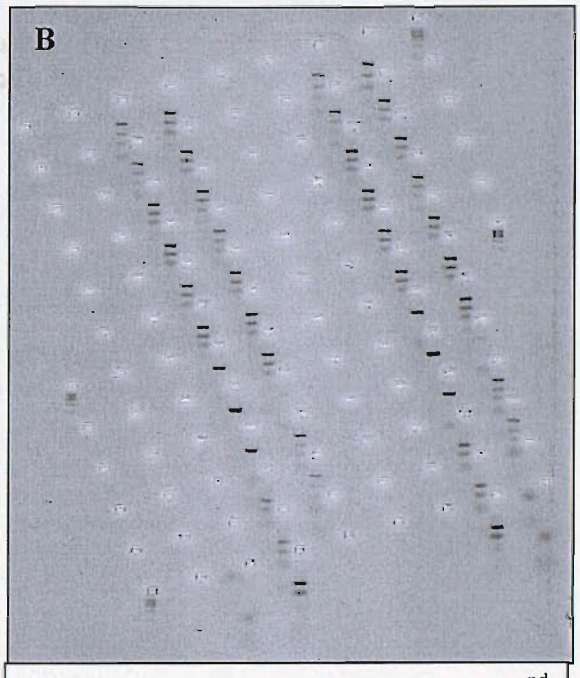


A 5% polyacrylamide gel containing two sets 1 & 2 (duplication) of L191L digestion with *Mnl 1* on double heterozygouus (for A1166C and L191L) cDNA templates.
3: Negative control, 4: 100bp ladder, 5: TT templates, 6: CC templates, 7: Water templates.

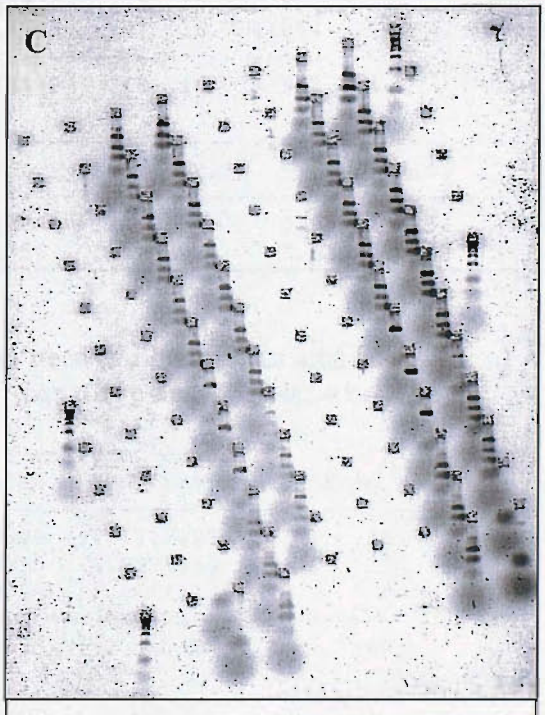
Figures 10: Gels Used for Ratiometric Analysis of L191L on cDNA



This is the same as Figure 9 after 1st step of destaining.



This is the same as Figure 9 after 2nd step of destaining.



This is the same as Figure 9 restained with Vistra Green™.

The above gels are the same gel as Figure 9 after one step (A) and two steps (B) of destainng and restating wih Vistra Green™.

ANOVA and Regression between genotypes and Delta Ct. of L191L

```
. oneway deltaCT Genotype, tabulate
```

Genotype	Summary of deltaCT Mean	Std. Dev.	Freq.
11	6.4609031	2.3627039	19
12	6.9114127	2.3857428	37
22	7.3871374	1.7046695	13
Total	6.8769886	2.2588832	69

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	6.7165348	2	3.3582674	0.65	0.5246
Within groups	340.257103	66	5.15541066		
Total	346.973638	68	5.1025535		

Bartlett's test for equal variances: chi2(2) = 1.8380 Prob>chi2 = 0.399

```
. regress Genotype deltaCT
```

Source	SS	df	MS		
Model	15.8782821	1	15.8782821	Number of obs	= 69
Residual	1124.5565	67	16.7844254	F(1, 67)	= 0.95
Total	1140.43478	68	16.7710997	Prob > F	= 0.3342
				R-squared	= 0.0139
				Adj R-squared	= -0.0008
				Root MSE	= 4.0969

Genotype	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
deltaCT	.213921	.2199404	0.97	0.334	-.2250818	.6529239
_cons	12.13756	1.590909	7.63	0.000	8.962095	15.31303

ANOVA and Regression between genotypes and Delta Ct. of A1166C

```
. oneway deltaCT genotypes, tabulate
```

genotypes	Summary of deltaCT Mean	Std. Dev.	Freq.
11	6.4458849	2.1712128	36
12	6.7456055	2.9824713	27
22	8.329569	1.8114118	6
Total	6.7269655	2.517407	69

```
                    Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------
Between groups      18.2636369       2   9.13181845      1.46     0.2395
 Within groups       412.67536      66   6.25265697
------------------------------------------------------------------------
    Total           430.938997      68   6.33733819

Bartlett's test for equal variances:  chi2(2) =   3.7853  Prob>chi2 = 0.151
```

```
. regress genotypes deltaCT

      Source |       SS       df       MS              Number of obs =      69
-------------+------------------------------           F(  1,    67) =    2.92
       Model |  26.2088138     1  26.2088138           Prob > F      =  0.0921
    Residual |   601.44336    67  8.97676657           R-squared     =  0.0418
-------------+------------------------------           Adj R-squared =  0.0275
       Total |  627.652174    68  9.23017903           Root MSE      =  2.9961

------------------------------------------------------------------------------
   genotypes |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
     deltaCT |   .2466129   .1443285     1.71   0.092     -.041468    .5346938
       _cons |   10.68887   1.035727    10.32   0.000      8.62155    12.75619
------------------------------------------------------------------------------
```

ANOVA and Regression between haplotypes and Delta Ct. of L191L

```
. oneway dct_l191l haplotype, tabulate

           |        Summary of dct_l191l
  haplotype |        Mean   Std. Dev.       Freq.
------------+------------------------------------
         11 |   7.3871374   1.7046695          13
         12 |   6.5261928   2.3447381          22
         13 |   7.4764019   2.4112397          15
         23 |   5.8956933    2.486434          12
         33 |   7.6460334   2.0054057           6
------------+------------------------------------
      Total |   6.8879349   2.2738337          68

                    Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------
Between groups      26.5757042       4   6.64392605      1.31     0.2765
 Within groups       319.83573      63   5.07675761
------------------------------------------------------------------------
    Total           346.411434      67   5.17031991

Bartlett's test for equal variances:  chi2(4) =   2.0399  Prob>chi2 = 0.728

. regress dct_l191l haplotype

      Source |       SS       df       MS              Number of obs =      68
-------------+------------------------------           F(  1,    66) =    0.07
       Model |  .350207935     1  .350207935           Prob > F      =  0.7969
    Residual |  346.061226    66  5.24335191           R-squared     =  0.0010
-------------+------------------------------           Adj R-squared = -0.0141
       Total |  346.411434    67  5.17031991           Root MSE      =  2.2898

------------------------------------------------------------------------------
   dct_l191l |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   haplotype |  -.0105745   .0409168    -0.26   0.797    -.0922676    .0711185
       _cons |   7.055261   .7044839    10.01   0.000     5.648714    8.461808
------------------------------------------------------------------------------
```

ANOVA and Regression between haplotypes and Delta Ct. of A1166C

```
. oneway dct_a1166c haplotype, tabulate

           |        Summary of dct_a1166c
  haplotype |        Mean   Std. Dev.       Freq.
------------+------------------------------------
         11 |   6.8020205   2.2664593          13
         12 |   6.2593047   2.1894053          22
         13 |   7.4256756    3.206075          15
         23 |   5.8955179   2.5541315          12
         33 |    8.329569   1.8114118           6
------------+------------------------------------
      Total |   6.7388196   2.5341832          68

                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------
Between groups      35.9038496      4   8.97596239      1.43     0.2332
 Within groups      394.375826     63   6.25993375
------------------------------------------------------------------------
    Total           430.279676     67   6.42208471

Bartlett's test for equal variances:  chi2(4) =   3.6651  Prob>chi2 = 0.453

. regress dct_a1166c haplotype

      Source |       SS       df       MS              Number of obs =      68
-------------+------------------------------           F(  1,    66) =    0.48
       Model |  3.10417985     1  3.10417985           Prob > F      =  0.4910
    Residual |  427.175496    66    6.472356           R-squared     =  0.0072
-------------+------------------------------           Adj R-squared = -0.0078
       Total |  430.279676    67  6.42208471           Root MSE      =  2.5441

------------------------------------------------------------------------------
  dct_a1166c |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
   haplotype |   .0314826   .0454599     0.69   0.491     -.059281    .1222462
       _cons |   6.240654   .7827043     7.97   0.000     4.677934    7.803373
------------------------------------------------------------------------------
```

HTR Results

1. L191L

```
Estimated haplotype frequencies:
1_1 :    0.26087
1_2 :    0.282609
2_1 :    0.456522
2_2 :    1.28372e-07

Max log-likelihood among 11 restarts: -109.628

Haplotype 1_1 mean:      6.29416
Haplotype 1_2 mean:      7.04222
Haplotype 2_1 mean:      7.10774
Permutation-based tests
Overall p-value is 0.102
Ind haplotype 1_1 :      p-value is 0.0319
Ind haplotype 1_2 :      p-value is 0.6015
Ind haplotype 2_1 :      p-value is 0.2545
```

2. A1166C

```
DELTA Ct of A1166C

Estimated haplotype frequencies:
1_1 :    0.26087
1_2 :    0.282609
2_1 :    0.456522
2_2 :    1.28372e-07

Max log-likelihood among 11 restarts: -109.628

Haplotype 1_1 mean:      6.11924
Haplotype 1_2 mean:      7.23298
Haplotype 2_1 mean:      6.76099
Permutation-based tests
Overall p-value is 0.0956
Ind haplotype 1_1 :      p-value is 0.046
Ind haplotype 1_2 :      p-value is 0.1423
Ind haplotype 2_1 :      p-value is 0.8769
```

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