The Inheritance of Atherosclerosis and Left Ventricular Structure: a Scottish Twin study

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Summary

Cardiovascular disease is a major cause of morbidity and mortality in Scotland. Coronary atherosclerosis alone accounts for 450 premature deaths per 100,000 males and 180 per 100,000 in females each year. The pathological processes underlying these clinical manifestations of cardiovascular disease are multifactorial in causation. However, it is unclear to what extent the Scottish population has a genetic predisposition to these disorders.

Twin studies are a highly informative method of delineating the varying genetic and environmental contributions to non-Mendelian parameters. Utilising a population of monozygotic and dizygotic twins allows the contribution of genetic factors to be quantified by comparing intra-class correlations between the monozygotic and dizygotic groups. Previous twin studies have primarily evaluated the genetic influences over known cardiovascular risk factors but little is known about the interplay of these factors in a population from the West of Scotland with its high prevalence of cardiovascular pathology. Few studies have focused on the inheritance of the actual extent of arterial disease. The determination of degree of heritability over a given phenotype is an important first step before embarking on the investigation of potential candidate genes.

The cohort investigated in this study consisted of 151 pairs of monozygotic and dizygotic twins aged 30-85 years old (82 monozygotic pairs and 68 dizygotic pairs). All pairs were recruited from the general population and

were of Caucasian origin. Using a classic twin methodology the heritability of a wide range of cardiovascular phenotypes were assessed.

Resting and 24-hour assessments of blood pressure were performed. 24-hour measures of diastolic and mean blood pressure were under a significant degree of genetic control. This was particularly true for night-time measurements (night-time mean and diastolic pressures - heritability of 0.53 ($p=0.03$) and 0.58 ($p=0.02$) respectively). The degree of heritability was stronger for these parameters than resting or 24-hour measures of systolic blood pressure.

Genes were important in the aetiology of left ventricular (LV) mass in this cohort. Heritability for LV mass was 0.69 ($p=0.008$). This genetic influence was not explained by similarities in age, sex, size or blood pressure and persisted following correction for these potential confounders ($h_2 = 0.53$; $p=0.006$).

Impaired lung function, as measured by spirometry, is a risk factor for cardiovascular events. Intra-class correlation coefficients were measured for FEV1 and FVC in the monozygotic and dizygotic groups. The baseline data and the data corrected for height both showed a significant percentage of the variability in these measurements were under genetic control. The heritability estimates for the height corrected spirometry variables were 0.61 ($p=0.02$) for FEV1 and 0.71 ($p=0.007$) for FVC. When these variables were

expressed as a percentage of their predicted value only the FEV1 result remained significant (h_2=0.61, p=0.002).

The genetic contribution towards the extent of atheroma formation was assessed using B-mode ultrasound of the carotid arteries. This technique measures carotid intima media thickness a marker of atherogenesis in the cerebral and coronary beds. None of the carotid measurements revealed any degree of genetic control. Environmental and familial influences appeared to be more important than narrow-sense heritability in the determination of carotid intima media thickness.

Numerous biochemical and haematological parameters were assessed in the twin cohort. A genetic basis was established for many of them including Lipoprotein A, HDL-Cholesterol, urate, haematocrit and platelet level.

In summary this population-based adult twin study, which recruited subjects from a high-risk catchment area (the West of Scotland) demonstrated that many important risk factors are genetically determined. Candidate gene investigations should be directed towards the study of phenotypes with the strongest heritability estimates. A clearer understanding of the aetiology of, and relationships between, the risk factors investigated in this study is necessary to enable effective targeting of preventative and therapeutic strategies.

Table of contents

List of Tables

List of Figures

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Chapter 1

Introduction

1.1 Cardiovascular Morbidity and Mortality in Scotland

Between 1970 and 1992 total cardiovascular disease mortality in Europe decreased annually by 1.5% but despite such improvements Scotland remains in the top 7 for cardiovascular deaths for both men and women (of 29 countries examined by the European Society of Cardiology Task Force on Cardiovascular Mortality and Morbidity Statistics in Europe). In 1990-1992 ischaemic heart disease death rates were 655 per 100,000 males in Scotland with the corresponding mortality rate in France of 142 per 100,000. A similar pattern is seen for cerebrovascular accidents with 139 and 67 per 100,000 for males and 107 and 35 for females (Sans S et al, 1997). The only countries with a higher mortality rate for coronary artery disease than Scotland were those of the former Soviet block, for example Latvia and Estonia, and ex-Czechoslovakia. In 1995 7609 men and 6167 females were recorded as dying from coronary heart disease in Scotland (Information and Statistics Division of the NHS in Scotland Review, 1997).

Scotland's predisposition to coronary disease is obvious even at a national level with a distinct North-South gradient in the UK. Mortality rates in Scotland are almost 50% higher than those seen in England and Wales. Death rates from ischaemic heart disease, in 1990-92, for women aged 45-74 were 273 per 100,000, compared with 190 for England and Wales. The corresponding mortality figure for France being only 36 per 100,000.

The prevalence of atherosclerotic related deaths in Scotland is not fully understood but this unenviable position persists despite increasing

awareness of the importance of primary prevention (Wallis EJ et al, 2000). It is still unclear the extent to which this population possesses a genetic predisposition to coronary disease. Other European countries such as Finland have made a significant impact on their mortality rates over the last 20 years (Rastenyte D et al, 1992). A fuller understanding of why the Scottish population is susceptible to such disease processes may permit a similar improvement in the health of the nation.

1.2 Genetic Epidemiology and the study of non-Mendelian disorders

1.2.1 Introduction

Familial clustering of coronary events is well recognised (Friedlander Y et al, 1985) as is the correlation amongst relatives of many of the intermediate risk factors associated with atherosclerosis (Hunt SC et al, 1989). A positive family history of cardiac events is known to be a strong independent risk factor for coronary artery disease (Snowden CB et al, 1982) and a parental history of myocardial infarction doubles the offspring's chance of having an infarct (relative risk of 2.2) (Colditz GA et al, 1991). In addition the risk of offspring myocardial infarction is inversely related to the age of the parent at the time of their infarction (Jousilahti P et al, 1996). Early cardiac events are, in particular, familial and there is evidence of diminishing genetic influence with age (Nguyen TD et al, 1998).

Even within the first 12 months of life inheritable differences in coronary anatomy are evident. Autopsy studies have shown luminal narrowing in left and right coronary arteries in infants with a family history of coronary

disease when compared with those with a negative family history (Kaprio J et al, 1993). This familial effect may exert its influence at various stages of the pathological development of atherosclerosis but little is known regarding the precise genetic loci or gene products modulating this effect.

Atherosclerosis, the process underlying 95% of coronary artery disease, is multifactorial in origin. The use of this phraseology may, however, disguise the incomplete understanding of the cellular triggers for this widespread pathological process. The recognition of modifiable and non-modifiable risk factors for atherosclerosis has greatly enhanced the understanding of cardiac disease and has led to new interventions that have already impacted on the clinical management of ischaemic heart disease. The West of Scotland Coronary Prevention Study (Shepherd J et al, 1995) and many other primary and secondary prevention lipid studies are a direct result of the identification of LDL cholesterol as a modifiable risk factor. Targeted intervention in the form of statin therapy was shown to improve both morbidity and mortality in a Scottish population (Capewell S et al, 1999). In the future a similar series of events may well occur with mediators such as fibrinogen (Lip GY, 1995) and homocysteine (Vollset SE et al, 2001).

As has been stated above there is evidence that the atherosclerotic process begins within the first few months of life. There is therefore a tremendous opportunity to identify and target the peri-natal triggers of this process. There is universal agreement that environmental factors such as smoking or lack of exercise have a major contribution to the occurrence of ischaemic

heart disease but it is equally true that there are also important inherent differences amongst individuals that also contribute. It is therefore necessary to unravel the relationships between and significance of these two facets (Chen CJ et al, 1984).

Human genetics has for many years been preoccupied with the study of single locus traits. There is increasing recognition, however, that a greater proportion of human diseases do not stem from such single gene defects but rather those in which the appearance of the disorder is the last stage in a long pre-clinical pathological prodrome. For this reason the attentions of genetic epidemiologists have turned towards diseases that are: 1. common and heterogeneous in nature; 2. variable in age of onset; 3. modulated by environmental or lifestyle factors and 4. which cluster in families. The major disease processes affecting the Scottish population e.g. ischaemic heart disease, hypertension, diabetes and many cancers, are potential targets for this form of work. It is the role of the genetic epidemiologist, in co-operation with the clinician, to concentrate not only on the genetic loci triggering these disease processes but to dissect out the complex tapestry of gene-gene and gene-environment interactions (Schork NJ, 1997).

There is now a vast amount of information on the epidemiology of cardiovascular disease. Large national and international population-based studies, such as the British Regional Heart Study (Shaper AG et al, 1981) and MONICA studies (Tunstall-Pedoe H, 1985) have identified a plethora of risk factors. The Scottish Heart Health Study documented a league table of

the 27 most important risk factors for cardiac events in men and women in a Scottish population (Tunstall-Pedoe H et al, 1997). The wealth of knowledge acquired from these types of studies show how environmental, familial and genetic factors all influence the clinical phenotypes of heart attack, angina and stroke. Further to these descriptive studies the subsequent steps involve determining the aetiology of these associations; the causality; the genetic determination; the clinical significance and the public health implications. The goal is therefore to decipher the genetic architecture responsible for the distribution of each risk factor trait by defining the number of genes involved, the frequency of alleles of each gene, the impact of the alleles on the level and variability of the trait and the inter-relationship between the traits. A genetic architecture involving many genetic loci each having polymorphic alleles, which in themselves contribute little effect, is not an unreasonable model for atherosclerosis and cardiovascular disease. It is thought, for example, that there are over 200 genes involved in the regulation of simple lipid metabolism; only one aspect of the atherosclerotic disease process (Goldbourt U et al 1986).

1.2.2 The "Top-down" unmeasured genotype approach

"Top-down" genetic epidemiology strategies are designed to answer the question of whither the segregation of unmeasured loci contributes to the variance in a trait in a population within a given environment (Schull WJ et al, 1980). This methodology includes studies of twins, nuclear families, and of extended pedigrees. "Top-down" analysis begins with establishing the impact of unmeasured genetic variation on inter-individual phenotypic

variability. A top-down biometrical study is useful in generating statistical evidence of a genetic input into a trait's determination (heritability). The estimation of heritability allows future investigation to be directed towards those traits, or disease processes, that are most likely to be under significant genetic influence. From this a functional candidate approach (e.g. studying the effect of a particular genotype such as the ACE genotype) or a genome wide search can be employed to identify individual genes, or gene products, that confer cardiovascular risk. These findings allow a clearer understanding of the cellular triggers of disease initiation and perpetuation.

When a "top-down" approach has studied a particular population's risk factor profile the information can be used to focus preventative health care resources. For example if such a study revealed that women from the West of Scotland had LDL cholesterol levels and body mass indexes that were highly inheritable and systolic blood pressure with a low heritability then it may be more fruitful to direct primary health care prevention towards the more modifiable risk factors.

1.3 Twin Studies - Theory

1.3.1 An introduction to twin studies

Twin studies are an informative method of delineating the varying genetic and environmental components of a non-Mendelian parameter (Hawkes CH et al, 1997). It was Galton, a cousin of Charles Darwin, who over a century ago first called attention to the usefulness of twin pairs in the study of the nature/ nurture debate (Galton F, 1869). The importance of monozygotic

twins comes from the fact that, barring mutation, identical twins have identical genotypes. Any dissimilarity, therefore, must be due to the influence of environmental stimuli. Most biological parameters are, to a greater or lesser degree influenced by both genetic and environmental factors, be that pre or post-natal. This genetic effect exerts itself in 2 ways. Firstly there is the "narrow sense heritability". Narrow sense heritability is the influence of various genetic loci exerting an equal effect of the parameter under study - the additive genetic effect (Kang KW et al, 1978). This reflects the contribution of heredity to a particular phenotype. Secondly there is the effect of the genotype-environment interaction where there is a genetically determined sensitivity to an environmental or lifestyle factor. An example of this latter form of inheritance is the recently identified interaction between cigarette smoking, the ecNOS gene locus on chromosome 7 and coronary artery disease (Wang XL et al, 1996). Figure 1.1 is a simplified model demonstrating the genetic, shared and non-shared environmental influences on a given phenotype.

1.3.2 Twinning

Twins are produced either from a single ovum (identical or monozygotic) or by two separate ova (non-identical, fraternal or dizygotic). 75% of twins are same sex pairs. Monozygotic twinning, which occurs in 2.3-4 per 1000 pregnancies, is independent of inherited factors, race or maternal age (Bulmer MG et al, 1970). Monozygotic twins, having resulted from the same ova and sperm, share a common genetic make-up. The rare exception to

this is when twinning has occurred very early and subsequently one twin develops a chromosomal error.

Dizygotic twinning is more common and occurs in about 1 in 80 confinements (Murphy M et al, 1997). It has a familial pattern with a maternal transmission and is influences by race and maternal age ((Tong S et al, 1997). Dizygotic twins are as genetically similar as siblings. Dizygotic twins have two chorions and either a fused or double placenta. In many cases inferences can be made about zygosity by careful examination of the placenta and membranes (Husby H et al, 1991).

The splitting of the single fertilised ovum can occur at several different time points. Early splitting before the morula stage (5 days after fertilisation) results in the two embryos having either fused or separate placentas. These embryos have separate chorions (dichorionic) and amnions and account for one third of identical twins. Division between day 5-10, after differentiation of the trophoblast but before amnion formation, results in one placenta and chorion but 2 amnions. This is the most common finding in monozygotic twinning. Occasionally later division, after amnion formation, results in a single placenta, chorion and amnion. Later twinning results in conjoint and incomplete twinning.

The peri-natal mortality rate for twins is 4-5 times that of singleton pregnancies (Glinianai SV et al, 2000). Twins experience an increased risk of intra-uterine growth retardation (Phillips DI et al, 1999), prematurity and fetal abnormalities (Spellacy WN et al, 1990). They are on average 900g

lighter than singletons (MacGillivray I et al, 1988). In addition monochorionic, monozygotic twins are lighter than dizygotic twins.

1.3.3 Twin Studies

There are 4 common forms of twin study analysis:

1. The Classic Twins Methodology

The Classic Twins Methodology utilises a population of monozygotic (MZ) and dizygotic (DZ) twins to determine the relative contribution of genetic factors to a given phenotype under investigation. Monozygotic twins share common genetic profiles where as dizygotic twins share only the same proportion of genes as normal siblings i.e. 50%. By comparing the rates of concordance for MZ and DZ groups an estimation of heritability can be determined. Heritability is the degree to which a trait is genetically determined. The classic twin study, one form of top-down analysis, determines the "narrow-sense" heritability i.e. a direct heritability effect that disregards the presence or absence of epistasis (gene to gene interactions) and interactions with the environment. Narrow sense heritability can be estimated by comparing the pair-wise concordance rates for mono and dizygotic pairs. The pair-wise concordance rate is the number of pairs of twins concordant for a given phenotype over the total number of pairs with at least one member with the given phenotype (Kringlen E, 2000). The proband-wise rate, an indication of a twin's risk of being affected by a disorder if its twin is also affected, can also be determined and is the number of affected twins in the concordant pairs over the number of probands (Kyvil KO, 1995).

2. *Co-twin control methodology*

Examination of a cohort of monozygotic twins discordant for a disease permits the "healthy" twin to act as a perfect matched pair control. Given an identical genetic profile, differences in disease manifestation are assumed to be due to intra-pair differences in environmental exposure. This model has been used to study twin pairs discordant for diseases such as diabetes mellitus (Dubrey S et al, 1994).

3. *Biometrical genetic methodology.*

Biometrical analysis involves the study of intermediate risk factors, for example metabolites or normal physical characteristics that may indicate the tendency to develop a particular disease. Genetic models may be fitted to the data to study the covariance and correlations between monozygotic and dizygotic twin pairs.

4. *Twins reared apart*

In theory studies of twins reared apart provide the "gold-standard" genetic model with a direct estimate of heritability uncluttered by environmental influences (Hayakawa K et al, 1987). In this setting residual variance not explained by heredity or a shared environmental rearing is due to non-shared environmental effects unique to the individual. However, in practice it is improbable that twins are placed in random dissimilar environments - an essential to the validity of such a model. In addition reared-apart models do not make amends for twins sharing a similar intrauterine environment and early postnatal experience both of which appear to be of importance

especially when studying a trait with its first sub-clinical manifestations present in the first year of life.

1.3.4 Estimates of Heritability

Heritability is an indicator of the proportion of a phenotypic trait or disease process that is determined by genotype. A myriad of formulae exist to estimate heritability and there is still much debate over the most suitable formula to apply to specific data-sets (Kang KW et al, 1978). As stated above heritability, as calculated from a classic twins methodology, is a measure of narrow-sense heritability and as such must comply to the following assumptions: 1) that the two types of twins have similar environmental influences 2) that the genetic and environmental influences on a trait are not correlated in an individual or between members of a twin set. 3) that there is assortative mating and 4) that the trait is continuously distributed with no dominance and no epistatic variance (i.e. no intra or inter-allelic interactions).

In the present study heritability was estimated using the equation proposed by Falconer (Falconer DS, 1995). The advantage this simple formula is that it eliminates the non-genetic familial effects on twins and provides an easily interpretable estimate of heritability. This methodology has been used extensively in twin studies including those from the NHLBI twin cohort (Reed T et al, 1993).

In Falconer's paper heritability was estimated using the equation:

$$h^2 = 2(rMZ - rDZ)$$

where rMZ is the monozygotic intra-class correlation and rDZ the dizygotic correlation. In this study results are given as intra-class correlations, heritability estimates on raw unadjusted data, and heritability estimates on residual data following correction for confounding variables.

1.4 Twin Studies - Bias and Assumptions

1.4.1 Ascertainment bias

Several recent classic twins studies have been appropriately criticised for lack of understanding of the limitations of twin models and for omitting a balanced discussion regarding the relevance of the study to the wider population (Kylik KO, 1996). Classic twin studies are subject to innate bias and in addition several assumptions are required to permit the functioning of twin study models. These limitations must be appreciated when reviewing twin data (Price B, 1950).

Large population based twin studies from national registers cross-referenced with disease registers allow the identification of large numbers of twins with no significant bias (Herskind AM et al, 1996). Unfortunately such well-documented established registers do not exist outwith a few major centres such as Scandinavia and some US states. Volunteer studies are less systematic but for logistic reasons are commonly employed. Modifications of

volunteer studies omitting to state either the nature of the phenotype, disease under study or the search for twin pairs help to minimise bias. Volunteer recruitment from twin societies or from hospital attenders both skew data - the first with an over-representation of MZ middle-class twins; the second with bias towards those with more severe disease states (Romanov L et al, 1990).

Approximately 60% of British twins born in 1992 were dizygotic (Murphy M et al, 1997). Non-registry based twin studies, however, tend to contain more monozygotic subjects than dizygotes. Lykeen et al, 1987, described the "Rule of 2 Thirds" bias that occurs in volunteer based twin studies. The rule states that 2/3 of patients recruited will be monozygotic and that there will be an over-representation of female twin pairs. This has 2 important implications. Firstly and most simply the study population bias means that the study group is non-representative of the wider population. Secondly a bias population of this nature leads to inaccuracies in heritability scores (Lykeen DT et al, 1987).

Lykeen modified the original self-selection model of Martin and Wilson (Martin NG et al, 1982). This model stated that the inclination to volunteer is a continuously distributed variable, *V*. This variable is known, in singletons, to vary with IQ, social class and numerous other parameters. An individual decides to participate in a study when V exceeds a threshold level. This threshold is determined by factors such as the interest in and rewards from such a study. A twin pair would only be recruited if for both individuals V

exceeded the threshold value. Different rates of MZ volunteering could be explained by either higher mean V for MZ (for example the belief that there is more to be gained from the study or because of a greater interest in twinning) or from a higher correlation for V for MZ than DZ. It may be that concordance to participate reflects concordance in social class, physical attractiveness or personality. If there are large within pair differences in any of these features twin pairs may not volunteer. This may lead to an over-estimation in DZ correlations.

An additional finding that may also explain the "over-representation" of monozygotic twins is the relative increase in MZ, compared with DZ, births. This has been documented in many different countries including much of Europe and Australia (Doherty JD et al, 1986). In England and Wales the monozygotic rate is almost a third higher than it was 50 years ago (Murphy M et al, 1997). Contemporary DZ and MZ twinning rates for the population under study are needed when assessing recruitment bias.

An assessment of sampling bias must be incorporated into twin studies to permit the reader to fully assess the data. When examining same-sex twin pairs the ratio of DZ to MZ twins should reflect that of the population i.e. approximately 1:1. In addition the parameter under investigation must not be significantly different between the 2 MZ and DZ populations and that the mean and variance of a given parameter should be comparable between the 2 groups. Failure to ensure this leads to an error in estimations of heritability. To permit the application of a twins study to a population in

general the twin population should be representative of the wider population and not be demographically skewed.

1.4.2 Zygosity ascertainment

The current gold standard for zygosity determination is the use if a group of microsatellite probes. Each twin has a limited "DNA fingerprint" performed with any dissimilarities in alleles indicating dizygosity. The error from such a method is $< 10^{-5}$ (Inglis GC et al, 1999). The use of blood grouping and secretor status is less accurate but is still correct in over 95% of cases. Many twin studies use validated questionnaires to assess zygosity. In Norwegian twin pairs the use of questions such as "Are you as similar as 2 drops of water?" (the Norwegian equivalent of as similar as 2 peas in a pod) have been reported as being accurate in up to 97% (Magnus P et al, 1983). Such questions commonly suffice for dizygotic twins but some MZ pairs are not completely similar physically and this is known to occasionally lead to misclassification. The determination of zygosity by the examination of fetal membranes is less accurate as both monozygotic and dizygotic twins may have separate placentas and separate chorions.

1.4.3 The Common Environment Assumption

One of the most important classic twin model assumptions is that monozygotic and dizygotic twins share a common environment and that that environment is not dissimilar to the population at large.

The Prenatal Environment

Forsdahl was amongst the first to report that death rates from cardiovascular disease are highest in areas with poor childhood living conditions and that past infant mortality is a marker for current adult cardiovascular disease (Forsdahl A, 1978). There is also a known geographic association between poor maternal health, poor fetal growth and later adult cardiovascular disease. Ischaemic heart disease mortality is associated with low birth weight, low ponderal index and low body mass index at 1 year of age (Eriksson JG et al, 2001). Similarly obstructive lung disease in later life is associated with birth-weight, infections in infancy and weight at 1 year of age (Barker DJ et al, 1991). Many of these associations persist even following correction for height and social class. Following these observations Barker hypothesised that retarded fetal and infant growth are strongly related to adult mortality from heart and lung diseases due to an "adverse intra-uterine environment" and poor fetal nourishment (Barker DJP et al, 1995). This adverse environment was thought to upset critical periods of fetal development when "programming", or crucial rapid growth, of a particular organ occurs therefore triggering the first step in the development of a degenerative disease process.

Barker's hypothesis has been used to form the foundation of much criticism against classic twins methodology. The intra-uterine environment of monozygotic twins may differ substantially from that of the dizygotic twin. Monozygotic twins, if occurring due to early separation, will have separate sets of membranes (dichorionic) and separate placentas as do dizygotic

twins. Monozygotic monochorionic twins are known to be 107-229g lighter at birth than dizygous or monozygous dichorionic twins even after correcting for their shorter gestation and Phillips used these facts to question the validity of twins studies (Phillips DI et al, 1993).

If impaired intra-uterine growth is associated with an increased risk of adult pathology, as suggested by Barker, then monozygotic monochorionic twinning would be associated with an increased risk of the diseases thought to be influenced by an adverse intra-uterine environment i.e. ischaemic heart disease, hypertension, diabetes, disorders of lipid metabolism, allergic and autoimmune conditions. This would mean a fundamental flaw in the classic twins methodology preventing the application of a twin study results to the larger non-twin population.

1.4.4 Defending the Classic Twins Methodology

There is, however, much evidence to refute the criticism above. Firstly monochorionic twins are actually less similar in birthweight than dichorionic monozygotic twins and the mean birthweights quoted may therefore be misleading (MacDonald AM, 1993). If this dissimilarity with regard to birthweight translated into differences in the incidence of various diseases monozygotic twins would be less alike. In a subsequent twins study this would if anything under-estimate, not over-estimate the importance of the genetic contribution to the parameter being studied. Secondly if the form of low birthweight associated with twinning is adverse in terms of long term health it would be expected that there would be an increased incidence of

these diseases in all twins and especially in monochorionic twins. This is not the case. Recent studies from Scandinavia have investigated the fetal origin hypothesis and its relationship to twins. Christensen studied 8495 twin individuals born in Denmark between 1870-1900 and found no significant difference in mortality between the twin cohort and the general population except in women aged 60-89. In females dizygosity, rather than monozygosity, was associated with a slight increase in mortality (Christensen K et al, 1995). Vagero followed 14786 Swedish twins and found a relative risk for ischaemic heart disease in twins of 0.99 for women and 0.85 for males (Vagero D et al, 1994). These studies suggested the fetal origins hypothesis is invalid for twins. This does not refute the hypothesis in itself but suggests that the growth retardation of twins is not analogous to the adverse intra-uterine environment and retardation associated with poor maternal nutrition described by Barker. More recently Phillips tested the fetal origin hypothesis in a cohort of twins and found no association between birth weight and either adult hypertension or glucose intolerance. This contrasted to his earlier work where he suggested that the intra-uterine retardation associated with twinning was akin to that associated with the fetal origins hypothesis (Baird J et al, 2001).

A further assumption made when constructing a twin model is that each of the phenotypic variables measured is the sum of several additive independent, normally distributed components which are attributable to additive genetic factors, general environmental factors not shared by the twins and common environmental factors shared by twins but not by

unrelated subjects. In this model heritability is defined as the ratio of the additive genetic variance to the total phenotypic variance. Estimates of heritability and the inter-relationship between genetic and environmental are environment specific. For example the same population migrated to a different environment will show different gene-environment interactions and different heritability scores. For this reason a population such as the West of Scotland with its high incidence of cardiovascular mortality is particularly informative.

1.5 Previous twin studies of cardiovascular events and mortality

The familial nature of life span and longevity has been investigated in several populations including sibling (Cohen et al, 1964), adoptees (Sorensen TI et al, 1988) and twins (Hrubec Z et al, 1981; McGue M et al, 1993 and Herkind AM et al, 1996). These have, in general, reported a familial aggregation in life span. Estimates of heritability have suggested only a weak genetic effect with estimates of about 0.26 for males and 0.23 for females. (Herskind AM et al, 1996). These estimates are consistent with previous theoretical studies (Fisher RA, 1930). Fisher's theory stated that characteristics related to longevity, fertility or fitness have already undergone strong natural selection. This results in the exhaustion of additive genetic effects. Therefore it is only the non-addition effects that remain. Selection has no effect on non-additive variance because genetic effects resulting from the interaction within or between loci are not transmitted from one generation to the next.

Treating life span as a simple biological entity is over simplification. Life span might be determined not only by direct inheritance of "longevity" genes but also by the presence or absence of frailty genes (Vaupel JW et al, 1988) or specific disease-prone genes. These groups of genes probably alter the risk of death at varying ages rather than directly determining age of death. Despite these genetic factors the role of the environment cannot be underplayed. A robust person may die before a "genetically frailer" individual following exposure to a specific environmental factor such as smoking.

Given that overall mortality is a composite end-point with numerous determinants interest has turned to more specific entities such as cardiac deaths and cardiovascular events. Once again cardiac events are known to aggregate in particular families. Twin, adoption and sibling populations have been utilised to quantify this effect. Sorensen's adoption study of 960 families gave a relative risk of 4.52 (1.32-15.4) for cardiovascular and cerebrovascular death if one's biological parent died from the same cause between the ages of 50 and 70. This compared with a risk of 3.02 (CI 0.72-12.8) for one's adoptive parent (Sorensen TI et al, 1988). This effect was not seen for cancer deaths. In the on-going study of the Norwegian Twin Panel concordance rates for coronary heart disease before the age of 60 are significantly higher in monozygotic than dizygotic (Berg K et al, 1983). A higher monozygotic than dizygotic concordance rate for ischaemic heart disease deaths was reported from the Swedish Twin Registry (de Faire U et al, 1975). The use of "death-discordant" twins allows cardiovascular mortality to be viewed as more than as "all-or-none" phenomenon (de Faire

U et al, 1976). Genetic effects over coronary heart disease deaths appear to be more significant in the young and diminish in the older age group (Marenberg ME et al, 1994).

There appears to be conflicting opinion on whether this genetic effect is different in males and females. De Faire suggested that the genetic effect over cardiac death was more significant in young males (de Faire U et al, 1975). Harvald and Hauge examined 352 coronary occlusion events registered from the Danish Twin Register. A more pronounced concordance was detected if there was a female/female twin pair or if the proband was female (monozygotic and dizygotic concordance rates of 0.39 and 0.26 for males compared with 0.44 and 0.14 for females). The risk for a female monozygotic co-twin dying from coronary occlusion was over 40% in the first 10 years after the proband death. The corresponding risk for a dizygotic co-twin was only 4%. The authors suggested that the effect of environmental factors affecting coronary occlusion outweighed the importance of genetic make up in males (Harvald B et al, 1970).

Care must be taken when interpreting twin study results particularly in relation to applying the results to other more general populations. The problems of using selected populations such as veterans were highlighted from the NHLBI Twin Study. Concordance rates were similar for both monozygotic and dizygotic twins due to selection at army enlisting which resulted in a decline in the number of concordant monozygotic pairs in the

later follow-up study (Reed T et al, 1990). Applying the results of such a study to a wider population could be misleading.

1.6 Aims

In conclusion twin studies, if performed in the light of the known innate limitations and bias of such a technique, provide the opportunity to assess the heritability of complex traits. This must precede the hunt for quantitative trait loci. In a population, such as that of the West of Scotland, with a high risk of cardiovascular events, it is particularly important to determine why that population is predisposed to develop disease. Twin cohorts provide a "superb natural experiment" to further investigate these predispositions (Martin N et al, 1997).

The aims of this thesis were:

1. To determine the heritability of resting and ambulatory blood pressures using a classic twin methodology in a population based study.

2. To determine the heritability of left ventricular structure, in particular left ventricular mass, and function.

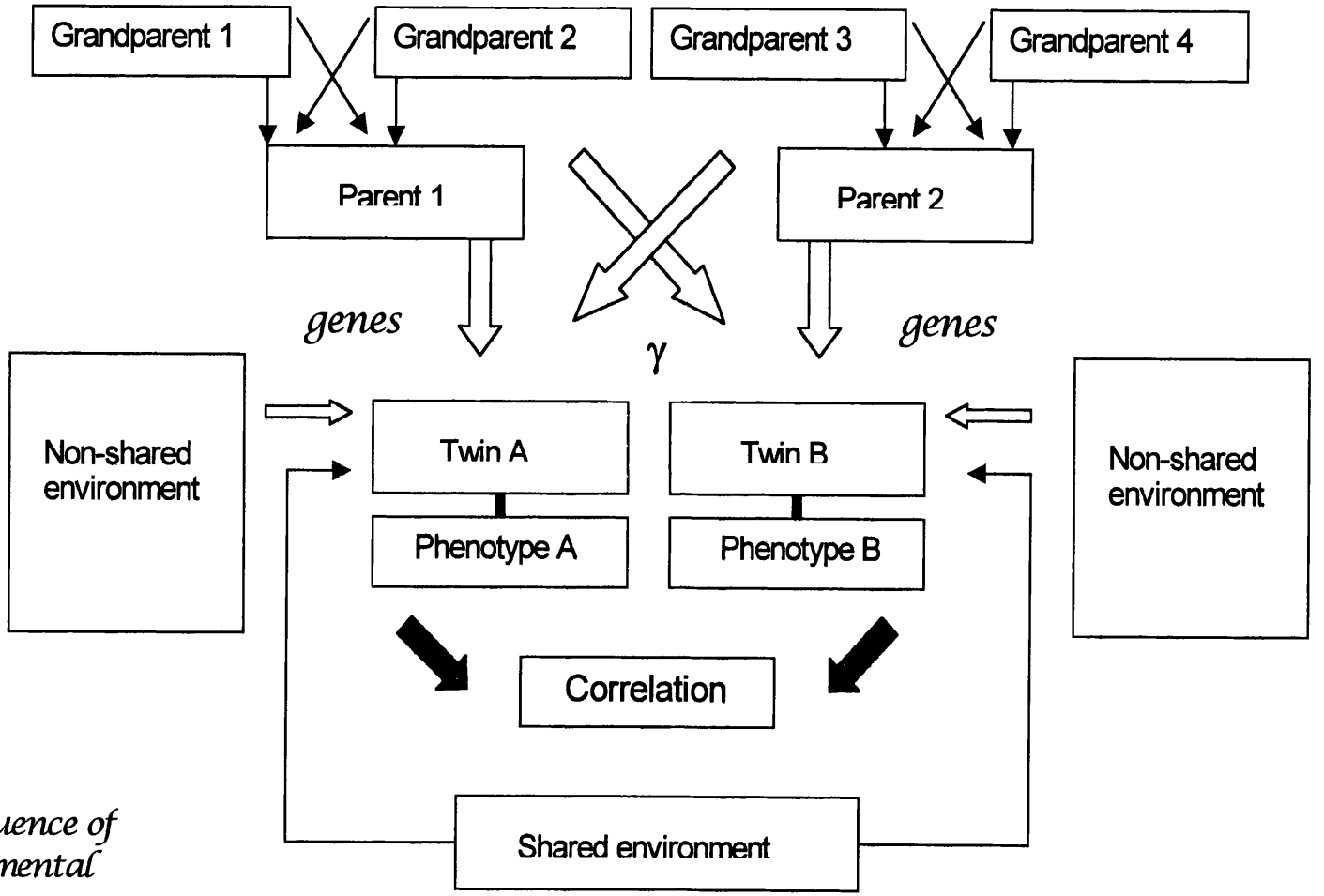
3. To determine the heritability of parameters of pulmonary function, a known risk factor for cardiovascular events.

4. To determine the heritability of the actual extent of atherosclerosis as measured by B mode carotid ultrasound.

5. To determine the heritability of known cardiovascular risk factors in this high-risk population.

6. To delineate the inter-relationships between these variables

7. To delineate the inter-relationships between genetic, familial and environmental factors in this population.



Note: the influence of gene-environmental interactions are not demonstated

- In monozygotic twins the genetic correlation between twins (γ) is 1.0.
- In dizygotic twins the genetic correlation between twins (γ) is 0.5.

Figure 1.1 Illustrative diagram of basic twin modelling

Chapter 2

Methodology

2.1 Twin Recruitment

The original twin database utilised by this present study contained pairs of twins recruited by Dr R Sutcliffe, Department of Medical Genetics, University of Glasgow in 1992. This cohort was recruited by media campaign in which the purpose of the research was not specified to avoid ascertainment bias. The original twin cohort was assembled to study the heritability of rheumatoid factor (Birnie D et al, 1995). In February 1995 160 of this original cohort from the West of Scotland were recontacted by letter and provisionally agreed to take part in the present study (The Scottish Twins Study).

Subsequent media campaigns between Dec 1995 -Dec 1996 yielded further volunteers. During these campaigns the cardiac aspects of the study were minimised to reduce bias. Articles and letters were placed in local and national newspapers. A broad spectrum of daily and weekly tabloid and broadsheet newspapers were approached to widen the population exposed to the recruitment campaign. Several twins volunteered to help with recruitment either by direct contact with other twins or by writing articles for local magazines. Adverts were also placed in local municipal libraries. In addition a brief item on the twins study was carried on a national television news programme (Reporting Scotland, BBC Scotland, December 1996).

All advertising stated that twin pairs were required between the ages of 30 and 85 years. Both twins were required to be seen. Initially the study specified that volunteers should live within a 30-mile radius of Glasgow later,

however this was widened to include any subjects living in Scotland. Articles stated that subjects with any state of health, past medical history or family history would be able to participate in the study. The only exclusion criteria stipulated was that pregnant women should be excluded from the study. This was done as it was felt that pregnancy itself would alter many of the haemodynamic and biochemical parameters under investigation. Following an initial pilot study 151 pairs of twins were recruited over a 2-year period.

2.2 Informed Consent

Volunteers were encouraged to contact the Scottish Twin Study either by phone (24 hour answer machine) or by letter. Subjects were then contacted by either nursing or medical staff and following an explanation of the study verbal consent was obtained. Subjects were asked to attend the study site for 2 visits. Written consent was obtained at their first visit. Occasionally, for logistic reasons the first visit was performed in the subject's home.

Ethical approval for all aspects of the study was obtained from the West Glasgow Hospitals NHS University Trust Ethics Committee.

2.3 Initial study visit

The purpose of the first study visit was 4 fold. Firstly informed written consent was obtained. Secondly patients were given study questionnaires to complete at home. Thirdly patients were fitted with ambulatory 24-hour blood pressure monitors (Spacelabs 90207) and finally the 24-hour urine collection was explained. Subjects were issued with written instructions and a urine

container. The initial study visit most commonly occurred during the 2-3 days prior to the main visit. Twins attending the study from a distance travelled on the day prior to study. On arrival in Glasgow they were given hotel accommodation near the hospital. On day 1 their initial visit was performed as above and commonly carotid scanning was performed the same day. The following day 24-hour blood pressure monitoring and urine collections continued through out the main visit and finished at the end of the collection period, just before twins departed home.

2.3.1 Twin Identification Numbers

All twins were allocated a twin study identification number which was used for all data collection. Twin pairs were numbered sequentially. The older twin was identified as Twin A; the younger Twin B (e.g. the older twin of the 25th pair studied was identified as TWI025A). Data was stored according to older and younger status of the twins ensuring pairs of twins were analysed separately.

2.3.2 Twin Questionnaire

Questionnaires were completed by all subjects, either at an initial home visit or following their first visit to the Western Infirmary. At all stages it was stipulated that these be completed in isolation, without conferring with their twin. Questionnaires were checked by the study nurse on the patients' second visit to the investigation unit. Any omissions were discussed at that stage. This again was done in isolation from the other twin. Previous

validated questions formed the basis of the exercise, diet, smoking, symptoms and zygosity aspects of the questionnaire.

2.3.3 Urine Specimens

All patients undertaking the study completed a 24-hr urine collection. This was performed either at home or, for the more distant twins, during their stay at a local hotel. A 25ml aliquot of this collection was then separated from the main urine collection for separate analysis. An early morning fasting urine specimen was also obtained.

2.4 Second Study Visit

The second study visit constituted the main period of investigation (Table 2.1). Investigations were performed in the Department of Sports and Exercise Medicine, Clinical Investigations Research Unit (CIRU), Department of Medicine and Therapeutics, Western Infirmary, Glasgow.

2.4.1 Clinical Examination

On arrival fasted volunteers were familiarised with the investigation unit and introduced to the remaining members of staff. They were then encouraged to rest for 5 minutes. Following this supine rest patient's lying and standing blood pressures and heart rates were recorded using a manual mercury sphygmomanometer. All readings were taken from the right arm. Blood pressure was taken using standard protocols with Karotkoff sounds 1 and 5. Pulse was taken over 30secs. Standing blood pressure measurements were made between 1 and 3mins from standing using the same methodology. All

blood pressure and heart rate recordings were performed by a single individual. Examination was performed by one of two individuals. Patients were examined for dependent oedema at both ankles. Chest auscultation was carried out looking for basal crepitations. Cardiac auscultation was performed at the apex, axilla, left lower sternal edge and bases of the heart, left and right. Abnormalities of the heart sounds and additional murmurs were documented. Auscultation of the carotid arteries for bruits was also performed. All peripheral pulses were examined including both femoral pulses, dorsalis pedis and posterior tibials. Height was measured using a Holtain stadiometer. The head was placed in the "Frankfurt plane" which is the optimum position in which to place the subject for height measurement. All height measurements were taken by the study nurse.

2.4.2 Blood Sampling

Blood sampling was performed following an overnight fast. Patients were instructed to fast from 9pm the previous evening and all initial blood samples were taken between 8.30 and 9.30am. Blood sampling was performed via an intravenous cannula placed in a vein in the antecubital fossa or the back of the hand. Documented in Table 2.2 is a list of the blood specimens taken (6 x 7 ml lithium heparin tubes, 3 x 10 ml EDTA tubes, 2 x 10 ml plain tubes, 1 x 5 ml fluoride tube, 1 x 10 ml SST tube, 1 x 5 ml EDTA tube). Following completion of this initial blood sampling, subjects were given a bolus of 250 micrograms of tetracosactrin in 1 ml (Synacthen, Ciba). This was then flushed with 5 ml of heparinised saline. Patients were rested supine for 30 mins, with only a brief interruption for an ECG and spirometry to be

performed. Thirty minutes after the initial blood sampling, a further 10 ml lithium heparin sample was taken for post-Synacthen cortisol levels. This was performed after the initial 10ml taken from the intravenous cannula had been discarded.

Time (hr)	Twins Study – Protocol
0815	Arrival at investigation unit
0830	Supine and erect blood pressures Height Questionnaire review
0900	Baseline bloods Administration of IV Synacthen bolus
0915	Resting 12 lead electrocardiograph Spirometry Clinical examination
0930	Completion of Synacthen Test
0945	Breakfast for subjects
1015	Echocardiography Bone scan
1200	Carotid ultrasound

Table 2.1 Timetable for the second study visit

2.4.3 Electrocardiography

Surface 12 lead electrocardiography (ECG) was performed at rest with the patient supine by an experienced physiological measurement technician or doctor. All ECGs were performed on the same machine (Quinton 4000) using standard chest and limb lead positions. ECGs were recorded initially at 25 mm/sec and then at 50 mm/sec. Patients were encouraged to relax and breathe normally during their ECG tracing. The calibration on all ECGs was equivalent to 1 mV = 1 mm.

2.4.4 Spirometry

Resting spirometry was performed using a Vitalograph COMPACT instrument. Before each pair of twins the Vitalograph was calibrating using a 1-litre calibration syringe. Calibration readings were only approved if they agreed within 3% of the standard. Ambient room temperature was recorded to the nearest degree Centigrade. The spirometry equipment did not accept ambient temperatures below 17°C. Results were related to body temperature, ambient pressure and saturation with water (BTPS). Spirometry was performed in the standing position without nose clips. Patients were instructed to inhale completely and then to "blast the air out of their lungs". It was the aim of the examiner to encourage patients to continue expiration for at least 6 seconds. Four readings were taken of forced expiratory volume, forced vital capacity and peak expiratory flow rate.

Data from these 4 forced vital capacity manoeuvres was recorded as a digital output from the COMPACT and as a hand -written copy. In addition

the COMPACT determined the "best " manoeuvre and stored this data. Volume-time and flow volume data from the "best" test print-out were examined according to the American Thoracic Society standards (Standardisation of spirometry 1994 Update, 1995). Unacceptable manoeuvres were not analysed further. Acceptable readings were obtained in 87.0% of the cohort. Lung function data were then double entered into an epidemiology database (EPI-INFO). Discrepancies were resolved by comparing the original documentation. The maximum values of FEV1 and FVC were used for further analysis. Chapter 7 discusses the analysis of the pulmonary function data.

2.4.5 Echocardiography

Echocardiography was performed using an Acuson 128 XP machine by a single experienced operator. Recordings were made on sVHS videotapes. Twin pairs were recorded on different tapes and were identified by their twin code numbers. Following explanation of the investigation patients were positioned on the examination couch at 45° in a left lateral position. ECG electrodes were applied and an ECG was recorded simultaneously to determine systole and diastole. Standard long-axis parasternal, short-axis parasternal, 4-chambered and 2-chambered views were all obtained.

Echocardiography analysis was performed at the end of the completion of all scans by one experienced individual. All echos were analysed on the same Acuson 128 XP machine. Echos were initially subjectively scored for valvular and structural abnormalities. M-mode analysis was performed as

per the American Society of Echocardiography (ASE) consensus statement using a "leading edge-to-leading edge" technique (Park SH et al, 1996). M-mode measurements were an average of at least 3 consecutive cardiac cycles. Ectopic beats and cycles following an ectopic were not analysed. Measurements of internal dimension and wall thickness were made at the end of diastole according to the methodology described by Devereux. Left ventricular mass was calculated as per the Penn-cube method which has been shown to correlate well with necropsy measurements of left ventricular mass. In addition corrected ASE-cube LV mass was calculated on-line using the Acuson software package. This corrected formula was used as the original ASE-cube formula leads to a systematic underestimation of LV mass (Devereux RB et al, 1986).

Penn-cube LV mass = 1.04 $(IVSd + LVDd + PWd)^3 - (LVDd)^3$ - 13.6 g

ASE LV mass = 1.04 $(LVDd + PWd + IVSd)^3 - LVDd^3$

ASE (corr) LV mass = 0.8 LV mass (ASE) + 0.6

The following additional left ventricular parameters were calculated using the on-line Acuson 128 XP software:

LV Fractional Shortening % = (LVDd-LVDs) / LVDd x 100%

IV Septal Fractional Thickening % = (IVSs-IVSd)/ IVSd x 100%

Post. Wall Fractional Thickening % = (PWs-PWd) / PWd x 100%

Where:

LVDd/LVDs	Left ventricular diameter diastole/ systole
IVSd/IVSs	Interventricular septum diastole/ systole
PWd/PWs	Left ventricular posterior wall diastole/ systole

Aortic and mitral valve Dopplers were averaged from at least 5 consecutive cardiac cycles using the Acuson Doppler software. Cardiac cycles with incomplete Doppler envelopes or cycles occurring adjacent to an ectopic beat were not analysed. The mean E and A wave amplitudes were determined from Doppler (Figure 2.1). With normal left sided pressures the early diastolic mitral velocity (E) exceeds the late wave of atrial systole (A). Ageing, left ventricular hypertrophy and ischaemia all result in a decease in the early velocity and an increase in the late velocity (Mantero A et al, 1995). Both abnormal ventricular relaxation and elevated left ventricular diastolic pressures have been implicated in these changes (Mulvagh S et al, 1992).

Reproducibility

A proportion of echocardiograms (10%) were re-analysed in order that an estimate of intra-observer and inter-observer variability could be determined. The correlation between analysers for left ventricular end-systolic diameter was 0.9 and for end-diastolic diameter 0.88.

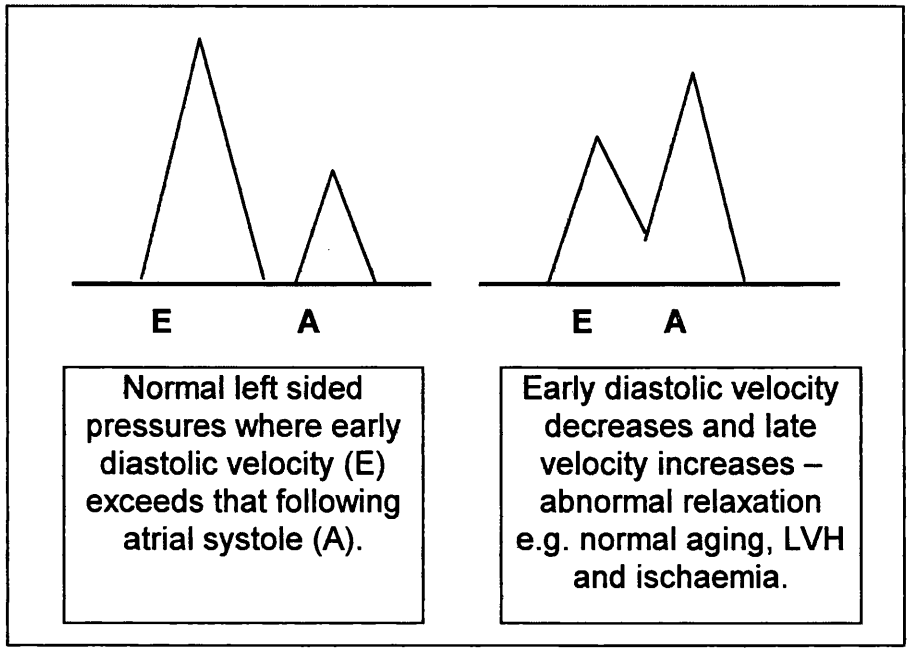


Figure 2.1 Mitral valve flow patterns

2.4.6 Bone Scan

All twins had bone densitometry performed at the spine and upper femur. The results of this are outwith the scope of this thesis.

2.4.7 B - Mode Carotid Ultrasound

Carotid ultrasound imaging was performed using a Biosound 2000 II (Biosound Inc., Indianapolis, USA) scanner based in the Department of Vascular Surgery, Gartnavel General Hospital. Scans were performed by 1 of 2 experienced technicians and were recorded on super-VHS videotape. Three segments of the carotid artery system were studied - the internal carotid (ICA), the bifurcation (BIF) and the common carotid (CCA). The right carotid was always imaged first locating the common carotid and recording it in the middle of the screen. The transducer was then moved cranially along the common carotid until the bulb was visualised. The sonographer then marked the crest of the bifurcation, which is usually positioned laterally or postero-laterally, with a marker to aid later analysis. At each site the sonographer attempted to record the images that best demonstrated the interfaces by rotating the transducer. The thickest sites were recorded for all segments of the artery.

Following imaging of the common carotid the bifurcation was placed in the middle of the screen. The transducer is rotated upwards to identify the flow-divider. The near and far walls are imaged at this site. Following this the transducer is rotated anteriorly and posteriorly to identify the internal carotid. A similar procedure is followed for the left carotid system. The scanning

protocol was similar to that used in the Atherosclerosis Risk in Communities (ARIC) study (Heiss G et al, 1991). A detailed description of the analysis of the scans is documented in chapter 6.

2.5 Zygosity Determination

Zygosity was determined by validated questionnaire and verified by analysing tandem repeat polymorphisms. Four markers were used – AFM238xd10, AFM288vb9, AFM273yfl and AFM199zb6 (Centre d'Etudes du Polymorphism Humain (Paris, France)). In addition 2 polymorphisms in the aldosterone synthase gene were typed. Monozygotic twins were homozygous for each marker where dizygotic pairs were heterozygous for at least 1 marker. The probability of inaccurate assessment of zygosity using this technique was less than 10^{-5}.

2.6 Statistical Analysis

Basic descriptive statistics were performed on all variables. These are described in terms of means and standard deviations. When comparing the relationships between variables, Pearson's correlation coefficients were used. Statistical significance was taken as a p value of < 0.05. Other comparisons were made using the student's *t*-Test and chi-square test, where appropriate. Data that was not normally distributed was log transformed.

Before studying monozygotic and dizygotic twin pairs, a test of equality was performed. This determined whether the means and total variance for both

of the groups were similar. Dissimilarity between the two groups would invalidate the use of a classic twin methodology. To determine equality, two sample t-tests were performed between the means of the two groups. F ratios, the ratios of large variance to the smaller, were assessed to look at the variance between the two groups.

A multiple regression analysis was then performed for each of the variables under investigation. Important intermediate determinants of the phenotype under question were identified by best subset regression. The combination that accounted for the large percentage of variability within each strait was used in the multiple regression analysis.

For each variable in the monozygotic and dizygotic groups intra-class correlations (r) were performed on Twin A versus Twin B using ANOVA. These were compared using Fischer's z test. Heritability estimates were performed on the raw unadjusted data and on the residual data following correction for confounding variables. The heritability was measured using the equation $h^2 = 2(r\text{-}MZ - r\text{-}DZ)$ where r-MZ and r-DZ were the intra-class correlation coefficients for the monozygotes and dizygotes respectively.

Parameters	Units	Laboratory
Electrolytes Urea Creatinine Calcium, phosphate Proteins Liver function Bilirubin Urate Glucose C- Reactive Protein Lipoprotein A Fibrinogen Triglyceride Cholesterol VLDL LDL HDL Cholesterol 25 Hydroxy Vitamin D	mmol/l mmol/l micromol/l mmol/l g/l U/l micromol/l mmol/l mmol/l mg/l mg/dl mg/dl mmol/l mmol/l mmol/l mmol/l mmol/l nmol/l	**Dept of Pathological Biochemistry, Gartnavel General Hospital**
Cortisol		**Dept of Medicine & Therapeutics**
Glucocorticoid receptor binding characteristics		**Dept of Medicine & Therapeutics**
DNA analysis		**Dept of Medicine & Therapeutics**
Haemoglobin White count & differential Platelets ESR	g/dl $x10^9/l$ $x10^9/l$ mm/hr	**Dept of Haematology, Western Infirmary**

Table 2.2 Blood specimens from twin study

Chapter 3

Demographics

3.1 Population Demographics

3.1.1 Age and Sex Distribution

The twin study cohort included 151 pairs of adult twins between the ages of 29 and 80 years (237 females (78.5%) and 65 males (21.5%)). There were 130 same sex pairs and 21 mixed sex pairs. Mean age at the time of investigation was 57.1 $\pm$ 11.4 years (Table 3.1). All twin pairs spent their early lives together and no twin pairs were separated before the age of 10 years. The mean number of years spent living in the same household was 22.7 years for monozygotes versus 22.9 years for dizygotes (p=0.08).

3.1.2 Zygosity

There were 82 pairs of monozygotic twins and 68 pairs of dizygotic twins. Most of these (145) zygosity determinations were performed by micro-satellite DNA probe and 5 by validated questionnaire. The zygosity of 1 pair of twins could not be determined and they were excluded from further analysis. When questionnaire determined zygosity was compared with DNA results 87.5% of twins accurately predicted their zygosity. As with other twin studies the majority of inaccuracies were due to twins falsely believing they were non-identical.

Age group	Pairs	Males	Females	Same sex pairs	Opposite sex pairs
under 35	11	8	14	9	2
35-39	9	4	14	7	2
40-44	19	13	25	14	5
45-49	22	4	40	20	2
50-54	24	10	38	20	4
55-59	18	5	31	17	1
60-64	18	5	31	17	1
65-69	14	8	20	12	2
70-74	8	1	15	7	1
75-79	7	5	9	6	1
80 and over	1	2	0	1	0
Total:	**151**	**65**	**237**	**130**	**21**

Table 3.1 Twin study population - by age and sex

3.1.3 Birthplace, nationality and current address

All twins in the cohort were Caucasian. 147 (97.4%) pairs of twins were born in Scotland. The places of birth of the remaining 4 pairs of twins were Donegal, Surrey, Peru and Nigeria. Within Scotland 79.6% (117 pairs) of the cohort were born in the West of Scotland. 145 (96%) pairs of twins had Scottish mothers; 3 (2%) Irish; and 3 (2%) English. 144 pairs (95.4%) had Scottish fathers; 5 (3.3%) Irish and 2 (0.67%) English. Only 1 pair of twins had no Scottish first-degree relatives. Table 3.2 displays grandparent nationality. The majority of the cohort lived in the Greater Glasgow area (61.6%) and 84.4% lived in the West of Scotland (Figure 3.1). The study

population was geographically stable with over 70% and 50% having lived within 10 miles of the same city for over 20 and 40 years respectively.

Country of birth	Maternal Grandmother	Maternal Grandfather	Paternal Grandmother	Paternal Grandfather
Scotland	128 (85)	128 (85)	132 (88)	135 (90)
Ireland	14 (9)	15 (10)	12 (8)	12 (8)
England/Wales	8 (6)	7 (4.7)	3 (2)	3 (2)
Other	0 (0)	2 (0)	1 (2)	0 (0)

Table 3.2 Ethnic origins of twin grandparents (number and percentage)

3.2 Past Personal and Family Medical History

3.2.1 Pre-existing medical history

A self-reported medical history was obtained for the major cardiovascular risk factors. Hyperlipidaemia was present in 40 individuals (13.2%); treated hypertension in 19 (6.2%); diabetes mellitus in 1.3% (only 1 was treated with insulin and 1 on oral hypoglycaemic agents); angina in 21 (7.1%); a history of previous myocardial infarction in 9 (3%); cerebrovascular disease in 3 (1%) and thyroid disease in 17 (5.7%).

3.2.2 Family History

Twins were asked to document the cause of death of their parents and siblings (if applicable) and describe their family's medical history. Cardiovascular disorders and cancers were the most common causes of parental death in the cohort. The self-reported family history confirmed the "high risk" nature of the population under study with 82.8% of the population

having a first degree relative with a cardiovascular diagnosis excluding hypertension (Table 3.3 and Table 3.4).

Causes of Death	Maternal Deaths	%	Paternal Deaths	%
Alive	**140**	**43.4**	**116**	**38.4**
Deaths				
Cancer	24	7.9	55	18.2
CVA	27	8.9	20	6.6
IHD/MI	42	13.9	62	20.5
Other cardiac	33	10.9	29	9.6
Other	29	9.6	13	4.3

Table 3.3 Causes of Parental Death

Family history	No. of Twins	%
Angina	126	41.7
MI	143	47.4
CVA	88	29.1
Hypertension	120	39.7
Cardiovascular disease	250	82.8

Table 3.4 Positive first-degree family history

3.2.3 Current medication

The most common medications being taken by the cohort were oral contraceptives/hormone replacement therapy (27 (8.9%)), aspirin (24 (7.9%)), diuretics (16 (5.3%)), non-steroidal anti-inflammatory drugs (12 (4%)), beta-blockers (11(3.6%)) and calcium channel blockers (10 (3.3%).

3.3 Social history

The majority of the cohort were married (73.6%); 11.7% single; 6% widowed and 8.7% divorced or separated. The cohort completed an average of 11.7 ± 2.6 years of full-time education after the age of 5. MZ twins completed 11.9 years compared with 11.4 years for DZ (p=0.09). More than 3/4 of the cohort (79.4%) lived in owner-occupier properties. This included those who had bought their local authority homes. Local authority housing accounted for 17.2% of the cohort and private rented properties for 3%.

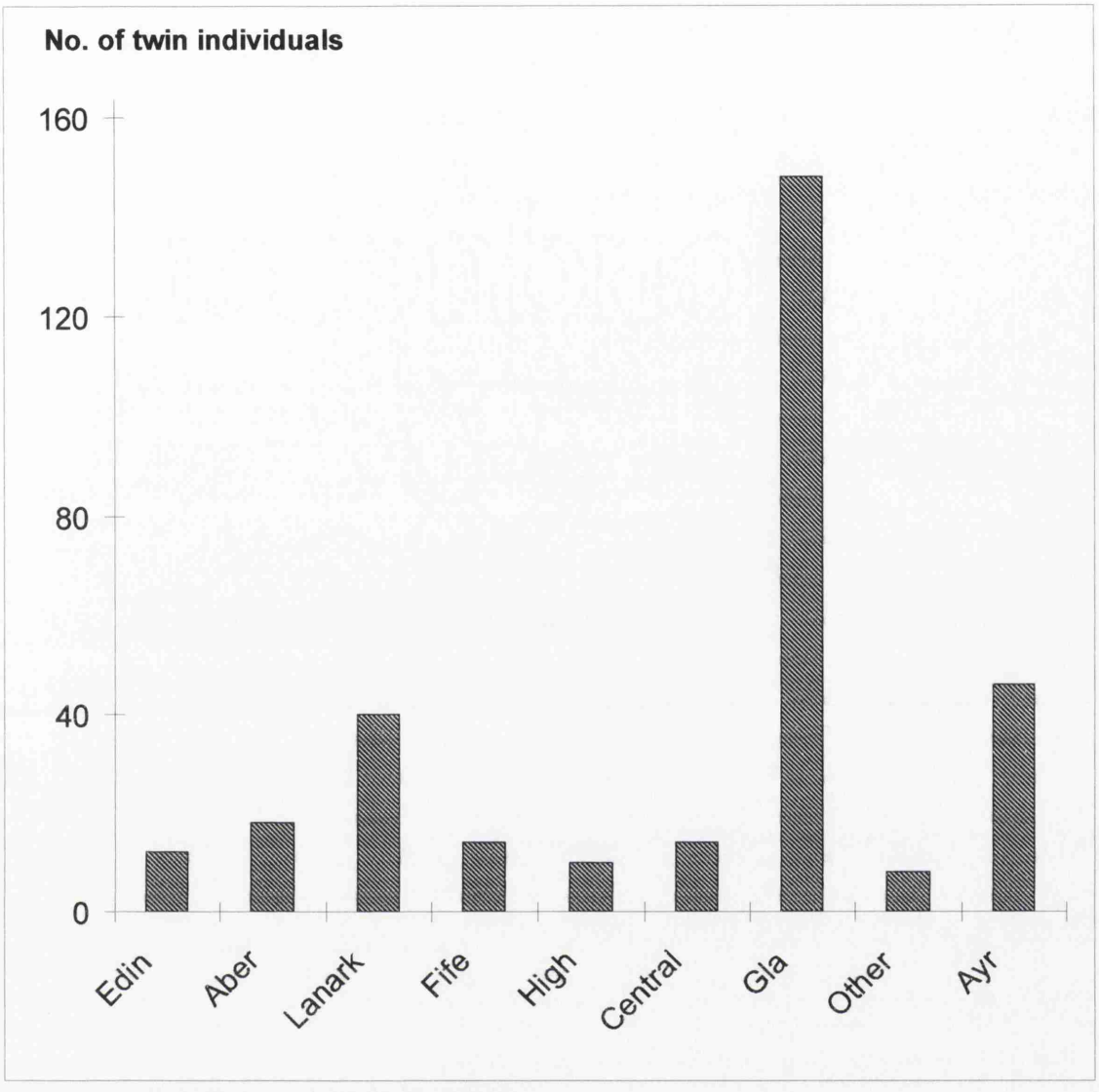


Figure 3.1 Birthplace of twin cohort

Edin – Edinburgh & Lothian
Aber – Aberdeen & Grampian
Lanark – Lanarkshire
Fife – Fife & Tayside
High – Highlands & Islands
Central – Central Region & Stirlingshire
Gla – Greater Glasgow
Ayr – Ayrshire

Chapter 4

Blood pressure

4.1 Introduction

4.1.1 Blood Pressure - a complex genetic trait

Essential hypertension imposes a significant burden of disease on the Scottish population with estimates that 35% of men and 50% of women over the age of 45 years are affected (Smith WC et al, 1990). The role of hypertension as a significant modifiable cardiovascular risk factor is unarguable (Stokes J et al, 1989) and thus effective treatment of blood pressure must form part of the strategy to improve the health of the Scottish nation.

The familial nature of blood pressure was proposed over 70 years ago (Veitz et al, 1923). In 1965 Pickering was the first to suggest that, contrary to contemporary belief, human blood pressure had a unimodal distribution resulting from the quantitative effect of a number of genes working together (Pickering G et al, 1965). This superseded the mechanism postulated by Platt who had suggested that hypertension was due to a single gene acting in a Mendelian-dominant fashion (Platt R et al, 1963). Pickering's work has triggered a plethora of animal and human studies confirming the important contribution of familial and genetic factors in the determination of varying aspects of blood pressure control.

4.1.2 Animal Studies

In 1963 Okamoto and Aoki (Okamoto K et al, 1963), through a programme of selective breeding, derived the spontaneously hypertensive rat (SHR). This was done by measuring the blood pressure in numerous non-inbred animals

and then crossing those with the highest blood pressures. The ability to produce a hypertensive animal by selective breeding provided evidence that genetic factors were important in the determination of hypertension in this species. The SHR is now the most commonly used animal model of essential hypertension enabling the investigation of multiple aspects of blood pressure regulation and end-organ damage. Animal models have been useful in suggesting potential candidate genes for the development of human hypertension. By performing co-segregation studies involving crosses of hypertensive rats and normotensive rats, several chromosome regions, which may contain genes important in the development of hypertension, have been identified. A locus on the rat chromosome 10, which was near the quantitative trait locus for ACE, was one such region (Deng AY et al, 1995). There are regions of homology between human and rodent genomes and for this reason candidate loci discovered in rat models may lead to identification of corresponding regions in the human genome (Levan G et al, 1991).

4.1.3 Rare hypertensive disorders

In humans there are several conditions inherited in a Mendelian manner that are associated with the development of hypertension. This includes some which are autosomal recessive e.g. 11-hydroxylase deficiency (de Simone G et al, 1985) whilst others are autosomal dominant e.g. glucocorticoid remediable aldosteronism (Luft FC et al, 1995). These are rare disorders that are not typical of hypertension prevalent within the wider community

however they illustrate how genes can directly influence the development of high blood pressure.

4.1.4 Family and Twin Studies of Human Hypertension

Hypertension far outshines other cardiovascular traits in the degree to which its genetic framework has been investigated (Luft FC et al, 1998). Inference that blood pressure was genetically influenced were originally made from studies tracking blood pressure in family groups. Family studies demonstrate familial clustering of several different levels of blood pressure arguing against the theory that specific genes separate patients into hypertensive or non-hypertensive extremes (Mitchell BD et al, 1996).

A positive family history of hypertension is associated with a subsequent risk to the proband (relative risk 1.8). In addition to have 2 or more relatives with early hypertension identifies a smaller set of families who are at substantial risk of developing hypertension (Hunt SC et al, 1986). Large epidemiology studies in varying populations have described correlations between parents and offspring (Hunt SC et al, 1991) and between siblings (Hennekens Ch et al, 1976).

Familial aggregation is not, however, evidence of a direct genetic contribution to a trait as these findings could be explained by familial sharing of common environmental factors. Two specific populations have been utilised to determine if this effect is mediated through genetic or familial factors - families with adopted children and cohorts of twins. Correlation

coefficients between related and non-related individuals in adoption studies have suggested a genetic effect. The inter-class correlation for systolic blood pressure between non-related siblings was 0.16 compared to 0.38 for natural siblings. In addition, parent-adoptee correlations were less than that noted for parent-offspring relationships (Annest JL et al, 1979).

Twins studies have examined various aspects of blood pressure determination and evidence of a genetic control over blood pressure can be demonstrated even in the first year of life (Levine RS et, 1982). Table 4.1 documents a selection of twin studies, from diverse populations, examining the heritability of various components of blood pressure. Twin studies heritability estimates are higher than those obtained from sibling or parent-offspring studies. Population-dependant differences in blood pressure determination are one proposed explanation for this difference. Hunt et al, however, examined the heritability scores for twins and siblings from the same Utah population and found a similar discrepancy (Hunt SD et al, 1989). He suggested that method of analysis had a significant impact on heritability estimates (Moll PP et al, 1983). An alternative explanation is that of age-specific genetic effects. This was highlighted in a Norwegian study of 43,751 parent-offspring pairs, 19,140 pairs of siblings and 169 pairs of twins (Tamms K et al, 1993). This showed that age effects the genetic contribution to a trait with phenotypic correlation between relatives decreasing as the age between them increases. In addition the same set of genes do not necessarily exert the same influence throughout the whole of an individual's life span (Van Hooft IM et al, 1988). Tamms postulated that

some of the discrepancies seen between high correlations from twin studies and lower parent-offspring studies could be explained by this phenomenon.

Author	Journal & Year	Twin study Group	Parameters	Conclusions
McIlhany	J Hopkins Med J, 1975	200 pairs, children	Cold pressor BP	Genetic contribution to resting and stressed levels.
Feinleib	Am J Epidemiol, 1977	514 NHLBI, male veterans	Resting BP	Familial rather than genetic input – high MZ and DZ correlations
Austin	Am J Epidemiol, 1987	434 pairs, females	Resting and stressed BP	SBP and DBP heritability not significant.
Miller	Am J Epidemiol, 1987	44 families with MZ offspring	Sodium restricted BP	Familial rather than genetic determination.
Hunt	Am J Epidemiol, 1989	154 Utah pairs, males	Resting and stressed BP	Heritability 39-63% for resting and stressed levels.
Wang	Genetic Epidemiology, 1990	110 Chinese pairs, children	Resting BP	Heritability estimated small, but significant, following adjustment
Carmelli	Am J Hypertension, 1991	101 pairs, male	Cold pressor & stressed BP	No heritability for cold-pressor BP.
Schieken	Circulation, 1992	253 children, MCV cohort	Resting BP	Genetic contribution SBP and DBP.
Tambs	Genetic Epidemiology, 1992	169 Norwegian pairs	Resting BP	MZ correlation 0.52 and 0.43 for SBP & DBP
Mosteller	Genetic Epidemiology, 1993	Female pairs, age 18-85	Resting BP	Familial clustering.
Colletto	Genetic Epidemiology, 1993	514 pairs, male	Resting BP	Heritability 0.5, changing contribution with age.
Tambs	Hypertension, 1993	169 pairs	Resting BP	Correlations decreased as age difference between relatives increases
Ditto	Psychophysiology, 1993	100 pairs, age 12-44	Cold pressor & stressed BP	Heritability SBP 0.63 & DBP 0.58
Carmelli	J Hypertension, 1994	1861 pairs, veterans	Resting BP	62% MZ co-twins hypertensive, 48% DZ hypertensive, 6% total cohort

Table 4.1 Previous twin blood pressure studies

4.1.5 Intermediate Blood Pressure Phenotypes

Hunt and Williams highlighted the confounding effects of intermediate phenotypes on the genetic determination of blood pressure (Williams RR et al, 1989). Obesity and in particular centrally distributed body fat are strong predictors of hypertension (Blair D et al, 1984). Body mass index, known itself to be under genetic control, is responsible for at least part of the familial aggregation seen in blood pressure (Schieken RM et al, 1992). Insulin resistance and lipid abnormalities are other phenotypes closely related to hypertension (Grundy SM et al, 1999). The existence of a major gene for any of these intermediates could partly explain the clustering seen with hypertension. These phenomena may not in themselves cause hypertension but as Hunt described "(may) make it easier for other interacting genes and environmental factors to overwhelm the body's normal control systems to a point where abnormal blood pressure is maintained to keep other more critical systems close to normal operating conditions".

Over the last decade the study of the genetics of resting blood pressures has advanced to the level of dissecting out individual genes involved in the control of both systolic and diastolic pressures. Identifying specific candidates (Nagy Z et al, 1999) and identifying the genetic contribution to specific mechanisms of blood pressure control (Nowson CA et al, 1992) have yielded a fresh insight into the complexity of blood pressure modulation.

4.1.6 The Genetic Effects of Non-Resting Blood Pressure

Thus far the discussion has concentrated on the genetic influence over resting clinic or casual blood pressures. However, blood pressure responses to various forms of stress e.g. exercise or mental arithmetic are also genetically determined and may be controlled by a different set of genes. Busjahn studied 123 pairs of German twins and suggested that different genes affected resting and cold-pressor blood pressures (Busjahn A et al, 1996). This finding has been reproduced by other groups who have also been unable to find a common genetic basis for both resting and cold-pressor blood pressures (Ditto B, 1993). If different genes regulate resting and stress blood pressure it may be that ambulatory blood pressure and circadian patterns of blood pressure are also affected by a different set of genes. Separation of these various facets of blood pressure control may allow us to understand its complexity and to target future studies towards those factors thought to be most detrimental in terms of end-organ damage and atherosclerosis development.

4.1.7 Twenty-four hour ambulatory blood pressure

Hypertension is a known adverse prognostic factor and indeed even a single measurement of blood pressure is known to be an independent determinant of outcome. However, it is becoming evident that more extensive characterisation of blood pressure, especially over a 24 hour period, is a more sensitive predictor of outcome (Liu JE et al, 1999). Left ventricular hypertrophy (Palmieri V et al, 1999), cardiac events and cardiac mortality

(Verdecchia P et al, 1994) are all more closely related to aspects of 24-hour blood pressures than to clinic readings. This may reflect the fact that 24-hour readings are a more accurate reflection of an individual's "true" blood pressure or that specific facets of blood pressure control over 24 hours are more important. The presence of end-organ damage, for example left ventricular hypertrophy, correlates better with nocturnal rather than day time blood pressure parameters (Fagher B et al, 1995). Commercially available 24-hour ambulatory blood pressure monitoring (ABPM) with measurement of blood pressure in the patient's home environment several times every hour has revolutionised the ability to study this further aspect of blood pressure regulation.

White-coat effect on blood pressure

The phenomenon that blood pressures measured in the clinic were often higher than the values obtained in the patient's own home was first noted in 1940 and attributed this the "excitement and tension associated with a visit to the clinic or doctor's office". This common finding may be present in up to 20% of patients with mild hypertension (Pickering TG et al, 1999). With the advent of ambulatory blood pressure monitoring it has been easier to define this "white-coat" phenomenon, which may not be as benign as was previously believed. White coat effect may carry a cardiovascular risk above that of the normal population. Microalbuminuria (Hoegholm A et al, 1994), abnormal left ventricular filling (Chang NC et al, 1997), metabolic abnormalities (Strandberg TE et al, 2000), carotid artery atheroma (Muldoon MF et al, 2000) and abnormalities in left ventricular structure (Muscholl MW

wt al, 1998) have all been shown to be abnormal in those with white coat effect. Over 35% of patients with white coat hypertension go on to develop persisting hypertension over a period of 6 years and it may well be that white coat effect represents the early stages of evolution towards frank hypertension (Verdecchia P, 1999).

Circadian rhythms in blood pressure

Invasive intra-arterial blood pressure monitoring has demonstrated that there is a normal reproducible diurnal blood pressure rhythm (Richards Am et al, 1986). This pattern remains even in the presence of bed rest indicating it is not purely a reflection of activity over the day (Gauquelin G et al, 1996). In addition cardiac pacing does not abolish this variability (Imai Y et al, 1990). This normal circadian pattern of blood pressure control is often lost in hypertensive patients particularly in secondary hypertension (Baumgart P et al, 1989). The term "non-dipping" has been given to those who have blunting or abolition of the 24- hour diurnal pattern. It appears that circadian pattern of blood pressure has significance with regards to outcome and the development of end organ damage. "Non-dippers", i.e. patients who do not have the normal drop in nocturnal blood pressure are at higher risk of end-organ damage e.g. left ventricular hypertrophy or cardiac death (Pickering TG et al, 1993). This concept of blood pressure pattern and 24-hour pressure load adds further facets to the understanding of the homeostatic mechanisms regulating blood pressure and its detrimental effects of on other body systems. It may well be that casual blood pressure level is only one

aspect and that investigation should be concentrating on other pathological intermediates such as circadian pattern or 24-hour blood pressure load.

Technical limitations of 24-hour monitors

The technical limitations of 24-hour ambulatory blood pressure monitoring (ABPM) need to be considered in any study. Motion artefact is a common problem and care must be taken to ensure the device used performs reproducible exercise measurements (Jacoby AC et al, 1993). In addition 24-hour monitors only sample a minority of blood pressures and cannot be reliably seen as a gold standard. There are approximately 100,000 heartbeats per day and given a measurement of 1 every 15 minutes, this gives a sampling of less than 1 per 1,000 heartbeats over-simplifying actual blood pressure patterns. In addition the definitions of day and night vary according to the method of analysis employed. If this does not closely correlate to the patient's activity pattern it may well cause artificial blunting of the 24 hr diurnal variability by artificially increasing the nocturnal blood pressure values. Nonetheless, despite these limitations 24-hour ABPM is an extremely useful tool. There are significant differences in the life event curves of patients with equivalent clinic blood pressures, but high versus low ambulatory blood pressure readings. Ambulatory blood pressures can, therefore, contribute additional precision over the predictive power of office pressures (Ohukubo T et al, 1997).

4.1.8 Familial and genetic determination of 24 hr ambulatory blood pressure

Twenty-four hour patterns of blood pressure show racial and familial differences. Black American patients have a different circadian blood pressure rhythm when compared to a similar White population with an increase in the tendency to lose normal diurnal variability in blood pressure - "non-dipping" (Harshfield GA et al, 1989). Wilson et al performed ambulatory blood pressure monitoring in a cohort of adolescent children; mean age 16 years. In these normotensive adolescents the presence of parental hypertension influenced blood pressure levels and variability especially during the hours spent at school (Wilson PD et al, 1988).

Only two previous twin studies have examined ambulatory blood pressure. In Degaute et al's study of 28 monozygotic and 16 dizygotic pairs of young male twins a significant genetic effect was demonstrated for 24 hr patterns of diastolic blood pressure and heart rate. Surprisingly it was found that monozygotic twins had a higher resting systolic blood pressure value than dizygotic twins (Degaute JP et al, 1994). The second study was also conducted in a small group of young healthy male twins (n=53 pairs) aged 18-38 years. It too suggested a genetic aetiology for 24-hr blood pressures (Fagard R et al, 1995). Both of these studies have been in small, highly selected populations.

The purpose of the present study was to investigate the heritability of clinic and varying 24-hour ambulatory blood pressure parameters in a population

between the ages of 30 and 85yrs (analogous to those who present clinically with hypertension).

4.2 Methods

Ambulatory blood pressure monitoring was performed out with hospital, either in the patient's home environment or during an overnight stay in a local hotel, using a Space Labs 90207 device fitted to the non-dominant arm. Patients were encouraged to have as normal a day as possible. Readings were taken every half hour during daylight hours of 08.00 to 23.00 and at hourly intervals during the rest of the time period. Recordings were excluded from analysis if there were less than 75% of acceptable readings. In addition, clinic blood pressures were recorded as per the methodology described in Chapter 2. Intra-class correlation coefficients were calculated on residual blood pressures. Residuals were calculated by regressing the reciprocal of BP on sex, age and weight. In addition 24-hr SBP and DBP were finally corrected for sitting blood pressures. Raw data were used when examining heart rate. Twin pairs were excluded from analysis if one 24-hour tape from the pair did not meet the pre-defined quality criteria or if a subject was on medication that would affect blood pressure.

4.3 Results

4.3.1 Descriptive statistics

Analysable recordings were obtained on 245 individuals. Average 24 hour, day and night systolic and diastolic results are shown in Table 4.2. There were no statistical differences between the monozygotic and dizygotic

groups. Within the cohort as a whole there was a strong correlation between lying and standing systolic and diastolic pressures (0.88 and 0.85 respectively, $p<0.01$ for both). The correlation between lying blood pressure and 24-hour pressures was 0.55 and 0.4 respectively ($p<0.01$ for both). Both systolic and diastolic blood pressure measured over 24 hours were significantly lower than resting values (systolic - 130.6 vs. 123.4; $p< 0.0001$ and diastolic - 81.1 vs. 76.7; $p<0.0001$).

The major correlates with 24-hour systolic blood pressure were resting pressures (systolic 0.46; diastolic 0.46; $p<0.01$) and age (0.23, $p<0.01$). For 24-hour diastolic pressures resting systolic (0.43,$p<0.01$), resting diastolic (0.42, $p<0.01$) and fasting blood glucose (0.22,$p<0.01$) were the closest correlates. There were no strong correlates with 24-hour heart rate. When diurnal variability was assessed 68 (28.2%) of the cohort were "non-dippers". This was defined as a less than a 10mmHg fall in both systolic and diastolic blood pressures overnight when compared to daytime values.

4.3.2 Heritability

Intra-class correlation coefficients and heritability estimates for resting blood pressure, 24-hour blood pressure and heart rate are displayed in Table 4.3. Casual blood pressures showed trends towards genetic control but only standing systolic pressures ($h^2= 0.68$, $p=0.006$) reached significance. There was no statistically significant genetic effect over any heart rate parameter or over the day-night variability. A genetic effect was detected over 24-hr diastolic ($h^2= 0.53$, $p=0.03$), 24-hr mean ($h^2= 0.58$, $p=0.02$) pressures,

daytime mean (h^2= 0.58, p=0.03) pressures, nighttimes diastolic (h^2= 0.62, p=0.02) and nighttimes mean (h^2= 0.55, p=0.05) pressures. Although there was a trend to a genetic effect over varying aspects of 24-hour systolic blood pressure none of these parameters reached statistical significance.

Variable	MZ	DZ	p
Numbers of pairs	42	45	-
Age (years)	56.0 (10.9)	54.4 (12.2)	0.13
Weight (kg)	65.2 (9.6)	68.3 (15.9)	0.35
Height (cm)	164.3 (5.8)	163.6 (10.6)	0.18
Systolic resting (mmHg)	130.4 (18.0)	130.0 (15.9)	0.26
Diastolic resting (mmHg)	80.4 (11.5)	78.4 (9.5)	0.71
Heart rate resting (bpm)	75.5 (10.3)	74.1 (9.9)	0.4
24-hour mean systolic (mmHg)	124.5 (24.4)	121.5 (10.6)	0.45
24-hour mean diastolic (mmHg)	78.7 (10.0)	74.6 (6.8)	0.63
24-hour heart rate (bpm)	76.5 (10.6)	74.1 (10.6)	0.12
Day mean systolic (mmHg)	130.9 (16.0)	124.5 (10.9)	0.85
Day mean diastolic (mmHg)	81.7 (11.1)	77.1 (7.1)	0.52
Day heart rate (bpm)	78.5 (11.4)	76.4 (8.1)	0.26
Night mean systolic (mmHg)	110.0 (22.0)	110.4 (11.7)	0.43
Night mean diastolic (mmHg)	66.8 (8.7)	65.2 (7.9)	0.92
Night heart rate (bpm)	68.9 (9.1)	65.7 (8.3)	0.15

Table 4.2 Descriptive data for MZ and DZ sub-groups

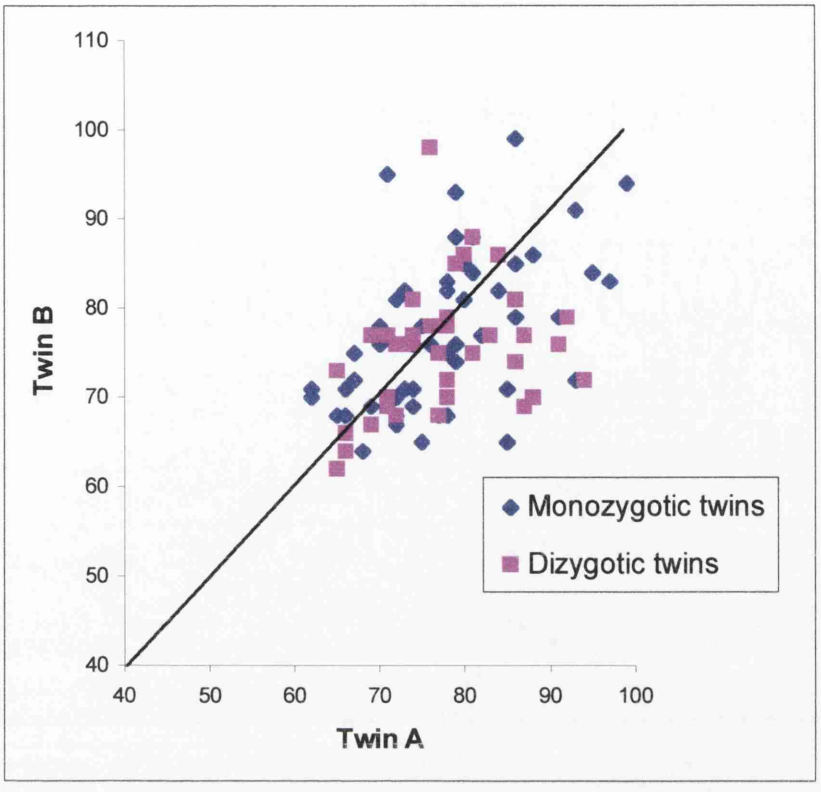


Figure 4.1 24-hr diastolic blood pressure (mmHg)– Twin A vs. Twin B

Variable	R-MZ	R-DZ	Z	h^2	p
24-hr SBP	0.55	0.27	1.55	0.55	0.06
24-hr DBP	**0.53**	**0.10**	**1.93**	**0.53**	**0.03**
24-hr MAP	**0.58**	**0.20**	**2.12**	**0.58**	**0.02**
24-hr HR	0.18	0.09	0.44	0.18	0.33
Day SBP	0.48	0.27	1.14	0.43	0.13
Day DBP	0.52	0.24	1.48	0.52	0.07
Day MAP	**0.58**	**0.24**	**1.92**	**0.58**	**0.03**
Day HR	0.11	0.11	-0.01	-0.004	0.50
Night SBP	0.59	0.39	1.21	0.40	0.11
Night DBP	**0.62**	**0.27**	**2.03**	**0.62**	**0.02**
Night MAP	**0.62**	**0.35**	**1.65**	**0.55**	**0.05**
Night HR	0.46	0.18	1.42	0.46	0.08
Day-night MAP	0.60	0.42	1.15	0.37	0.13
Lying SBP	0.67	0.43	1.59	0.48	0.06
Lying DBP	0.56	0.36	1.19	0.42	0.12
Lying HR	0.20	-0.06	1.18	0.52	0.12
Standing SBP	**0.77**	**0.43**	**2.48**	**0.68**	**0.006**
Standing DBP	0.64	0.43	1.32	0.42	0.09
Standing HR	0.20	0.08	0.56	0.20	0.29

Table 4.3 Intra-class correlations and heritability estimates

Variable	R-MZ	R-DZ	p	Conclusion
24-hr SBP	0.55	0.27	0.06	trend for genetic effect
24-hr DBP	0.53	0.16	0.03	genetic effect
24-hr MAP	0.58	0.20	0.02	genetic effect
24-hr HR	0.18	0.09	0.33	no genetic effect
Day SBP	0.48	0.27	0.13	trend for genetic effect
Day DBP	0.52	0.24	0.07	trend for genetic effect
Day MAP	0.58	0.24	0.03	genetic effect
Day HR	0.11	0.11	0.50	no genetic effect
Night SBP	0.59	0.39	0.11	trend for genetic effect
Night DBP	0.62	0.27	0.02	genetic effect
Night MAP	0.62	0.35	0.05	genetic effect
Night HR	0.46	0.18	0.08	trend for genetic effect
Day-night MAP	0.60	0.42	0.13	trend for genetic effect

Table 4.4 Intra-class correlation coefficients - 24hr values

Where:	
24-hr SBP/ DBP/ MAP	mean 24 hour blood pressures
Day SBP/ DBP/ MAP	mean daytime blood pressures
Night SBP/ DBP/ MAP	mean night-time blood pressures
HR	heart rate
Day-night MAP	measure of diurnal variability

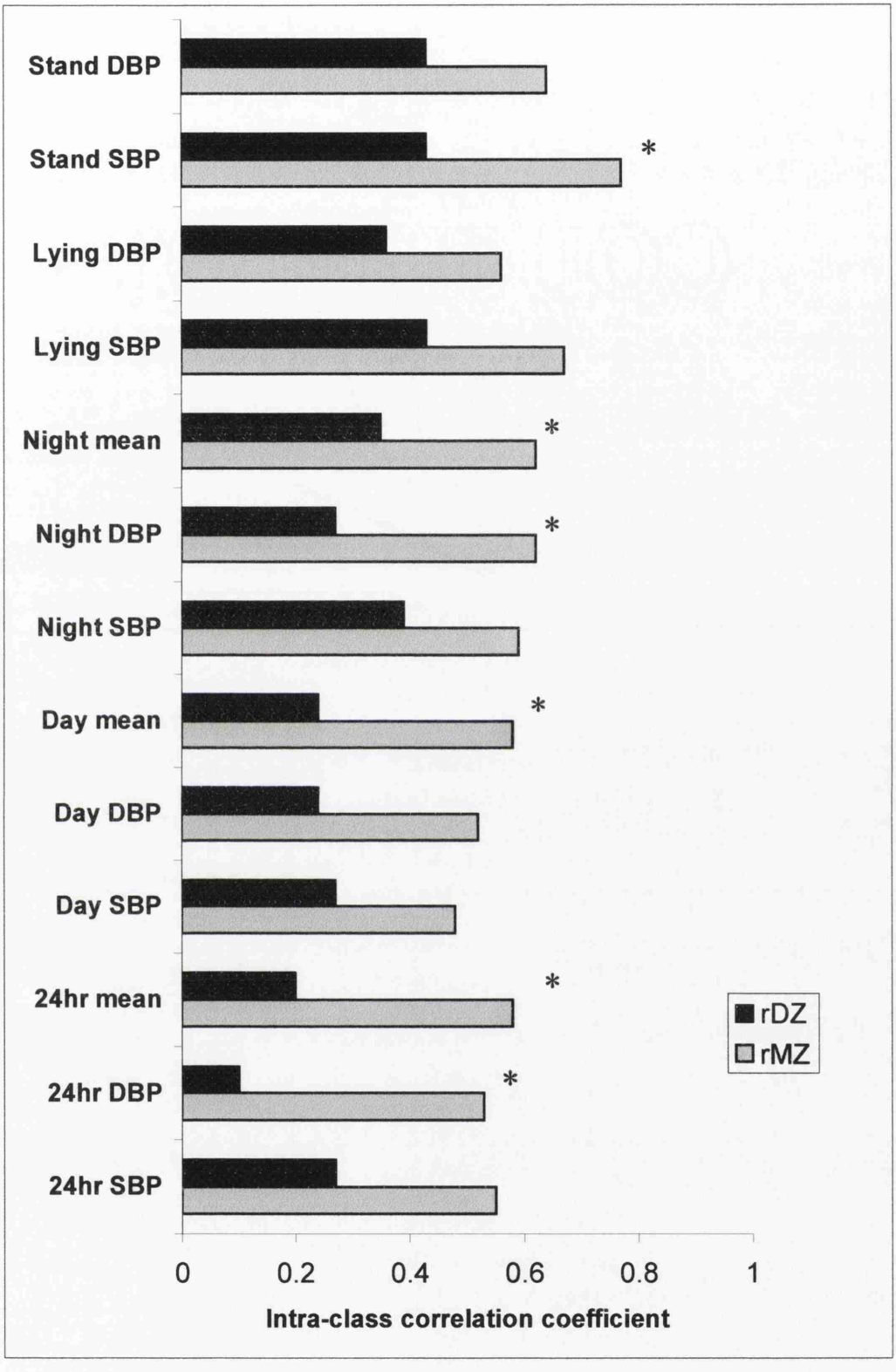


Figure 4.2 Summary of intra-class correlation coefficients (*p<0.05)

4.4 Discussion

This study examined the degree to which varying aspects of clinic and 24-hour blood pressures demonstrated familial and probable genetic determination. Unlike the smaller twin study performed by Degaute et al there were no significant differences in blood pressure parameters between the two twin groups (Degaute JP et al, 1994). In Degaute's study there was a statistically significant increase in the casual systolic blood pressures of the monozygotic group. This finding has not been borne out in the numerous larger twin studies of resting blood pressures and was not a finding in this cohort suggesting that Degaute's cohort may not be representative of the general population. In this study the strongest correlates with 24-hour blood pressures were the casual blood pressures obtained at rest. However these correlations were not strong suggesting that the ambulatory recording is not merely an "extended" version of the same end-point and indeed the heritability estimates would confirm this view.

This study demonstrated a significant genetic effect over ambulatory mean and diastolic blood pressures rather than systolic pressure with over half (53%) of the variability in 24-hour diastolic pressures being genetically determined. However, diastolic blood pressures are a derived measure from the Spacelabs ambulatory device. Systolic and mean pressures are measured directly with diastolic blood pressures being subsequently derived from an algorithm. Therefore caution should be exercised in the interpretation of the diastolic heritability data. Mean blood pressures are more robust and reliable measures and as such their heritability is one of the

most important findings from this study. There were no systolic parameters that had a significant genetic basis. Systolic parameters did show a trend towards a genetic pattern with monozygotic correlations being larger than that of the dizygotic group. These correlations, for example those of lying systolic blood pressure (rMZ=0.67, rDZ=0.43, p=0.06) are highly suggestive of a familial influence. A larger, more powerful, cohort would have been more able to detect a genetic influence.

The pattern of these findings mirrors that of Degaute who found a genetic basis for 24-hour diastolic pressures rather than systolic pressures. Interestingly this pattern was not borne out on the casual standing blood pressures where the genetic input was greater to systolic pressures. These findings strengthen the belief that differing blood pressure parameters e.g. a random clinic pressure vs. a nocturnal pressure may have different genetic influences.

In Fagard's twin study of young males (Fagard R et al, 1995) the heritability estimates obtained were higher (70% for 24-hour systolic pressures and 73% for diastolic pressures). The potential reasons for these discrepancies are multiple. Firstly both previous studies were performed in selected young, healthy male subjects. The present study contained predominately middle-aged female subjects. Unfortunately the present study is not large enough to subdivide subjects into age groups or sexes. Again the phenomenon of age-specific genetic effects (Tambs K et al, 1993) comes into play. These factors could partly explain the differences between the present study and that of

Fagard but could not explain the difference between Fagard and Degaute. Methods of analysis, differing populations and sample sizes are more likely to be the underlying cause of these variations. To date the present study is the largest adult twin study on 24-hour ambulatory blood pressures and is the only study involving female subjects.

A simple measure of diurnal variability was assessed. The intra-class correlations for the monozygotic group and the dizygotic group were 0.6 and 0.42 respectively (p=0.126) suggesting a familial input rather than a genetic one. Again shared influences, particular in the peri-natal period and in early infancy may be important determinants of blood pressure later in life.

Heart rate either at rest or within the duration of the 24-hour recording was not apparently heritable. Previous studies have provided conflicting results regarding the heritability of resting heart rate. Several report a measurable genetic effect (Havlik RJ et al, 1980) where others could not demonstrate such an influence (Carmelli D et al, 1991). Ambulatory heart rate has only been investigated in Degaute's and Fagard's studies. Degaute showed a trend towards a genetic effect but his 24-hour heart rate measurements did not reached statistical significance (R-MZ 0.72 vs. R-DZ 0.39, p=0.15). It would be relatively simple to postulate that environmental factors could overwhelm a small genetic influence over heart rate. In addition it may well be that resting heart rates allow some of these variables to be controlled where as a period of "free-range" ambulation introduced overwhelming environmental stimulation.

Both this study and that of Degaute have suggested a greater genetic input to ambulatory diastolic rather than systolic blood pressures. There are several potential explanations for this pattern. There was a trend towards a genetic and familial control in the systolic phenotypes but this study would suggest that environmental determinants are also important in this group. In older subjects systolic blood pressure, to a degree, reflects arterial vessel stiffness (compliance). A single casual resting measure of systolic blood pressure represents the interplay between changes in cardiac output and total peripheral resistance. Multiple measurements such as the mean values from 24-hour systolic recordings may be confounded by these competing factors. In addition external influences such as physical activity and cathecolamine stress, which occur over the 24-hour period, affect systolic blood pressure more than diastolic pressure and may "swamp" the underlying genetic contribution to these phenotypes. Large twin studies of resting pressures have not shown a consistent trend towards diastolic pressure determination. Further studies in differing twin populations would be useful to assess if this pattern is accurate and reproducible.

Meanwhile in a Scottish population 24-hour mean and diastolic pressures may provide useful phenotypes for the investigation of genetic factors involved in blood pressure regulation. The finding that the genetic influences over resting pressures is different to that of 24-hour measurements is important. Different loci may be relevant to ambulatory pressures but not relevant to resting values. In this regard, potential candidate genes need to be investigated not only with regard to resting clinic values but also with

other blood pressure phenotypes, as not doing so may lead to important associations being overlooked.

Chapter 5

Left ventricular structure

5.1 Introduction

5.1.1 Prognostic significance of left ventricular hypertrophy

Regardless of the underlying aetiology ventricular hypertrophy, defined by either electrocardiography (Kannel WB et al, 1969) or echocardiography (Casale PN et al, 1986), is a well-established predictor of cardiovascular morbidity and mortality. The Framingham cohort revealed that an increased left ventricular mass was associated with adverse events. Following adjustment for confounding factors, the relative risk of cardiovascular disease was 1.49 (CI 1.2-1.85) for increments of 50 g/metre in left ventricular mass corrected for height in male subjects and 1.79 (CI 1.2-2.04) for female subjects. Left ventricular mass was associated with cardiovascular disease, cardiovascular death, all cause mortality, and in men, sudden death (risk factor adjusted relative risk of sudden death 1.7 (C.I.1.02-2.83) for each increment of 50 g/metre in left ventricular mass) (Levy D et al, 1990). The adverse prognostic effects of LVH occur both in normotensive and hypertensive individuals (Casale PN et al 1986). In addition left ventricular mass is known to be associated with coronary artery disease incidence (Levy D et al, 1989) and stroke disease (Bikkina M et al, 1994).

The reasons for these adverse associations are not clearly defined. Increased myocardial oxygen consumption, leading to a reduction in coronary blood flow reserve and a tendency to ventricular arrhythmia are commonly quoted hypotheses. Alternatively left ventricular mass may simply be a marker of the cumulative effect of other cardiovascular insults such as hypertension and obesity (Urbina EM et al, 1995).

5.1.2 Determinants of left ventricular mass

The growth of left ventricular myocytes, left ventricular hypertrophy, occurs in early life until adulthood and later in life in response to hypertension, volume overload, left ventricular contractile inefficiency and non-haemodynamic variables such as hormonal influences. These factors taken together explain more of the variation in LV mass than blood pressure alone. Population studies have attempted to further delineate these variables. For example the Framingham Investigators reported that advanced age, male sex, hypertension, obesity and history of myocardial infarction were all associated with left ventricular hypertrophy (Levy D et al, 1988). The Tecumseh Blood Pressure Study performed echocardiography in 851 subjects, with no cardiac history, who lived within a 30-mile radius of the village of Tecumseh, Michigan. In men left ventricular hypertrophy was associated with indices of enhanced sympathetic nervous system reactivity, e.g. plasma epinephrine and nor-epinephrine levels in response to the stress of mental arithmetic. In women obesity and body weight were more important determinants (Marcus R et al, 1994). In contrast several authors cast doubt over the role of catecholamines in the pathogenesis of LVH. They cite the example the left ventricular hypertrophy associated with phaeochromocytoma which is independent of norepinephrine levels (Fouad-Tarazi FM et al, 1992).

Race has a significant effect on left ventricular mass even following correction for other known associations. Left ventricular mass, when examined in 13,990 subjects in the Atherosclerosis At Risk in the Community

Study, was higher in black subjects than in their white counterparts. These figures were adjusted for systolic blood pressure but did not take into account the fact that the black population is more prone to early hypertension and the difference in left ventricular mass may, to some degree, reflect cumulative exposure (Arnett DK et al, 1994). Alternatively African-Americans may be more sensitive to the adverse affects of hypertension or be genetically pre-disposed to the development of hypertrophy (Dunn FG et al, 1983).

In many individuals left ventricular hypertrophy proceeds in the absence of any recognised predisposing factor suggesting other, as yet unrecognised, causative factors. In addition different ventricles response to the same stimulus, e.g. increased load or more specifically wall stress, in varying ways again suggesting the importance of other influences such as local growth factors. It is likely that ventricular growth and remodelling are determined by an interaction of a large number of different influences, some of which are genetically determined.

5.1.3 Twin Studies on Left Ventricular Hypertrophy

Despite the interest in the genetic determination of left ventricular mass there are relatively few human studies that have quantified the extent of heritability in non-hypertensive subjects. Prior to the advent of well-defined echocardiography techniques heart volumes from chest x-rays were examined by Klissouras (Klissouras K, 1971) demonstrating as much intra-pair variability in monozygotic twins as in dizygotic twins suggesting no

significant genetic influence. In 1985 Landry examined 10 pairs of monozygotic twins who underwent echocardiography before and after a 20-week endurance training programme. Post-training, intra-pair differences were more similar when compared with the pre-test data suggesting a possible homogenising effect (Landry F et al, 1985). Adams et al examined 41 sets of college-aged twins in addition to 6 pairs of same-sex siblings and a group of non-related male subjects. Echocardiography was performed before and after a 14-week period of training. Intra-pair differences for left ventricular dimensions were compared between the four groups, i.e. monozygotic, dizygotic, sibs and non-related subjects. Inter-pair differences of left ventricular end diastolic dimensions were less in group 1 and group 2 when compared with the non-related subjects. This observation persisted following training. This study suggested that although familial influences may be important in determining cardiac size, there were no specific genetic effects (Adams TD et al, 1985). Beilen in 1990 studied 53 pairs of healthy male twins. The mean age at time of study was 21.7yrs in monozygotic twins and 23.8yrs for dizygotic twins. He concluded that there is a significant genetic component determining left ventricular structural features but not for cavity dimensions (Beilen E et al, 1991). In a further study of young healthy male twins aged 18-31 (n = 24) they could not detect a significant genetic effect on left ventricular mass independent of body size (Fagard R et al, 1995). Harshfield studied a small cohort on black twins (n = 22). There intra-class correlations for monozygotic and dizygotic twins were 0.9 and 0.33 respectively (Harshfield GA et al, 1990). The use of highly selected

populations and under-powering of studies goes some way to explain these contradictions.

The largest twin study to date was performed on 254 twin pairs living in the Commonwealth of Virginia (Verharen HA et al, 1991). The mean age of the subjects, who were identified from school rosters, was 11.2 years. This study attempted to answer the questions of how much of left ventricular mass variation was under genetic control; how much of this determination persisted following correction for weight and sexual maturity and what proportion of this genetic input was common to both weight and left ventricular mass. The author concluded that following removal of the effects of weight and sexual maturity, genes remained important determinants of left ventricular mass in this population of children. Bivariant genetic analysis, confirmed that genes common to left ventricular mass and weight significantly influenced the covariation of these variables and that greater than 90% of the correlation was due to common genes. To date there have been no large adult study examining left ventricular structure in a population-based twin study.

5.1.4 Potential candidate genes

The existence of rare familial disorders of left ventricular structure and function has provided several potential candidate genes in the study of left ventricular hypertrophy. Hypertrophic cardiomyopathy, a familial disorder associated with asymmetrical septal hypertrophy and sudden death, has been mapped to several gene loci in different family groups (Bonne G et al, 1998). In 1990 the Beta-myosin heavy chain gene 14q 11-12, which codes

for a protein of the myofilament in the sarcomere, was identified. Since then 10 different genes and over 140 different mutations have been shown to be responsible for the disease in various family groups (Bonne G et al, 1998). The prognostic significance and phenotypic expression of individual mutations are the subject of ongoing studies (Karibe A et al, 2001). Other familial disorders of myocardial structure and function such as the group of familial dilated cardiomyopathies also suggest potential genetic loci for investigation - for example the "pure" dilated familial cardiomyopathy associated with the cardiac actin gene and two chromosomal loci 9q 13-22 and 1q32 (Olson TM, 1998). Understanding of the basic pathophysiology of hypertrophy and remodelling, in these disease states, permits the dissecting out of other potential candidates for the more common forms of left ventricular hypertrophy and dysfunction occurring within the community. This is often a slow process involving the investigaton of a large numbers of potential candidates (Tiret L et al, 2000).

The left ventricle consists of myocytes and interstitial tissue, including collagen, and both types of tissue are involved in hypertrophy of the ventricle. Genes encoding proteins involved in the processes of muscle and collagen turnover such as angiotensin II, insulin-like growth factor I, cytokines, and metalloproteinases, provide plausible candidates (Busjahn A et al, 2000). The ACE (angiotensin converting enzyme) genotype, with its associated deletion and insertion alleles, has been investigated more fully that most and will be discussed in greater detail.

The genetic polymorphism in intron 16 was first described by Rigat et al in 1990 (Rigat B et al, 1990). This polymorphism is characterised by an insertion (I) or a deletion (D) of a 287 base-pair sequence. The ACE I/D polymorphism is closely related to the plasma ACE level with increased levels in subjects with the D allele. Schunkert in 1994 suggested that subjects with homozygosity for the DD allele were more prone to cardiac hypertrophy (Schunkert H et al, 1994). However, this association was not evident in Lindpaintner's larger Framingham cohort (Lindpaintner K et al, 1996). Controversy persists but more recent consensus is that the ACE genotype may be of only borderline significance. Many of these initial studies were in small populations and publication bias may have clouded the issues further.

The ACE locus may, however, be more important in particular situations where the renin-angiotensin system is under stress. One such example was Montgomery's study of 140 male army recruits undergoing a period of intensive training. The DD allele was associated with the greatest increase in LV mass in this group (Montgomery HE et al, 1997). Further investigation of this locus continues and identification of particular facets of LV mass that are affected by the ACE gene (Busjahn A et al, 1997).

This area of genetic research has blossomed over the last 5 years with a series of potential candidate genes being investigated in human and animal subjects. However, as discussed above, a prerequisite to detailed teasing

out of the genetic architecture of a phenotype is to firstly determine that the parameter is under a significant degree of genetic control.

5.2 Aims

The aim of this chapter was to determine:

1. To determine factors associated with left ventricular mass in the twin cohort
3. To identify, following adjustment for these factors, to what degree left ventricular mass was inherited in this population.

5.3 Methods

Trans-thoracic echocardiography was performed and analysed on the twin cohort as described in Chapter 2 Methodology. Left ventricular mass was calculated using the Penn-cube LV mass equation. The data from a subject was disregarded if a good quality M-mode long-axis view could not be obtained.

5.4 Results

5.4.1 Analysable M-mode data

Fully analysable echocardiograms were obtained in 220 patients (72.8%). Those without acceptable M-mode measurements were excluded from further analysis. This group were older 58.7 (12.5) vs. 51.2 (11.7), $p<0.0001$ and had higher systolic blood pressures 136.8 (16.2) vs. 129.1 (15.0), $p=0.0004$ than those with satisfactory data. There was no differences in weight 65.9 (16.5) vs. 66.0 (14.8), p=NS or height 163.4 (9.35) vs. 161.5

(7.94), p=NS between those with analysable data and those with data that could not be used.

5.4.2 Descriptive data

There were no significant differences between the MZ and DZ groups with regards to baseline echocardiographic parameters. This data is displayed in Table 4.1. The most important determinants of E/A ratio, that is the ratio of the 2 peaks of mitral forward flow (Figure 2.1), were age (correlation coefficient of –0.53, $p<0.01$) and resting diastolic blood pressure (correlation –0.62, $p<0.01$) (Table 5.2). There was a significant difference in age (50.6 vs. 53.7, p=0.012) and systolic blood pressure (123.6 vs. 112.1, p=0.04) between the lowest and highest quartile of E/A ratio. There was no difference in LV mass between these 2 quartile groups (p=NS).

5.4.3 Determinants of LV mass

The most important determinants of left ventricular mass in the cohort were weight (correlation 0.43, $p<0.01$) and sex (-0.41, $p<0.01$). Day-time systolic blood pressure was the most important blood pressure parameter with a correlation of 0.26; $p<0.01$. Blood pressure parameters, clinic and 24 hour measurements, were more closely related to wall thickness than internal diameters (Table 5.4).

5.4.4 The heritability of left ventricular structure

The intra-class correlation coefficients for the monozygotic and dizygotic groups and their comparison (z) are displayed in Table 5.3. Left ventricular

internal dimensions in diastole and left ventricular mass were both under a significant degree of genetic determination (heritability estimates of 0.61 and 0.69 respectively). Two thirds of the variation in LV mass could be explained by a genetic influence. Following correction for age, sex, blood pressure and weight (Corrected LV mass (Corr LVM*)) there was still a strong genetic component to left ventricular mass (heritability estimate of 0.53, p=0.006). Although there was a trend towards a genetic contribution to diastolic wall thickness this did not reach significance.

	MZ	DZ	p value
Sex	**22M; 98F**	**28M; 72F**	-
Age (yrs)	57.8 (10.5)	54.1 (11.5)	0.14
SBP (mmHg)	133.8 (16.0)	131.6 (17.6)	0.94
Weight (kg)	68.2 (20.0)	64.1 (13.1)	1.0
IVSd (cm)	1.1 (0.2)	1.08 (0.21)	0.7
LVDd (cm)	4.67 (0.5)	4.72 (0.5)	0.51
PWd (cm)	0.81 (0.14)	0.81 (0.13)	0.33
LV mass (Penn)	178.4 (57.9)	180.4 (59.6)	0.43
E wave (m/s)	0.76 (0.16)	0.78 (0.15)	0.48
A wave (m/s)	0.66 (0.14)	0.68 (0.16)	0.48
FT (IVS)	33.11 (18.5)	33.19 (17.7)	0.94
FS	38.28 (7.1)	37.83 (6.1)	0.72
E/A	1.18 (0.3)	1.19 (0.31)	0.43

Table 5.1 Descriptive data: means (SD)

Where:
SBP = systolic blood pressure
IVSd = end-diastolic septal thickness in cm
LVDd = end-diastolic internal diameter in cm
PWd = end-diastolic posterior wall thickness in cm
FT = fractional thickening (%)
FS = fractional shortening (%)
E/A = ratio of E to A wave height

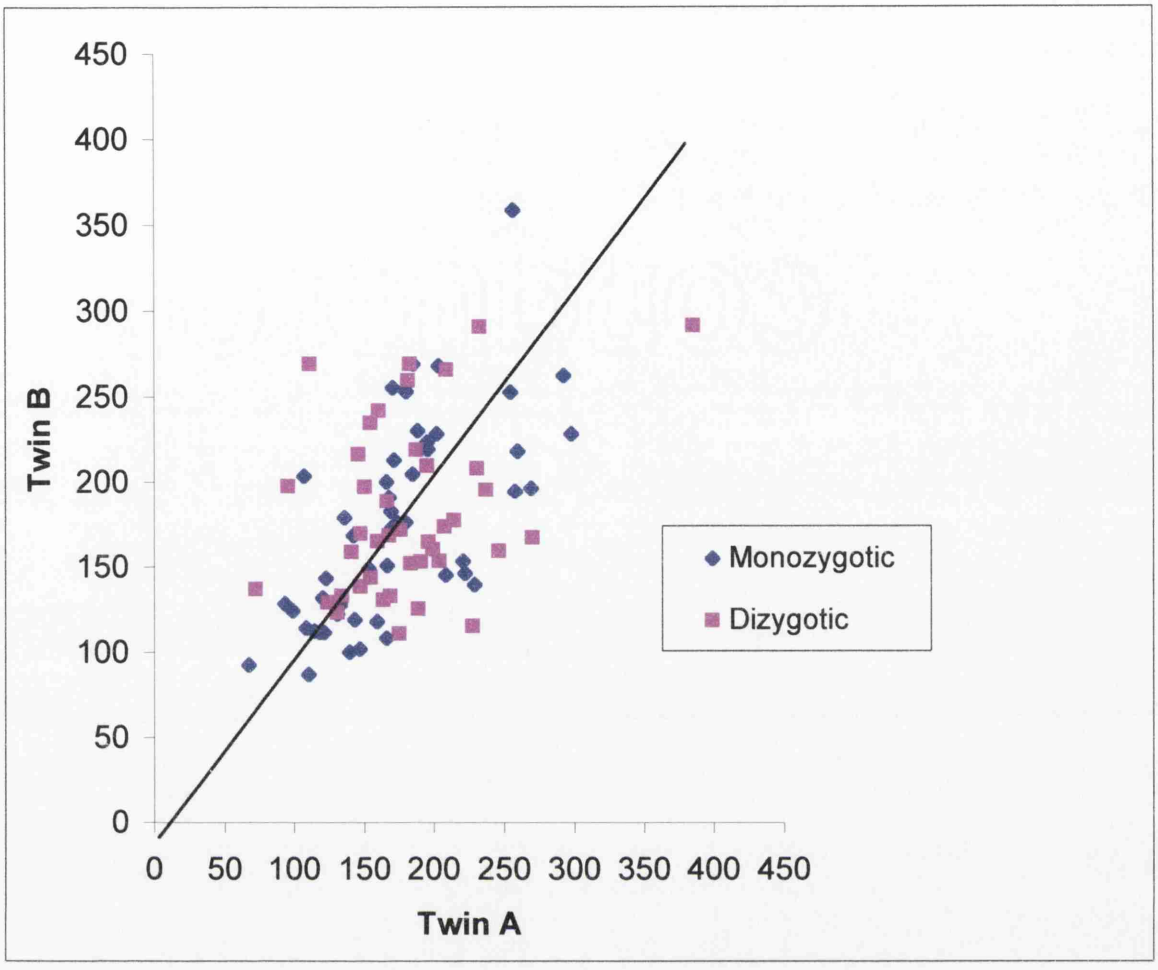


Figure 5.1 Left ventricular mass (Twin A vs. Twin B)

5.5 Discussion

The major determinants of left ventricular mass in this population were weight, sex and age. Of the 24-hour blood pressure parameters investigated daytime systolic pressures were most closely linked to left ventricular mass. Gosse also suggested this parameter affected myocardial hypertrophy more than nocturnal or diastolic measures of blood pressure (Gosse P et al, 1989) although as discussed in chapter 4 this is not a uniform finding.

	LV mass	E/A ratio
Age	0.20**	-0.53**
Sex	-0.41**	0.01
Weight	0.43**	0.05
Height	0.29**	0.09
Body surface area	0.14*	-0.04
Resting systolic BP	0.14*	-0.15*
Resting diastolic BP	0.08	-0.62**
Smoking (packs)	0.01	0.04
24hr systolic BP	0.17**	-0.27**
24hr diastolic BP	0.18*	0.29**
24hr mean BP	0.19**	-0.33**
Day systolic BP	0.26**	-0.3**
Day diastolic BP	0.16*	-0.27**
Day mean BP	0.19**	-0.3**
Night systolic BP	0.17*	-0.26**
Night diastolic BP	0.22**	-0.23**
Night mean BP	0.21**	-0.29**

Table 5.2 Correlations with LV mass and E/A ratio

(*p<0.05, **p<0.001)

Previous twin studies in children and young adults have given conflicting results regarding the heritability of left ventricular structure and function. In the largest twin study to date left ventricular mass was under genetic control in a group of 11-year-old children (Verharen HA et al, 1991). However, it is known that the degree of genetic influence over a given phenotype varies with age and different genes are important at various stages in life. In adult life, the age at which many of these disease processes are clinically relevant, the effects of genes may be diminished or "watered-down" by the cumulative effect of environmental and lifestyle factors (Tambs K et al, 1993). The aim of this study was to investigate if this heritability persisted into adulthood.

This study confirms a genetic influence over many aspects of left ventricular structure. Left ventricular mass, uncorrected, demonstrated a significant degree of heritability with approximately 70% of LV mass being genetically determined. A familial effect and a trend towards a genetic effect are noted in the measurements of septal and posterior wall thickness. It is, however, left ventricular internal dimensions, rather than wall thickness that were influenced by genetic factors. The heritability of mass remained significant following correction for basic anthropometric and blood pressure measurements with approximately half of the variation being due to genes.

Functional assessments of the left ventricle such as fractional shortening and fractional thickening did not reveal a significant genetic component, environmental factors being more important.

	n-MZ	R-MZ	Z-MZ	n-DZ	R-DZ	Z-DZ	Z	*p*	h^2
LVDd	50	**0.69**	0.85	41	**0.39**	0.41	2.04	**0.02**	**0.61**
LVDs	49	0.56	0.63	41	0.42	0.45	0.82	0.20	0.27
FS	49	0.20	0.20	41	0.28	0.28	-0.376	0.64	-0.15
FT	50	0.06	0.06	41	0.31	0.33	-1.22	0.89	-0.50
IVSd	50	0.38	0.40	41	0.21	0.21	0.88	0.19	0.34
IVSs	49	0.29	0.29	42	0.36	0.38	-0.38	0.65	-0.15
PWd	50	0.41	0.44	41	0.11	0.11	1.54	0.06	0.61
PWs	48	0.46	0.50	42	0.35	0.37	0.63	0.26	0.23
IVS/PW	49	0.14	0.14	42	0.15	0.15	-0.05	0.52	-0.02
PENN	50	**0.69**	0.85	41	**0.32**	0.33	2.39	**0.008**	**0.69**
Corr LVM*	48	**0.53**	0.59	39	**0.03**	0.03	2.52	**0.006**	**0.53**
LA	45	0.68	0.82	39	0.51	0.56	1.19	0.12	0.34
E	45	**0.49**	0.54	40	**0.13**	0.13	1.84	**0.03**	**0.49**
A	44	0.36	0.37	39	0.12	0.12	1.01	0.14	0.36
E/A	44	0.24	0.25	40	0.50	0.55	-1.36	0.91	-0.52

Table 5.3 Intra-class correlation coefficients and heritability estimates for echocardiographic parameters

Mitral valve E wave forward flow showed similar genetic determination. The mitral wave E wave reflects the early diastolic filling of the left ventricle which flows down a pressure gradient. The relative contribution of early and late filling (E and A wave) is a reflection of diastolic left ventricular function. As in Bielen's study early diastolic filling, but not late filling was under genetic control. The determinants of the E/A ratio in this population were age and systolic blood pressure. LV mass was not a determination of E/A ratio in this population. Bielen suggested that the E/A ratio may be "more related to qualitative than to quantitative cardiac characteristics" - in other words a reflection of myocardial function rather than myocardial structure.

Twin estimates of heritability are often higher than those of sibling and family cohorts and indeed the estimates from this study were higher than those obtained from the Framingham family study which estimated adjusted LV mass at between 0.24-0.32 (Post WS et al, 1997). In the largest twin echo study in children the estimates of heritability varied from 63-77%. This study reveals that this genetic influence persists into adulthood and identifies LV mass in adults as a potential target for further genetic study with corrected left ventricular mass being a valuable phenotype for the further study of potential candidate genes. It would be of interest to assess if the proportion of genetic control over LV mass in this "high risk" Scottish population was significantly different from other adult populations studied using a twin methodology.

	LV mass	IVSd	IVSs	PWd	PWs	LVDd	LVDs
24hr systolic	0.17*	0.27**	0.31**	0.20**	0.22**	-0.04	-0.15*
24hr diastolic	0.18*	0.25**	0.29**	0.22**	0.29**	-0.05	-0.10
24hr mean	0.19**	0.26**	0.31**	0.24**	0.32**	-0.05	-0.12
24hr heart rate	-0.15*	0.02	-0.02	-0.02	-0.10	-0.24**	-0.14
Day systolic	**0.26****	**0.32****	**0.34****	0.26**	0.33**	0.01	-0.09
Day diastolic	0.16*	0.25**	0.27**	0.19**	0.25**	-0.04	-0.07
Day mean	0.19**	0.28**	0.30**	0.22**	0.29**	-0.04	-0.11
Day heart rate	-0.24**	-0.07	-0.07	-0.09	-0.15*	**-0.26****	**-0.16***
Night systolic	0.17*	0.16*	0.19**	0.18*	0.39**	0.05	-0.03
Night diastolic	0.22**	0.18*	0.27**	0.25**	0.35**	0.06	-0.03
Night mean	0.21**	0.18*	0.29**	**0.27****	**0.40****	0.05	-0.05
Night heart rate	-0.13*	-0.05	-0.01	0.04	-0.03	-0.19**	-0.14

Table 5.4 Pearson's correlation coefficients for 24hr blood pressure and LV structure

(*p<0.05, **p<0.01)

Chapter 6

Carotid Intima Media Thickness

6.1 Introduction

6.1.1 Atherosclerosis

The process of atherosclerosis places a considerable financial burden on Western civilisation with its clinical manifestations of ischaemic heart disease, ischaemic heart failure, stroke, renovascular and peripheral vascular disease (Currie CJ et al, 1997). The formation of atheroma is the end-point of a long pathological prodrome involving a complex interaction between the endothelium, intimal smooth muscle cells, macrophages, T-lymphocytes, collagen elastic fibrous protoglycans and numerous circulating factors including lipids. The precursors of this process are detectable even in early childhood. Lipid-laden macrophages and lipid-filled smooth muscle cells causing fatty streaks are apparent in children even in the first decade of life (Stary HC et al, 1989). At the cellular level the initiation of pathological genetic and molecular processes may be activated even earlier by pre-natal factors such as intra-uterine growth retardation (Barker DJ, 1991). A multitude of studies are attempting to elucidate the individual cellular and genetic triggers underpinning these processes and the way in which factors culminate in the development and maintenance of clinically relevant atheroma.

Until recently the routine assessment of the extent of atherosclerosis has been limited to documenting end-points such as myocardial infarction or through the indirect examination of risk factor profiles. Only a minority of individuals with suspected established disease are then subjected to invasive quantification such as coronary angiography. Risk factor assessment has

been extremely useful not only in identifying patients at particular risk of vascular endpoints but also in aiding our understanding of the triggers involved in the initiation of atherosclerosis. The Framingham scoring system (Anderson KM et al, 1991), and its simpler clinical equivalents such as the Sheffield (Haq IU et al, 1997) Primary Prevention Tables, allow patients to be given an estimate of atherosclerotic risk. Such assessment, which will be discussed in detail in chapter 8, is, however, only a surrogate for direct measurements.

Angiography of the coronary circulation, peripheral vascular tree, carotid or renal artery beds is the current invasive approach for the assessment for atherosclerosis and provides the key to both diagnosis and to further intervention. However, the invasive nature of angiography prevents its application to the general population as a screening tool and limits its usefulness in charting disease progression. In an ideal world it would be possible to screen an individual prior to the development of clinically relevant atheroma and also to follow the progression of disease using a simply non-invasive test. Such a test would also permit the effects of therapy, such as lipid lowering drugs, to be monitored individually as opposed to relying on average response rates from large-scale clinical trials. Imaging of the carotid tree by B-mode ultrasound fulfils many of these requirements.

6.1.2 B-mode carotid ultrasound

Doppler ultrasound assesses velocity of flow in an artery allowing conclusions to be reached regarding cross-sectional area and function. In

contrast B-mode ultrasound relies on acoustic characteristics of different tissue types to generate a cross-sectional image of an artery. This can be used to estimate lumen diameters, but more interestingly, is its use in imaging the arterial wall and in particular the intima and media (Salonen JT et al, 1993). It was Pagnole who pathologically demonstrated that the two interfaces measured in B-Mode ultrasound correspond to the adventitial peri-adventitial line and the intimal lumen interface (Pignoli P et al, 1986). B-mode estimates of wall thickness (Figure 6.1) are reproducible and are superior to angiographic assessment when compared to pathological specimen results (Ricotta JJ et al, 1987).

The normal intima at birth consists of a thin layer of connective tissue consisting of basement membrane and the occasional smooth muscle cell. With age the amount of connective tissue within this layer increases due to thickening of the basement membrane, and the appearance of elastic fibres and collagen fibrils. In addition, there is a concentric increase in the number of smooth muscle cells. In patients who develop clinically relevant atherosclerosis there is an asymmetrical thickening of the intima which eventually encroaches upon the lumen of an artery, leading to functional alterations of blood flow. A second form of thickening is recognised which is associated with a degree of dilatation of the artery so that there is little in the way of change in lumen size. This second form commonly remains clinically silent. The ideal non-invasive investigation would permit the assessment of asymptomatic intimal medial thickening within the vascular bed studied but

also be a reflection on the manifestations of atheromatous disease throughout the vascular tree (Geroulakos G et al, 1994).

Carotid B-mode ultrasound assessments of intimal medial thickness (IMT) have been advocated as reflecting the "total body atheroma burden". Autopsy studies suggest a close correlation between carotid and coronary atherosclerosis (Holme I et al, 1981). B-mode carotid IMT measurements are known to be markers for events within that vascular territory, for example cerebral infarction (Touboul PJ et al, 2000) (Chambless LE et al, 2000), but also for events in remote sites (Burke GL et al, 1995) (Craven TE et al, 1900). In a cohort of 1300 Finnish men the presence of structural abnormalities in the carotid tree was associated with a 3.29 (CI 1.31-8.29) relative risk of acute myocardial infarction suggesting a close relationship between carotid artery atheroma and coronary atheroma (Salonen JT et al, 1991). The Rotterdam Study was a prospective follow up study in 7,983 elderly subjects living in the suburb of Rotterdam. The analysis was based on the occurrence of 98 myocardial infarcts and 98 strokes during a mean follow up of 2.7yrs. The risk of stroke increased with increasing IMT - the odds ratio for an increase of 1 standard deviation in IMT was 1.41 (CI 1.25; 1.82) for stroke and 1.43 (1.16-1.78) for myocardial infarction. These findings persisted following correction for cardiovascular risk factors and excluding whose with previous events (Bots ML et al, 1997).

Unsurprisingly IMT measurements correlate with known cardiovascular risk factors for example smoking (Ebrahim S et al, 1999) and systolic

hypertension (Salonen R et al, 1991) are both associated with an increased prevalence of carotid plaques. An interesting association exists between carotid atherosclerosis and another well-known risk for cardiac events - left ventricular hypertrophy. Roman et al studied 486 asymptomatic adults following observations from the Framingham study of an association between stroke and LV mass. They observed that individuals with LVH were twice as likely to have carotid atheroma although the mechanism for this association was unclear (Roman MJ et al, 1995).

6.1.3 B-mode Methodology

There has been much debate over a standard methodology for measuring and scoring intima medial thickness (Crouse JR et al, 1993). Initial scoring systems were complex with attempts to measure IMT at the internal and common carotid segments and at the bifurcation. When defining a protocol it is important to determine which measurements are 1) easily obtained on the majority of the cohort 2) are the most reproducible and 3) which are the best markers for future events. The common carotid artery can be visualised in greater than 95% of individuals as compared to the internal carotid at only 62% (Crouse JR et al, 1995). Measurements of the far wall, rather than the near wall, are easier to obtain and more reproducible. In addition analysis of the internal carotid has the additional disadvantage of being hampered by the more frequent occurrence of plaques. For these reasons measurements solely from the common carotid segments have been used in recent studies. These studies have confirmed the relation between results from this methodology and clinical end-points (Touboul P-J et al, 2000). The sum of

the maximal thickness at several sites has been shown to be more reproducible than individual measurements and for this reason averaged measurements are used (Riley WA et al, 1996). Attention should be paid to reproducibility data when accessing a study's conclusions as marked variations exist between centres and methodologies. A reproducibility 3 or 4 times the annual progression rate of carotid IMT would cast major doubt over the validity of the resulting conclusions.

In this present study near and far wall common carotid artery segments were analysed. Maximum and mean intimal medial thickness measurements were used in the analysis.

6.1.4 Previous heritability studies

As discussed in chapter 1 cardiovascular and cerebrovascular events are known to cluster in families and previous twin and family studies have attempted to quantify this degree of heritability (Berg K et al, 1989) (Reed T et al, 1991). Whether this is mediated through the actual extent of atherosclerosis and the burden of plaque disease within a vascular bed is unclear.

When jejunal arteries are transplanted between WKY normotensive rats and spontaneously hypertensive rats (SHR) medial hypertrophy occurred only in the vessels transplanted into the hypertensive rats and not the normotensive group suggesting the factors at work were independent of a genetic influence (Pang SC et al, 1985). This contrasts with studies of inbred mice that show a

genetic influence over the development of aortic atheroma calcification (Qiao JH et al, 1994). Conflicting results are also seen in human studies. Hamsten investigated a group of young men and found no relationship between a positive family history of vascular events and the extent of atheroma (Hamsten et al, 1987).

Race influences intimal medial thickness. Subjects of an oriental extraction have lower IMT measurements than those of a Caucasian American or Australian ethnicity (Avolio A et al, 1995). Structural abnormalities in the abdominal aorta, such as a tendency to aneurysm formation, are also known to show a familial pattern (Baird PA et al,1995). Case-reports have described similarities in coronary angiographic appearances between pairs of twins but these are isolated examples that cannot be considered as anything more than anecdotal (Samuels LE et al, 1999). No studies to date have focused on the formal quantification of actual atheroma load within a population based twin study. This study examined the heritability of carotid intimal medial thickness within such a population using B-mode ultrasound.

6.1.5 The aims of this study were:

1) To assess the relationship between known cardiovascular risk factors and intimal medial thickening in this population.
2) To assess the relationship between left ventricular hypertrophy, 24 hr ambulatory blood pressure and IMT measurements
3) To assess the heritability of IMT measurements in this population.

6.2 Methodology

Carotid scans were performed by experienced ultrasonographers using a Biosound 2000II scanner (Biosound Inc., Indianapolis, USA) with an 8 MHz transducer. Scans were recorded on Super VHS videotape. Following the acquisition of the scan the ultrasonographer grabbed the optimal images which best demonstrated near and far wall intimal thickening. These grabbed frames were acquired in diastole. Both near and far walls were visualised on the same scan to ensure the transducer was transecting the artery at 90°. The ultrasonographer grabbed the frames, demonstrating the maximal intimal medial thickness (Digital Time Base corrector FA-310 P, FOR-A Co. Ltd., Japan). All carotid scans were subsequently read by a single blinded observer and images were analysed using a quantitative analysis package (Siemens). This software gives an axial resolution of 0.001mm. Grabbed frames were calibrated against a 10 mm reference marker. IMT measurements were made to the nearest 1 pixel (0.0769 mm) and quoted to one-hundredth of 1mm. Measurements of intimal-medial thickness were made at 1mm intervals over a 10mm segment of artery. Sites were defined according to the ARIC study protocol (Burke GL et al, 1995). No value was entered if the interfaces were not clearly visible. The maximal and mean IMT value were determined for each segment.

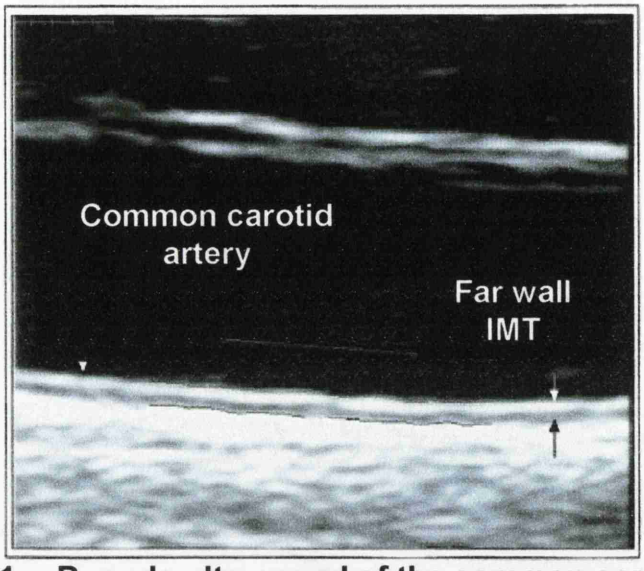


Figure 6.1 B-mode ultrasound of the common carotid artery

6.3 Results

6.3.1 Descriptive data

There were 270 carotid scans with at least one analysable segment in either the left or right common carotid segments. As previously reported, quality far wall images were more frequently obtained than near wall images. Analysable far wall measurements of the right common carotid segment (RCCA) were obtained in 93% and RCCA near wall measurements in 79%. In the left common carotid artery (LCCA) the far wall was measured in 86% and the near wall 74%. Intimal medial thickness measurements in all 4 segments were normally distributed. There were no statistically significant differences between the monozygotic and dizygotic sub-groups (Table 6.1).

	MZ	DZ	p
Age	54.3	51.7	0.08
Sex	**24 males 118 females**	**31 males 91 females**	
RCCA - Far wall mean	0.73 ± 0.13	0.72 ± 0.14	0.43
RCCA - Far wall max	0.83 ± 0.14	0.81 ± 0.15	0.26
RCCA - Near wall mean	0.78 ± 0.13	0.79 ± 0.15	0.8
RCCA - Near wall max	0.87 ± 0.15	0.88 ± 0.16	0.54
LCCA - Far wall mean	0.74 ± 0.13	0.73 ± 0.13	0.67
LCCA - Far wall max	0.84 ± 0.15	0.82 ± 0.14	0.42
LCCA - Near wall mean	0.81 ± 0.13	0.79 ± 0.15	0.34
LCCA – Near wall max	0.91 ± 0.15	0.88 ± 0.16	0.23

Table 6.1 Intimal medial thickness - descriptive data

6.3.2 Correlations with IMT

The strongest correlations with intimal medial thickness (right common carotid far wall mean) were age (0.59, $p<0.001$), systolic blood pressure (0.42, $p<0.001$), and diastolic blood pressure (0.32, $p<0.001$). Smoking history was not significantly correlated with IMT (-0.06, $p=0.34$). In addition there was no statistical difference between the intima medial thickness of "never" smokers (RFW-mean 0.74±0.13) versus "ever" smokers (RFW-mean 0.72±0.14, $p=0.28$). Total cholesterol was weakly associated with carotid IMT (0.24, $p<0.001$). Ambulatory blood pressure measurements were not any more sensitive predictors than clinic blood pressures. In addition pulse pressure, which has been suggested as a marker for wall stress, was not as strong a correlate as systolic blood pressure (Table 6.2).

Intima medial thickness was compared with left ventricular parameters. There was an association with septal thickness (0.19, p=0.01) but not with left ventricular mass itself (0.12, p=0.1). Carotid intima medial thickness was significantly higher in those with a history of vascular disease, hypertension or diabetes (0.76±0.13 versus 0.72±0.14, p=0.014). When those with ischaemic heart disease were looked at in isolation there was still a positive association with an increased intimal medial thickness (0.79±0.12 vs. 0.72±0.14, P=0.01).

6.3.3 Heritability

Intra-pair correlation coefficients and heritability estimates were calculated for maximal and mean intimal medial thickness for all 4 segments. A significant genetic effect was not demonstrated for any of the measures of intimal medial thickness. There was a trend towards a genetic effect with larger intra-class correlation coefficients for the monozygotic group than the dizygotic group but this did not reach statistical significance (for example RFW-mean MZ r=0.54 vs DZ 0.39, p=0.15). Following correction for known cardiovascular risk factors this trend continued but correlations were diminished. Heritability data is displayed in Table 6.3.

	RFW-mean	p
Age	0.55	**<0.001**
Sex	-0.14	**0.03**
Height	-0.02	0.71
Weight	0.005	0.94
Creat	0.22	**0.001**
Total cholesterol	0.16	**0.02**
Smoking	-0.06	0.39
Fibrinogen	-0.06	0.38
SBP	0.38	**<0.001**
DBP	0.30	**<0.001**
24hr SBP	0.28	**<0.001**
24hr DBP	0.16	**0.02**
PW	0.02	0.81
IVS	0.17	**0.02**
Penn LV mass	0.12	0.12

Table 6.2 Correlation Coefficients - RFW-IMT

6.4 Discussion

The heritability of intimal medial thickness was investigated because of the belief that IMT is an accurate assessment of total body atheroma burden. B-mode ultrasound is a safe non-invasive test that can be used repeatedly to detect sub-clinical abnormalities or to plot disease progression and is easily applicable to a large population study. In family or genetic studies if IMT was demonstrated as showing a significant degree of heritability then these

measurement could be used in the assessment of potential candidate genes not only targeting cardiovascular risk factors but also assessing the actual presence of disease. This study did not, however, reveal a significant difference between the correlations for monozygotic and dizygotic groups. There were familial similarities between the 2 groups and a trend towards a genetic contribution but no statistically significant heritability. This methodology focuses on narrow-sense heritability and it may be that some of the within pair correlations noted may have a genetic basis – granted a weak one.

As discussed in chapter 1 some sub-sets of twins are known to share similar intra-uterine and early post-natal environments. It may be that these factors are important in the familial similarities observed in this study. This would be in keeping with Barker's suggestions that early peri-natal programming is a key factor in the development of atherosclerosis. Twin studies on the occurrence of myocardial infarction show the strongest genetic component in those infarcts that occur at an early age (de Faire U et al, 1975). This suggests that environmental factors may in later life "overwhelm" a pre-existing genetic tendency to disease or that different stages of the process of atherosclerosis are under differing degrees of genetic control.

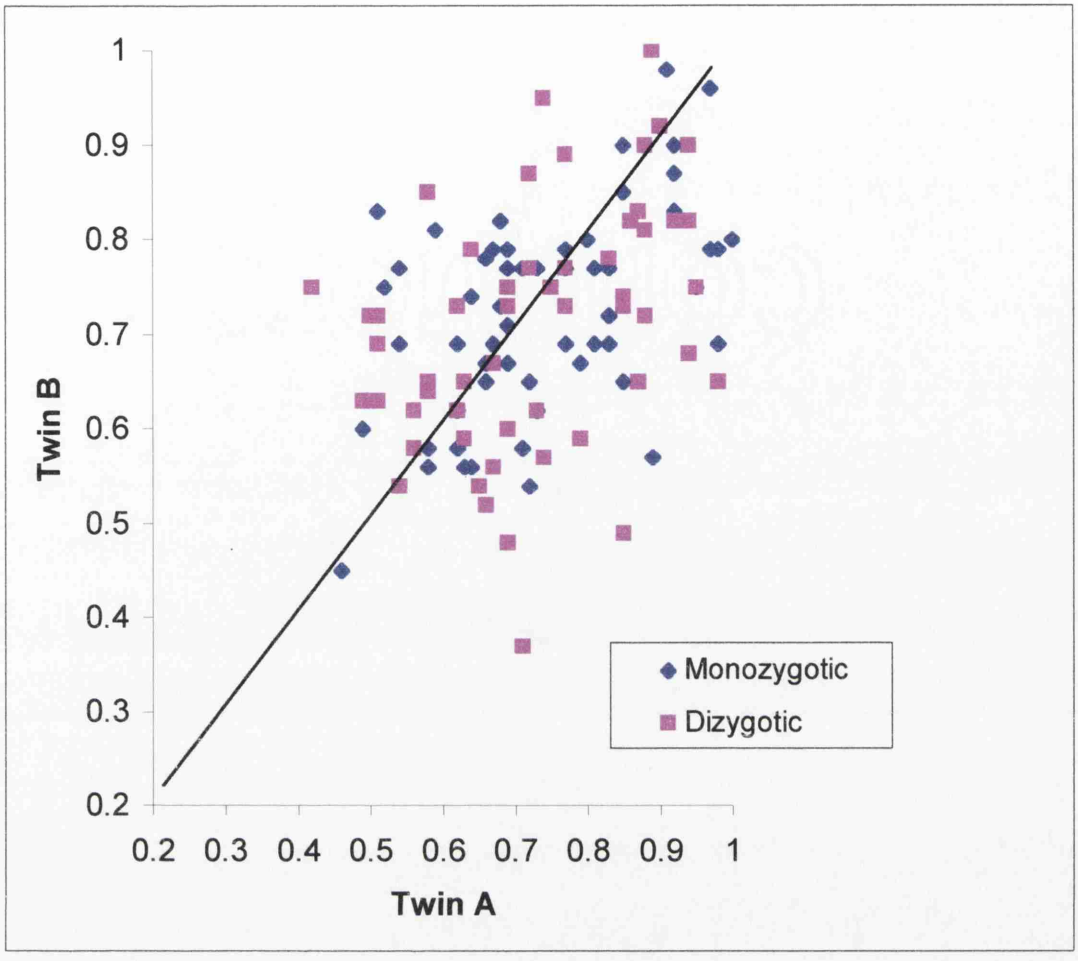


Figure 6.2 Graph of intimal medial thickness (RCCA)– Twin A vs. Twin B

Figure 6.2
As can be seen pictorially there is no apparent difference between the monozygotic and dizygotic groups i.e. the monozygotes are no closer to the ideal line of Twin A=Twin B than the dizygotes.

Although many authors believe that the presence of early atheroma has a strong genetic basis there remains several conflicting studies. As discussed above Hamsten found no relationship between a positive family history of vascular disease and the extent of atheroma or the number of haemodynamically significant stenoses (Hamsten A et al, 1987). In this present study there was a positive association between an increased intimal medial thickness and vascular events. This was not just true for stroke

disease but for ischaemic heart disease i.e. events in a vascular bed remote from the area being scanned. One possible hypothesis from this study, and that of Hamsten, is that the genetic contribution to cardiovascular events is not mediated through the degree of atherosclerotic load. Our focus would therefore turn to the mediators of plaque rupture and thrombosis. A proposed area of future research would be to focus on the genetic contribution to these "dynamic" determinants of vascular events rather than the extent of atheroma in the vessel wall.

In this study middle-aged women predominate - the majority of whom have no specific past history of atheromatous disease. This population may be not at sufficiently high risk of vascular disease to show extremes of intimal medial thickening. This may partly explain the lack of associations with risk factors such as cigarette smoking. Important differences exist between cardiovascular event rates in males and females of this age and it may be that a twin study restricted to males would have had a different outcome.

This study did not reveal a significant genetic component to the early development of atheroma in the carotid artery wall. It would be interesting to repeat this study in a large group of male subjects to assess these relationships further. This study is not large enough to completely exclude a weak relationship (chapter 9). However, it can be said that when compared to left ventricular mass (chapter 4) and various blood pressure parameters (chapter 5) any genetic input is trivial. Whither this is true for other ethnic groups remains to be determined. In conclusion the use of intimal medial

thickness in further investigation of potential candidate genes may be less rewarding than investigating other parameters such as blood pressure or prothrombotic tendencies.

	n-MZ	R-MZ	Z-MZ	n-DZ	R-DZ	Z-DZ	z	h^2	p
RFW mean	60	0.54	0.61	55	0.39	0.41	1.05	0.31	0.15
RFW max	60	0.44	0.48	54	0.35	0.37	0.58	0.19	0.28
RNW mean	43	0.46	0.49	41	0.50	0.55	-0.27	-0.09	0.60
RNW max	44	0.45	0.48	41	0.46	0.50	-0.07	-0.02	0.53
LFW mean	53	0.42	0.45	49	0.41	0.44	0.07	0.02	0.47
LFW max	53	0.44	0.47	48	0.31	0.32	0.75	0.26	0.23
LNW mean	43	0.60	0.70	34	0.49	0.53	0.70	0.23	0.24
LNW max	44	0.60	0.69	33	0.37	0.39	1.27	0.45	0.10
RFW corr*	63	0.30	0.31	52	0.15	0.15	0.88	0.31	0.19

Table 6.3 Intra-class correlation coefficients / heritability estimates for carotid IMT

RFW mean/ RFW max Right common carotid far wall - mean & maximum measurements
RNW mean/ RNW max Right common carotid near wall - mean & maximum measurements
RFW corr* Right far wall measurement corrected for confounding variables

Chapter 7

Pulmonary function

7.1 Introduction

7.1.1 Pulmonary function tests

Simple tests of forced expiration have been used for many years in the routine clinical assessment of patients with lung disease. The physiology of these tests is complex but provided plateau flow conditions are achieved then these tests provide a simple measure of the mechanical characteristics of the airways and lungs independent of the pressures applied. These parameters are most commonly measured as changes in volume versus time using spirometry. Standard measurements are the forced expiratory volume in one second (FEV_1) and the forced vital capacity (FVC). Abnormalities in these variables tend to be in one of two distinct patterns: 1) "obstructive" spirometry, seen in illnesses such as chronic obstructive pulmonary disease, where the reduction in FEV_1 is greater than the reduction in FVC (the ratio of these 2 variables (FEV_1/FVC) is therefore also reduced) and 2) "restrictive" spirometry, for example in pulmonary fibrosis, where a small FVC is associated with a normal or even increased FEV_1. The simplicity of measuring these non-invasive parameters has permitted their widespread use in epidemiological and genetic studies (Ashley F et al, 1997).

7.1.2 The prognostic value of measures of forced expiration

A diminished FEV_1 is a well-established prognostic factor in pulmonary disease and is a marker for pulmonary mortality (Ebi-Kryston KL et al, 1989). However more unexpectedly all cause mortality, and more specifically cardiovascular death, correlate with FEV_1 and FVC. In 1989 Tockman et al stratified a group of 884 men who had undergone spirometry into those with

an FEV_1 < 70%, 70-89% and > 90% of predicted. Across age and cause of death, those with the impaired lung function had the poorest survival. Cardiovascular mortality followed pulmonary function more closely than smoking status. The link between an impaired FEV_1 and mortality was independent of smoking status, but its risk combined with smoking identified a sub-population at even greater risk of cardiac events. The odds ratio for a life-long non-smoker with an FEV_1 of 90% of predicted was 1.52 compared with 17.8 for a current smoker with an FEV_1 of <65% (Tockman MS et al, 1989). FEV_1 was associated with mortality from non-respiratory causes in a study of 3,452 male civil servants aged between 40-64 (Strachan DP et al, 1994). The odds ratio per litre decrease in FEV_1 was 1.44 (confidence interval of 1.19-1.73). In a population from Baltimore, all cause mortality during 24 years of follow up was significantly related to FEV_1 (Beaty TH et al, 1985). Similar findings have been reported for forced vital capacity among healthy non-smokers in the Framingham study (Kannel WB et al, 1983) and the Copenhagen City Heart Study (Lange P et al, 1991). Strachan suggested that FEV_1 adjusted for age was almost as strong a predictor of death as systolic blood pressure (Strachan DP et al, 1992).

These associations are also present in the Scottish population. The Renfrew and Paisley Study (Hole DJ et al, 1996) was a longitudinal study of 15,000 adults aged 45-64yrs who were first examined between 1972 and 1976. At base-line spirometry measurements of respiratory function were made and FEV_1 was estimated relative to a predicted value. Over the subsequent 15-year follow-up period mortality from all causes, ischaemic heart disease,

cancers, stroke and respiratory disease were examined. There appeared to be significant trends of increasing risk for all cause mortality with diminishing FEV_1. This persisted following adjustment for age, smoking, diastolic blood pressure, cholesterol, body mass index and social class. The relative hazard ratios for all cause mortality in subjects in the lower fifth of FEV_1 were 1.92 (1.68-2.2) for men and 1.89 (1.63-2.2) for women. The corresponding hazards for ischaemic heart disease were 1.56 and 1.88 and for stroke 1.66 and 1.65. These findings mimic those in the Whitehall 10-year mortality study (Ebi-Kryston KL et al 1988).

7.1.3 Height and pulmonary function

It is known that men of short stature are at an increased risk of death from coronary heart disease (Watt GC et al, 1995). Pre-natal malnutrition, social deprivation and childhood illness have all been postulated as playing a potential role in this relationship. Controversy has surrounded the relationship between FEV_1, cardiac mortality and height with several authors suggesting that the relationship between FEV_1 and cardiac disease is explainable solely by confounding variables such as build (Marcus EB et al, 1989). In contrast the British Regional Heart Study, which examined a representative sample of 7,735 middle-aged men, suggested the relationship was independent of height. After baseline spirometry the cohort was followed for a mean of 7.5 years with documentation of fatal and non-fatal ischaemic events. The heart attack rate within this group of patients increased from 5.6/1,000 per year in the tallest group to 10.7/1,000 in the shortest group in a linear fashion. FEV_1 was strongly related to height and also associated with ischaemic heart

disease. In this study the risk in the bottom quintile of FEV_1 distribution was 1.8 times greater than at the top quintile after adjustment for age, smoking, systolic blood pressure, cholesterol and height. It was the investigator's conclusion that height had no independent effect on the risk of major ischaemic events and that reduced lung function could have a direct causal role in myocardial infarction (Walker M et al, 1989). In the Whitehall study height adjusted FEV_1 was a stronger predictor of death than height, body mass index or plasma cholesterol (Strachan DP et al, 1992).

7.1.4 Proposed mechanisms of association

The reasons for the association between pulmonary function and cardiovascular disease are unclear with suggestions that lung function may simply be a marker of physical activity and general physical fitness (Higgins et al, 1991). This was supported by the Framingham population where the strongest correlation with FVC was handgrip strength suggesting that FVC may be a measure of general health and vigour (Kannel WB et al, 1983). However, habitual activity does not importantly determine respiratory function in adults. Forced vital capacity is a poor predictor of physical fitness (Blair SN et al, 1989) and does not correlate with measures of physical activity.

An alternative theory is that airway obstruction can lead to lowered partial pressure of oxygen (PaO_2) and this additional stress in combination with the reductions seen in cardiac output with age may be responsible for the excess in cardiovascular end-points. Tockman et al suggested that the reduction seen in pulmonary function reflects early sub-clinical cardiac failure

(Tockman MS et al, 1989). In early cardiac failure a "restrictive" spirometry would be present. It appears, however, that both obstructive and restrictive patterns are prognostically significant. Other theories postulated to explain this relationship include confounding phenotypes such as an excess of centripetal fat and insulin resistance (Weiss ST et al, 1991) or tendency to ventricular arrhythmia (Engstrom G et al, 2001). However, thus far no satisfactory explanation or understanding on causality have been determined between decreased pulmonary function and cardiac disease but the validity of the relationship now appears beyond doubt.

7.1.5 Previous genetic and familial studies on pulmonary function

The genetics of pulmonary function determination is an area of ongoing investigation (Chen Y, 1999) (Barnes PJ et al, 1999). Early family studies demonstrated an aggregation of varying levels of pulmonary function measurements (Astemborski et al, 1985) and a degree of heritability in lung function has been shown even in children under 10 years old (Lewitter FI et al, 1984). Abnormalities in lung function are more prevalent in the blood relatives of those with obstructive airways disease than in their spouses (Larson RK et al, 1970).

Conflicting results have, however, been obtained from studies of twins. Following correction for height Ghio et al could not detect any measure of pulmonary function that was heritable (Ghio AJ et al, 1989). In contrast to this the NHLBI Twin Study pulmonary function study, performed on 268 pairs of white male twins aged between 42 and 56 years, detected a significant

genetic variance in pulmonary function. After adjustment heritability was 0.91 and 0.77 for FVC and FEV_1 respectively. The subsequent analysis indicated the observed heritability of FVC resulted from the effect of years of smoking as well as genetic factors relating to body size. These findings suggest that there were no other significant determinants of FVC. In contrast heritability of FEV_1 could not be explained by constitutional factors such as height, weight or smoking (Hubert HB et al, 1982).

7.2 Aims of this chapter

The aims of this chapter were as follows:

1. To determine the strongest correlates with parameters of forced expiration in this population.
2. To determine if these parameters were related to other cardiovascular risk factors including 24hour blood pressure variables, left ventricular mass and Framingham risk scores.
3. To determine the heritability of FEV_1, and FVC in this population.

7.3 Methods

The methodology of the pulmonary function testing is as documented in chapter 2. All data was double entered into an epidemiology database - EPI-INFO. Data that did not meet the American Thoracic Society quality control guidelines were excluded from further analysis (Gardner RM et al, 1988). The maximum measurements of FEV_1 and FVC for each individual were used for further analysis. FEV_1 and FVC were then corrected for height (i.e. FEVH = $FEV_1/height^2$ and FVCH = $FVC/height^2$) and a second measure of FEV_1 and

FVC was corrected for age, sex, height, blood pressure, smoking and cholesterol (FEV1Corr+ and FVCCorr+). In addition FEV_1 and FVC were expressed as a percentage of the expected FEVH and FVCH for their age. Expected values were obtained from published normative data (Docherty DW et al, 1985). Docherty's study provides data for ages between 25-75 years and therefore twins older than 75 were excluded from this part of the analysis. As discussed in chapter 2 correlations are quoted as intra-class correlation coefficients. Smoking exposure was quoted as total packs smoked.

7.4 Results

7.4.1 Descriptive results

Removal of individuals with incomplete data or data that did not fulfil the American Thoracic Society Standards left 268 twins with analysable data. Means and standard deviations were compared for monozygous and dizygous groups. There were no statistically significant differences in baseline FEV_1, FVC, FEVH, FVCH or values expressed as a percentage of their predicted values between the 2 groups (Table 7.1). Smoking exposure was not significantly different between the two groups. In addition age (53.1$\pm$11.9 years vs. 51.6$\pm$11.5 years, p=0.3) and height (1.63$\pm$0.09 m vs. 1.64$\pm$0.1m, p=0.19) were not significantly different between the groups.

	MZ	DZ	p
Number	149	119	
FEV_1	2.48 ± 0.66	2.61 ± 0.64	0.12
FVC	3.19 ± 0.88	3.4 ± 0.81	0.06
FEVH	0.93 ± 0.19	0.95 ± 0.16	0.36
FVCH	1.19 ± 0.23	1.23 ± 0.19	0.13
%PFEVH	0.95 ± 0.15	0.95 ± 0.18	0.95
%PFVCH	0.99 ± 0.13	0.99 ± 0.16	0.92
Smoking – total packs	2074 ± 4010	2655 ± 4088	0.23

Table 7.1 Descriptive lung function data

7.4.2 Correlation of FEV_1 with other phenotypic variables

Age (-0.4, $p<0.001$), sex (-0.58, $p<0.001$) and height (0.66, $p<0.001$) correlated strongly with FEV_1. The residual values for FEV_1 following regression on age, sex, height, systolic blood pressure, cholesterol and smoking (FEVCorr+) were calculated for further analysis in addition to FEV_1 corrected for height (FEVH) and FEV_1 expressed as a percentage of predicted value (%PFEVH). FEV_1 corrected for height (FEVH), as discussed above, has been investigated by many authors as a marker of cardiovascular outcome. The strongest statistically significant correlates with FEVH from this cohort were fasting serum cholesterol (-0.21, $p=0.002$), fibrinogen (-0.22, $p=0.002$), clinic systolic blood pressure (-0.24, $p<0.001$), diastolic blood pressure (-0.133, $p=0.047$) and cigarette smoking (-0.164, $p=0.014$) (Table 7.2). Additionally a score of cardiovascular risk, based on the Framingham risk tables, was strongly correlated with FEVH (0.46, $p<0.001$). There was no significant correlation with 24hr parameters of blood pressure control. The

strong correlation of left ventricular mass with FEV_1 (r=0.63, p=0.03) disappeared when corrected for height (Figure 7.2).

These associations with FEV_1 and FEVH were studied again for FEVCorr+ and for %PFEVH. The only association with %PFEVH was cigarette smoking (-0.344, p<0.001). There was a non-significant trend towards a weak association with fibrinogen (-0.123, p=0.091).

7.4.3 Correlation of FVC with other phenotypic variables

The associations with FVC are displayed in Table 7.3. Again many intermediate cardiovascular risk factors, such as cholesterol, lipoprotein A, fibrinogen and blood pressure were correlated to FVC itself and to FVC corrected for height. This was also true for Framingham risk score (the correlation coefficient with FVCH was -0.374, p<0.001). In contrast to the FEV_1 as expressed as a percentage of predicted FEV_1 (%PFEVH) there was a persisting relationship between %PFVCH and fibrinogen (-0.164, p=0.022). Even following extensive correction for confounders a relationship remains between FVCCorr+ and fibrinogen (Table 7.4).

	FEV_1		FEVH		%PFEVH		FEV1corr+	
	Correlation	p	Correlation	p	Correlation	p	Correlation	p
Creatinine	0.16	**0.02**	-0.03	0.68	-0.04	0.63	-0.10	0.15
Cholesterol	-0.23	**0.001**	-0.21	**0.002**	0.02	0.79	-	-
Lipo A	-0.12	0.08	-0.08	0.24	-0.06	0.37	-0.13	0.06
Fibrinogen	-0.19	**0.007**	-0.22	**0.002**	-0.12	0.09	-0.20	**0.04**
SBP	-0.20	**0.003**	-0.24	**<0.001**	0.03	0.62	-	-
DBP	-0.12	**0.05**	-0.13	**0.05**	0.10	0.17	-	-
24hr SBP	0.04	0.56	-0.04	0.61	0.03	0.74	-	-
24hr DBP	0.16	**0.03**	0.08	0.28	0.08	0.30	-	-
LV mass	0.63	**0.03**	-0.04	0.64	-0.40	0.22	-0.02	0.81
Smoking	-0.04	0.52	-0.16	**0.01**	-0.34	**<0.001**	-	-
Risk score	-0.36	**<0.001**	-0.46	**<0.001**	-0.02	0.84	-	-

Table 7.2 Correlations with FEV_1 parameters

	FVC		FVCH		%PFVCH		FVCCorr+	
	correlation	p	correlation	p	Correlation	p	correlation	p
Creatinine	0.23	**0.001**	0.08	0.23	0.01	0.86	-0.07	0.32
Cholesterol	-0.21	**0.002**	-0.21	**0.003**	0.04	0.56	-	-
Lipo A	-0.14	**0.02**	-0.16	**0.02**	-0.13	0.07	-0.18	**0.01**
Fibrinogen	-0.19	**0.007**	-0.23	**0.001**	-0.16	**0.02**	-0.23	**0.001**
SBP	-0.15	**0.02**	-0.19	**0.004**	0.05	0.48	-	-
DBP	-0.13	**0.04**	-0.15	**0.03**	0.04	0.51	-	-
24hr SBP	0.03	0.65	-0.04	0.59	0.001	0.10	-	-
24hr DBP	0.11	0.15	0.03	0.73	-0.04	0.64	-	-
Penn	0.20	**0.007**	0.02	0.75	-0.07	0.35	-0.02	0.77
Smoking	0.02	0.70	0.06	0.33	-0.26	**<0.001**	-	-
Risk score	-0.29	**<0.001**	-0.37	**<0.001**	0.01	0.86	-	-

Table 7.3 Correlations with FVC parameters

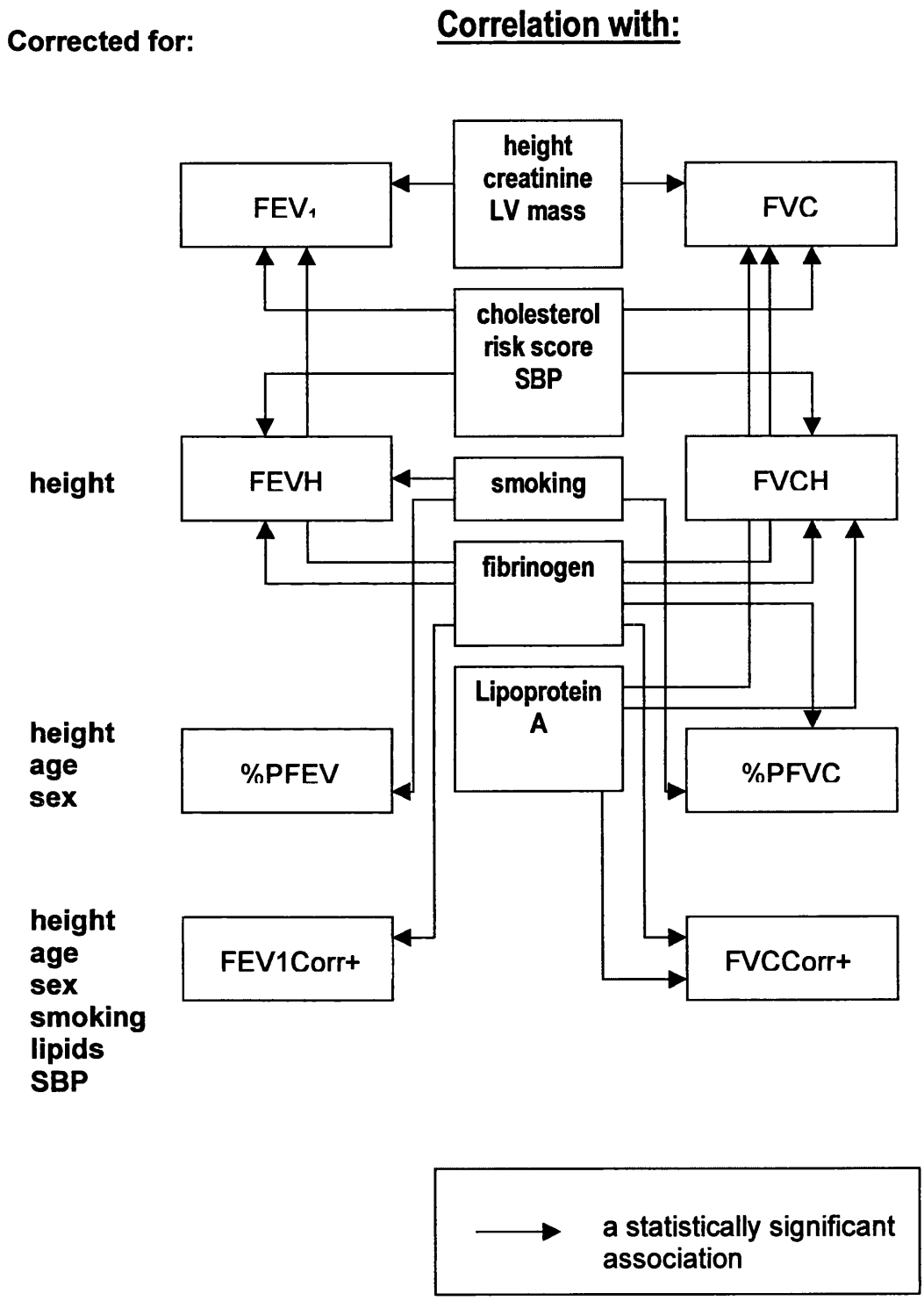


Figure 7.1 Associations with pulmonary function

7.4.4 Heritability of pulmonary function

FEV_1 and FVC are both under a significant degree of genetic control with estimates of 0.82 and 0.84 respectively (both $p<0.0001$). This association persisted following correction for height (FEVH and FVCH – h^2 of 0.61 and 0.71). When expressed as a percentage of the expected pulmonary function for a subject of that age, sex and height the genetic contribution to FEV_1 remained statistically significant (Table 7.4). When FVC was expressed in this format the MZ correlation remained greater than that of DZ (0.58 and 0.43) but this result was not significant ($p=0.16$). A similar non-significant trend was seen for FEVCorr+ and FVCCorr+, that is lung function that has been fully corrected for the additional compounding effects of blood pressure, lipids and smoking history.

Variable	R-MZ	R-DZ	h^2	P
Height	0.82	0.35	0.82	**>0.0001**
FEV_1	0.82	0.31	0.82	**>0.0001**
FVC	0.84	0.31	0.84	**>0.0001**
FEVH	0.68	0.38	0.61	**0.02**
FVCH	0.71	0.35	0.71	**0.007**
%PFEVH	0.61	0.10	0.61	**0.002**
%PFVCH	0.58	0.43	0.31	0.16
Smoking	0.65	0.10	0.65	**>0.0001**
FEVCorr+	0.56	0.39	0.23	0.25
FVCcorr+	0.53	0.33	0.39	0.14

Table 7.4 Heritability estimates for lung function

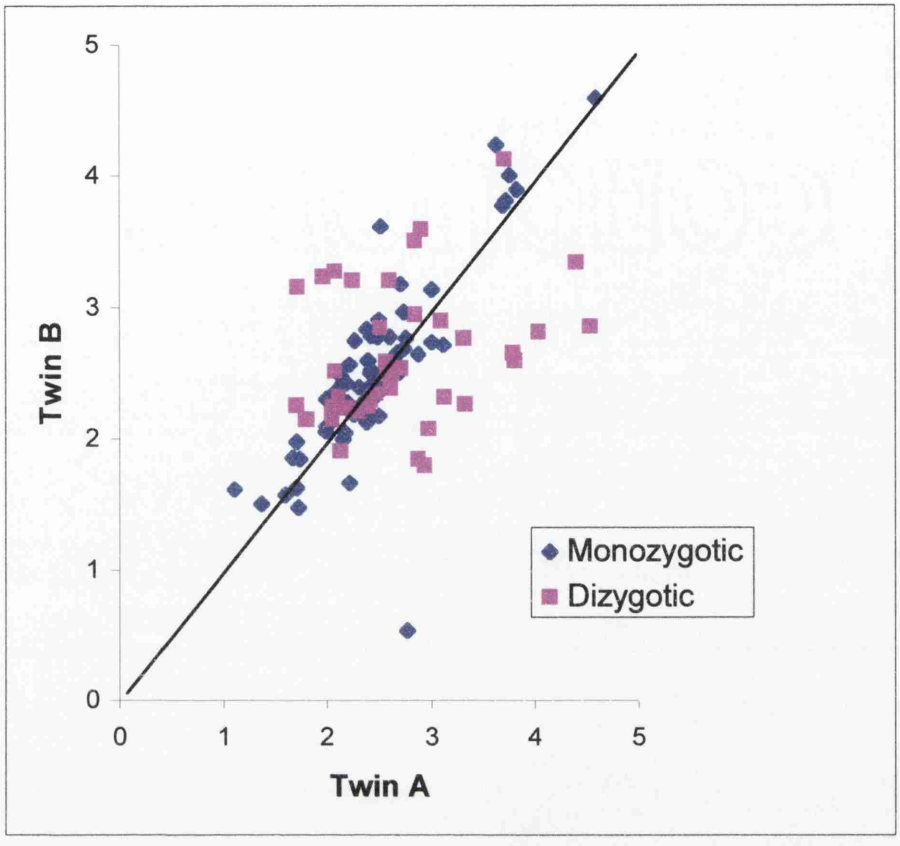


Figure 7.2 Graph of FEV_1 - Twin A against Twin B

Figure 7.2
As can be seen pictorially the monozygotic group are nearer the ideal line of Twin A=Twin B than the dizygotes. This demonstrates the stronger correlation between the monozygotes than the dizygotes.

7.5 Discussion

FEV_1 and FVC are reported to be risk factors for cardiovascular events and this study demonstrates a degree of genetic control over their determination (heritability estimates of 0.82 ($p>0.0001$) and 0.84 ($p>0.001$) respectively). This genetic effect is not explained by similarities in height as both FEVH and FVCH also have a significant genetic determination. However, expressing these lung function markers as a percentage of a predicted value diminishes

the importance of this contribution. Indeed with FVC although the MZ correlation is still considerably higher than the DZ this finding is not significant in a cohort of this size. This is also true once FEV_1 and FVC are fully "corrected" for smoking history, lipid profile and blood pressure.

The genetic framework of these measures of lung function is strongly related to the genetic architecture of other risk factor phenotypes. This does not negate the degree to which there is a genetic influence over these factors but suggests the effect of common genes and common mechanisms of action.

In this study FEVH and FVCH are shown to correlate with other well-established risk factors such as cholesterol (correlation with FEVH –0.21, $p=0.002$) and systolic blood pressure (-0.24, $p<0.001$). To adequately assess the relationship between cardiac end-points and lung function these confounding variables would need to be accounted for as in the British Regional Heart Study (Walker M et al, 1989). If a persisting relationship was not present following correction for these variables then FEV_1 and FVC would be no more than a marker of the exposure to, and influence of, other well-known intermediate cofounders.

The only persisting correlations with lung spirometry, when expressed as a percentage of the predicted value, are smoking, lipoprotein A and fibrinogen (Table 7.2 and 7.3). The relationship with lipoprotein A and fibrinogen persist following correction for smoking and other risk factors (FEVCorr+ and FVCCorr+). Lipoprotein A and fibrinogen are both known cardiovascular risk

factor in the Scottish population (chapter 9). Little is known of the effect of fibrinogen on the association between lung function and cardiac events and this is an area requiring further study. Whither lung function is simply a marker of another variable that modulates fibrinogen and lipoprotein A or whither there is an unknown mechanism linking the two directly is unclear.

In this cohort carotid intima medial thickness was not associated with FEV_1 and FVC even when corrected for height (RFW-mean and FEVH correlation of -0.1, p=0.17) suggesting no independent association with the presence of atheroma in this cohort.

The associations between lung function and other cardiovascular risk factors have potentially important aetiological, clinical and public health implications. The persisting relationship between lung function and fibrinogen and the lack of association with carotid intima media thickness suggest that thrombogenesis, rather than atherogenesis, may be the pathway through which impaired lung function exerts its influence. This relationship is independent of smoking history.

The clinical implications of the role of parameters of lung function are yet to be determined. The Framingham authors have not explained why they did not include FEV_1 or FVC in their risk assessment calculations. Whither this was because it did not add to the predictive value of the cohort, or whither for logistic reasons is not clear.

The public health implications of the influence of impaired lung function are in need of further study. The population attributable risk for cardiovascular mortality due to a low FEV_1 is greater than that of hyperlipidaemia (Strachan DP et al, 1992). If this was a causal relationship it would be hoped that intervention to improve FEV_1, including smoking cessation, would impact on cardiovascular mortality.

In conclusion there appears to be a genetic framework to the determination of pulmonary function, as measured by spirometry, in this population. However much of this is explained by shared influences. Simple non-invasive measures of spirometry are known markers of cardiovascular events and as such a marker should not be overlooked. The assessment of pulmonary function in this manner is an ideal tool for large epidemiology studies, screening large groups of individuals. The associations of FEV_1 and FVC with fibrinogen, and not with intima media thickness, may suggest a mechanism other than atherogenesis.

Chapter 8

Metabolic risk factors

8.1 Introduction

8.1.1 Metabolic cardiovascular risk factors

Over the last 60 years the understanding of the complex interactions that form the molecular basic of atherosclerotic disease has increased year upon year. Simple observations have progressed through to proven associations, mechanistic hypotheses and finally targeted intervention. This is seen most clearly in the field of lipid metabolism.

A great deal has been learned since Carl Muller's simple observation of the association between markedly elevated levels of serum cholesterol and premature ischaemic heart disease (Muller-Harbitz disease or familial hypercholesterolaemia described in 1939). Since then a plethora of lipid risk factors have been identified and the ability to intervene in their synthesis has revolutionised the concept of preventative cardiovascular medicine. Modification of adverse lipid profiles, usually by HMCoReductase Inhibitors, has been shown to reduce cardiovascular mortality and morbidity. The West of Scotland Primary Prevention Study (WOSCOPS) demonstrated that even in a population with no previous evidence of ischaemic heart disease (primary prevention) reduction in serum cholesterol resulted in improved cardiovascular mortality (Shepherd J et al, 1995). These findings have been reproduced in many patient groups including diabetics (Pyorala K et al, 1997), females (Byington RP et al, 1995) and those with previous ischaemic disease - secondary prevention (CARE study - Pfeffer MA et al, 1995). Although many other intermediate risk factors have been identified few have

made this progression through to the randomised clinical trials proving efficacy of therapeutic intervention.

8.1.2 Other cardiovascular risk factors

Derangement of haemostatic factors, for example plasma fibrinogen (Woodward M et al, 1998), Factor VII (Broadhurst P et al, 1990) and D-Dimer (Folsom AR et al 2001), is associated with atherogenesis. As with lipid metabolism interest has focused on assessing how these factors exert their influence and how these processes can be modified.

Fibrinogen is a large glycoprotein that has an important influence on characteristics of blood flow and platelet aggregation (Lip G et al, 1995). As the precursor to fibrin it has an integral role in the coagulation system. Elevation of plasma fibrinogen levels, above that needed for the effective operation of the clotting cascade, are thought to represent a pro-thrombotic tendency. The Scottish Heart Health Study examined plasma fibrinogen in a large cohort of middle-aged Scots. They demonstrated fibrinogen as an adverse risk factor and revealed its associations with other risk factors such as cigarette smoking and hypertension. They suggested that fibrinogen may be one of the mechanisms through which these risk factors promote ischaemic heart disease but tempered their findings with the statement that other risk factors explained less than 10% of the total variance in fibrinogen levels in the general population (Lee AJ et al, 1990).

Lipoprotein A, which is structurally similar to LDL, is another independent risk factor for cardiovascular disease (Price JF et al, 2001). Lipoprotein A is

known to be under significant genetic control. Boerwinkle suggested that 90% of the variability in lipoprotein A concentration was due to a genetic influence (Boerwinkle E et al, 1992). Further generations of risk factors such as CRP and homocysteine are being investigated as potential targets for future intervention. Unfortunately the identification of a risk factor and vehicles to modify that factor do not quickly translate into beneficial therapies. One such example is homocysteine which can be modulated by folic acid supplementation but this therapy has yet to demonstrate mortality benefit in large clinical trials.

8.1.3 Risk scores

The knowledge of the multi-facetted nature of atherogenesis has revolutionised the approach to clinical risk assessment. No longer are single risk factors taken in isolation, or treated independently, as risk factors are now known to interact - often additively. The concept of total risk, usually quoted as an annual risk or as a risk relative to others of a similar age without adverse features, is widespread in primary prevention programmes. There are now a myriad of easy to use risk assessment tables most of which are based on modifications of the original Framingham equations (Anderson KM et al, 1991). These tables have been validated in populations though out the world and are now used to determine the appropriateness of intervention such as the prescription of statins. The Framingham tables use age, sex, fasting blood glucose, total cholesterol, HDL cholesterol, systolic blood pressure and smoking history to calculate a global risk score. Accurate risk assessment has been one of the factors leading to the success of primary

prevention which, in the Scottish population has been estimated to have saved many lives over the last 10 years (Capewell S et al, 1999).

In this study risk scores were calculated for the individual twins. The modification of the Framingham equation used was proposed by the American Heart Association and the American College of Cardiology and published in 1999 (Grundy SM et al, 1999). In this scoring system diabetes was defined as a fasting plasma glucose level of >126mg/dL to conform to the recent American Diabetic Association guidelines (American Diabetic Association, 1997). The total risk score sums the points for each risk factor (Figure 8.1).

Each total score corresponds to a relative and absolute risk level. These levels are specific for age and sex groups and are quoted as 10-year risk values for total coronary heart disease. This includes angina pectoris, recognised and unrecognised myocardial infarction, unstable angina and coronary heart disease deaths. For example a 46-year old hypertensive, hyperlipidaemic male smoker, with no history of diabetes, would have a total risk score of 12 points. This score corresponds to a relative risk of 12.3 compared to a "low risk" individual with no adverse risk factors or to a 10-year absolute risk of 37%.

Risk factors	Male	Female
Age, in years		
<34	-1	-9
35-39	0	-4
40-44	1	0
45-49	2	3
50-54	3	6
55-59	4	7
60-64	5	8
65-69	6	8
70-74	7	8
Total cholesterol, mg/dL		
<160	-3	-2
169-199	0	0
200-239	1	1
240-279	2	2
≥ 280	3	3
HDL-cholesterol, mg/dL		
<35	2	5
35-44	1	2
45-49	0	1
50-59	0	0
≥60	-2	-3
Systolic BP, mmHg		
<120	0	-3
120-129	0	0
130-139	1	1
140-159	2	2
>160	3	3
Diabetes		
No	0	0
Yes	2	4
Smoker		
No	0	0
Yes	2	2
Add up the points – total risk score		

Table 8.1 AHA/ACC Risk Assessment Scores

8.2 Aims

The aims of this chapter were:

1. To assess the correlations between simple blood parameters and other known risk factors within the twin cohort.
2. To assess the heritability of these metabolic and haematological parameters.
2. To assess the heritability of cardiovascular risk factor scores.

8.3 Methods

Fasting blood samples were taken from the twin subjects between 0830–0930hrs. Chapter 2 describes the methodology in greater detail. Those analysing and reporting twin results were blinded to information regarding zygosity and other study parameters. Biochemical data was collected on 139 pairs of twins and haematological data on 94 pairs. For each parameter measured correlations and heritability estimates were calculated.

8.4 Results

8.4.1 Blood parameters

The descriptive data for the biochemical parameters is displayed in Table 8.1. There was a statistical difference between the monozygotic and the dizygotic groups for fasting glucose, total cholesterol, VLDL and LDL. Fasting glucose, for example was statistically higher in the dizygotic group than in the monozygotic group. These differences persisted even following the correction for age and sex. For this reason little can be read into estimates of heritability for these parameters. To highlight this heritability scores for these variables are included in the subsequent tables in sets of brackets "( )".

There was no evidence of a significant genetic effect over serum sodium, potassium, calcium or urea concentrations. This was also the case for total protein levels, albumin and phosphate (Table 8.3). Several of these parameters did, however, reveal a degree of familial control. Urea for example showed strong intra-class correlations for both monozygotic and dizygotic twin groups. A genetic contribution was evident in the variability in blood glucose levels (h^2 = 0.49, p=0.035). This was true for all twin subjects not solely those with elevated glucose levels. Serum creatinine (h^2 = 0.49, p=0.048), urate (h^2 = 0.54, p=0.007) and vit D (h^2 = 0.72, p=0.0003) levels also revealed a genetic determination (Table 8.3).

In this cohort fibrinogen levels were controlled neither by genetic nor familial influences. The intra-class correlations were low in both the monozygotic and dizygotic groups (MZ 0.23 and DZ 0.07 respectively). There was also no significant heritability for haemoglobin. In contrast approximately 64% of the variability in haematocrit (h^2 = 0.64, p=0.013) and 66% of the variability in platelet levels (h^2 = 0.66, p=0.014) were genetically determined.

	MZ	DZ	p
Smoker	2197	2398	0.6
Age	58 (10.2)	56.3 (12.4)	0.44
Weight	66.8 (19.2)	68.0 (15.93)	0.69
SBP	133.3 (16.6)	133.1 (16.72)	0.96
Na	139.7 (2.17)	140 (2.1)	0.47
K	4.23 (0.3)	4.14 (0.36)	0.15
Urea	6.1 (1.6)	5.9 (1.45)	0.58
Creat	81.1 (16.2)	84.6 (14.5)	0.23
Calcium	2.25 (0.12)	2.25 (0.12)	0.84
Glucose	**5.04 (0.5)**	**5.44 (1.3)**	**0.04**
Urate	0.24 (0.09)	0.24 (0.08)	0.83
TG	1.19 (0.53)	1.18(0.59)	0.95
T Chol	**5.88 (1.03)**	**5.36 (0.93)**	**0.006**
VLDL	**0.92 (0.47)**	**0.7 (0.34)**	**0.02**
LDL	**3.6 (0.93)**	**3.26 (0.8)**	**0.05**
HDL-C	1.36 (0.31)	1.32 (0.3)	0.45
LipoA*	**37.5 (45.7)**	**22.2 (23.27)**	**0.03**
Fibrinogen	349.8 (80.0)	352.8 (98.7)	0.88

Table 8.2 Metabolic variables - Descriptive data

(*following correction for age and sex, p=0.36).

	n-MZ	R-MZ	Z-MZ	n-DZ	R-DZ	Z-DZ	z	h^2	p
Sodium	73	0.30	0.31	62	0.50	0.55	-1.39	-	0.92
Potassium	73	0.25	0.26	62	0.23	0.23	0.17	-	0.43
Urea	72	0.51	0.56	67	0.50	0.55	0.10	-	0.46
Creatinine	74	0.50	0.55	65	0.26	0.26	1.67	**0.49**	**0.05**
Total protein	71	0.38	0.40	67	0.32	0.33	0.37	-	0.35
Albumin	72	0.67	0.80	67	0.58	0.66	0.80	-	0.21
Glucose (fast)	68	0.49	0.53	65	0.21	0.21	1.81	**(0.49)**	**0.04**
Phosphate	70	0.56	0.63	65	0.41	0.43	1.11	-	0.13
Calcium (corr)	73	0.67	0.82	64	0.60	0.69	0.74	-	0.23
Urate	74	0.54	0.61	65	0.19	0.19	2.43	**0.54**	**0.007**
Vit D	72	0.71	0.90	67	0.30	0.31	3.42	**0.72**	**0.0003**

Table 8.3 Heritability estimates for biochemical parameters

	n-MZ	R-MZ	Z-MZ	n-DZ	R-DZ	Z-DZ	Z	h^2	p
Cholesterol	69	0.69	0.85	66	0.44	0.47	2.18	**(0.51)**	**0.02**
LDL	56	0.73	0.93	55	0.29	0.30	3.27	**(0.73)**	**<0.001**
VLDL	50	0.52	0.57	50	0.37	0.39	0.90	-	0.18
HDL-Chol	67	0.62	0.78	66	0.43	0.46	1.53	**0.39**	**0.06**
Lipoprotein A	68	0.90	1.49	63	0.64	0.77	4.10	**(0.52)**	**<0.0001**
Triglyceride	69	0.42	0.45	66	0.24	0.24	1.18	-	0.12

Table 8.4 Heritability estimates for lipids

	n-MZ	R-MZ	Z-MZ	n-DZ	R-DZ	Z-DZ	Z	h^2	p
Fibrinogen	69	0.23	0.23	61	0.07	0.07	0.95	-	0.17
Haematocrit	52	0.64	0.75	42	0.27	0.28	2.23	**0.64**	**0.01**
Platelets	52	0.69	0.84	42	0.36	0.37	2.21	**0.66**	**0.01**
Haemoglobin	52	-0.004	-0.003	42	0.28	0.29	-1.37	-	0.92

Table 8.5 Heritability estimates for haematological parameters

8.4.2 Cardiovascular risk scores

Risk scores were calculated for the individual twins. The mean risk score for the 128 MZ twins and 111 DZ twins were 6.2 ± 5.7 and 4.9 ± 5.6. There was no statistical difference between the 2 groups (p=NS). Risk scores were related to measures of the presence of carotid atheroma i.e. the larger the cardiovascular risk from the ACC/AHA equation the thicker the carotid intimal medial thickness (correlation with RFW-max of 0.43, p<0.001). In addition the cardiovascular risk scores were higher in those who had a history of a cardiovascular event (mean of 7.7 vs. 5.3, p=0.002).

	Correlation with risk score	**p**
FEV	-0.36	**<0.001**
FVC	-0.29	**<0.001**
%PFEVH	-0.02	0.84
%PFVCH	0.02	0.80
FEV/FVC	-0.22	**0.001**
Peak flow	-0.18	**0.01**
RFW-max	0.43	**<0.001**
RFW-mean	0.42	**<0.001**
Height	-0.09	0.16
Lipoprotein A	0.10	0.15
Fibrinogen	0.23	**<0.001**
Smoking	0.06	0.43

Table 8.6 Correlations with risk scores

The intra-class correlation coefficients for monozygotic and dizygotic groups were calculated. The correlations for both groups were high (0.81 and 0.70 respectively). This is unsurprising given the fact that the risk scores are derived values from the combination of subject age, sex, total cholesterol, HDL-cholesterol, systolic blood pressure, the presence of diabetes and a history of cigarette smoking. These close correlations are depicted in Figure 8.1.

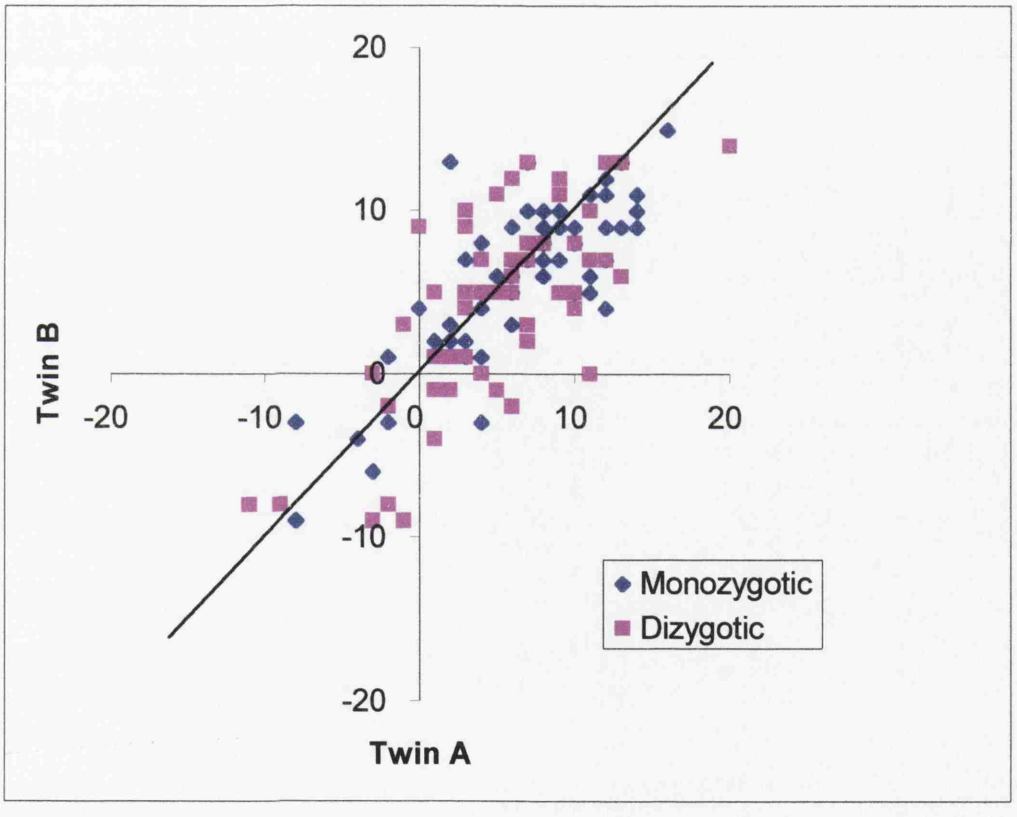


Figure 8.1 Graph of risk scores (Twin A against Twin B)

Figure 8.1
The graph reveals the close correlations between Twin A and Twin B for both the monozygotic and dizygotic groups (i.e. the data points are near to the ideal line of Twin A=Twin B).

8.5 Discussion

This study reveals a significant genetic contribution to several known metabolic risk factors that predispose individuals to cardiovascular disease. The genetic framework of many of these factors has been established and it is of interest to review the results from this cohort in that wider context. For example the heritability of cholesterol (Christian JC et al, 1977) and various lipoprotein sub-groups (Friedlander Y et al, 2000) have been well characterised in other populations. These studies report the importance of variations in heritability for different cohorts and for different subjects - Chen et al's study of Chinese adolescents revealed important differences between male and female subjects in the degree to which traits are genetically determined (Chen CJ et al, 1984). These differences re-iterate the importance of studying different populations and racial groups. Again the Scottish population, with its relatively "adverse" lipid profile, is a particularly informative cohort. This study confirms that in this population a significant degree of heritability is important in many of the major cardiovascular risk factors.

As previously discussed using a classic twin methodology, examining narrow-sense heritability, has the potential risk of minimising the importance of familial influences over the phenotypes under investigation. For example in this cohort both plasma urea and albumin concentrations show close intra-class correlations for both the MZ and DZ sub-groups suggesting the importance of familial similarities between the twins. Age is known to play a role in the genetic

determination of both urea and albumin concentrations and in older cohorts this contribution is known to weaken (NHLBI cohort - Kalousdian S et al, 1987).

The reasons for the significant differences between means for MZ and DZ sub-groups, for certain parameters, are unclear. This phenomenon has been noted in other studies of clinical chemistry genetics (Lamon-Fava S et al, 1991). These differences remained following correction for potential confounders such as age, sex or built. Innate biases in recruiting twin cohorts is one potential explanation. Differences in peri-natal environment and early fetal growth may be alternative confounders. These limitations are discussed in greater detail in Chapter 9.

The heritability estimate for lipoprotein A was lower than that expected from previous studies (0.52, $p<0.0001$) (Boomsma DI et al, 1993). However as Table 8.3 demonstrates the intra-class correlation coefficients within the monozygotic and dizygotic groups were high (0.9 and 0.64 respectively). This high dizygotic correlation diminishes the pure narrow sense heritability estimate. This reiterates the importance of other non-additive genetic determinants and the variation that can result in study results using different heritability formulae. For example if heritability had been calculated using an equation that makes an assessment of non-additive effects such as $h^2= (rMZ-rDZ)/(1-rDZ)$ then the heritability estimate would have been 0.72.

Fibrinogen, which has a stronger association with cardiac events than systolic blood pressure in the Scottish population (Tunstall-Pedoe H et al, 1997), has minimal MZ and DZ intra-class correlations in this cohort. Several previous studies have suggested a genetic control over this variable (Hamsten A et al, 1987) but this was not evident in this cohort - a finding in keeping with the NHLBI cohort which showed only a minimal genetic contribution to this trait (Reed T et al, 1994). Fibrinogen concentrations are modified by numerous external influences such as hormonal status and cigarette smoking and it may be that these influences are overwhelming a small genetic effect.

In chapter 7 FEV_1 and FVC were both related to plasma fibrinogen concentrations. This association was present even following correction for cigarette smoking. This interesting correlation requires further investigation. Confounders such as social class and forms of chronic inflammation such as chronic dental sepsis have been proposed as important modulators of plasma fibrinogen concentrations and it is yet to be established if the association between lung function, fibrinogen and cardiovascular events can be solely explained by these factors.

Risk factors scores show a striking intra-twin pair correlation. This is unsurprising given that the score is simply a measure of the effect of several other known risks that correlate between the twin pairs – in particular age and sex. As such the heritability of risk factor scores is not of particular interest out with their association with other measures of disease in this population (for

example the presence of carotid atheroma or a history of a cardiovascular event). Risk scores had no independent association with FEV_1 and FVC when expressed as percentages of predicted values. There was, however, an association with the ratio of FEV_1/FVC a measure of "obstructive" spirometry.

In conclusion several metabolic parameters, that are important in the pathogenesis of atheroma, are to a greater or lesser extent modulated by the effects of genes. A myriad of different influences such as lipid concentrations, haematocrit, plasma glucose and urate concentrations are known to adversely affect the vascular system. The study of the genetics of atherogenesis and its clinical end-points must involve the assessment of these metabolic parameters and their own "genetic structures" in the particular population of interest. Other influences, outwith the effect of narrow-sense heritability, are also important (such as fibrinogen concentrations) and should not be ignored when attempting to dissect out the relationships between end-points and factors that pre-dispose to risk.

Chapter 9

Conclusions

9.1 Conclusions

9.1.1 Study conclusions

Although there have been numerous studies examining the genetics of varying cardiovascular parameters within differing populations the question of heredity was of particular interest to a Scottish population. The reasons for Scotland's high cardiovascular morbidity and mortality are unclear and the aim of this present study was to attempt to cast light on the contribution of heredity to some of the mediators of this pathological process. A cohort of adult twins was recruited from the West of Scotland - region that has a particularly high incidence of cardiovascular events even when compared to other Scottish regions (Smith WCS et al, 1988).

Of the phenotypes investigated there appears to be a significant genetic contribution to many of the classical cardiovascular risk factors. Blood pressure, and in particular 24-hour ambulatory measurements, show a significant degree of heritability in this population. This was also true for hypertrophy of the left ventricle and simple measures of left ventricular function. When actual presence of atheroma in a vessel wall was investigated familial and environmental influences appeared to be more important determinants. The relative degree of heritability of these parameters is shown in Figure 9.1. Lung function, a marker of an increased risk of ischaemic cardiac events, is modified by genes. However, much of this genetic influence is in common with other variables such as height. Despite the above there are still importance risk

factors in the Scottish population that appear to be under a minimal degree of genetic control such as fibrinogen concentrations.

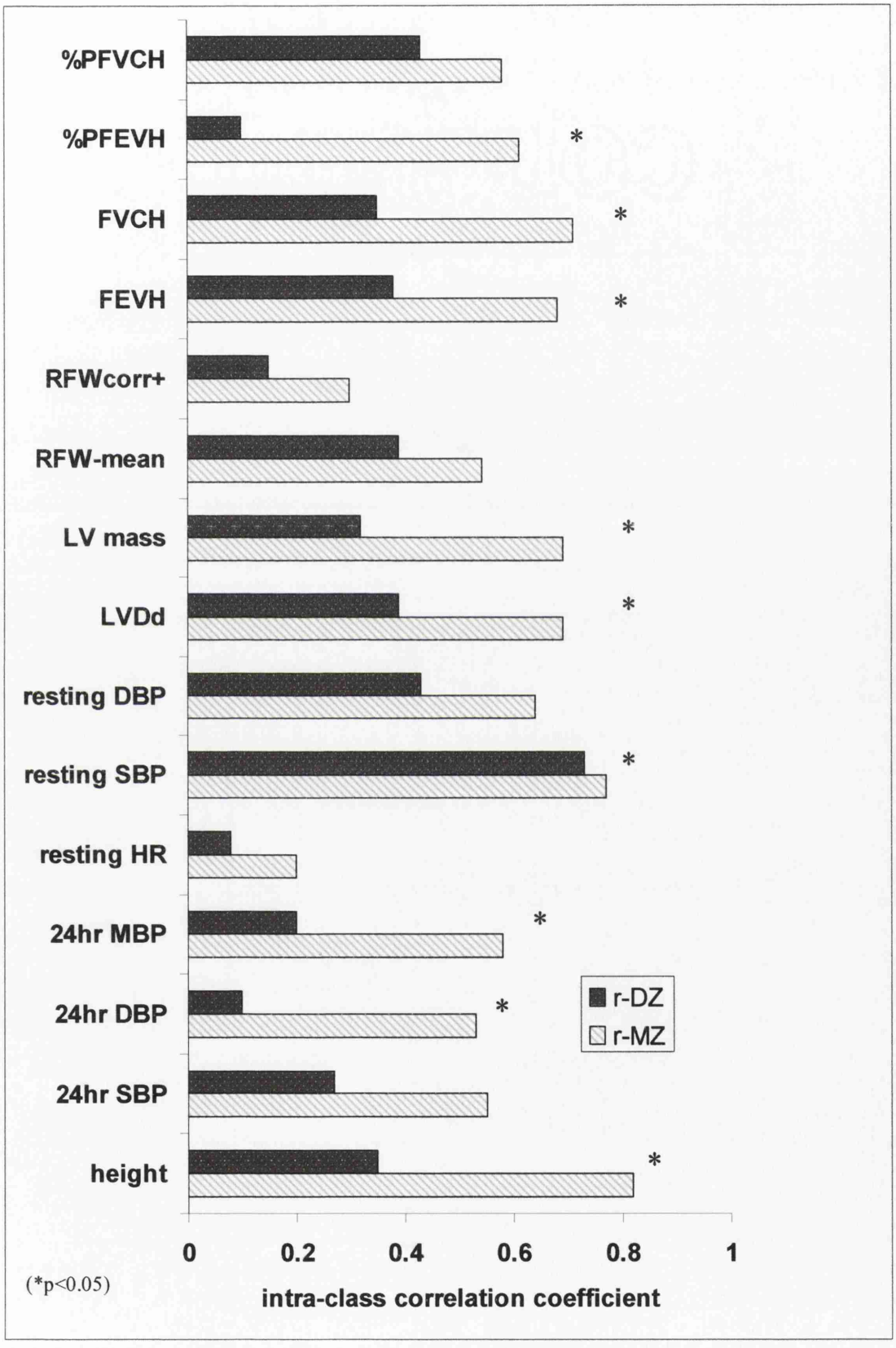


Figure 9.1 Summary of intra-class correlations

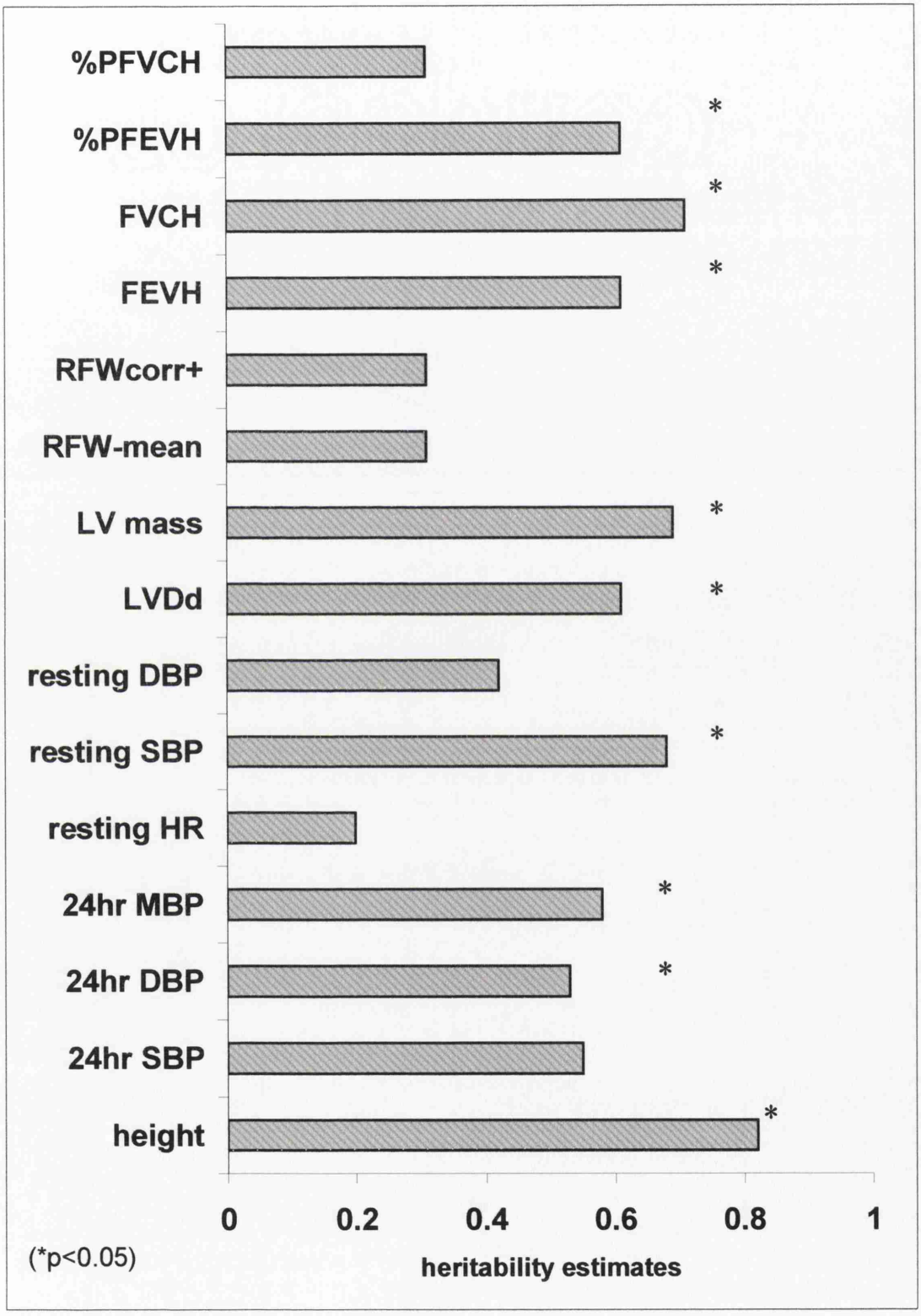


Figure 9.2 Summary of heritability estimates

The Scottish heart health study compared the importance of 27 factors in predicting coronary risk in a population of 11629 men and women aged 40-59 (Tunstall-Pedoe H et al, 1997). Factors were ranked according to age adjusted hazard ratios between highest and lowest categories. In Table 9.1 the top 20 risk factors for all coronary heart disease in women (and men) are displayed.

Rank		*male*				*male*	
1	Previous angina	*(1)*		11	Bortner score	*(26)*	
2	Triglycerides	*(6)*	*	12	Work inactivity	*(19)*	
3	HDL-cholesterol	*(3)*	*	13	Diastolic BP	*(4)*	*
4	Serum cotinine	*(7)*		14	Ascorbic acid	*(11)*	
5	Total cholesterol	*(2)*	*	15	Urine sodium	*(23)*	
6	Systolic BP	*(5)*	*	16	Height	*(13)*	*
7	Diabetes	*(21)*	*	17	Alcohol	*(12)*	
8	Cigarette smoking	*(9)*	*	18	Uric acid	*(20)*	*
9	Plasma fibrinogen	*(8)*	*	19	Leisure inactivity	*(16)*	
10	Housing tenure	*(15)*		20	Urine potassium	*(23)*	

Table 9.1 Ranking of risk factors - all coronary events (women) and *(men)*.

Table 9.1 reveals the differences between men and women regarding coronary risk factors.

Serum cotinine = measure of exposure to tobacco smoke
Bortner score = tests for type A personality

Parameters investigated in the twin cohort represented by *

The above table demonstrates the initial unifactorial rankings of various risk factors. Given that no allowance is made for potential interactions or confounders caution should be exercised in their interpretation. Uric acid is, for example, shown to be a risk factor. However when studied in other populations most of this association is explained by confounders (Culleton BF et al, 1999).

The importance of potential genetic contribution to this disease process in the Scottish population can be seen by the parameters that have been investigated in this study. There is, however, a long-way to go in translating this knowledge into the prevention of premature cardiac deaths. The variety of factors in Table 9.1 demonstrates the multi-armed primary prevention approach that must be employed - on one hand tackling the management of hypertension and on the other the problems of inadequate housing provision.

9.2 Familial influences

As have been discussed in of the above chapters there are strong familial similarities between many of the twin phenotypes investigated. These similarities are not explained by narrow sense heritability. Additive and epistatic genetic phenomenon or alternatively gene-environment interactions are all possible explanations for this finding. A shared environment, particularly in early life, is however another crucial component to this familial pattern.

In a cohort of twins reared together for, an average, the first 22 years of their lives, it is not difficult to see how important environmental, social, geographical and lifestyle influences would tend to be very similar. Similarities in pre-natal intra-uterine influences, believed to be important in the long term pathophysiology of many multifactorial diseases such an ischaemic heart disease, chronic pulmonary diseases and cancers, are also potentially contributing to this effect. As mentioned in chapter 1 this is not a simple association as twin pairs may experience marked differences in intra-uterine conditions for example placental flow and nutrition.

Discovering that many of the parameters that determine cardiovascular health are familial, rather than genetic, is important. Public health issues and targeting early environmental "pathogens" must also form part of a comprehensive strategy to reduce cardiovascular morbidity and mortality.

In conclusion although much of the Scottish burden of cardiovascular disease is pre-determined by genetic make-up this should not dampen any enthusiasm to improve the health of the nation. There is much work to be done from the detection of potential candidate genes that may reveal targets for intervention to addressing of truly modifiable social and lifestyle risks.

9.2 Limitations of the present study

As discussed in chapter 2 the classic twin study methodology has several inherent limitations. From the outset this study has attempted to minimise the impact of these biases as discussed below.

9.2.1 Ascertainment bias

The lack of a national twin registry means that a degree of ascertainment bias is unavoidable when planning a twin study in Scotland. When designing the present study precautions were taken to attempt to minimise this bias. A wide range of sources, encompassing as many social groups as possible, was used to recruit twin pairs. This included a broad section of the media with tabloid and broadsheet newspapers and television coverage. The nature of the disease processes being investigated was not specified to minimise selective uptake from twins with a vested interest in cardiovascular disorders. However despite these precautions the eventual twin cohort has several of the predicted biases. Firstly there is a preponderance of female twins (78.5% of the cohort). Secondly although social class was not formally assessed there appeared to be a tendency towards a middle-class population. Over 75% of the cohort lived in owner-occupier accommodation – a figure that is not representative of the wider West of Scotland population and 74% were married. Finally, a volunteer-based cohort has the innate biases described in Chapter 2. Highly discordant twins or those with significant illnesses are unlikely to volunteer for such a study.

There was, however a more realistic balance of monozygotic to dizygotic twins (55% vs. 45%) and the "Rule of 2/3" was not applicable to this cohort. Ascertainment bias must be taken into account when drawing extrapolating conclusions to the wider Scottish population. However, in the absence of a national database the current cohort is probably the nearest to an unbiased population-based sample of twins as could be obtained.

The study of a group of predominantly middle-aged females has potential advantages. In the area of cardiovascular research, particularly clinical studies, there is an under-representation of female subjects. A more detailed study, of this nature, in a group of females highlights potential areas of difference between the sexes. For example the lack of a significant genetic influence over carotid intima medial thickness in the cohort may be a manifestation of differences in the genetic framework of atheroma formation on males and females. A cohort of higher risk male subjects may have resulted in a different conclusion. If atheroma formation, per se, is less heritable in the female population it may be that lifestyle adaptation and secondary prevention have more to offer the female population than their male counter-parts. This is, however, speculation.

9.2.2 Study size

Previous twins studies as well as being performed in selected cohorts (such as college children or veterans) have suffered from lack of power. Cohorts of small groups of twins suffer from Type 2 error with insufficient power to detect weak

associations between variables. The present study, although smaller than many of the Scandinavian database studies, is the largest Scottish twins study to date. In addition it compares favourably with other UK cohorts. The data chapters of this study reveal several examples of trends towards a genetic influence over a trait - for example clinic measurements of diastolic blood pressure (rMZ=0.64 and r-DZ=0.43, p=0.09). This study is not sufficiently powered to exclude a significant genetic input to this parameter. However, the aim of this study was to investigate a wide range of cardiovascular phenotypes to determine which of these were the most likely to be of use in the further investigation of potential candidate genes. As such the actual heritability estimate is a limited end-point and of more interest is the relative position of these estimates when assessed as a whole (Figure 9.2).

Due to the classic twins methodology employed confidence intervals were not quoted in this study. However using this methodology confidence intervals are relatively large again cautioning against the over-emphasis on the heritability estimate as a lone end-point.

9.2.3 Additional data

In retrospect one deficiency of the current study is the lack of an inclusion of an assessment of social class. Social class is of course known to be of vital importance with regards to cardiovascular morbidity and mortality. However, an easy measure of these parameters was not available from this cohort. Postal code scoring would have been possible on a limited number of pairs but data on

other measures of social class, such as numbers of household cars, was not available. As social class correlates between both MZ and DZ pairs and as subjectively the cohort appears to be pre-dominantly middle-class it is hoped that the exclusion of this variable did not significantly bias the results.

The addition of birth-weight and chorionic status to the data set would have been invaluable. However in studying a cohort of adults with a mean age of 57 years accurate information on both of these variables is not available. It was felt that verbal reports of birth-weight would add an unacceptable degree of inaccuracy to the analysis.

9.3 Future work

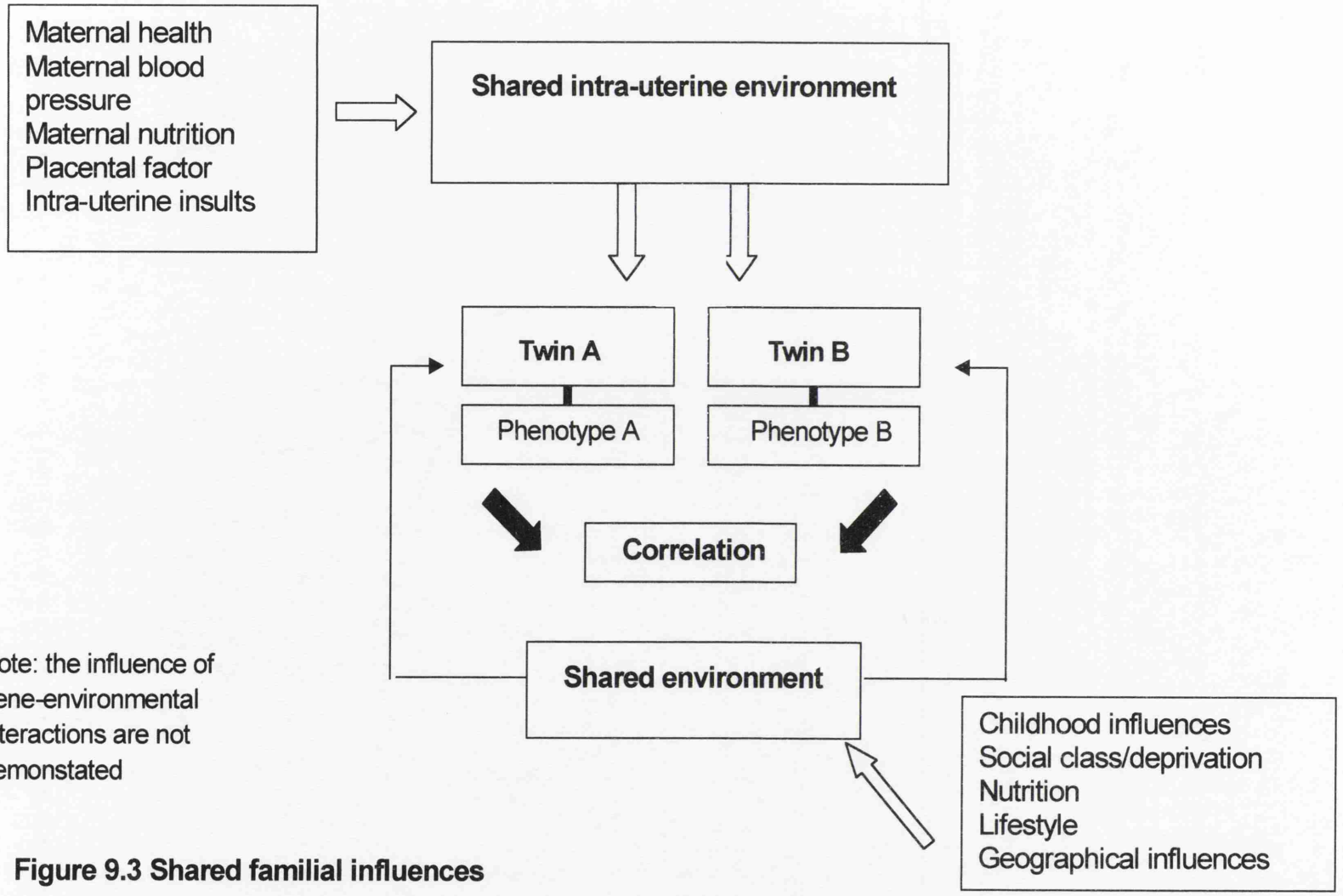
As has been alluded to in previous chapters the twin cohort provides an excellent resource for the investigation of potential candidate genes directed towards the parameters under the greatest genetic influence.

Although outside the score on this thesis work has already begun on potential candidates in the determination of left ventricular mass. The first set of genotypes to be investigated include ACE, G-protein beta3, Beta-1 adrenoceptor and aldosterone synthase. Genotypes of Beta-1 adrenoceptor show some potentially interesting correlations with left ventricular hypertrophy.

The group are also undertaking further investigation into the heritability of a series of metalloproteinases. It is hoped that this work will complement the data

on left ventricular structure and function permitting the further assessment of the role of metalloproteinases in the vascular system.

The twin study has revealed a plethora of associations between known and relatively unknown risk factors. However, a simple association is not an indication of causality. The mechanisms of these relationships require further investigation. In particular the relationship between lung function, fibrinogen and cardiac events is one of particular interest in the West of Scotland. As discussed in the methodology of Chapter 2 additional information was collected on the twin cohort including more detailed assessments of lifestyle, steroid parameters and bone densitometry. Although these areas are outwith the scope of this work it is hoped that future work will examine the relationships between these variables and those already investigated.



Note: the influence of gene-environmental interactions are not demonstated

Figure 9.3 Shared familial influences

Abbreviations

ACE	Angiotensin converting enzyme
%PFEVH	FEV_1 as expressed as percentage of expected value
%PFVCH	FVC as expressed as percentage of expected value
ABPM	Ambulatory blood pressure monitoring
ASE	American Society of Echocardiography
CCA	Common carotid artery
Corr LVM	Left ventricular mass - corrected for confounders
DBP	Diastolic blood pressure
DZ	Dizygotic
E/A	E wave /A wave ratio
ECG	Electrocardiograph
FEV_1	Forced expiratory volume in 1 second
FEVCorr+	FEV_1 corrected for confounders
FEVH	FEV_1 corrected for height
FS	Fractional shortening
FT	Fractional thickening
FVC	Forced ventilatory capacity
FVCCorr+	FVC corrected for confounders
FVCH	FVC corrected for height
h_2	Heritability
IMT	Intima media thickness
IVSd	Inter-ventricular septum thickness in diastole
IVSs	Inter-ventricular septum thickness in systole

Continued,

LVDd	Left ventricular internal diameter in diastole
LVDs	Left ventricular internal diameter in systole
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
MZ	Monozygotic
NS	Not significant
PWd	Posterior wall thickness in diastole
PWs	Posterior wall thickness in systole
QTL	Quantitative trait locus
r-DZ	Intra-class correlation coefficient for dizygotic pairs
r-MZ	Intra-class correlation coefficient for monozygotic pairs
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rat

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