LUND UNIVERSITY

Genetic Factors and Dietary Salt Intake as Determinants of Blood Pressure and Risk of Primary Hypertension

Wowern, Fredrik

2006

Link to publication

Citation for published version (APA):
Wowern, F. (2006). *Genetic Factors and Dietary Salt Intake as Determinants of Blood Pressure and Risk of Primary Hypertension*. [Doctoral Thesis (compilation), Translational Muscle Research]. Medical Faculty, Lund University.

Total number of authors:
1

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Download date: 07. Oct. 2026

Genetic Factors and Dietary Salt Intake as Determinants of Blood Pressure and Risk of Primary Hypertension

ACADEMIC DISSERTATION

Fredrik von Wowern

Lund University
Department of Clinical Sciences
Diabetes and Endocrinology
Malmö University Hospital

FACULTY OF MEDICINE
Lund University

With the permission of the Medical Faculty of Lund University, to be presented for public examination in the Grand Hall at the Medical Research Center, Entrance 59, Malmö University Hospital, on February 17, 2006, at 09.00 a.m.

Faculty Opponent

Professor Jan Staessen
Division of Hypertension and Cardiovascular Revalidation
University of Leuven
Leuven
Belgium

"He that saves one person saves the world entire"

The Torah

© 2006, Fredrik von Wowern, Lund University, Department of Clinical Sciences, Diabetes and Endocrinology, Malmö University Hospital

ISSN 1652-8220

ISBN 91-85481-44-0

Printed by Media-Tryck, Lund University, Lund, Sweden

Table of Contents

LIST OF PAPERS 6

ABBREVIATIONS 7

ABSTRACT 8

BLOOD PRESSURE 9

DEFINITION AND DIAGNOSIS OF HYPERTENSION 9
EPIDEMIOLOGY OF PRIMARY HYPERTENSION 10
ETIOLOGY AND PATHOPHYSIOLOGY OF PRIMARY HYPERTENSION 12
GENETICS OF PRIMARY HYPERTENSION AND BLOOD PRESSURE 12
FINDING THE GENETIC CAUSE OF PRIMARY HYPERTENSION 13
ENVIRONMENTAL FACTORS FOR PRIMARY HYPERTENSION AND BLOOD PRESSURE 14
INTERRELATION BETWEEN BLOOD PRESSURE AND INSULIN RESISTANCE 14

SALT INTAKE AND BLOOD PRESSURE 16

DEFINITION OF SALT SENSITIVITY 17
MARKERS FOR PREDICTING SALT SENSITIVITY 18

GENETICS OF COMPLEX DISEASES IN GENERAL 19

STRATEGIES FOR IDENTIFYING THE GENETIC COMPONENT OF COMPLEX DISEASES 19
THE IMPORTANCE OF THE PHENOTYPE 19
THE CANDIDATE GENE APPROACH 19
THE LINKAGE APPROACH 20
LINKAGE DISEQUILIBRIUM 22
THE GENOME-WIDE ASSOCIATION APPROACH 22

ONE GENE OR MANY GENES? 24

INSIGHTS FROM ANIMAL MODELS 24

MONOGENIC BLOOD PRESSURE REGULATION 24

LIDDLE´S SYNDROME 25
PSEUDOHYPOALDOSTERONISM 26

RENIN-ANGIOTENSIN-ALDOSTERON SYSTEM 27

CANDIDATE INTEGRATORS OF INSULIN AND ALDOSTERONE ON NA^+ TRANSPORT 28

THE SYMPATHETIC NERVOUS SYSTEM 29

THE ADRENERGIC RECEPTORS 30
THE α-ADRENERGIC RECEPTORS 31

POLYMERASE CHAIN REACTION 31

PRESENT STUDY 32

AIMS 32

METHODS 32

STUDY I 32
STUDY SUBJECTS AND PHENOTYPING 32
GENOTYPING 33
STATISTICS 33
STUDY II 34
STUDY SUBJECTS 34
STATISTICS 34
STUDY III 34
STUDY SUBJECTS 34
TABLE 1 35
MEASUREMENT OF BLOOD PRESSURE AND DIAGNOSIS OF PRIMARY HYPERTENSION 35
BIOCHEMICAL ANALYSIS 35
GENOTYPING 35
STATISTICS 35
STUDY IV-V 36
STUDY SUBJECTS 36
"Malmö family collection for the study of Macrovascular and Hemodynamic Genetics" 36
"Malmö Diet and Cancer" 36
BLOOD PRESSURE MEASUREMENTS 37
BIOCHEMICAL ANALYSIS 37
GENOTYPING 37
STATISTICS 37
STUDY VI 37
STUDY SUBJECTS 37
BLOOD PRESSURE MEASURMENTS 37
BIOCHEMICAL ANALYSIS 38
PROCEDURES AND MEASUREMENTS OF SALT SENSITIVITY 39
STATISTICS 39

RESULTS 40

STUDY I 40
STUDY II 40
STUDY III 41
STUDY IV 42

STUDY V 43
STUDY VI 44

DISCUSSION 46

STUDY I AND II 46
STUDY III 47
DESIGN ISSUES OF STUDY III 48
RELEVANCE OF STUDY III FOR PRIMARY HYPERTENSION AND BLOOD PRESSURE 48
STUDY IV-V 48
DESIGN ISSUES OF STUDY IV-V 49
RELEVANCE OF STUDY IV-V FOR THE PATHOPHYSIOLOGY OF PRIMARY HYPERTENSION 49
LIMITATIONS AND STRENGTHS OF STUDY IV-V 50
IMPLICATIONS OF THE FINDINGS OF STUDY III-V 51
STUDY VI 51
SUMMARY AND CONCLUSIONS OF STUDIES I-VI 53
FUTURE IMPLICATIONS 54

ACKNOWLEDGEMENTS 55

POPULÄRVETENSKAPLIG SAMMANFATTNING 56

REFERENCES 58

Genetic Factors and Dietary Salt Intake as Determinants of Blood Pressure and Risk of Primary Hypertension

List of Papers

I. von Wowern F, Melander O, Bengtsson K, Orho-Melander M, Fyhrquist F, Lindblad U, Råstam L, Forsblom C, Lindgren C, Kanninen T, Almgren P, Burri P, Ekberg M, Katzman P, Groop L, Hulthén UL; *"A genome wide scan for early onset primary hypertension in Scandinavian families"* (Hum Mol Genet. 2003 Aug 15; 12(16): 2077-81)

II. Koivukoski L, Fisher SA, Kanninen T, Lewis CM, von Wowern F, Hunt S, Kardia SL, Levy D, Perola M, Rankinen T, Rao DC, Rice T, Thiel BA, Melander O; *"Meta-analysis of genome-wide scans for hypertension and blood pressure in Caucasians shows evidence of susceptibility regions on chromosomes 2 and 3*" (Hum Mol Genet. 2004 Oct 1;13(19):2325-32)

III. von Wowern F, Bengtsson K, Lindblad U, Råstam L, Melander O*; " A Functional Variant in the α_{2B} Adrenoceptor Gene, a Candidate on Chromosome 2, Associates With Hypertension"* (Hypertension. 2004 Mar;43(3):592-7)

IV. von Wowern F, Berglund G, Carlson J, Månsson H, Hedblad B, Melander O *"Genetic Variance of SGK-1 Is Associated with Blood Pressure, Blood Pressure Change over Time and Strength of the Insulin-Diastolic Blood Pressure Relationship"* (Kidney International. 2005 Nov; 68(5):2164-2172)

V. Fava C, von Wowern F, Berglund G, Carlson J, Hedblad B, Rosberg L, Burri P, Almgren P, Melander O; *"24-hour Ambulatory Blood Pressure is Linked to Chromosome 18q21-22 and Genetic Variation of NEDD4L Associates with Cross-Sectional and Longitudinal Blood Pressure in Swedes"* (Submitted to Kidney International)

VI. Melander O, von Wowern F, Frandsen E, Burri P, Willsteen G, Aurell M, Hulthén L; "Moderate Salt Restriction Effectively Lowers Blood Pressure And Degree Of Salt Sensitivity Is Related to Baseline Concentration of Renin and N-terminal ANP in Plasma" (Submitted to JAMA)

Papers I-IV are reproduced with permission from the publishers.

Abbreviations

HT – ***Hypertension***
PHT – ***Primary Hypertension***
EOHT – ***Early Onset Hypertension***
SBP – ***Systolic Blood Pressure***
DBP – ***Diastolic Blood Pressure***
BP – ***Blood Pressure***
CVD – ***Cardiovascular Disease***
AHT – ***Antihypertensive Treatment***
SS – ***Salt Sensitivity***
IR – ***Insulin Resistance***
BMI – Body Mass Index
RAAS – ***Renin Angiotensin Aldosterone System***
ANP – ***Atrial Natriuretic Peptide***
SNP – ***Single Nucleotide Polymorphism***
LOD – ***Logarithm of the Odds***
PHA – ***Pseudohypoaldosteronism***
SNS – ***Sympathetic Nervous System***
AR – ***Adrenergic Receptors***
MSDR – ***Metabolic Syndrome***
GSMA – ***Genome Search Meta-Analysis approach***
MZ – ***Monozygotic***
DZ – ***Dizygotic***
DNA – ***Deoxyribonucleic Acid***
ENaC – ***Epithelial Sodium Channel***
SGK-1 – ***Serum and Glucocorticoid regulated Kinase type-1***
NEDD4L - ***Neural precursor cell Expressed Developmentally Down regulated type 4-Like***
SGK-1 risk – ***Carrier-ship of the intron 6 CC and exon 8 CC/CT genotypes***
NEDD4L risk - ***Carrier-ship of the exon 1 GG and intron 6 CC/CT-genotypes***
LD – ***Linkage Disequilibrium***
TDT – ***Transmission Disequilibrium Test***

DOKUMENTDATABLAD enl SIS 61 41 21

Organization	Document name
LUND UNIVERSITY Department of Clinical Sciences Diabetes and Endocrinology Malmö University Hospital Sweden	DOCTORAL DISSERTATION
	Date of issue Februari 17, 2006
	Sponsoring organization
Author(s) Fredrik von Wowern	

Title and subtitle

Genetic Factors and Dietary Salt Intake as Determinants of Blood Pressure and Risk of Hypertension

Abstract

Blood pressure (BP) and development of hypertension (HT) are determined by both genetic and environmental factors. The specific genetic etiology of these two entities has remained enigmatic despite large efforts. In addition, daily salt intake seems to predispose for certain individuals to develop HT. The aims of the present study were to: (i) reveal where in the genome BP and early onset hypertension (EOHT) susceptibility genes reside by using linkage analysis and meta-analysis of several such studies (study I-II); (ii) investigate the effect on BP or HT of polymorphisms in genes selected either on basis of position derived from study I and II (alpha2B-Adrenergic Receptor gene (ADRA2B)), function (Serum and Glucocorticoid regulated Kinase 1 gene (SGK-1)) or both (Neural precursor cell Expressed Developmentally Down regulated 4 like gene (NEDD4L)) (study III-V); (iii) investigate the role of dietary salt restriction on BP as well as to explore the possibility to predict this BP change by measuring plasma concentrations of renin (P-renin) and N-terminal pro-Atrial Natriuretic Peptide (P-Nt-proANP). Regions on chromosome 2, 3, and 14 revealed loci that are likely to harbor genes of importance for BP regulation and subsequent development of HT (study I-II). The ADRA2B, that resides directly underneath the peak on chromosome 2, contains a functional insertion/deletion (I/D) polymorphism of which the DD-genotype was found to be associated with EOHT and HT (study III). Two polymorphisms in the SGK-1 gene (SGK-1 risk) and two in the NEDD4L gene (NEDD4L risk) were found to be associated with elevations in BP and BP change over time (study IV-V). The SGK-1 risk did also lead to a strengthening of the insulin-diastolic BP correlation (study IV). Finally, we observed that by reducing daily salt intake from 150 to 50 mmol per day BP decreases significantly and this decrease correlated with base-line levels of P-renin and P-Nt-proANP (study VI).These results suggest that: (1) loci on chromosome 2, 3 and 14 could harbor genes of importance for BP regulation or the development of HT; (2) carriers of the DD versus II genotype of the a2B-adrenoceptor are at increased risk of HT; (3) SGK-1 risk carriers and NEDD4L risk carriers are at increased risk of HT and SGK-1 risk carriers are more sensitive to the BP elevating effects associated with hyperinsulinemia; (4) salt restriction from 150 to 50 mmol per day induces significant BP reductions and that P-renin and P-NtproANP, measured with subjects on their habitual diets, could be useful biomarkers to identify individuals benefiting most from reduced salt intake.

Key words: Genetics, Linkage, Association, Hypertension, Blood Pressure, Cardiovascular Medicine, Salt Intake.

Classification system and/or index termes (if any):

Supplementary bibliographical information:		Language English
ISSN and key title: 1652-8220		ISBN 91-85481-44-0
Recipient's notes	Number of pages 140	Price
	Security classification	

Distribution by (name and address) Fredrik von Wowern, UMAS, Entrance 46, 205 02 Malmö, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature _____ Date 5/1 - 2006

Blood pressure

Blood Pressure (BP) is calculated as the product of stroke volume of the left ventricle of the heart, heart rate and peripheral resistance in the vessels of the body. During the contraction of the heart, systole, BP increases to its maximum i.e. the systolic blood pressure (SBP) and during relaxation of the heart, diastole, BP decreases to its minimum i.e. diastolic blood pressure (DBP).

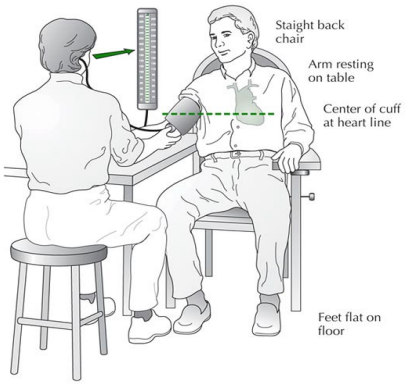

Figure 1. *To obtain a reliable measurement of BP the personnel who measure BP need to have adequate knowledge and skills required for accurate BP measurement and use the correct techniques. The patient should be lying on his back or sitting in a chair with the arm supported and aligned with the body in a quiet surrounding. The arm should be positioned so that the cuff is at heart level. The observer should also inquire about factors that might affect BP now: pain, tobacco or caffeine use, medication, full bladder or strenuous exercise. (Adapted with permission from Grim C. Atlas of Heart Diseases: Hypertension)*

BP can be measured invasively through intra-arterial canylation but is in clinical practice measured non-invasively by sphmygmomanometric methods over the brachial artery. By this method the SBP is defined as the pressure when the first beating sound is heard over the antecubital fossa when slowly lowering the pressure inside the cuff (Korotkoff phase I). The DBP is defined as the pressure when the beating sounds disappear (Korotkoff phase V). (Figure 1)

Definition and diagnosis of hypertension

Levels of BP are normally distributed[1] in the population and hypertension (HT) refers to the upper tail of this distribution. The definition of HT has changed over the last two decades. National guidelines in Sweden proposed in 1987 that HT should be referred to individuals over the age of 20 with DBP more than or equal to 90 mmHg independent of level of SBP.[2] New international guidelines were proposed in 1999 where cut-off values for HT was agreed upon to be more than or equal to 140 mmHg SBP or more than or equal to 90 mmHg DBP.[3] These cut-off values together with total cardio vascular disease (CVD) risk assessment have since been the golden standard for when to commence pharmacological therapy. In the latest recommendations from the Joint National Committee 7 and European Society of HT evidence is laid forth that individuals with BP surpassing 130/85 mmHg are at increased risk for CVD and should be regarded as pre-hypertensive as lowering BP below 130/85 mmHg encompasses significant benefits in terms of cardiovascular morbidity and mortality.[4,5]

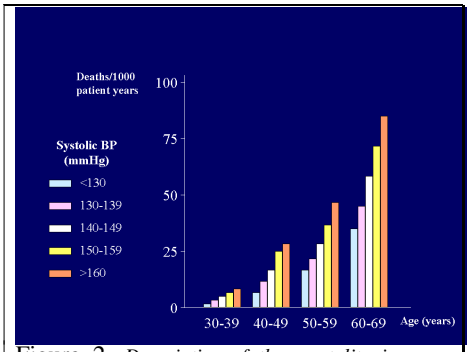

Figure 2. *Descriptive of the mortality increase occurring in every age group with increasing blood pressure. (Adapted with permission from Build and Blood Pressure Study of US Society of Actuaries)*

When evaluating a hypertensive BP recording it has to be established that the elevated BP is sustained by re-measurement of BP on at least two additional occasions with the exception of BP exceeding 180/110 mmHg on the first occasion where treatment for HT should be initiated as soon as possible.[4,5] Besides a thorough physical examination, an evaluation of additional risk factors should be performed as risk of cardiovascular complications is highly correlated to the number of risk factors coinciding on one patient.[4,5] Furthermore, even though not accounting for more than 5-10%, secondary forms of HT should be ruled out. Despite relentless efforts aimed at dissecting the etiology of primary hypertension (PHT) we still know little about what causes the chronic elevation in BP.

Epidemiology of primary hypertension

Maintaining BP is essential for the adequate perfusion of organs. HT refers to chronic elevation of BP beyond levels known to increase the risk of CVD-related morbidity and mortality and elevated BP is the greatest contributor to impaired health in the developed world[6] (Figure 2). It has long been known that elevated BP and HT increases the risk of both stroke[7] and myocardial infarction[8] independent of other known risk factors. Also, there is a continuous relationship between BP and risk of incident CVD[7,9] (Figure 3).

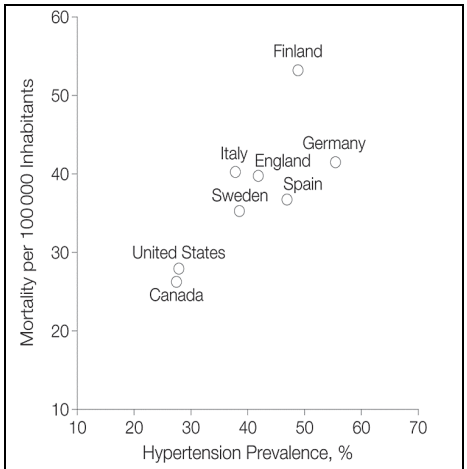

Figure 3. *Hypertension Prevalence vs. Stroke Mortality in 6 European and 2 North American Countries, Men and Women Combined (35-64 Years), Age Adjusted. (Reproduced with permission from JAMA(2003;289:2363-2369))*

SBP increases with increasing age throughout life whereas DBP increase reaches a plateau around 50-60 years or age and even decreases somewhat after age 60.[10,11] This discrepant progression of SBP and DBP makes DBP a stronger predictor of cardiovascular risk below age 50 and pulse pressure a stronger predictor from age 60. Between 50-59 years of age both SBP and DBP as well as pulse pressure are equal in predicting cardiovascular risk[12] (Figure 4).

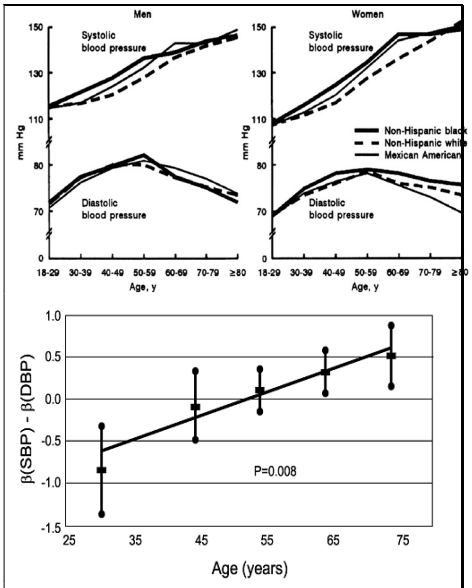

Figure 4. *Upper picture: Changes in systolic and diastolic blood pressure with age. SBP and DBP by age and race or ethnicity for men and women over 18 years of age in the US population. Data from NHANES III, 1988 to 1991. (Adapted with permission from Burt VL et al. Hypertension 1995;23:305–313) Lower picture: Difference in CHD prediction between systolic and diastolic blood pressure as a function of age. The strength of the relationship as a function of age is indicated by an increase in the β coefficient. (Adapted with permission from Circulation(2001;103:1247))*

The definition of HT has varied internationally as well as over time and have therefore provided an obstacle in epidemiological studies when assessing prevalence. A recent multi-center study investigating the prevalence of HT in North America and six European countries found the prevalence to be uniformly distributed in the European countries observed when using BP over 140/90 mmHg or current use of antihypertensive medication (AHT) as definition of HT.[11] The prevalence in the age group 35-44 was 14% in North Americans and 27% in Europeans, increasing to 53% and 78% respectively, among persons aged 65-74 years (Figure 5). Ethnicity does also tend to play a part for the susceptibility of BP elevation and its associated risks as African Americans[13] have higher and Mexican Americans[14] lower BP and HT-related mortality than Caucasians.

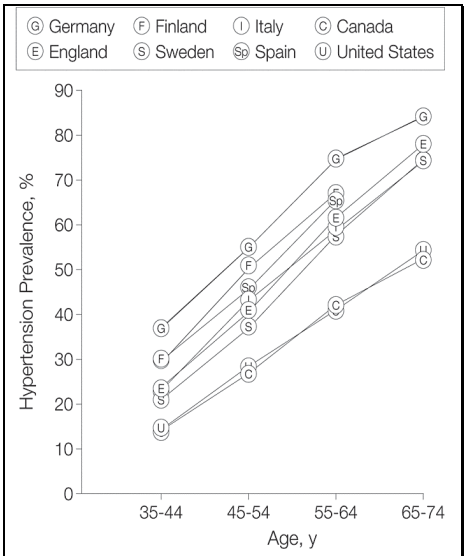

Figure 5. *Hypertension Prevalence in 6 European and 2 North American Countries, Men and Women Combined, by Age Group. (Reproduced with permission from JAMA (2003;289:2363-2369))*

The 1988-2000 NHANES study showed that the prevalence of HT is increased in males through mid life whereas prevalence in the elderly is increased in females.[15] It has also been showed that merely 5.5% - 28.6% of those with HT in the population had a satisfying BP less than 140/90 mmHg.[15,16] Furthermore do country of residence, socioeconomic status, education and psychosocial factors seem to influence the tendency of developing HT.[17-19]

Etiology and pathophysiology of primary hypertension

PHT is by definition HT where the specific causes underlying the disease cannot be determined. To enable an explanation for how PHT develops it is essential to understand how BP is regulated. It is likely that several disturbances in many different pathways are needed for the pathogenesis of PHT to occur (Figure 6).

Genetics of primary hypertension and blood pressure

The normal distribution of BP in the population suggests a multifactorial etiology behind PHT. An overwhelming amount of evidence supports the presence of a substantial genetic component in BP regulation. Twin studies have showed greater concordance of BP in monozygotic (MZ) twins than dizygotic (DZ) twins.[20] Population studies have demonstrated greater intra-family similarity of BP than between families[21] and adoption studies have shown that the familial aggregation of BP is not simply due to shared environment.[22,23] Individuals with two or more first degree relatives with HT develop HT four times as often by the age of 40, three times as often by the age of 50 and twice as often by the age of 60 compared to individuals with no family history of HT further stressing the importance of the genetic heritage especially at early age at onset of the disease. After the age of 70 the predictive value of a family history of HT is negligible.[24] This implies that common genetic susceptibility variants also exist in normotensive subjects, although less frequently than in hypertensives. In all monogenic forms of HT described the culprit has been genes involved in the process of renal sodium reabsorbtion[25-27] implying that the causative mutations in the genes involved in the development of PHT are intimately related to the genes resulting in the monogenic forms, either themselves or in the signaling pathways of specific genes. The genetic background of PHT is more complex and considerably harder to dissect than monogenic HT as the number of genetic variants and their impact are unknown, resulting in a "non-Mendelian" inheritance pattern. However, feasibility of such a gargantuan task has become real due to recent development in molecular genetics[28], genetic statistics[29,30] as well as clinical research. Even though the genetic component is substantial, finding the genetic variants responsible for BP elevation is quite cumbersome. Several pitfalls in this search can be identified. Studies of BP variation in the general population are complicated by multifactorial determination, with a variety of demographic, environmental, and genetic factors contributing to the trait in any single individual.[31] Absence of even rough estimates of the number of genes that influence the trait and the magnitude of the effect imparted by any single locus has made optimal study design in the general population a matter of conjecture. The heritability of BP (i.e. the proportion of BP variance that can be explained by genetic factors) has been shown to be between 50 – 70 %[32-37] in twin studies and around 35% in adoption studies in which shared environment is taken into consideration..[38-40] Recently, Fava and colleagues found that the heritability of 24-hour ambulatory BP was ~30%.[41]

Finding the genetic cause of primary hypertension

To identify genes predisposing for PHT one can focus on either functional candidate genes in pathways known to be involved in BP regulation or positional candidate genes located at loci identified by genome wide linkage analysis. The majority of studies performed so far have aimed at functional candidate genes comparing the prevalence of PHT or BP levels among individuals with contrasting genotypes at the candidate locus. Above all, these studies have revealed that the effect of individual variants are modest at best and findings have been very hard to replicate across study populations.[42-44] It is therefore likely that each individual common variant that has been found to be associated with PHT or BP account for a very small fraction of BP variation at the population level. Several genome wide linkage analyses on BP and PHT have been published with positive results at the genome wide significance level. However, consistency of results between studies have been lacking probably due to underpowered designs and a less rigid phenotype. Pooling studies together in a meta-analysis could increase power to detect true linkage. So far, only one meta-analysis has been performed on PHT without success in identifying candidate loci for HT.[45] However, the currently available genetic data in humans strongly reinforce the concept that regulation of extra-cellular fluid volume by the kidneys is essential in the pathogenesis of PHT as well as salt sensitivity (SS) and stress the crucial role of tubular sodium transport in this process.[46]

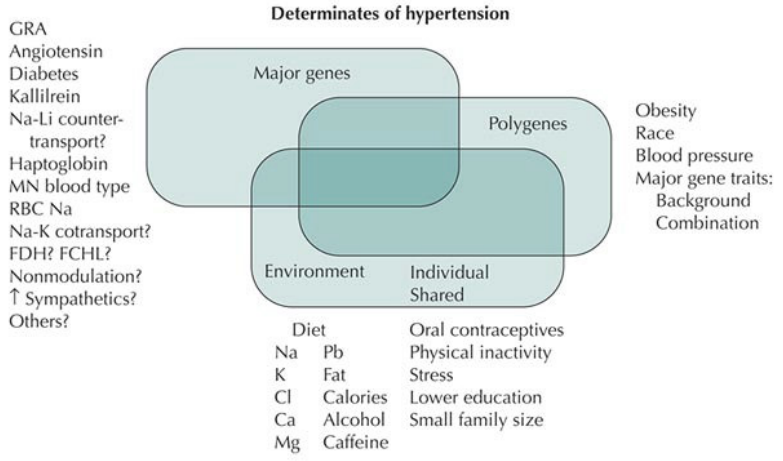

Figure 6. *PHT could result from the combined effects of individual major genes that have a large impact on blood pressure, blended polygenes with small individual contributions, and environmental effects operating on individuals or within families. FCHL—familial combined hyperlipidemia; FDH—familial dyslipidemic hypertension; GRA—glucocorticoid-remediable aldosteronism. (Adapted with permission from Atlas of Heart Diseases:Hypertension)*

Environmental factors for primary hypertension and blood pressure

Several environmental factors have been shown to contribute to HT even in subjects without family history of HT. Such factors are long term high sodium intake[47]; inadequate dietary intake of potassium and calcium[48,49]; excessive alcohol intake[50] and sedentary life style.[51] Given the BP elevating effects of these environmental factors it is rational to speculate that if genetically predisposed individuals were exposed to these factors the BP elevation would be substantial. Apart from environmental factors with independent ability to cause HT several conditions or intermediate phenotypes reflect an interaction between environment and genes (Figure 6). Intermediate phenotypes, i.e. phenotypes that are present already before the development of HT, represent factors, which together with a series of events lead to disease providing a less heterogeneous phenotype. SS[52]and insulin resistance (IR)[53] are examples of important intermediate phenotypes of PHT which will be discussed below. Other common intermediate phenotypes of PHT is increased sympathetic nervous system activity; overproduction of sodium-retaining hormones[54] and vasoconstrictors[55]; deficiencies of vasodilators such as prostacyclin,[56] nitric oxide[57] and natriuretic peptides[58]; alterations in the kallikrein-kinin system affecting vascular tone and salt handling[59]; obesity[60]; increased activity of vascular growth factors[61]; endothelial dysfunction,[57] increased oxidative stress[62] and finally vascular remodeling.[63] However, the heritability of intermediate phenotypes is not yet well documented and they may not always be causally related to the development of HT.

Interrelation between blood pressure and insulin resistance

It has been known for some time that certain co-morbidities have the ability to exaggerate the severity of PHT and display synergistic effects on target organ damage. Several interrelating phenotypes with PHT have been described of which the most common ones are the components of the metabolic syndrome i.e. IR with concomitant hyperinsulinemia, central obesity and dyslipidemia[64] (Figure 7). The absence of cut-off values for the quantitative traits comprising the syndrome has obscured the advent of a clear-cut definition of the metabolic syndrome (MSDR). Adding further complexity to the problems of a definition of the MSDR comes from the fact that its components are reciprocally interrelated with incomplete knowledge of their individual contribution to the pathophysiology of the MSDR. Definitions of the syndrome have been suggested by many groups such as the WHO, the European Group for the Study of IR and the National Cholesterol Education Program.[65-67] These definitions are fairly well correlated giving a prevalence of the MSDR around 25 % in the general population.[68] It has been suggested that IR and compensatory hyperinsulinemia underlie the clustering of the metabolic disturbances and that the syndrome itself is an important risk factor for cardiovascular disease.[69] In the clinical setting obesity and HT or dyslipidemia are most commonly seen together with type 2 diabetes (50%).[70] Commonly, derangements in fibrinolysis, coagulation and inflammation adds to the cardiovascular risk of the MSDR.[71-74]

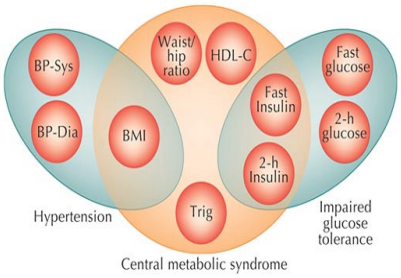

Figure 7. *Risk factors for the metabolic syndrome. There are three distinct domains of the metabolic syndrome: one related to hypertension that includes measurement of blood pressure measures and body mass index (BMI), a second related to glycemia that includes fasting and measurement of postprandial glucose and insulin, and a third related to waist/hip ratio, high-density lipoprotein cholesterol (HDL-C), triglycerides (Trig), fasting and postprandial insulin, and BMI. (Adapted with permission from Wilson P. Atlas of Heart Diseases: Atherosclerosis)*

There is a large body of experimental evidence that IR and compensatory hyperinsulinemia are increased in patients with PHT, and similar changes can be seen in first-degree relatives of patients with PHT.[53,75,76] However, these studies have not been able to establish the causality of the observed link. It is likely that the elevated insulin levels cause the rise in BP as prospective studies have shown that high insulin levels, even within normal ranges, is a strong independent risk factor for developing subsequent PHT.[77,78] About 50 % of hypertensive subjects[79,80] are insulin resistant and it should not be obscured that even though IR and hyperinsulinemia does not contribute to the etiology of PHT in some individuals it does in others, most likely due to genetic differences in bio-molecular pathways linking insulin to BP. One such pathway, involving the serum glucocorticoid regulated kinase type 1 (SGK-1) gene and the neural precursor cell expressed developmentally down regulated type 4 like (NEDD4L) gene, has recently been identified providing a link between hyperinsulinemia and over-activity of the epithelial sodium channel (ENaC) in the collecting ducts of the kidney.[81] This pathway will be explained in more detail below. In a study of Mexican-Americans and non-Hispanic whites 13.8 % of obese subjects (BMI > 30 kg/m^2) were hypertensive compared to 6.3% among non-obese subjects. The same study showed that hyper-insulinemia as a marker of IR increases the prevalence of PHT from 6.9% in normo-insulinemic subjects to 13.4% in hyper-insulinemic subjects. In general, around 50% of type 2 diabetics display PHT,[82] which is considerably higher than in non-diabetic subjects, thus clearly showing that PHT clusters among the components of the MSDR. Furthermore, it has been shown that subjects fulfilling the criteria for the MSDR[65] have a greatly increased risk of cardiovascular mortality.[70]

Salt intake and blood pressure

Throughout evolution humans have consumed a diet consisting of less than 1 gram of salt/day,[83] implying the evolutionary importance of the ability to retain salt. Five-thousand years ago humans started to add salt to their diet leading to, on average, a daily consumption of more than 10 grams of salt/day today.[83] The kidney plays a pivotal role in linking salt intake to BP as supported by renal cross-transplantation in humans.[84] In addition, several hypertensive rat strains have been shown to retain significantly more sodium compared to the normotensive Wistar-Kyoto rat further pointing at the importance of the kidney in BP regulation.[85-88] Guyton described through the pressure-natriuresis curve that sodium induced volume expansion and increased BP causes the kidneys to excrete more water and salt in the urine than are entering the body, thereby decreasing the extra-cellular and blood volumes and subsequently the BP decreases.[46] The ability of the kidney to excrete sodium, SS, is heritable as shown by the fact that normotensive first degree relatives of patients with PHT react with less sodium excretion and increase in BP upon saline infusion compared to normotensive controls.[89-92] Furthermore, the ability to excrete sodium declines with increasing age in the human, probably due to a decrease in glomerular filtration rate,[93,94] thus leading to increased SS in the elderly[95,96] (Figure 10). Although there are many hypotheses on how salt can increase BP most of them converge on that the rise in pressure is dependent on an increase in volume, although evidence in both rat and man has been inconsistent.[85,87,88,97-99] An alternative explanation could be that increases in plasma sodium that occurs in hypertensive subjects can elicit a rise in BP by an increase in hypothalamic sympathetic nervous system activity due to elevated concentrations of sodium in the cerebro-spinal fluid.[100-105] The observation that PHT and dietary salt intake seem to co-segregate has been passionately debated for hundreds of years. Several studies have shown that HT and its co-morbidities like cerebral hemorrhage is virtually absent in societies where salt intake is very low [106,107] and the opposite is true in populations where salt intake is very high.[108,109] However, a clear-cut relationship between salt intake and BP in populations with moderate salt intake has been difficult to establish and has been the root of ambivalence. Figure 8 depicts the role of the kidney in sodium reabsorbtion.

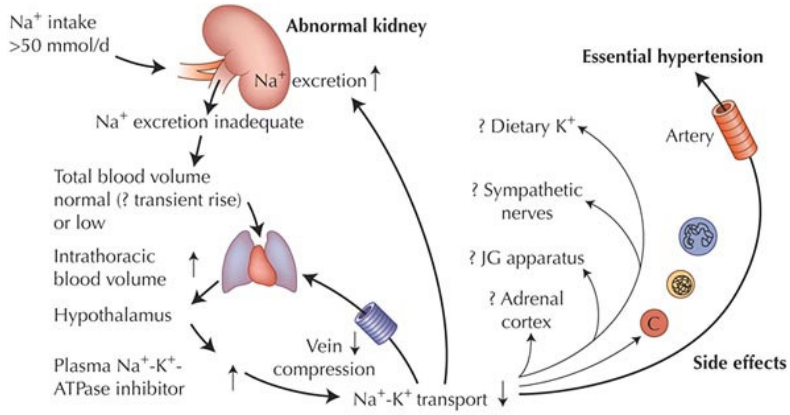

Figure 8. *Hypothetical sequence of events showing the role of sodium retention in hypertension. (Adapted with permission from Siragy H, Carey R. Atlas of Heart Diseases: Hypertension)*

The hardships of finding a link between salt intake and BP in these populations are due to both the narrower range of salt intake and substantial day-to-day within-individual variation in urinary salt excretion due to differing daily salt intakes[110,111] leading to error prone estimations of salt excretion.[112,113] The necessity of multiple 24-hour urine collections to overcome this problem can sometimes be impractical and only a few studies have verified the phenotype by this measure. Both the INTERSALT study and the CARDIAC study showed that levels of 24-hour sodium excretion was associated with BP.[114-117]

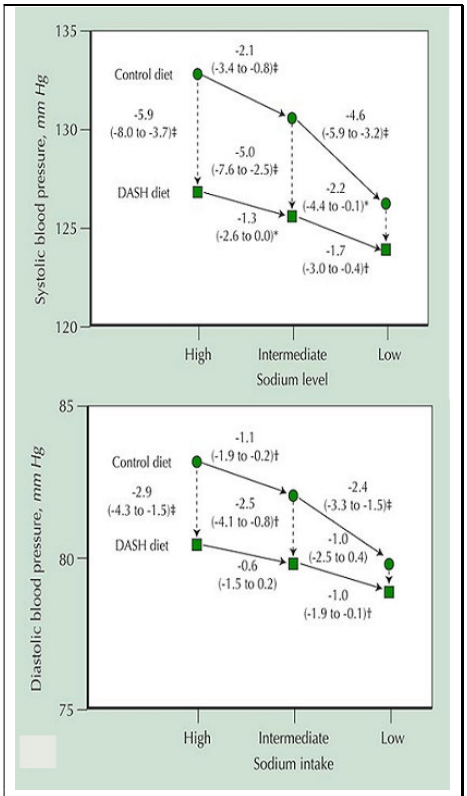

Figure 9. *Reduction of dietary sodium augments the reduction in blood pressure produced by the DASH diet. (Adapted with permission from British Medical Journal (1988;297: 319-328))*

Studies on moderate salt restriction have shown that lowering salt intake from around 10 g/day to 5 g/day during 4 weeks effectively lowers BP in both hypertensive[118] as well as normotensive subjects.[119] Evidence that the BP elevating effects of sodium intake is not due to ethnicity has come from migration studies showing that BP rises in individuals from low salt-eating countries when turned to a more salt rich diet.[120-122] The DASH trial investigated the effect of salt intake[47] as well as the effect of a diet rich in vegetables, fruits and low-fat dairy products known to lower BP.[123] When lowering salt intake from 9 to 3 grams per day BP dropped significant independent of ethnicity, being on the DASH or habitual diet or being hypertensive or normotensive[47] (Figure 9). Several primary preventive programs, stressing the deleterious effects of excessive salt intake, have shown that lowering salt consumption decreases the incidence of HT and CVD mortality.[119,124-126]

Definition of salt sensitivity

SS has historically been regarded as an individual's response to an acute sodium load. Salt sensitive individuals respond to such treatment with an abrupt increase in BP whereas salt resistant individuals respond with a minute change in BP. In practice SS is measured by either the "the acute SS test" developed by Weinberger et al which is performed over 48 hours or "the chronic SS test" developed by Sharma et al performed over the course of 12-14 days. Both test the degree of BP change in response to salt load. The two methods are correlated with each other.[52,127,128] According to the protocol put forth by Weinberger and colleagues,[52] 51% of all hypertensives and 26% of all normotensive are considered salt sensitive. An attractive alternative to the exhaustive tests mentioned above would be to measure

biochemical markers predicting the level of SS.

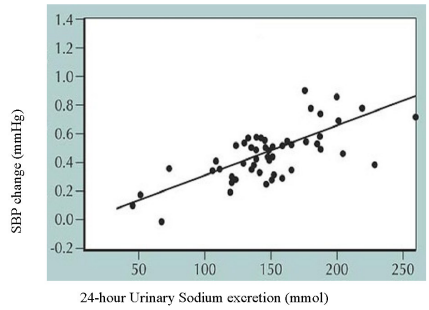

Figure 10. *Averages for urinary sodium excretion (adjusted for age, sex, body mass index, and alcohol consumption) and blood pressure rise with age are shown. Each point represents one center. Derived from the regression line (0.0034 ± 0.00006 mm Hg/y/mmol Na^+) the magnitude of the effect of urinary sodium excretion shows that by reducing sodium intake by 100 mmol/d the rise in systolic blood pressure is reduced by 3.4 mm Hg for a period of 10 years. (Adapted with permission from British Medical Journal (1988;297: 319-328))*

Markers for predicting salt sensitivity

Given the difficulties of determining SS in large populations the idea of being able to measure levels of biochemical markers that predict SS in blood or plasma is appealing. Perhaps the most intriguing potential marker of SS is the atrial-natriuretic peptide (ANP) that have been shown to induce a natriuretic effect associated with a decrease in BP.[129] ANP is produced mainly by cardiomyocytes in the atria of the heart in response to wall stress associated with volume expansion. Furthermore, ANP is also produced locally in the kidney where they act in a paracrine fashion to elicit natriuresis. The effects of this peptide seems to be mediated primarily through guanyl-cyclase coupled receptors although several other unidentified receptors seem to be involved. ANP knock-out mice have been shown to have increased BP and become hypertensive when put on an intermediate salt diet thereby implicating a role for the ANP protein in the regulation of sodium balance.[130,131] Melander et al found that levels of N-terminal ANP was strongly correlated to salt induced change in BP in subjects going from extremely low levels of salt in their diet (10 mmol/day) to very high levels (240 mmol/day).[132] Taken together, these findings suggest that levels of ANP could be a candidate biomarker for SS. It is well established that increased salt intake suppresses the activity of the renin-angiotensin-aldosterone system (RAAS) and consequently a corresponding reduction in salt intake will cause a similar increase in RAAS activity. It has been shown that plasma renin activity could act as a biomarker for SS when measured under short-term extreme salt restriction (going from 240 to 10 mmol/day).[133] The elevation of BP upon oral salt load could be caused by substances that inhibit the Na+-K+-ATP-ase, leading to inhibition of the Na-Ca exchanger in vascular smooth muscle.[134,135] Marinobufagenin[136,137] and oubain[137,138] have been proposed to be responsible for this inhibition and could therefore be markers for SS. The gastrointestinal mucosa synthesizes a peptide, uroguanylin, that when knocked out impair excretion of sodium when put on a high oral salt load thus providing an explanation to why BP increases more when salt is ingested orally compared to when infused as saline.[139]

Genetics of complex diseases in general

Strategies for identifying the genetic component of complex diseases

Finding the underlying genetics for rare monogenic diseases has provided valuable insight into fundamental biological processes. In contrast, the genes giving rise to complex diseases, such as PHT, which contribute to the overwhelming majority of HT related morbidity and mortality in the population, can be expected to be more plentiful but each with a substantially lesser individual impact on BP and development of CVD.[140,141] The small contribution of each genetic anomaly and the fact that it is likely to be common and present in both affected and unaffected individuals mediates a very modest risk increase at the individual level. These plentiful genetic variants are likely to interact in complex fashions representing a major obstacle in unraveling the genetic background of complex disorders. Also, the same phenotype may arise as a result of abnormalities in any one of a combination of several genes giving rise to genetic heterogeneity and heavy environmental exposures alone can in certain instances produce the same phenotype, thus giving rise to phenocopies. Incomplete penetrance obscures the predictive value of genetic variants and carrying a risk variant might increase the overall risk but may heavily depend on gene-gene and gene-environment interactions. Apart from genetic considerations, clinical classification of complex diseases and ascertainment of families for genetic studies does also often represent major obstacles.[142,143] A final consideration of studying complex disorders is the eventuality of ethnic heterogeneity giving rise to the possibility of genetic variants being differentially important in different populations.[144] The above-mentioned considerations highlight the importance of correct study design.

The importance of the phenotype

The onset of PHT is relatively late and of varying severity. The genetic contribution to disease is greater at an earlier age at onset of disease.[24] Due to phenotypic heterogeneity within HT it is necessary to restrict the phenotype by rigid definitions. Studying subjects with an early onset of disease will minimize the numbers of phenocopies as these increase with increasing time of environmental exposure. Life-style factors or co-morbidities like diabetes, especially with microalbuminuria, could lead to HT by alternate routes. Thus, the "cleaner" the phenotype, the greater the probability of finding true associations. An advantage of studying diagnosed hypertensive subjects is that HT, if diagnosed according to established guidelines, is normally a very reliable phenotype as opposed to HT defined by "epidemiological" criteria.

The candidate gene approach

Association studies of candidate genes compare frequencies of alleles or genotypes of particular variants between cases and controls and can be divided into 2 categories: functional and positional candidate gene studies. The functional candidate gene approach has historically been the most commonly applied strategy for discovering genes involved in the development of disease and has identified many of the genes that are known to contribute to susceptibility to common disease.[145-148] Here, a gene is selected on the basis of known biological function and screened for variants, which are then tested in a case-control material or a population for association with the disease thereby requiring the correct prediction of the identity of the gene based on a biological hypothesis. Candidate genes can also be selected based on position in the genome. This approach aims at applying linkage

without any á priory hypothesis and then investigating obvious candidate genes within the region of linkage or applying a LD-based approach to pinpoint the location of the susceptibility gene. Common for both approaches is that if association is found, conclusions are drawn that the variant itself or a variant in linkage disequilibrium (LD) with it contributes to the phenotype. This conclusion could be erroneous due to type 1 errors or population stratification.[149] Population stratification can be avoided by using family based materials, e.g. parent – offspring trios in a transmission equilibrium test (TDT)[150] or by investigating representative population based materials (Figure 11). The TDT investigates whether one variant from heterozygous parents is overtransmitted to affected offspring and thus provides information on both association and linkage. A problem of the TDT derives from the difficulties of collecting large numbers of informative trios required for detecting small genetic effects in late onset diseases like HT were many of the parents of affected subjects have passed away.[145] A limitation of candidate gene studies is that they will only identify a part of the genetic risk factors for diseases in which the pathophysiology is relatively well understood and even less when the pathophysiology is unknown.

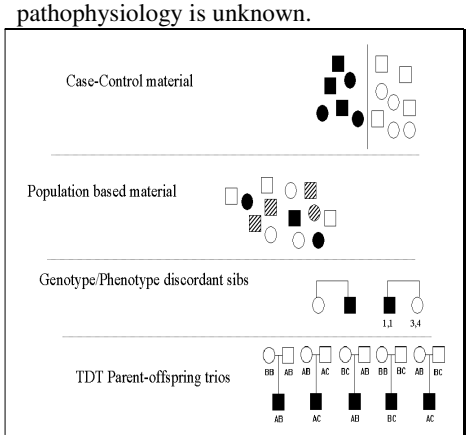

Figure 11. *Different design strategies for finding genes of relevance for complex disorders.*

The linkage approach

During meiosis alleles on different chromosomes (chr) are distributed randomly to gametes due to independent segregation i.e. they are unlinked. However, the relationship of alleles on the same chr will be determined by recombination which is highly affected by physical distance between alleles making alleles in close proximity more likely to co-segregate i.e. being linked. During meiosis, 30–40 recombinations occur thus dramatically increasing the genetic diversity of the organism (Figure12).

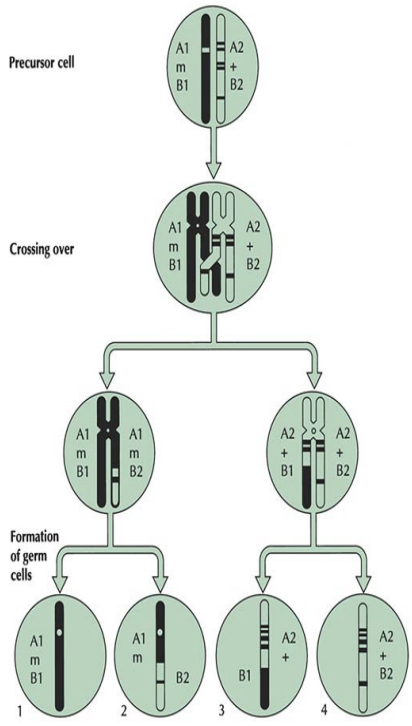

Figure 12. *Schematic of recombinatory events during meiosis. (Adapted with permission from Atlas of Hypertension: Genetics)*

Exploring linkage can be used to locate disease genes[151] by observing the segregation of polymorphic markers at known chromosomal locations together

with the segregation of the disease in families thereby providing a likelihood of chromosomal regions harboring susceptibility loci. Linkage strategies have traditionally been applied to monogenic diseases with Mendelian inheritance patterns, characterized by low population frequency of disease causing mutations, high penetrance, low numbers of phenocopies and little genetic heterogeneity. However, it can also be used in unraveling the genetics behind complex diseases although requiring larger numbers of small nuclear families because the statistical model does not recognize phenocopies, incomplete penetrance, genetic heterogeneity or high frequency of the disease causing mutation.[140,152] Also, uncertain inheritance patterns of complex diseases warrants application of "non-parametric" model-free statistical methods.[29] Incomplete penetrance does not affect the model as un-affected family members are left out of the analysis. The small to modest effect of genetic variants on complex diseases limits the power of the linkage analysis. Linkage analysis of continuous variables such as BP investigates whether sib-pairs discordant for the marker displays greater phenotypic difference than sib-pairs that are concordant for the marker.[153] Linkage studies can be performed at the genome wide level by dispersing markers across the genome. A limitation of this strategy is that multiple markers are tested without any á priory hypothesis of where in the genome disease susceptibility genes are located thereby possibly giving rise to a number of false positive results requiring stricter than ordinary significance levels.[140] As complex diseases is determined by the sum of, and/or interactions between, multiple genetic and environmental factors,[154] any individual genetic variant will have a relatively small effect on disease risk and will be difficult to identify by a linkage approach due to the poor power of linkage analysis to detect common alleles with low penetrance. It is therefore very important to study subjects having a higher genetic contribution to their disease such as subjects with early onset HT. Applying linkage at the genome wide level on complex traits is likely to generate an approximation of the position (10-30 cM) of the susceptibility gene.[30,155] Keeping in mind that 20 cM equals about 20 million base pairs and harbors on average 200 genes and contains around 60000 common single nucleotide polymorphisms (SNP´s). However, tremendous variation in coding sequences and number of SNP´s exists between different chromosomal regions. Finding a causal variant is therefore a monumental task. Addition of more markers in a second step will refine the resolution of the marker map and improve the localization of the linkage peak. Genes known to be involved in the etiology of the disease studied are then selected for further scrutiny from the region indicated by the refined linkage peak. For common complex diseases, linkage analysis has achieved only limited success[156] due to the relatively low heritability of most common diseases, lack of standardized sets of markers to be used across studies[157,158], diffuse definition of the phenotype[159] and inadequate power of the studies.[160] Pooling together results from several linkage studies on the same phenotype in a meta-analysis could partially circumvent these problems. The genome-search meta-analysis method (GSMA)[161,162] is a powerful statistical tool for identifying regions producing weak but consistent linkage signals in multiple genome scans. The GSMA incorporates the logarithm of the odds (LOD) score for each marker used in each individual scan participating in the analysis. The results are placed into bins stretching 30 cM thus giving an opportunity to discover concordance between scans. Each study is weighted for sample size as reliability of linkage analysis is much dependant on power. The limitations of the GSMA are the issue of multiple testing and that markers and their distribution can differ

between studies, thus introducing analytical bias. The GSMA method provides estimation to the location of the susceptibility gene (> 10 cM) still requiring extensive candidate gene studies to find the causal gene.

Linkage disequilibrium

The understanding of the structure of linkage disequilibrium (LD) across the genome is imperative in unraveling the genetics of complex traits as it is used to track down variation that has produced a linkage signal as well as in association studies in which disease variants can be detected through the presence of association at nearby sites.[163,164] LD refers to the fact that alleles at nearby sites co-occur on the same haplotype more often than is expected by chance.[165,166] The main difference between LD and linkage is that linkage investigates the segregation of loci within families and LD analysis investigates the frequency patterns of alleles within populations.[140] LD is created by either natural selection, "bottleneck" events that modify the genetic composition of the population, genetic admixture or genetic drift and can therefore also be used to investigate the evolutionary history of humans.[167] However, LD is constantly destroyed by recombination and *de novo* mutations throughout generations.[164,168] Also, the pattern of LD is quite unpredictable as markers many kilobases apart may be in complete LD whereas nearby markers may not. The extent of LD vary between genomic regions[169], a fact that can be explained by differing recombination rates and population specific factors such as population history and structure since LD differs significantly between African and non-African populations.[165,168,170] The structure of LD can be described by using carefully selected markers designated into discrete haplotype or LD-blocks[171] which are separated by regions of numerous recombination events.[171] The size of any individual block varies depending on the frequency of recombinatory events at those specific loci. However, the typical block averages 5-20 kb but is highly dependant on the resolution of the marker map used. Similar to linkage, the probability of LD is strongly influenced by the physical proximity between alleles since disruptive recombination events will thus have been limited and can be used in the localization of disease susceptibility genes.[172,173] However, regions of LD are often small (tens of kilobases) compared to regions of linkage (usually megabases) providing a much more precise localization of the disease gene.[168,169,174]

The genome-wide association approach

The genome-wide association approach (GWAA) is the association study equivalent of genome wide linkage studies, surveying the entire genome for genetic susceptibility variants without any *á priory* hypothesis of where the variants may be. Due to cost and laboriousness this approach has until recently been unfeasible. Large-scale genotyping is now reaching below 0.01US$ per genotype making the GWAA feasible. GWAA requires knowledge of common genetic variants and the ability to perform extensive genotyping in large sets of patients. Nearly 11 million SNPs with a frequency larger than 1 % have been identified.[175] The objective of the HapMap project[176] is to determine the pattern of LD across the genome which is crucial for the selection of markers for GWA studies. Many obstacles must be overcome before GWA studies become reality. Genotyping all SNP´s in the genome is not feasible, however, since genotypes that are in close physical proximity are usually in LD it is theoretically possible to investigate the entire genome utilizing a much smaller set of markers with only a modest loss of power.[177] Tag SNP´s have the ability to act as surrogates for other SNP´s and can be obtained by using the statistical program

Tagger.[178] Present speculations indicate that between 300.000 to 500.000 carefully selected SNP´s is adequate to provide information about most of the common variations in the genome even in regions showing low LD. The statistical tools for handling the copious amounts of data generated and tests for correction for multiple comparisons are currently being developed. The limitations of the GWAA is the likelihood of producing large amount of false positives or false negative findings[147,179] as well as risking to miss detection of rare variants that could be important for development of disease as only common variants are selected. Replication of GWAA results across studies will therefore be warranted. Also, the GWAA is sensitive to population history, evolutionary selection, disease architecture and mutation rates.[180-182] Recently, studies have shown promising results of LD mapping of complex traits within large genomic regions[181,183,184] thus offering great promise to test common genetic variation across the entire genome in common disease and complex traits.

One gene or many genes?

Historically it has been debated whether HT is the product of the derangement of one gene or many. Under the impression that HT seem to follow the Mendelian laws of inheritance after studying multiple generations Platt proposed that HT was a monogenic disorder in the middle of the 20th century.[185,186] However, the concept of HT was later revised by Pickering who argued that HT merely represented the upper distribution of the BP distribution in the population and as such was more of a quantitative trait than qualitative.[187-189] This description of HT allows for the possibility that common variants in several genes can increase the likelihood of developing HT and is further substantiated by the normal distribution of BP in the population. Recent studies support the argument that common diseases such as primary HT are the product of common variation (frequency > 1%) in many different genes.[168,190,191] Since most of the sequence differences between any two chromosomes are accounted for by common variants[192,193] it is plausible that common variants might contribute to common diseases in which susceptibility alleles might not be under intense negative selection.[168] Several common variants have been shown to contribute to common disease, most of which increase the risk of disease by two-fold or less when examined in large populations[147,194] adding further to the demands on study size and design.

Insights from Animal Models

The study of hypertensive animals has provided understanding of long-term regulation of BP. Quantitative trait linkage analysis in inbred animals have identified numerous loci potentially harboring susceptibility genes for PHT that could be transferred to the genetic map of humans. Genetic knock-out or knock-in mice have added to the knowledge of BP control.[195] Knocking out the α_{2B}-AR in mice produces a salt sensitive hypertensive phenotype[196] making this gene a suitable candidate gene for human PHT. Furthermore, mice lacking the SGK-1 are unable to decrease sodium excretion when fed a low salt diet[197] making it plausible that a gain of function mutation in this gene could precipitate a blood pressure elevation. However, far from all gene alterations produce BP change indicating that other physiologic systems compensate for alterations in gene function.[198] Transferring the results from initial studies in humans into mouse models provides an opportunity for investigations that would be impossible to perform in humans.[195] Interestingly, manipulation of genes involved in renal sodium handling is commonly accompanied by hyper- or hypotension.[199-201]

Monogenic blood pressure regulation

Seventeen genes have so far been found causing monogenic forms of hyper- or hypotension in humans, 8 of which cause HT. Generally, the phenotype is dramatic as the gene mutated affects BP substantially, as apposed to the small effects of genetic variants in PHT. A common feature in monogenic HT is that the genes mutated all are involved in renal sodium reabsorbtion (Figure 13). It can be speculated that genes involved in monogenic forms of HT might harbor variants of importance in the development of PHT.

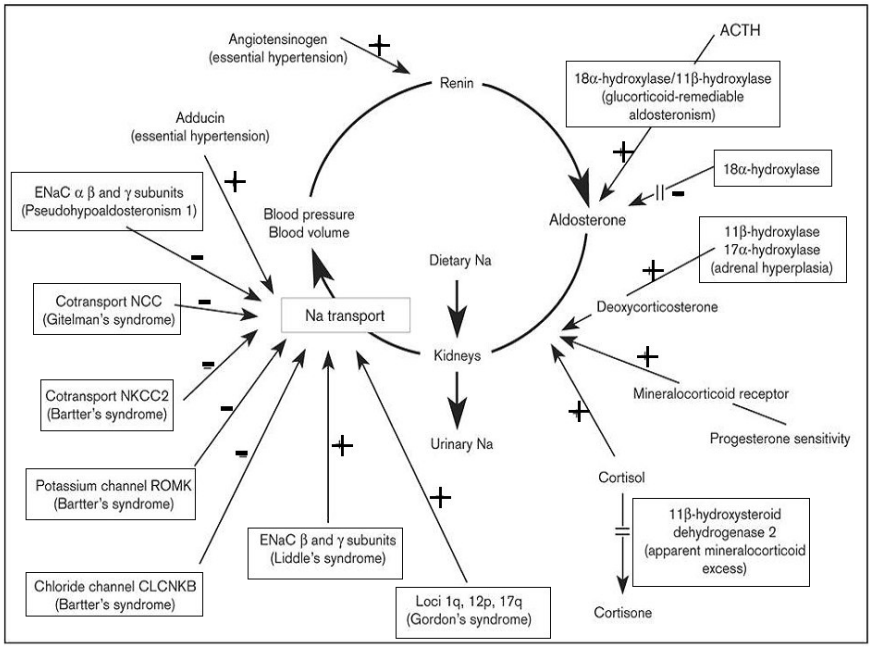

Figure 13. *All mutations and polymorphisms giving rise to monogenic forms of blood pressure alterations in humans are in genes involved directly or indirectly in the control of renal sodium reabsorption. ACTH, adrenocorticotrophic hormone; ENaC, epithelial sodium channel; Na, sodium. (Reproduced with permission from Seminars of Nephrology (2001;21(2):81-93)*

Liddle´s syndrome

Liddle´s syndrome is a rare autosomal dominant disorder with variable penetrance, characterized by HT, sodium retention, hypokalemia and low plasma renin activity. Aldosterone levels are undetectable and treatment with mineralocorticoid receptor (MR) antagonists is without effect. However, amiloride, which blocks sodium reabsorbtion and potassium excretion by MR-independent mechanisms, ameliorate the syndrome.[27,202] Liddle´s syndrome results from constitutive activation of ENaC in the collecting ducts of the nephron causing excess sodium reabsorbtion. ENaC consists of three subunits and is normally regulated by aldosterone. The mutations causing Liddle´s syndrome have been localized to the genes of the β- and γ- subunit of ENaC resulting in changes in the amino acid sequence[203] of a significant portion of a proline rich segment of the C-terminal part of either the β- or γ- subunit called the PY-motif[27] which is essential for its interaction with down-regulatory proteins (Figure 14). The half-life of ENaC is prolonged resulting in increased channel density.

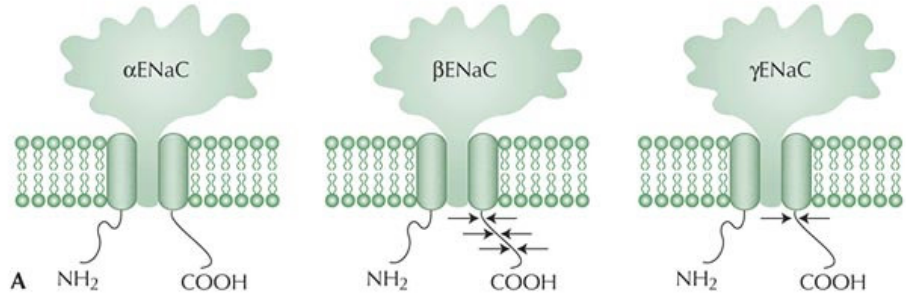

Figure 14. *The mutations in the beta and gamma subunit of the ENaC responsible for Liddle´s syndrome are thought to impair removal of active channels from apical cell membranes, resulting in excessive renal sodium and water reabsorption, and ultimately in hypertension. These mutations keep the gate open, and thus increase sodium current as the ENaC cannot be down regulate by NEDD4L. (Adapted with permission from Weder A. Atlas of Heart Diseases: Hypertension.)*

Pseudohypoaldosteronism

There are two types of pseudohypoaldosteronism (PHA). Type 1 (PHA-1) is the clinical inverse of Liddle´s syndrome caused by homozygous loss-of-function mutations in any one of the ENaC subunits[204,205] (Figure 15). It is characterized by hypotension, renal salt wasting and hyperkalemic metabolic acidosis, despite raised renin and aldosterone levels.[206] Both autosomal recessive and dominant inheritance patterns have been described both presenting in the first weeks of life. The recessive form of PHA-1 presents the most severe symptoms with sodium wasting from the colon, the sweat and salivary glands as well as the kidney causing recurrent life-threatening episodes of salt wasting and hyperkalemia, requiring lifelong sodium supplementation and treatment with potassium-binding resins. Outcome for these patients are often very poor with even minor illness bringing rapid deterioration with hypotension and hyperkalemia leading to nausea and vomiting and further acceleration of clinical decline. In the dominant form of PHA-1, caused by heterozygous mutations in the MR gene,[207] sodium wasting is limited to the kidney. These patients often have a much milder course, responding well to salt supplementation.

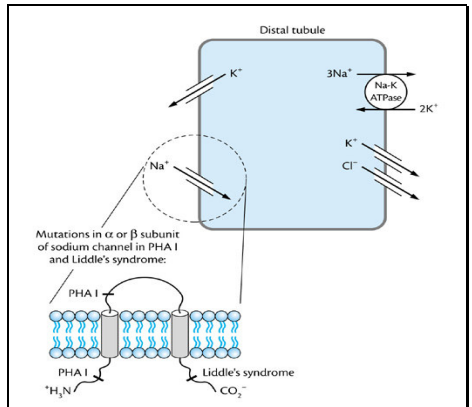

Figure 15. *Genetic mutations responsible for PHA I occur in the alpha and beta subunits of the ENaC. Mutations in the amino terminal or extracellular loop of either subunit disrupt the integrity of the sodium channel, resulting in loss of channel activity. Sodium reabsorbtion fail and volume depletion and activation of the RAAS ensues together with hyperkalemia and metabolic acidosis. Interestingly, when mutations occur in the carboxyl terminal, ENaC activity is increased and Liddle´s syndrome is observed. (Adapted with permission from Osorio F, Linas S. Atlas of Diseases of the Kidney: Disorders of Water, Electrolytes, and Acid-Base*

Renin-angiotensin-aldosteron system

Renin was discovered more than a century ago subsequently followed by discovery of the RAAS, which has shown to be remarkably complex, both from a biochemical point of view as well as from evolutionary aspects. The RAAS has very diverse physiological and pathophysiological involvements and hence important therapeutic implications. To describe the RAAS briefly, renin digests its substrate angiotensinogen, thus producing angiotensin I. Angiotensin I is digested by angiotensin converting enzyme (ACE) to form the main active component of the system, angiotensin II. A substantial proportion of circulating renin is in an inactive form, prorenin. The proportions of active renin and prorenin vary in different circumstances and diseases. The main sources of renin and angiotensinogen are respectively the renal cortical juxtaglomerular cells and the liver. Figure 16 depicts the signaling pathway of the RAAS.

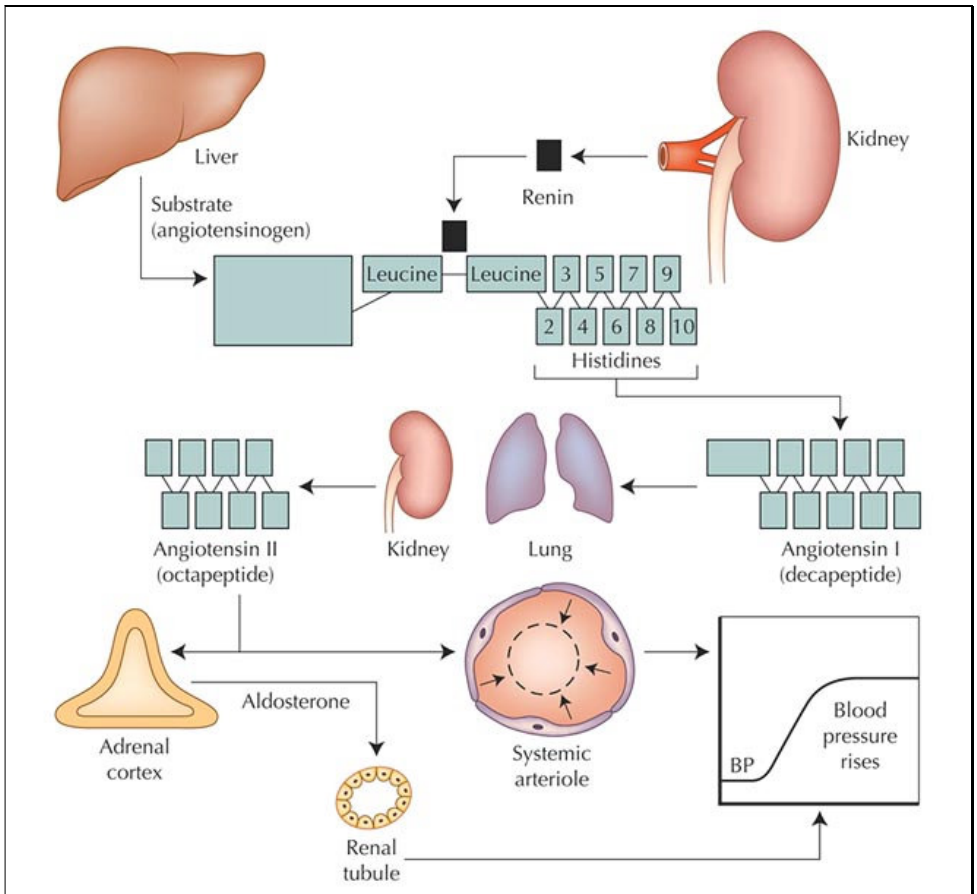

Figure 16. *The circulating RAAS plays a role in body fluid regulation, electrolyte homeostasis, and BP control. (Adapted with permission from Siragy H, Carey R. Atlas of Heart Diseases: Hypertension)*

Candidate integrators of insulin and aldosterone on Na^+ transport

IR seems to influence the level of BP partly via elevated insulin levels associated with IR. Recently, a novel biological signaling pathway for insulin has been discovered, connecting the sodium retentive features of aldosterone signaling to insulin signaling in the collecting ducts of the kidney. Aldosterone is the main regulator of Na+ transport in the collecting duct of the kidney. However, insulin has also been shown to stimulate reabsorbtion.[208] Thus, this may at least partly, explain the BP elevation associated with hyperinsulinemia. A serine/threonine kinase, SGK-1, has been identified as a mediator of aldosterone action in the colon and distal nephron.[209] The activated MR increases SGK-1 gene transcription and SGK-1, in turn, strongly stimulates the activity of the ENaC via indirect actions. Interestingly, insulin appears to stimulate SGK-1 activity through the phosphatidylinositol-3-kinase signaling pathway.[210] Hence, SGK-1 could integrate the effects of insulin and aldosterone on Na+ transport and thereby play a key role in volume homeostasis and it could be speculated that polymorphisms in the SGK-1 gene might be implicated in several medical conditions such as the MSDR, PHT and congestive heart failure. Further more, SGK-1 acts as a negative regulator of the ubiquitin ligase NEDD4L.[211] NEDD4L was originally identified in the mouse brain. Although its function in neural development remains uncertain, it has been shown to act as an interacting protein for the C-terminal tail of the ENaC-β subunit. NEDD4L harbors a tryptophane-rich region (WW domain), which stably interacts with a proline- and tyrosine rich amino stretch on ENaC termed the 'PY' motif.[212] Following NEDD4L-dependent ubiquitination, channel proteins are removed from the plasma membrane and degraded. Liddle´s syndrome results from ENaC mutations that disrupt the PY motif and inhibit NEDD4L ubiquitination of ENaC.[212] The signaling cascade elicited by insulin and aldosterone is shown in figure 17.

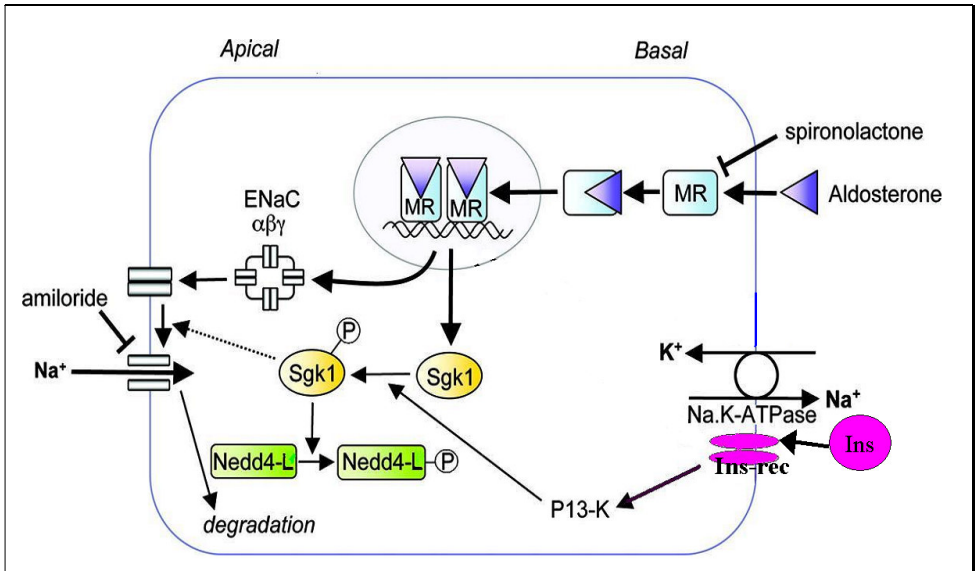

Figure 17. *Representation of an aldosterone/insulin responsive epithelial cell. (Adapted with permission from American Heart Association (Hypertension. 2005;46:1227-1235))*

One previous study has investigated and found an effect of genetic SGK-1 variants on BP.[213] Two studies have found positive association between genetic variations in the NEDD4L gene and BP phenotypes.[214,215] A polymorphism in exon 1 of NEDD4L, leading to the deletion of a C2 domain crucial for Ca2+ dependant intracellular localization, have been under intense scrutiny as deletion of this domain leads to a substantial gain of function of the NEDD4L protein in removing ENaC from the luminal membrane.[216] Furthermore, the locus on chr 18 harboring the NEDD4L gene has been implicated in several linkage studies for various BP phenotypes making the NEDD4L gene a highly interesting positional candidate gene for PHT.

The sympathetic nervous system

The sympathetic nervous system (SNS) is central in cardiovascular medicine. The importance of the sympathetic activation in progression of heart failure and renal insufficiency and mortality is well established and in PHT evidence has accumulated that sympathetic over-activity is a key factor for pathology to occur. SNS mediates primarily acute changes in BP through increases in arterial and venous vasoconstriction and cardiac output but does also contribute importantly to long-term BP regulation trough renal vasoconstriction, sodium retention via Na+/K+ ATPase inhibition, thickening of blood vessel walls with subsequent increased vascular resistance (Figure 18). General SNS overactivity promotes, not only BP increase, but also development and progression of HT-related cardiovascular and metabolic complications, such as left ventricular hypertrophy, vascular hypertrophy, endothelial dysfunction, cardiac rhythm disturbances and IR. The detrimental effects of SNS over activity make the genes partaking in SNS signaling attractive candidate genes for explaining human PHT. Polymorphisms in adrenergic receptors have been the issue of great interest for determining the genetic etiology of PHT and BP elevation

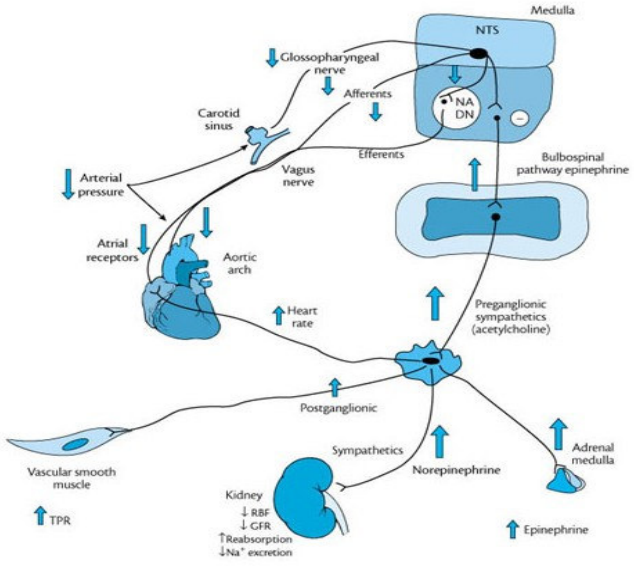

Figure 18. *Neural and sympathetic influences on blood pressure regulation. (Adapted with permission form Navar L, Hamm L. Atlas of Diseases of the Kidney: Hypertension and the Kidney)*

The adrenergic receptors

The adrenergic receptors (ARs) are divided into 2 pricipal types: α and β. At present, 10 subtypes of ARs have been discovered, namely the $\alpha_{1A,B,C,D}$, $\alpha_{2A,B,C}$ and $\beta_{1,2,3}$. All are 7-transmembrane receptors containing amino acids in the carboxy terminus susceptible to phosphorylation and desensitization and all bind to heterotrimeric G-proteins acting as second messengers. The diffents ARs have differing affinity for epinephrine and norepinephrine as well as eliciting different responces upon stimulation depending on which type of G-protein it preferentially binds, resulting in a broad range of physiological responses upon agonist binding.[217] The β_1-AR is preferentially expressed in the myocardium stimulating inotropy and chronotropy, in adipose tissue stimulating lipolysis and in the kidney stimulating renin release whereas the β_2-AR relaxes smooth muscle and is located in bronchi, blood vessels, uterus, gut and bladder.[218] Genetic variants in both the β_1-AR[219-222] and the β_2-AR[223-225] have been associated with response to AHT, myocardial infarction, PHT and BP elevation.

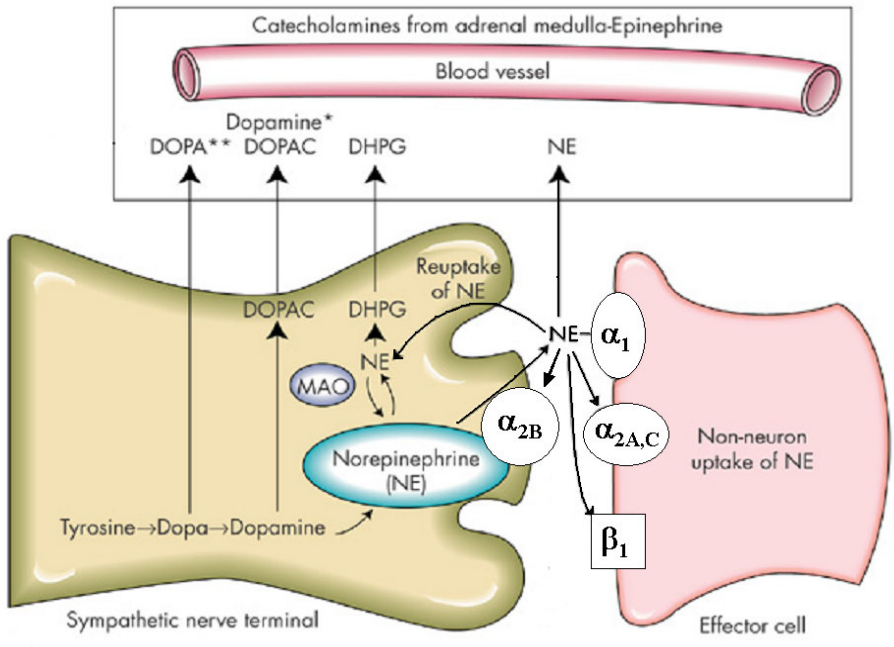

Figure 19. *Description of sympathetic nervous system signaling. DOPAC—dihydroxyphenylaceticacid; DHPG—3,4-dihydroxyphenolgycol; MAO—monoamine oxidase. (Adapted with permission from Raja S. Atlas of Anesthesia: Pain Management)*

The α-adrenergic receptors

α_1-adrenergic receptors (α_1-ARs) are postsynaptic receptors expressed in smooth muscle, heart, vas deferens, prostate and the brain involved in the contraction and relaxation of vessels and smooth muscle. α_1-ARs also exert inotropic effects on the myocardium although less than the β-1 receptor. When administering the α_1-AR antagonist doxazosin to hypertensive patients BP decrease, firmly establishing the importance of α_1-ARs in PHT. However, no study has been able to demonstrate a statistically significant association between genetic variations in any of the four α_1-ARs and cardiovascular disease or BP levels.[226]

The α_2-ARs on the other hand are principally located presynaptically in postganglionic nerve terminals. The α_{2A}-AR and α_{2C}-AR act via negative feedback and when stimulated inhibit further norepinephrine release thus reducing sympathetic outflow from the central nervous system (Figure 19). When blocked with clonidine sympathetic outflow is increased and peripheral vasoconstriction occurs. The α_{2B}-AR has the direct opposite effect from the α_{2A}-AR and α_{2C}-AR. A fraction of α_{2B}-ARs are located postsynaptically in the peripheral nervous system mediating vasoconstriction. However, this effect is counterbalanced by the vasodilatory effect of the centrally located receptors.[227] Several studies have found that a deletion of 3 glutamic acid residues in the α_{2B}-AR, that increases the function of the receptor, is associated with several cardiovascular anomalies including PHT and reduced agonist induced desensitization.[228,229]

Polymerase chain reaction

The development of the PCR technique has in many ways revolutionized molecular biology. It allows selective amplification of tiny amounts of DNA by more than a millionfold via exponential replication of double-stranded DNA. A PCR consists of cycles of three steps: denaturing, annealing, and extension. The starting material is double-stranded DNA which is heated to 94ºC, resulting in separation of the strands. Two primers, each complementary to one of the two strands, a few hundred nucleotides apart, are present in the reaction. During annealing, the temperature is decreased and these primers anneal to the now single-stranded DNA. During extension, the temperature is taken to 72ºC, the optimum temperature for the DNA polymerase, which extends the primers, assembling a second strand on each single-stranded DNA molecule resulting in a doubling of the total amount of DNA in the area flanked by the primers. The whole process is then repeated and after 22 cycles a single strand of DNA is amplified 1 millionfold. This number is particularly impressive considering that each cycle takes less than 2 minutes (Figure 20.

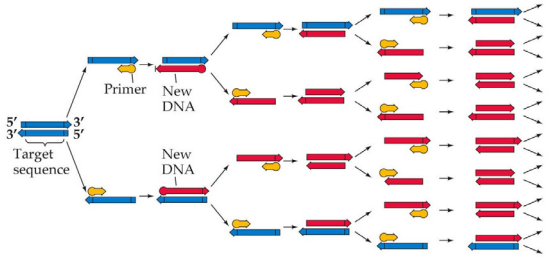

Figure 20. *Schematic of the PCR allowing selective amplification of tiny amounts of DNA by more than a millionfold. (Adapted with permission from Durieux M. Atlas of Anesthesia: Scientific Principles of Anesthesia)*

Present study

Aims

The aims of this thesis were:

- To localize genetic susceptibility loci for early onset PHT by genome wide linkage analysis. (Study I)
- To localize regions of the genome harboring susceptibility loci for PHT and BP variation across multiple studies applying a genome search meta-analysis method. (Study II)
- To test if genetic variation of the α_{2B}-AR, a positional candidate gene in study I and II, affects the risk of early onset PHT and PHT at the population level. (Study III)
- To investigate if variants in the SGK-1 and NEDD4L genes influence BP levels, BP change over time and the insulin-BP correlation. (Study IV and V)
- To test if moderate salt restriction lowers BP and if SS can be predicted by measuring levels of N-terminal atrial natriuretic peptide or renin. (Study VI)

Methods

Study I

Study subjects and phenotyping

This study population was composed of subjects from southern Sweden and southern Finland. Six different health care centers in Sweden and Finland were involved in the collection of subjects. Hypertensive probands were identified from health care files and invited to a re-investigation and included according to the following criteria: (i) age at diagnosis of PHT (at least three consecutive BP measurements of >160 mmHg SBP and/or >90 mmHg DBP on different occasions) ≤ 50 years; (ii) initiation of chronic AHT at age ≤50 years; and (iii) the proband should have at least one affected sibling fulfilling criteria (i) and (ii). Altogether, 243 affected patients from 91 families (91 sib-ships with a mean of 2.7 and a range of 2–6 affected members per sib-ship) were ascertained. Criteria (i) and (ii) comprised the definition of early onset HT (EOHT). Genotype information of both parents was available for 8 sib-ships; for 38 sib-ships, genotype information was available in one parent. The remaining 45 sib-ships lacked parental genotype information. To enable estimation of parental genotypes and determination of identity of descent of genotypes an additional 129 unaffected siblings, contacted through the probands, were genotyped. Eight-point-six percent of all 243 patients had type 2 diabetes. Given that subjects with PHT often are insulin resistant or have type 2 diabetes we allowed type 2 diabetics to take part in the study provided that they were normoalbuminuric and had normal serum creatinine values. Patients showing any of the following signs were investigated for secondary forms of HT and subsequent exclusion: elevated serum creatinine, hypokalemia, albuminuria, hematuria, inability to control BP with ≥2 antihypertensive agents and symptoms of pheochromocytoma. Heavy drinkers were excluded from the study by self-reported consumption >70 cl of 40% liquor per week or elevated serum amino-transferases. BP was measured three times in the supine position by trained nurses after 10 minutes rest by a mercury sphygmomanometric method with the arm positioned at the level of the heart. The mean of the three readings was used to determine BP. BMI was calculated as the ratio of the weight in kilograms to the square of the height in meters. The age at onset of PHT was 40.0±7.7 years (mean ± SD), age at the time of the study was 57.9±10.1 years and BMI was 27.4±4.4 kg/m^2. SBP measured at the study examination was 153±21 mmHg and DBP was 90±11 mmHg and represent "on

treatment" values as all affected patients were on chronic AHT.
Serum and urinary electrolytes, blood glucose and serum creatinine were measured by standard biochemical methods at the Department of Clinical Chemistry, Malmö University Hospital. Microalbumiuria was measured by the MICRAL test.[230]

Genotyping

Genomic DNA was prepared from whole blood and amplified via PCR. Markers had to have an average heterozygosity index of at least 0.75 to be chosen for genotyping. Indices and marker position were obtained from Marshfield Center for Medical Genetics (http://www2.marshfieldclinic.org/RESEARCH/GENETICS/Map_Markers/maps/IndexMapFrames.html). Mean sex average distances between juxtapose markers were 10 cM in the initial scan and 4.6 cM in fine-mapped regions. For detection, PCR-primers were labeled with either a yellow, green or blue fluorescent dye (DNA Technologies, Denmark and ABI kit, Applied Biosystems, USA) allowing allelic discrimination for same-size fragments. PCR mix preparations were conducted via automated pipetting stations and overlaid with mineral oil after which PCR was performed. Each PCR product was pooled with others from the same individual allowing for detection of multiple fragments in the same subject. Pooled mixes were then loaded onto a denaturing polyacrylamide gel and fragments where separated via electrophoreses. Detection of the fluorescent products was performed using an ABI 377 sequencer (Perkin Elmer) and data was processed via the software Genescan/Genotyper (Perkin Elmer). Each run was read by two independent investigators and discrepancies in allele calling was subject to extended scrutiny and re-running the fragment in question for the entire family. Data was subsequently checked for errors of Mendelian segregation using the Pedmanager software. Individuals showing evidence of errors of Mendelian segregation by miss match in identical by descent status were excluded from the study. Candidate genes were located through NCBI Entrez Genome.

Statistics

Evidence of linkage was assessed with a non-parametric method using GENEHUNTER software version 2.0.[29] Complete multipoint analysis of the statistical significance of allele sharing identical by descent among all affected sib-pairs at each location in the genome was performed. The contribution of each sib-pair was weighted to compensate for the difference in the size of the sib-ships. The strength of the linkage was expressed as LOD score and P-value. If parental genotypes were not available they were estimated from offspring using GENEHUNTER v 2.0.[29] Marker allele frequencies used in the analysis were those of the founders of the families in the study.[29] If the founder genotypes were not available, allele frequencies were estimated from allele frequencies of their offspring using PEDMANAGER (Whitehead Institute for Biomedical Research, MIT, Cambridge, MA). Allele frequencies did not differ from that of a control material consisting of 1200 Finns (data obtained from the Finnish Genome Center). Full-sib status of all siblings was confirmed using the computer program RELATIVE.[231] The marker map used in the present study was more sparse and lacked total informativeness compared to the marker map upon which Lander & Kruglyak based their estimations for thresholds for genome wide significance.[232] Therefore, to establish appropriate thresholds for genome wide suggestive and significant linkage for our particular set of data, 1000 simulations were performed by generation of artificial genotypes in our particular set of families [GENSIM software (M. J.

Daly, unpublished data)]. According to this simulation a LOD score of 2.7 corresponds to a genome wide significant linkage result and 1.7 to genome wide suggestive linkage.

Study II

Study subjects

At the time of the initiation of this study 11 published studies fulfilled the criteria for inclusion. Only genome wide linkage scans in Caucasian population for the study of PHT or BP were included in an attempt to decrease the genetic and environmental heterogeneity with the exclusion of subjects originating from genetic isolates. In studies where other ethnicities had been included results for Caucasians were dissected out and incorporated into the meta-analysis.[159,233-240] Genome wide scans investigating PHT were only included if the phenotype was early age at onset HT (<60 years of age) in order to ensure a significant genetic component. Nine out of 11 eligible studies agreed to participate.[159,233-240] Of these, 5 studies were BP scans[159,233,237-239] and four HT scans.[234-236,240] Two HT scans had maximal age at onset <50 years of age[235,240] while the other 2 relied on an age at onset < 60 years of age.[234,236]

Statistics

The autosomes were divided into 120 bins with an average width of 29.1 cM according to the Marshfield map. Markers and marker maps differed between studies and were adjusted to the Marshfield map by way of transforming each marker location into Marshfield map locations on the basis of the first and last genotyped marker of the chr.[241] This transformation was applied to all studies except two[237,238], in which a physical map had been used. These maps were divided into bins with the help of the KTL cartographer (http://www.bioinfo.helsinki.fi/cartographer/) and the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway).

Bins were ranked within each study, giving the bin with the highest LOD score the best within study rank (R_{study}) value. A weighting factor, defined as the square root of the number of affected/included subjects, was also introduced to adjust for differences in size between studies. The weighting factor was divided by the average value of the studies, giving a mean weight of 1.0 giving a range between 0.5 and 1.7 indicating that the largest study contributed 3 times more to the results than did the smallest study. In both the weighted and unweighted analyses, the R_{study} values were summed and a point-wise P-value for each bin was calculated. Point-wise P-values were determined from the theoretical distribution of the GSMA in the unweighted analysis[162] and by simulation of the observed ranks for the weighted analysis.[161] Point-wise P-value of $P<0.0004$ was defined as genome-wide significant and $P<0.008$ as genome-wide suggestive evidence of linkage taking into account corrections for multiple comparisons. Correlations between the summed ranks of the analysis of HT and those of diastolic and SBP, respectively, were calculated using Kendall/Spearman non-parametric rank correlation coefficients.

Study III

Study subjects

The material was derived from two collections of individuals from Skara in southwest Sweden. The material has been described in detail previously.[242] In brief, all known patients with PHT or type 2 diabetes in Skara were surveyed, including blood sampling for DNA, between 1992-3. A corresponding survey was performed on an age-stratified sample of the Skara population aged 40 years or older between the years 1993-4. Patients suffering from

PHT and type 2 diabetes with signs of incipient or overt diabetic nephropathy (i.e. microalbuminuria) were excluded (n=72) in accordance with the phenotypic inclusion criteria in our previous genome-wide scan.[240] A total of 943 patients with PHT (772 of whom were non-diabetic) and 817 population controls were included. Of the patients with PHT 311 (260 of whom were non-diabetic) had been diagnosed at an early age defined as 50 years or younger, similar to the phenotype utilized in our genome wide scan for EOHT.[240] A subset (n=261) of the population controls was normotensive as defined by the Joint National Committee 7[4] (i.e. BP less than 120/80 mmHg) thereby allowing us to compare the upper and lower tails of the BP distribution and theoretically maximizing the power of finding a genetic effect. A description of clinical characteristics is shown in Table 1.

Table 1

	Population Controls (n=817)		Hypertensive Patients Without Type 2 Diabetes (n=772)			Hypertensive Patients With or Without Type 2 Diabetes (n=943)		
	Mean	(SD)	Mean	(SD)	*P* Value	Mean	(SD)	*P* Value
Age (y)	60.4	(12.8)	65.7	(12.2)	<0.0001	65.5	(11.3)	<0.0001
Age at onset (y)	NA	NA	53.9	(11.7)	<0.0001*	54.1	(11.3)	<0.0001*
BMI (kg/m^2)	26.1	(4.0)	27.7	(4.6)	<0.0001	28	(4.6)	<0.0001
AHM (%)	NA	NA	81			81		
Sex (% male)	49		40		0.001	41		0.002
SBP (mm Hg)	132	(19.2)	155	(19.1)	<0.0001	157	(19.7)	<0.0001
DBP (mm Hg)	75	(9.6)	84	(9.3)	<0.0001	84.5	(9.3)	<0.0001

BMI indicates body mass index; AHM, antihypertensive medication; SBP, systolic blood pressure; DBP, diastolic blood pressure; NA, not analyzed.

P values refer to the difference between patients and population controls.

*Difference between age at onset for patients and age of population controls.

Measurement of blood pressure and diagnosis of primary hypertension

BP was measured three times in the supine position by trained nurses after 10 minutes rest by a mercury sphygmomanometric method with the arm positioned at the level of the heart. The mean of the three readings was used to determine BP. PHT was diagnosed as BP exceeding 160/90 mmHg on three separate occasions, subsequently followed by administration of anti-hypertensive medications.

Biochemical analysis

Serum and urinary electrolytes, blood glucose and serum creatinine were measured by standard biochemical methods at the Department of Clinical Chemistry, Malmö University Hospital. Microalbumiuria was measured by the MICRAL test.[230]

Genotyping

The 9 base-pair difference between the D and I alleles in the α_{2B}-AR was detected by UV light after DNA electrophoresis on a 3% agarose gel with ethidium bromide after PCR. All genotypes were read by two independent investigators who were unaware of the phenotypic status of the study subjects.

Statistics

Data were analyzed with NCSS statistical software (version 6.0.21, Statistical Solutions Limited, Cork, Ireland). Frequency differences were analyzed by

chi^2 test and differences in continuous variables by t-test and ANOVA or Mann-Whitney and Kruskal-Wallis test, depending on whether the variable was normally distributed. The genotypic effect of the I/D polymorphism on risk of PHT was analyzed by multiple logistic regression. All statistical tests were two-sided and P<0.05 was considered statistically significant.

Study IV-V

Study subjects

"Malmö family collection for the study of Macrovascular and Hemodynamic Genetics"

118 Caucasian families from Malmö, Sweden, were collected from September 2000 until March 2002. Probands were ascertained from the "Malmö Diet and Cancer Study" (MDC) and the "Malmö Preventive Project" (MPP).[243,244] One-hundred-twenty-one sib-ships free from AHT were included in the quantitative trait locus (QTL) analysis of ABP in the present study. Sib-ships with one or two parents with diagnosed and pharmacologically treated PHT were primarily included for enrichment of genetic predisposition and 91% of the included subjects had ≥1 first-degree relative with diagnosed PHT. The mean age of the 260 included subjects was 38.3 ± 8.6 years, BMI 25.2 ± 4.0 kg/m^2, heart rate 68.6 ± 10 beats per minute and 49% were male. Two-hundred-and-one, non-phenotyped, additional family members were genotyped in order to increase phase information, i.e. identity by descent (IBD), in the QTL analysis.

"Malmö Diet and Cancer"

The study population in the present study is derived from the "MDC" which is a cohort study on diet and cancer in Malmö, Sweden described in detail elsewhere.[244] A random 50% (n=11456) of those who entered the study between November 1991 and February 1994 were invited to take part in a study of the epidemiology of carotid artery disease.[245] Of the 5540 subjects who accepted to participate full phenotypic data, required for inclusion and successfully extracted DNA-samples, was obtained from 4830 subjects. BP was only studied in subjects free from AHT (n=4001) whereas the patients on AHT (n=829) were included when genotype frequencies were compared between "hypertensives" and "normotensives". Two-thousand and seventy-one untreated subjects in "MDC" had been investigated ~11 years previously in the "MPP"[243] allowing for the study of BP change over time. Clinical characteristics of all subjects free from AHT at "MPP" and "MDC" and those on AHT at "MDC" are shown in Table 2.

Table 2

	MPP without AHT (*N* = 2171)	MDC without AHT (*N* = 4001)	MPP to MDC (*N* = 2171)	MDC with AHT (*N* = 829)
Age *years*	47.2 ± 5.7	57.4 ± 6.0		59.5 ± 5.5
Body mass index *kg/m²*	24.2 ± 3.2	25.7 ± 3.7		27.7 ± 4.3
Gender % *male*	54	41		45
Systolic blood pressure *mm Hg*	123 ± 13.2	140 ± 18.1		152 ± 19.2
Diastolic blood pressure *mm Hg*	81.9 ± 8.4	86.1 ± 9.0		92.1 ± 9.6
P-insulin *mIU/L*[a]	8.8 (5.0–9.0)	7.0 (5.0–9.0)		9.0 (6.0–12.0)
Diabetes mellitus % *yes*	0.5	6.3		19.1
Follow-up time *years*[a]			12.5 (7.79–14.51)	
ΔSystolic blood presure *mm Hg/year*[a]			1.56 (0.69–2.63)	
ΔDiastolic blood pressure *mm Hg/year*[a]			0.37 (0.00–0.95)	
ΔSystolic blood pressure %/*year*[a]			1.27 (0.54–2.24)	
ΔDiastolic blood pressure %/*year*[a]			0.78 (0.00–1.21)	

Abbreviations are: MPP, Malmö Preventive Project; MDC, Malmö Diet and Cancer; AHT, antihypertensive treatment. All variables except frequency variables are shown as mean ± SD.
[a]Median and interquartile range (IQR).

Blood pressure measurements

BP was measured, in the supine position after a 5-minute rest, twice over the brachial artery using a sphygmomanometer. The mean of these two readings was used as a measurement of BP.

Biochemical analysis

Serum and urinary electrolytes and blood glucose were measured by standard biochemical methods at the Department of Clinical Chemistry, Malmö University Hospital. Insulin was measured by a non-specific radioimmunoassay at the Department of Clinical Chemistry, Malmö University Hospital.

Genotyping

Genotyping was performed using the Sequenom Mass ARRAY™ system (Sequenom Inc., San Diego, USA), according to manufacturer's recommendations. Following an initial PCR reaction residual dNTPs and primers were inactivated using Shrimp Alkaline Phosphatase (SAP) and the hME (Mass Extend) primer mix, GTP/ddACT terminator mix and Mass Extend Thermo Sequenase (32 U/µl) (Amersham International) were added. After subsequent thermocycling the hME reaction products were cleaned with resin and were robotically applied to analysis chips. Genotyping analysis was performed using Sequenom software on the MassArray Maldi-Tof mass spectrometer. Rs1743964, rs1743966 and rs1057293 were genotyped in study IV. In study V rs4149601, rs502450, rs2288774 and rs2075403 were genotyped.

Statistics

Data was analyzed with SPSS Statistical Software (version 11.5) (SPSS Inc., Chicago, IL, USA). Frequency differences were analyzed by chi-2 test or Fisher's exact test where appropriate. Differences in continuous variables were tested by t-test and analysis of variance (ANOVA) or Mann-Whitney and Kruskal-Wallis test, depending on normality of the variable tested. Multiple linear regression and multiple logistic regression analysis were used to test if genotypic effects on BP and HT were independent of covariates. The independent effect of genotype on change in BP over time was analyzed by applying the general linear model. Spearman's test for correlations was used to calculate correlations. Fisher's R to Z transformation was used to test differences in correlations depending on genotype using a one-sided test. All other tests were two-sided and throughout $P < 0.05$ was considered statistically significant.

Study VI

Study subjects

Forty-six subjects free from anti-hypertensive medication and without family history of PHT, diabetes or kidney disease were included. Recruitment was conducted via local newspaper advertisements. A total of 39 subjects (20 men and 19 women) completed the study whereas 7 individuals were excluded due to either feverous infections (n=2) or incompliance to ingest the capsules used in the study (n=5). All subjects had day-jobs and followed the ordinary day-night cycle. Based on the results in non-black normotensive subjects of the DASH trial,[47] at least 25 study subjects would be required to detect a mean SBP reduction of 4 mmHg with 80% power at a significance level of 5% assuming a SD of 7 mmHg of the SBP lowering effect of a daily 100 mmol reduction in NaCl intake. (STATA, STATA corp, College Station, Texas).

Blood pressure measurments

Office BP (OBP) was measured after 30 minutes of rest in the supine position three times in the right arm at heart level using an ISO-STABIL 5 device (Speidel & Keller, Jungingen, Germany) with a tricuff manchett (AJ Medical, Lidingö, Sweden) and the mean of the three office BP measurements was used as definition of office BP. ABP was measured using an ABPM 90207 device (Spacelabs Medical Inc, Redmond, WA, USA) applied on the left arm. Two different cuff sizes were used depending on the arm circumference (24-32 cm and 32-42 cm, respectively) of the study subjects. Daytime (one recording every 20 minutes) was defined as 06 a.m.-10 p.m. and nighttime (one recording every 60 minutes) as 10 p.m.-06 a.m. Study subjects were advised to maintain the left arm relaxed along the body during each measurement. During the 24-hours of ABP measurements, 24-hour urinary collections were also obtained. The following day when subjects came back to return the ABP device and the 24-hour urine collection, a second OBP (OBP2) was obtained as described. OBP in all studies was determined by the same nurse especially educated in BP measurements. Study design is illustrated in Figure 21.

Biochemical analysis

Serum and urinary electrolytes, blood glucose and serum creatinine were measured by standard biochemical methods at the Department of Clinical Chemistry, Malmö University Hospital. Plasma renin concentration (P-renin) was measured with a RIA diagnostic kit (Abbot laboratories) and plasma Nt-ANP concentration (P- Nt-ANP) was measured by radioimmunoassay (RIA) using antiserum from Peninsula Laboratories.

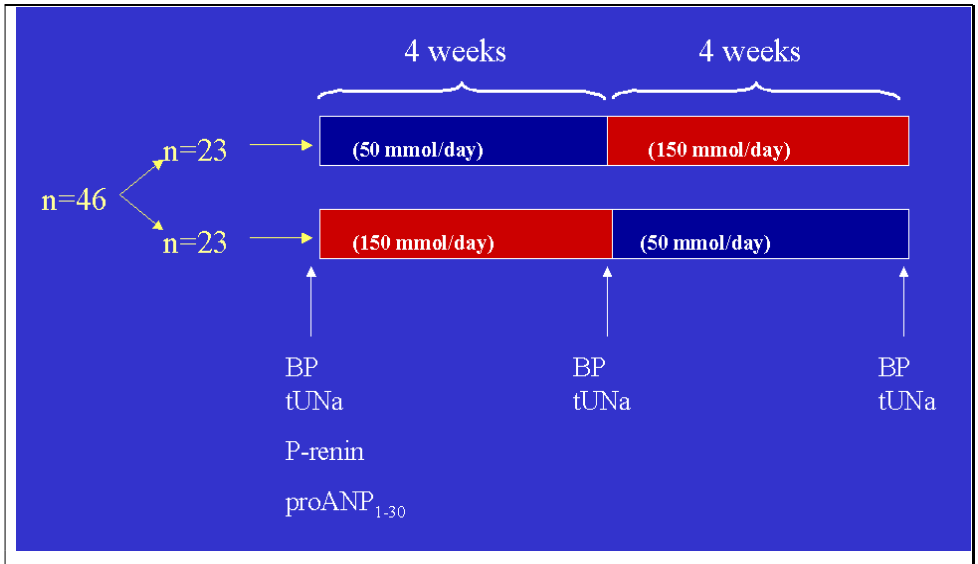

Figure 21. *Design of study VI. tUNa = total urinary sodium*

Procedures and measurements of salt sensitivity

Subjects were investigated with ABP measurements as well as office BP measure at baseline and at the end of each low and high salt period. Subjects were instructed to not alter their lifestyle during the course of the study. The standardized meals and drinks contained 50 mmol NaCl/day and were provided by our metabolic ward. At baseline subjects were randomized to a high salt diet (150 mmol NaCl/day) or low salt diet (50 mmol NaCl/day) respectively for 4 weeks and subsequently switched to the other diet for another 4 weeks. Subjects were randomly given capsules containing 100 mmol NaCl or placebo depending on being on high- or low salt diet. A dietician composed the meals, which were then adjusted regarding energy content to accommodate for gender and weight differences (caloric content ranging between 2000-2600 kcal/day). Degree of SS was defined as the difference in 24-hour systolic ABP after high-salt and 24-hour systolic ABP after low-salt.

Statistics

Data was analyzed with SPSS statistical software (version 11.5, SPSS Inc. Chicago, Illinois, USA). Frequency differences were analyzed by χ^2-test. Significance of differences in paired variables was tested by paired t-test or Wilcoxon´s paired rank test, where appropriate. Pearson or Spearman´s test for correlations was used to calculate correlations. All tests were two-sided and $P< 0.05$ was considered statistically significant.

Results

Study I

In the 10 cM initial genome wide scan, nominal P-values ≤0.016 (LOD score ≥1) were found in regions on chr 1 at 81 cM, chr 2 at 115 cM, chr 3 at 108 cM, chr 14 at 45 cM and at 99 cM, chr 17 at 42 cM and chr 19 at 89 cM which where subsequently fine mapped with additional markers within the 1-LOD drop interval.

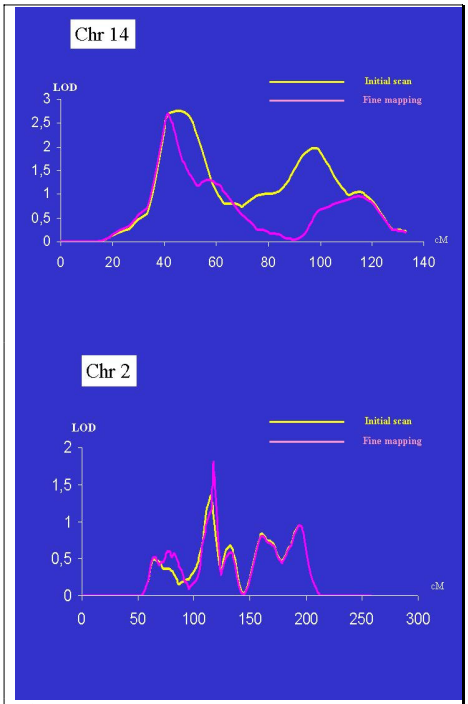

Figure 22. *Results from study I on chromosome 14 (upper) and chromosome 2 (lower).*

Final average marker density in fine mapped regions was 4.6 cM (range 3–5 cM) with average marker heterozygosity index of 0.78. Genome wide thresholds for significant and suggestive linkage was determined by 1000 simulations to be a *P*-value of ≤0.0002 and *P* ≤0.003 respectively (LOD ≥ 2.7 and LOD ≥ 1.7) at the 5% level based on 4.6 cM marker density. A region on chr 14 at 41 cM reached genome wide significance and a peak on chr 2 (Figure 22) reached suggestive evidence of linkage after fine mapping (LOD = 2.7 and 1.8 respectively corresponding to non-parametric linkage scores of 3.5 and 2.9).

Study II

A region on chr 3p14.1–q12.3 (bin 3.4) obtained genome-wide significant evidence of linkage to HT in both unweighted and weighted analysis and suggestive evidence of linkage to DBP in unweighted analysis. In combined analysis of HT and BP the same region showed genome-wide significant evidence of linkage in the unweighted analysis. A region on chr 2p12–q22.1 (bin 2.5) showed suggestive evidence of linkage to HT as well as to HT and BP and nominal evidence of linkage to DBP.

In an ordered rank analysis bin 3.4 and 3.5 showed significant and suggestive evidence of linkage respectively, confirming that these bins are likely to harbor a susceptibility gene for BP variation and/or HT. Bin 2.5 showed suggestive evidence of linkage (Figure 23)

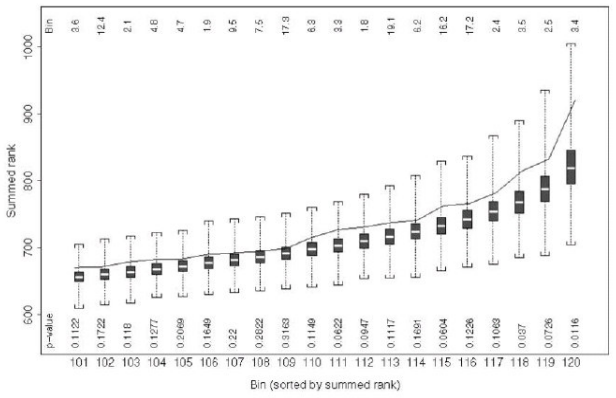

Figure 23. *Results of the ordered rank analysis for the combined weighted analysis of HT + DBP, showing observed results (solid line) and the distribution of simulated results for the highest (120), second highest (119), etc, summed rank in the GSMA simulation. Also shown for each bin is the simulated distribution with median, interquartiles and range of values.*

To refine the location of the locus on chr 3 a fused bin analysis on HT + DBP was performed in which bins 3.4 and 3.5 were divided creating one bin where the outer bin halves of bins 3.4 and 3.5 were merged and one new central bin with the original boundary marker of the two bins in the middle. In this analysis the central bin was the most significant (P=0.00013) indicating that the susceptibility gene on chr 3 is located somewhere in the medial parts of bins 3.4 and 3.5. The HT and DBP ranks correlated significantly (r=0.20, P=0.03) whereas those of HT and SBP did not (r=0.07, P=0.42).

Study III

DD-genotype carriers of the α_{2B}-AR I/D polymorphism had significantly increased risk for EOHT independent of age, sex and BMI whether or not concomitantly afflicted with type 2 diabetes compared to II- or ID-genotype carriers (Figure 24). When comparing non-diabetic hypertensives of all ages and population controls, carriers of the DD genotype had a significantly increased OR for PHT (Figure 24).

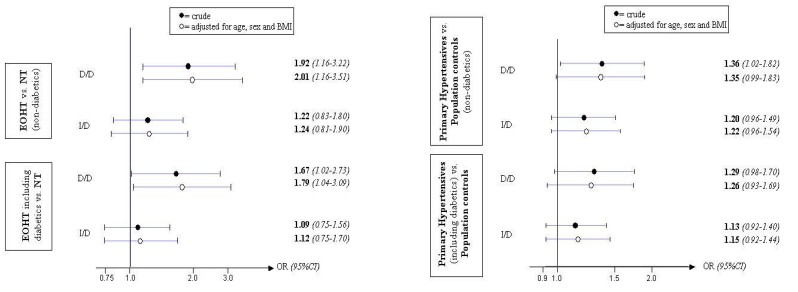

Figure 24. *Odds ratios (OR) and 95% confidence intervals (95% CI) for EOHT (with and without diabetes) vs. NT and for primary hypertension (with and without diabetes) vs. population controls in carriers of the DD and ID genotypes in carriers of the DD and ID genotypes. The II genotype is defined as the reference (i.e., OR=1.0)*

However, after adjustment for age, sex, and BMI, the association was of borderline significance (Figure 24). When type 2 diabetic subjects where included in the hypertensive group the effect of the DD genotype became non-significant (Figure 24). The I/D polymorphism did not seem to influence the levels of BP in subjects free from anti hypertensive medication; SBP (134±19.4 mm Hg versus 133±20.1 mm Hg, P=0.89) and DBP (75.1±8.9 mm Hg versus 76.3±9.8 mm Hg, P=0.25) (n=130 with DD genotype and n=295 with II genotype).

Study IV

We found that carriers of the CC-genotype of the intron 6 polymorphism had higher DBP than carriers of the CT- and TT-genotype and carriers of at least one C-allele of the exon 8 polymorphism had higher SBP than carriers of the TT-genotype. Simultaneous carrier-ship of both these risk-polymorphisms (SGK-1 risk carriers) conferred elevation of both SBP and DBP compared to non-carriers (Table 3).

Table 3

	SGK-1 risk ($N = 128$)	Other genotype carriers ($N = 3688$)	P value
Intron 6 CC + exon 8 CC/CT (SGK-1 risk) vs. all other subjects ($N = 3816$)			
Systolic blood pressure *mm Hg*	143 ± 18	139 ± 18	0.03
Diastolic blood pressure *mm Hg*	88.2 ± 8.3	86.0 ± 9.0	0.009

Analyses were made on subjects free from antihypertensive medication.

The effect of the SGK-1 risk was independent of age, sex and BMI for both SBP (P=0.02) and DBP (P=0.01). Additionally, carrier-ship of the SGK-1 risk increased the BP change over time from MPP to MDC, both for SBP and DBP, independent of the effects of covariates (Table 4).

Table 4

	SGK-1 risk ($N = 65$)	Other genotype carriers ($N = 2005$)	P value
Intron 6 CC + exon 8 CC/CT (SGK-1 risk) vs. all other subjects ($N = 2070$)			
ΔSystolic blood pressure *mm Hg/year*	2.1 (1.2–3.4)	1.6 (0.7–2.6)	0.002
ΔDiastolic blood pressure *mm Hg/year*	0.8 (0.2–1.3)	0.4 (0.0–0.9)	0.001
ΔSystolic blood pressure *%/year*	1.6 (1.1–2.7)	1.3 (0.5–2.2)	0.002
ΔDiastolic blood pressure *%/year*	0.9 (0.2–1.7)	0.4 (0.0–1.2)	0.001

Data are given as median (interquartile range) (IQR). Analyses were made on subjects being free from antihypertensive medication.

Furthermore, carrier-ship of the SGK-1 risk was associated with HT (OR=2.0; 95% CI=1.3-3.1) independent of age, sex and BMI. The proportion of patients with poorly controlled HT was significantly higher in carriers as compared to non-carriers of the SGK-1 risk (27.8% vs. 8.9%; P=0.02) and carriers of the SGK-1 risk had higher on-treatment DBP (median, IQR) compared to non-carriers (99.0, 89.5-102.5 vs. 90.0, 85.0-98.5; P=0.05) whereas on-treatment SBP did not differ (150, 137-170 vs. 150, 140-164; NS). The correlation between fasting insulin concentration and DBP, but not SBP, was stronger in carriers of the SGK-1 risk compared to non-carriers (R=0.32 vs. R=0.17; P=0.03) (Figure 25).

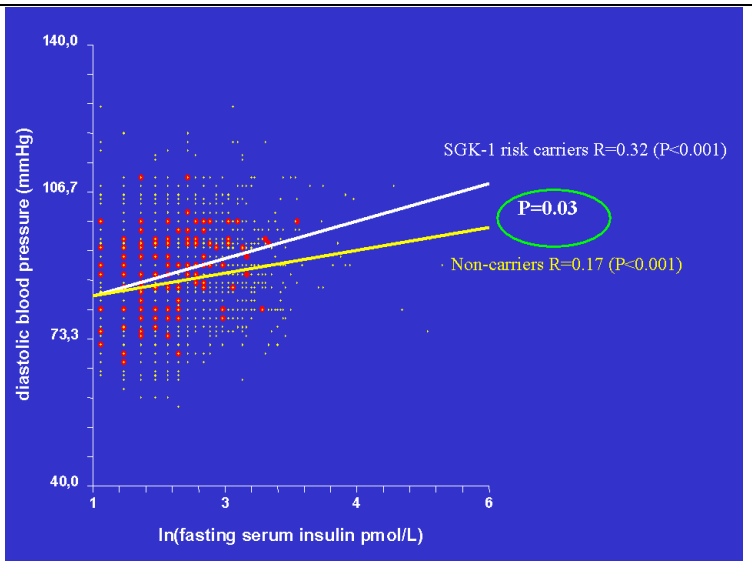

Figure 25. *Correlation between fasting insulin and DBP in subjects free from anti-hypertensive medication at "MDC"*

Study V

We found evidence of linkage between a region on chr 18q21-22 (82.25) and variation in daytime systolic ABP (LOD=1.95, P=0.0014), 24-hour systolic ABP (LOD=1.84, P=0.002), nighttime systolic ABP (LOD=0.77, P=0.03), daytime diastolic ABP (LOD=0.59, P=0.05), 24-hour diastolic ABP (LOD=0.59, P=0.05) (Figure 26). Two markers in the NEDD4L gene at exactly 82.25 cM (rs4149601 and rs2288774) pinpointed the position and contributed substantially to the peak indicating that much of the linkage peak in this region can be explained by variance in the NEDD4L gene. A simulation provided us with an exact *P*-value for this study (*P* for significance ≤ 0.007) indicating that the risk for type I errors is quite small at least for daytime systolic ABP and 24-hour systolic ABP.

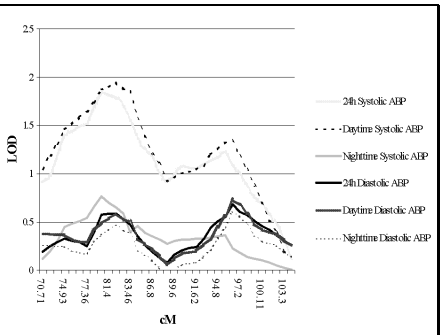

Figure 26. *Multipoint linkage analysis of chromosome 18. The Y-axis displays LOD score and the X-axis displays chromosome length in cM.*

QTDT analysis of the SNP-markers in the NEDD4L gene revealed a significant association for the rs4149601 and daytime, nighttime and 24-hour systolic ABP thus further adding to the evidence pinpointing the NEDD4L gene as the culprit responsible for the linkage results in this study.

Table 5

Blood pressure at "MDC" according to the rs4149601, rs2288774 and a NEDD4L risk-genotype combination

rs4149601 G/A polymorphism (n=3836)

	GG (n=1646)	GA (n=1740)	AA (n=450)		*P* GG vs AA
SBP (mmHg)	140.1 ± 18.6	139.8 ± 17.8	139.3 ± 18.2		0.45
DBP (mmHg)	86.4 ± 8.8	86.1 ± 9.2	85.3 ± 8.9		0.02
rs2288774 C/T polymorphism (n=3577)					
	CC (n=807)	CT (n=1591)	TT (n=1179)	CC/CT (n=2398)	*P* CC/CT vs TT
SBP (mmHg)	140.0 ± 18.5	140.1 ± 18.3	138.6 ± 17.9	140.1 ± 18.4	0.03
DBP (mmHg)	86.1 ± 9.0	86.4 ± 9.1	85.8 ± 8.9	86.3 ± 9.0	0.16
rs4149601 GG + rs2288774 CC/CT (NEDD4L-risk) vs. all other subjects (n=3550)					
	NEDD4L-risk (n=1088)		Other genotype carriers (n=2462)		*P*
SBP (mmHg)	140.7 ± 18.9		139.1 ± 17.8		0.01
DBP (mmHg)	86.6 ± 9.0		85.9 ± 9.0		0.04

Blood pressure change from MPP to MDC according to rs4149601 and a NEDD4L risk-genotype combination

rs4149601 exon 1 G/A polymorphism (n=2093)

	GG (n=898)	GA (n=952)	AA (n=243)	AA//AG (n=1195)	*P* GG vs AA
ΔSBP (mmHg/year)	1.61 (0.73-2.73)	1.52 (0.67-2.60)	1.56 (0.59-2.51)	1.53 (0.66-2.56)	NS
ΔDBP (mmHg/year)	0.39 (0.00-1.01)	0.35 (0.00-0.95)	0.30 (0.00-0.79)	0.34 (0.00-0.91)	0.042
rs4149601 GG + rs2288774 CC/CT (NEDD4L-risk) vs. all other subjects (n=1953)					
	NEDD4L-risk (n=592)		Other genotype carriers (n=1361)		*P*
ΔSBP (mmHg/year)	1.70 (0.74-2.93)		1.53 (0.68-2.51)		0.026
ΔDBP (mmHg/year)	0.42 (0.00-1.03)		0.34 (0.00-0.94)		0.047

Blood pressures are shown as means ± standard deviations or as median (interquartile range). Analysis performed on individuals free from antihypertensive medication. SBP, systolic blood pressure; DBP, diastolic blood pressure

When testing four SNP´s in the NEDD4L gene for association with BP levels we found that carrier-ship of the GG-genotype of the rs4149601 polymorphism conferred significantly higher DBP, but not SBP, compared to carriers of the AA-genotype (P=0.02) and carriers of the CC- or CT-genotype of the rs2288774 polymorphism had significantly higher SBP, but not DBP, than carriers of the TT-genotype (P=0.006). These effects were independent of the effects of age, sex and BMI. Furthermore, carrying the GG-genotype of the rs4149601 polymorphism resulted in an increased rate of DBP change over time compared to the AA-genotype carriers (*P*=0.01). Simultaneous carrier-ship of the rs4149601 GG-genotype and the rs2288774 CC- or CT-genotype (NEDD4L risk-genotype combination) conferred increased SBP (*P* = 0.02) and DBP (*P* = 0.002) as well as increased progression of both SBP (*P*=0.004) and DBP (*P*=0.002) from MPP to MDC compared to non-carriers independent of covariates (Table 5).

Study VI

24-hour urinary sodium excretion after 150 mmol (high-salt) and 50 mmol (low-salt) salt intake per day indicated excellent compliance. Mean 24-hour urinary excretion of sodium was significantly higher at baseline than after the high- (P=0.03) and low-salt (P<0.0001) periods. All indices of blood pressure decreased significantly from high- to low-salt and the (mean, 95% confidence interval) reduction in 24-hour systolic ABP (salt sensitivity) was 5.8, 3.4-8.2 mmHg (P<0.0001) (Figure 27). High- vs. low salt diet was accompanied by significant changes in P-

renin and in serum sodium ($S\text{-}Na^{+}$) and creatinine (S-creatinine) concentration.

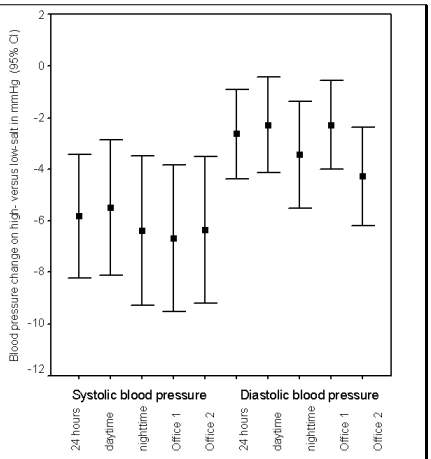

Figure 27. *Change in BP indices in study VI after change from the high-salt diet to the low-salt diet.*

Additionally, blood pressure was lowered by moving from unstandardized salt intake at baseline to the high-salt level by 2.6, 0.48-4.8 mmHg (P=0.02) for 24-hour systolic ABP and 1.4, 0.11-2.6 (P=0.03) for 24-hour diastolic ABP. This change in SBP and DBP also correlated significantly with change in 24-hour urinary sodium excretion (R=0.55, P<0.001 and R=0.48, P=0.002). Salt sensitivity correlated inversely with P-renin (r = -0.50, *P* = 0.001) (Figure 28) and directly with P-Nt-proANP (r = 0.33, *P* = 0.04) with both hormones measured at non-standardized conditions of salt-intake at baseline.

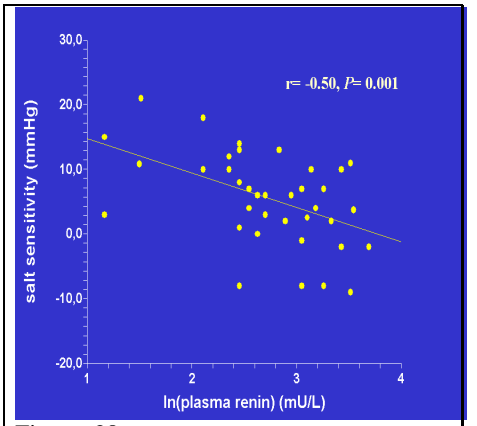

Figure 28. *Correlation between salt sensitivity and level of plasma renin concentration in study VI.*

Baseline P-renin was significantly inversely correlated with change in most ABP phenotypes and P-Nt-proANP was significantly directly correlated with 24-hour systolic ABP and night-time systolic ABP. Salt sensitivity in subjects with baseline P-renin of <9mU/L (lowest quintile) was 13.5, 8.5-18.5 mmHg (P<0.0001) compared with 3.8, 1.3-6.2 mmHg (P=0.004) mmHg in other subjects and salt sensitivity differed significantly between these groups (*P*=0.001). Salt sensitivity in subjects with baseline P-Nt-proANP >400 pmol/L was 7.0, 4.3-9.6 mmHg (P<0.0001) compared with 0.57, (-4.4) -5.5 mmHg (P=0.79) in subjects with Nt-proANP <400 pmol/L (lowest quintile) and salt sensitivity between groups differed significantly (*P*=0.03). P-renin and P-Nt-proANP did not predict salt-induced change in OBP1 and OBP2. Baseline 24-hour systolic ABP was directly correlated to salt sensitivity (r=0.32, P<0.05).

Discussion

Study I and II

We performed a genome wide linkage scan, followed by fine-mapping of regions with LOD scores ≥ 1.0, with the aim to identify susceptibility loci for early onset PHT. This study was then incorporated into a meta-analysis together with 8 other scans for HT (n=3) and BP variation (n=5) using the GSMA method. In order to enrich the genetic component of disease we only included patients with an age at onset of PHT ≤ 50 years in our genome wide scan thereby also minimizing the risk of inclusion of phenocopies. The detection of true linkage relies heavily on the reliability of the phenotype thus providing an advantage to studying the dichotomized phenotype of diagnosed HT since the diagnosis is based on repeated BP measurements at different occasions as opposed to BP as a continuous trait with single measurements, knowing that intra-individual BP variability is pronounced.[246] However, having access to a reliable BP distribution provides greater power to the study. All patients in our scan were diagnosed with EOHT. In the meta-analysis 2 of the participating HT scans had a upper cut-off age at diagnosis of 50 years[235,240] and 2 had a cut-off age at diagnosis of 60 years.[234,236]

In study I we found significant evidence of linkage to EOHT at 41 cm on chr 14. This region (37-46 cM) has not been previously significantly linked to any BP related phenotype. Additionally, this region did not prevail when investigated in the meta-analysis and could perhaps represent a false positive result. However, one of the separate scans for BP participating in the meta-analysis reported nominal evidence of linkage to this region for DBP, keeping in mind that diagnosis of PHT in our scan was based on DBP, implying some importance to this region.[247] Direct inspection of the region of the 1-LOD drop interval of the peak on chr 14 (37-46 cM) does not reveal any obvious candidate gene for HT or BP thus requiring additional fine mapping to clarify the relevance of this finding. We also found suggestive evidence of linkage on chr 2 at 118 cM, a region that has been repeatedly found in genome scans for BP variation[238,247,248] and HT[235,249] thus highlighting this region as a plausible container of a susceptibility gene for BP regulation or development of HT. Further evidence of this hypothesis came from the GSMA showing suggestive evidence of linkage to bin 2.5, which covers the 2p12-q22.1 locus also found in the scan. This region on chr 2 harbors one interesting candidate gene for HT, the α_{2B}-AR gene, a key component in the sympathetic nervous system. Using the GSMA method, we also found the strongest evidence of linkage to chr 3p14.1-q12.3 with bin 3.4. This region was not significantly linked to EOHT in our own scan. Ordered rank analysis showed that the same bin was significantly linked to the combination of PHT and BP at the genome wide level and that bins 2.5 and 3.5 showed suggestive evidence of linkage of PHT and DBP. All three bins reached the 99% significance threshold in the combined weighted analysis as well as in the weighted analysis of HT and BP. The range of linkage can extend more than 30 cM from the exact position of the susceptibility gene[250] and bins (3.5 and 2.4), which were adjacent to the 2 most significant bins (3.4 and 2.5), also showed at least nominal significance further strengthening our finding. It has been shown that when significant *P*-values, generated from the GSMA, are present in both summed rank analysis and ordered rank analysis false positives are very rare[161] and as the individual scans consists of unique sets of individuals that can be regarded as independent samples replicating each other, the possibility of our findings being those of chance is very low. Interestingly, the other individual scans showed no or only modest linkage to the loci on chr 3 and 2, suggesting that the

genetic effect mediating the linkage is too weak to be identified by any of the individual scans. The GSMA is designed to identify regions that show consistent linkage signals in multiple genome scans, most useful when applied to traits with many weakly linked loci such as is likely to be the case for BP,[161] and does not recognize linked regions present only in a subset of scans. As earlier stated it is important to avoid study populations with deviating genetic structure. We therefore only included studies of Caucasians from genetically non-isolated populations. Comparing the different scans revealed that ranks of the HT scans correlated with the DBP linkage patterns in the BP scans but not with those of SBP, implying a greater overlap between HT and DBP genes than between PHT and SBP genes. A possible explanation for this could be that, in the PHT-scans, the diagnosis of PHT was based primarily on DBP. Considering the relatively low prevalence of isolated systolic HT before the age of 60 years, a majority of participants in the PHT scans had only elevated DBP or elevations in both SBP and DBP. However, part of the discrepant BP linkage findings could be attributed to age related arterial stiffening considering that DBP starts to decline after 60 years of age whereas SBP continues to rise thus perhaps describing a differential pathophysiological mechanisms for SBP and DBP.

One study have previously utilized a meta-analytical approach for combination of results of HT and BP genome-wide scans[45] however with the application of a different method.[251,252] Although this study did not find any significant evidence of linkage, a modest peak was found on chr 2 overlapping the region identified in our GSMA (2p12-q22.1). The major limitation of pooling individual scans of HT and BP using the GSMA method is that markers and their distribution differ between studies thus introducing difficulties in interpretation of results. It is likely that the same genes that regulate BP are also involved in the pathogenesis of HT. An explanation for discrepant findings in studies of BP and HT could be due to that different genes could be differentially active depending on whether the individual belongs to the upper or lower BP distribution because of, for instance, differing capacities in counter-regulatory systems and vascular function. It should also be mentioned that the largest genome wide scan for HT performed thus far, the BRIGHT study[253] was not included into the meta-analysis. This study showed nominal evidence of linkage to the chr 3 region corresponding to bin 3.4,[253] adding further support to this region as a HT susceptibility locus.

Study III

Here we explored the role of a polymorphism in the α_{2B}-AR gene for a potential involvement in the etiology of PHT and BP regulation. We found that the DD genotype of the α_{2B}-AR gene was associated with both EOHT as well as with PHT at the population level. The α_{2B}-AR gene is an interesting positional as well as functional candidate gene for PHT and BP as it belongs to a locus highlighted in both our own[2] and several previous genome wide scans for PHT and BP variation[3-6] as well as the results of our meta-analysis[254] and is preferentially expressed presynaptically within the central portions of the sympathetic nervous system and works by enhancing sympathetic outflow upon stimulation and thereby increases BP. The I/D polymorphism has previously been associated with PHT,[255] myocardial infarction[256] and peripheral vasoconstriction,[257] making it highly interesting for investigation in the context of EOHT. One study has failed in finding an association between the I/D polymorphism and PHT.[258] However, this study was primarily designed for detecting linkage in sib-pairs. The observed frequency of the DD genotype was very low (n=3) and genetic dependence between

family members may have obscured the results of the association study.

Design issues of study III

The study was performed in two stages. In the first stage we compared contrasting groups corresponding to the upper (EOHT) and lower (NT) parts of the BP distribution in the population. The EOHT phenotype was derived from our previous genome wide scan[240] that guided us to the α_{2B}-AR gene locus. Due to the fact that genetic factors contribute more strongly to the PHT the earlier the age at onset,[24] EOHT should have a greater load of genetic variants contributing to PHT compared to PHT with later age at onset. Furthermore, a control group of subjects with low normal BP should have fewer genetic variants contributing to PHT than a control group consisting of a population without diagnosed PHT.

To reduce the risk for genetic heterogeneity hypertensive type 2 diabetic patients with micro- or macroalbuminuria were excluded as HT in these patients could be secondary to nephropathy. Due to the potentially differential pathogenesis of PHT in of hypertensives with type 2 diabetes compared to those without type 2 diabetes, despite absence of clinically detectable incipient diabetic nephropathy, we performed analyses both with and without normo-albuminuric type 2 diabetics in the EOHT group. In the second stage we investigated the effect of the same polymorphism at the population level by comparing genotype frequencies in all patients with PHT in the Skara population and those in the population controls. A limitation of this study is that BP in the normotensive subjects in the population was only measured at a single occasion and therefore the obtained BP could be subjected to some inaccuracy.[246] This could be one explanation why we failed to find association between the I/D polymorphism and BP in the non-hypertensive population.

Relevance of study III for primary hypertension and blood pressure

The effect of the DD-genotype of the α_{2B}-AR gene on PHT diminished with increasing age at onset and inclusion of type 2 diabetics as would be anticipated[24] as the load of BP elevating environmental factors accumulate with increasing age as well as the possibility that BP elevation in diabetics works via a partially different pathway. Given the observed small effect of the DD genotype on the development of PHT it is unlikely that the I/D polymorphism is of any predictive or prognostic relevance at the individual level by itself. However this is analogous to the notion that the genetic component of PHT is expected to be composed of many genes, of which the individual effect is likely to be small to moderate. The relevance of the α_{2B}-AR in the hypertensive context, especially salt sensitive PHT, have been shown in mice[259,260] fortified by studies showing that delivery of α_{2B}-AR anti-sense oligonucleotides into areas of the brain in mice decreases BP and abolishes the tendency for salt dependent HT.[259] Apart from its actions within the central nervous system the α_{2B}-AR also seem to mediate peripheral vasoconstriction thus further establishing a role for this gene in the development of PHT and BP control.[261] In vitro studies have shown that D-variant of the α_{2B}-AR is less susceptible to agonist-induced desensitization indicating that this polymorphism is functional.[228] Considering the role of the α_{2B}-AR in SS HT, it should be kept in mind that SS in humans is both associated with a positive family history of PHT[262] and is a characteristic of many patients suffering form PHT.[52]

Study IV-V

In study IV-V we explored the role of polymorphisms in SGK-1 gene and NEDD4L gene and their potential

involvement in the etiology of BP regulation and PHT. The SGK-1 and NEDD4L genes are both expressed in the kidney and are involved in the same signaling pathway downstream of aldosterone and insulin working as indirect stimulants (SGK-1) and direct inhibitors (NEDD4L) of ENaC thus affecting sodium reabsorbtion and volume homeostasis thus representing themselves as excellent functional candidate genes.

We found that a genotype combination in the SGK-1 gene, SGK-1 risk, was associated with SBP, DBP and change in SBP and DBP over time and HT as well as being associated with increased strength of the insulin-DBP correlation. In study V we found significant evidence of linkage to a locus on chr 18 harboring the NEDD4L gene and indeed we found that a genotype combination in the NEDD4L gene, NEDD4L risk, was associated with SBP, DBP and change in SBP and DBP over time.

One previous study has investigated the impact of genetic variants in the SGK-1 gene in German twins concluding that the SGK-1 risk mediates a stronger association with BP elevation than any of the two SGK-1 risk SNP´s alone[213] thereby corroborating our findings. However, that study was performed in a relatively small set of DZ twins and their parents (n=232) and considering that false positive associations are common in genetic studies of complex traits, particularly if cases and controls are related, replication studies are warranted.

The NEDD4L gene resides at ~ 82 cM on chr 18 and is, in addition to being an excellent functional candidate,[263] an interesting positional candidate gene for PHT and BP as several linkage studies,[247,249,264,265] including our own findings of linkage at 82 cM in study V, have highlighted a region on chr 18 between 70-115 cM as potentially harboring a susceptibility gene for BP and HT. Two previous studies have investigated the role of genetic variation in the NEDD4L gene with regards to BP phenotypes and HT.[214,215] Both studies found positive association between the marker rs4149601 and the phenotype studied indicating that especially this variant is of importance for BP regulation.

Design issues of study IV-V

In both study IV and V we excluded patients on AHT, who theoretically should have the highest frequency of risk genotypes, in the analyses of cross sectional and longitudinal BP due to the fact that the small single gene effects on BP could be obscured by the effect of AHT. Exclusion of treated subjects could thus only lead to, if anything, type II errors. When defining HT according to the ESH/ECC[5] the prevalence of HT in the "MDC" was 65%. This number corresponds well to those found in Europeans in the same age span (65-70%) in a population survey using a similar definition of HT.[266] It is likely that HT prevalence is overestimated since the bulk of subjects were not clinically diagnosed with HT but merely exceeded the ESH/ECC criteria[5] for HT.

Relevance of study IV-V for the pathophysiology of primary hypertension

As evident from Liddle´s syndrome it is clear that the ENaC is crucial in the regulation of renal sodium reabsorption and BP and that SGK-1 and NEDD4L are important regulators of ENaC[267,268]. In vitro[269] and animal studies[197,270] have shown that knocking out SGK-1 function clearly alters renal sodium handling and evokes a tendency for salt wasting despite elevated aldosterone levels and decreased glomerular filtration rate.[197] The Dahl salt sensitive rat, on the other hand, is unable to down regulate SGK-1 on a high salt diet.[270]

A recent study on the NEDD4L gene revealed a common G/A variant at the last nucleotide in exon 1 (rs4149601) which was part of the NEDD4L-risk studied in

the present report. The A-variant of this polymorphism generates a protein lacking exons 2-6 which code for an important C2 domain.[216] Deletion of the C2 domain in non-human systems seem to enhance NEDD4L´s capability to down-regulate ENaC[271,272] supporting the notion that genetic variants altering expression or activity of SGK-1 or NEDD4L could be of importance in the pathogenesis of human HT. A potential pathogenetic mechanism could be through interaction with insulin, which is consistently correlated with BP at the population level.[76] We found that SGK-1 risk, but not NEDD4L, was associated with increased strength of the insulin-DBP correlation. Several studies have shown that SGK-1 expression is induced by aldosterone[273-276] and activated by insulin via its classical signaling pathway.[210,277,278] Increased expression or activity of the SGK-1 gene and of the NEDD4L gene could thus explain the link between hyperinsulinaemia and elevated BP. As a majority of hypertensive subjects are insulin resistant[79,80] it is possible that hyperinsulinaemia, as a consequence of selective insulin resistance in muscle, fat and liver, but not the kidney, results in increased insulin-induced sodium retention in the kidneys[69] through prolongation of ENaC half-life by SGK-1 mediated phosphorylation of NEDD4L which decreases NEDD4L capacity to ubiquitinate and remove ENaC from the cell membrane.[211,279] The finding that carriers of the SGK-1 risk have stronger correlation between insulin-DBP could be due to the fact that the SGK-1 risk leads to either increased phosphorylating capacity, insulin sensitivity or longevity of the SGK-1 protein or that these polymorphisms positions SGK-1 in a more favorable intra-cellular position for phosphorylating NEDD4L. However, the statistical strength of this finding was quite weak, warranting replication of this result in another study. Even though none of the variants studied in NEDD4L seemed to affect the insulin-BP correlation it cannot be entirely excluded that NEDD4L could harbor SNP´s of physiological importance for the insulin-BP relationship, as the effect of the SNP´s studied in the NEDD4L gene on BP was very small. Carriers of the SGK-1 risk also had elevated treated DBP and less well-controlled HT implying that conventional AHT perhaps is not adequate and that agents such as amiloride and even dietary salt restriction could be more successful in such patients. None of the SNP´s forming the SGK-1 risk or NEDD4L risk genotype combination alter amino acid sequence of the respective protein, thus the most likely explanation for their effect is altering protein expression or that they are in LD with other variants that alters expression, activity, localization or sensitivity to stimulation/inhibition. Supporting that these variants are in LD with other causative variants is the detection of significant evidence of linkage to systolic ABP at the locus harbouring the NEDD4L gene in the MMHG material as linkage studies have an inherent ability of reflecting co-inheritance between large genomic regions harbouring many genetic variants and BP levels within families which could explain why we were able to detect linkage to this region considering the modest effect of the NEDD4L-risk on BP in the MDC. Also, ABP, the BP phenotype used in the MMHG, is a more exact measurement of BP and substantially more heritable than office BP,[41] thus yielding a greater power to detect genetic effects possibly further increased by the fact that in the MMGH > 90% of the probands had first-degree relatives with diagnosed PHT and therefore probably higher prevalence of PHT causing variants than the MDC.

Limitations and strengths of study IV-V

In study IV and V the study participation rate was quite low (48.4%) implying non-optimal representation of the Malmö population. The individuals that participated in the investigation were

healthier, came from higher social standards and were less exposed to hazardous environmental factors than the average citizen.[280] The lower load of environmental factors could however, decrease the frequency of phenocopies thereby providing greater power to detect an effect of genetic variants. Further, these studies were not primarily designed to investigate the effect of genetic variation on HT and HT-treatment control, making those results uncertain and warranting replication in studies specifically designed for this matter. Also, some of the groups in the sub-analysis were quite small thus limiting the power to detect true association. It should be kept in mind that subjects carrying the A-allele of the rs4149601 polymorphism lack the C2 domain[216] in a facultative fashion making it difficult to predict at what extent the C2 domain is expressed in individual subjects carrying the A-allele.

Implications of the findings of study III-V

Study III highlights the benefits of studying contrasting phenotypes such as EOHT and NT, which represent the tails of the population BP distribution, in the detection of small single gene effects.
Study IV and V have indicated the potential clinical importance of the molecular interaction between insulin, SGK-1, NEDD4L and ENaC encouraging further investigation if carriers of these "risk-genotypes" could benefit more from therapies specifically targeted to the "insulin, SGK-1, NEDD4L, ENaC system" such as treatment with amiloride, dietary salt restriction, drugs and insulin sensitivity improving regimens that lower insulin concentration and, in the future, selective SGK-1 antagonists and NEDD4L agonists. Given the findings of study III-V and the biological evidence found in the literature it could be speculated that the DD-genotype of the α_{2B}-AR gene, the SGK-1 risk and the NEDD4L risk-genotype combination could have more pronounced effects on sub-phenotypes of BP dependent on renal sodium reabsorption, such as salt sensitivity. In concurrence with our findings, the effect of individual SNP´s in a highly multifactorial phenotype like BP of HT is expected to be small, thus inferring little clinical relevance at the individual level. However, once a number of single gene effects have been discovered, gene-gene and gene-environment interactions can be tested in population based samples. Hopefully this may result in a battery of interacting risk-genotypes, which significantly affect the risk of PHT also at the population level and thus can be applied in clinical practice.

Study VI

The since long known greater benefits of restricting salt intake in hypertensive subjects than in normotensives[52] was substantiated by our finding that salt sensitivity was directly related to the level of baseline BP. The potential of dietary salt restriction to lower BP has been a matter of great conjecture, probably due to the poor ability of various studies to control salt intake and having clinically irrelevant short term designs where extreme differences in salt-intake have been observed.[119,281] This study applied a double-blind, placebo-controlled design allowing us to draw the conclusion that the clinically significant 24-hour BP lowering effect attained is mainly attributable to reducing daily salt intake from 150 mmol to 50 mmol *per se*.
Considering that urinary sodium excretion is a non-optimal measure for control of salt intake, providing all meals and thereby controlling salt intake conferres a great strength to our study compared to most other previous studies. Both our study and the DASH-study[47] clearly show that reducing daily salt intake from 150 mmol to 50 mmol produces a highly clinically relevant BP decrease. Interestingly, baseline urinary sodium excretion indicate that the subject's habitual salt intake

exceeded that of the high-salt diet and 24-hour SBP and DBP decreased significantly from baseline to high-salt. It could be speculated that the drop in BP from baseline to BP at high-salt was induced by decreased stress as subjects was familiarized with the study surroundings. However, HR did not correlate with 24-hour BP when going from baseline to high-salt strongly arguing against such a claim, thus supporting that the drop in BP observed was mainly attributable to the reduction in sodium intake. A possible limitation of this study could be that even though 4 weeks can be regarded at long-term we cannot extrapolate our findings to BP levels after life-long salt restriction.

In addition to reducing salt intake for achieving BP reduction lowering body weight with 5.1 kg have been shown to decrease SBP with 4.4 mmHg.[282] However, the BP decrease due to caloric restriction seen in normotensive individuals is dependent on concomitant salt restriction[283] indicating that reducing salt intake from 150 mmol to 50 mmol per day is a more effective regimen for BP reduction than sole caloric restriction.

Lowering BP through increased sodium excretion with thiazide diuretics, working via a mechanism similar to dietary salt reduction, effectively decreases cardiovascular risk[284] making it reasonable to hypothesize that dietary salt restriction is equally effective at lowering cardiovascular risk. Considering the attention given body weight lowering interventions despite the lack of evidence on cardiovascular benefits it is rational that greater efforts in cardiovascular primary prevention aimed at reducing dietary salt intake receives recognition. Salt restriction is quite difficult to translate into clinical practice as well as into primary preventive programs considering that 89% of all salt is inherent or added to the food already before the individual person starts to prepare it.[285] Also, the fact that 77% of all dietary salt is added during industrial food processing[285] indicates that the industrial food producing companies could have a great impact on cardiovascular disease in the population if managing to lower salt content in foods during processing.

High salt intake suppresses and low salt intake increases RAAS activity as the RAAS attempts to restore blood pressure and total body sodium to normal levels. Analogously, we found that P-renin increased on low-salt compared with salt intake at baseline and high-salt. Importantly, serum potassium concentration was not affected by the increased RAAS activity, suggesting that the observed increase in activity has only very minute global side effects. Furthermore, treatment with thiazide diuretics, which has beneficial cardiovascular effects, conferres similar increases in RAAS activity speaking against the increased RAAS activity being harmful.[284] Being able to predict the most salt sensitive individuals would allow for a more narrow and intense focus on subjects benefiting mostly from dietary salt restriction. Melander et al. previously showed that plasma renin activity and P-Nt-proANP can predict response to short term, severe salt restriction.[132,133] In the present study we showed that P-renin and P-Nt-proANP at baseline, with subjects on their habitual salt intake as they would be in the clinical setting, correlates significantly, respectively inverse and direct, with degree of salt sensitivity (Figure 29). This finding implies that P-renin and P-Nt-proANP could potentially be used as biomarkers to identify individuals with the greatest benefit from dietary salt restriction intervention. SS was most pronounced in subjects belonging to the lowest quintile of P-renin displaying dramatic BP decreases on salt restriction. A smaller corresponding effect was also seen with P-Nt-proANP.

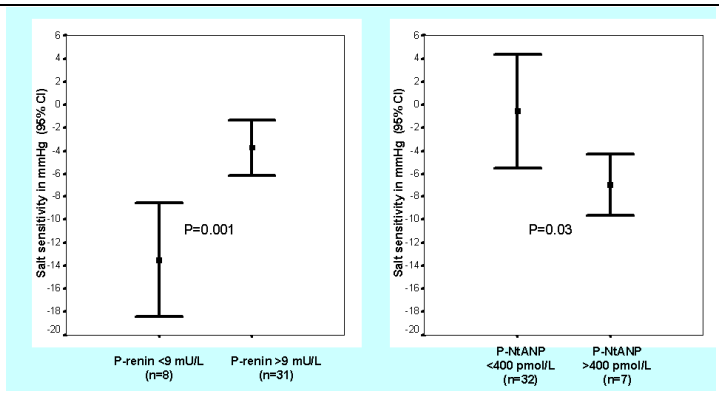

Figure 29. *Comparison, in study VI, of degree of salt sensitivity between subjects with the lowest quintile of P-renin and all other and subjects with the highest quintile of P-NtANP compared to all other.*

Summary and conclusions of studies I-VI

1. Via a genome wide linkage approach and subsequent fine mapping in Scandinavian families we found significant evidence of linkage between a region on chr 14 at 41 cM and EOHT. Furthermore, suggestive evidence of linkage was found between a region on chr 2 at 118 cM and EOHT, thus implying that these regions harbor susceptibility genes for EOHT.
2. By incorporating the results of our own genome wide scan for EOHT into a meta-analysis, employing the GSMA method, together with 3 other PHT-scans and 5 BP-scans, two regions on chr 3p14.1-q12.3 and on chr 2p12-q22.1 showed consistent inter-study linkage thereby indicating that these regions harbor genes of importance for the development of PHT.
3. By comparing genotype frequencies of the I/D polymorphism in the α_{2B}-AR gene, which resides at a locus corresponding to the ones found in study I and II on chr 2, between patients with EOHT and normotensive subjects with low blood pressure we found that the DD genotype was associated with EOHT. Furthermore, we found that the DD genotype is significantly more prevalent in the non-diabetic hypertensive population regardless of age at onset indicating a role in the pathophysiology of PHT.
4. We found that a combination of two SNP´s in the SGK-1 gene was associated with increased SBP, DBP and change in SBP and DBP over time. Additionally, the combination was associated with strength of the insulin-DBP correlation. Given the results of this study it is rational to believe that this gene is important in BP regulation and in the interplay between circulating insulin and BP.
5. In study IV we found significant evidence of linkage between a locus at ~ 82 cM on chr 18 and systolic ABP phenotypes. We also found that a genotype combination in the NEDD4L gene, residing within the locus on chr 18, was associated with SBP, DBP and change of SBP and DBP over time. These findings indicate that the NEDD4L gene is of importance in the regulation of BP.
6. By providing participants with all meals during 8 weeks we were able to control dietary salt intake and

monitor the BP reduction achieved by lowering salt intake from 150 mmol to 50 mmol per day. Significant decreases were observed in all BP indices when going from high-salt to low-salt indicating that salt restriction conferres clinically significant BP reduction. Also, we were able to show that P-renin and P-Nt-ANP, predict those responding with most vigilance to the salt restriction.

Future implications

The clinical implications for genetics of complex disorders are still in its cradle. However, the technical and methodological development is moving forward at an incredible velocity making studies of whole genome association feasible in the near future. These studies will enable the detection of genetic variants in genes with currently unknown function. Furthermore, the field of epigenetics is an interesting one beginning to attract attention among cardiovascular researchers and could explain phenotypic variation not found in pure genetic studies. As genetic statisticians develop tools for analyzing massive amounts of genotypic data in conjuncture with environmental factors, thereby allowing for the identification of risk genotypes as well as gene-gene and gene-environment interactions, risk batteries of genetic variation and environmental factors can be developed for determination of cardiovascular risk and subsequent choice of therapeutic strategy. Hopefully, further research will reveal additional biomarkers of salt sensitivity enabling detection of subjects benefiting most from dietary advice and salt restriction.

Acknowledgements

Several persons have made this thesis possible

Olle Melander – Thank you for teaching me all that I know about genetics, for being the greatest supervisor ever and a good friend. This thesis would not have been possible without you.

Theresé Swensson – For your love and patience with me on my 4-year long ego-trip. I love you so much that it hurts – AJ!

My Mother Gun von Wowern and Father Göran von Wowern – for raising me up to more than I ever thought was possible. I have been standing on the shoulders of giants. I love you with all my heart.

My Sister Katarina von Wowern – I love you.

Johan Immelgård – for being the best friend anyone ever could have. I love you grabben.

Leif Groop – for having faith in me and for being a great role model.

Johan Holmkvist – for being a great roommate and the second best badminton player at the lab.

Marju Orho-Melander – for always sharing your knowledge.

Cecilia Lindgren – for always helping, sharing knowledge and being a great friend.

Lena Rosberg – for your excellent technical skills and help.

Phillipe Burri – for always making sure that the lab wins the EM and for being a MacAlister-connoisseur.

Jenny Fredriksson, Marketa Sjögren, Maria Carlsén and Camilla Cervin – for making the years at the lab more amusing as each year passes.

Anna Berglund and Margareta Svensson – for teaching me all I know about lab-technology.

Lennart Hulthén – for being my supervisor.

Göran Berglund and Joyce Carlson – for sharing your knowledge and for believing in me.

Nael Shaat – for great collaboration and interesting talks.

My fellow classmates in med-school – for covering for me when I didn´t have time to attend classes.

Henrik Månsson – the best computer gimp at UMAS.

Peter Almgren – you are the hub around which the wheel spins.

All the people working with the Skara material and Malmoe Diet and Cancer Material especially Kristina Bengtsson, Ulf Lindblad and Lennart Råstam.

Homer Simpson – for always inspiring me.

Gizmo, Grålle and Goliat – for cheering me up when days seems gray.

I owe gratitude towards the Medical Faculty at Lund University, Bosse Hedblad; Gunilla Willsten, the rest of the people at the Wallenberg Lab – the hardest working group in science, all friends and family.

Populärvetenskaplig sammanfattning

Högt blodtryck (hypertoni) är en av de vanligaste sjukdomarna i västvärlden idag. Att leva med hypertoni innebär att man har en ökad risk för att drabbas av slaganfall och hjärtinfarkt. Därför är det synnerligen viktigt att sänka blodtrycket hos personer med hypertoni till nivåer som är mindre skadliga med hänseende till dessa ovan nämda allvarliga följder. Inom det medicinska området skiljer man mellan primär hypertoni, dvs hypertoni där orsakerna är okända och sekundär hypertoni där man känner till de bakomliggande orsakerna. Primär hypertoni är den i särklass vanligaste formen (ca: 90%). Trots att man sedan länge kunnat mäta blodtryck och insett de skadliga effekterna av hypertoni så vet vi förhållandevis lite om varför vissa personer drabbas medan andra verkar vara skyddade. Vissa miljömässiga faktorer spelar roll för utvecklandet av primär hypertoni. Den kanske viktigaste faktorn är den dagliga dosen salt som vi intar via vår kost. Sänker man saltdosen så sänker man också blodtrycket. Denna effekt är mer uttalad hos personer som har primär hypertoni vilket talar för att dessa individer kan ha genetiska förutsättningar att samla på sig salt och därmed också vätska som höjer deras blodtryck. Ett flertal genetiska faktorer förklarar tillsammans minst en tredjedel av vilken blodtrycksnivå man har. Vi tror att de enskilda genetiska varianterna har en mycket liten effekt på individens blodtryck men tillsammans med andra genetiska varianter och ofördelaktiga miljöfaktorer kan de bidra till att individen utveckla primär hypertoni.

Målsättningen med denna avhandling var att identifiera och lokalisera genetiska varianter som bidrar till förhöjt blodtryck och hypertoni och att utvärdera den blodtrycksänkande effekten av att skära ner på det dagliga saltintaget. En ytterligare målsättning var att utvärdera ifall den blodtrycksänkande effekten av saltrestiktion kunde förutspås av nivåer av biologiska markörer i blodet.

I delarbete I & II försökte vi identifiera kromosomavsnitt som innehåller gener som kan vara viktiga för utvecklandet av primär hypertoni genom sk. kopplingsanalys i vilken man observerar samnedärvning av gensegment mellan familjemedlemmar som har hypertoni. Delarbete I resulterade i att två segment identifierades på kromosom 2 och 14 med förhoppning om att gener som är viktiga för utvecklingen av primär hypertoni kan finnas inom dessa regioner. I delarbete II slogs flera studier samman för att kunna analyseras som en enda stor studie och därmed ge större möjlighet att identifiera intressanta regioner. Denna studie indikerade att en region på kromosom 3 och regionen på kromosom 2 som återfanns i delarbete I med stor sannolikhet innehåller någon eller några gener av betydelse för blodtrycksreglering och hypertoni.

I delarbete III följdes fynden på kromosom 2 från studie I & II upp genom att studera en genetisk (insertion/deletion) variant i genen för den adrenerga receptorn α_{2B} som ligger i det aktuella området på kromosom. Denna gen har visat sig vara viktig för blodtrycksreglering hos både människor och djur. Vi fann att de personer som bar deletion-varianten på båda sina kromosom 2 har påtagligt ökad risk för att utveckla primär hypertoni vilket talar för att denna genvariant kan vara av betydelse för om man ska utveckla primär hypertoni eller ej.

En mycket viktig komponent i regleringen av blodtrycket är den så kallade epiteliala natrium kanalen (EnaC). Denna kanal är viktig för njurens förmåga att återuppta salt och vatten från urinen och därigenom viktig för blodtrycket. Det är därför möjligt att om man har genetiska varianter i beståndsdelarna som påverkar EnaC så kan man ha en ökad risk för förhöjt blodtryck. I

delarbete IV och V undersökte vi ifall genetiska varianter i två gener som är regulatorer av ENaC (SGK-1 och NEDD4L) innebar risk för förhöjt blodtryck. Vi fann att personer som bär en kombination av två genetiska varianter in antingen SGK-1 genen eller NEDD4L genen har förhöjt blodtryck jämfört med personer som inte har någon av dessa varianter.
Det har visats att insulin kan påverka EnaCs förmåga att återta salt och vatten för att höja blodtrycket och eftersom en stor del av de personer som har primär hypertoni har också bekymmer med blodsockerreglering och nivåer av insulin i blodet finns möjligheten att SGK-1 och NEDD4L kan underlätta insulinets blodtrycksförhöjande effekt. Blodtryck och nivåer av insulin i blodet uppvisar ett beroende av varandra där höga blodtryck ofta går med höga insulinnivåer i blodet. Varför det är på detta sätt är dock okänt. Vi fann att kombinationen av genetiska varianter i SGK-1 verkade förmedla delar av insulinets blodtrycksförhöjande effekt. Fynden i delarbete IV & V antyder att SGK-1 och NEDD4L är viktiga för regleringen av blodtrycket och att SGK-1 kan förmedla delar av insulinets blodtryckseffekter.

Då det förfaller att det dagliga intaget av salt är av betydelse för blodtrycket undersökte vi i delarbete VI effekten av att sänka den dagliga dosen salt från 9 gram till 3 gram under 4 veckor. Alla deltagarna i studien fick all mat från den metabola avdelningen på universitetssjukhuset MAS vilket innebar att vi kunde kontrollera hur mycket salt deltagarna fick i sig. Den genom saltrestriktion förmedlade blodtryckssänkning som deltagarna uppvisade var av hög klinisk relevans. Det är känt att inte alla individer är känsliga för salt och det är därför angeläget att finna biologiska markörer som kan förutspå vilka individer som kommer att vara mest betjänta av att dra ner på sitt dagliga saltintag. I vår studie fann vi att genom att mäta koncentrationen av renin i blodplasma kan man förutspå vilka som kommer att sjunka mest i blodtryck om det dagliga saltintaget sänks. Dessa resultat tyder på att nivån av det dagliga saltintaget spelar en påtaglig roll för blodtrycket och att man kan identifiera vilkaindivider som är lämpade för en sådan terapi genom att mäta koncentrationen av renin i blodplasma.

Sammanfattningsvis indikerar fynden i delarbete I och II att regioner på kromosom 2, 3 och 14 innehåller gener som kan påverka risken för att insjukna i hypertoni. Vi visade i delarbete III-V att tre gener: den α_{2B}-adrenerga receptorn, SGK-1 och NEDD4L innehåller varianter som påverkar blodtrycksregleringen eller ökar risken för hypertoni. Slutligen visade vi i delarbete VI att om man sänker sitt dagliga intag av salt med 6 gram så sjunker också blodtrycket påtagligt. Denna blodtrycksänkning kunde förutspås av mätning av biologiska blodmarkörer.

References

1. Pickering G. Hypertension. Definitions, natural histories and consequences. *Am J Med*. 1972;52:570-83.
2. National Board of Health and Welfare DIC. Treatment of mild hypertension. *Vol. 1. Stockholm: National Board of Health and Welfare, Drug Information Commitee*. 1987.
3. Subcommitee W-IG. 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. *J Hypertens*. 1999;17:151-83.
4. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr., Roccella EJ. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *Jama*. 2003;289:2560-72.
5. Committee ESoH-ESoCG. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens*. 2003;21:1011-1053.
6. Mosterd A, D'Agostino R, Silbershatz H, Sytkowski P, Kannel W, Grobbee D, Levy D. Trends in the prevalence of hypertension, antihypertensive therapy, and left ventricular hypertrophy from 1950 to 1989. *New England Journal of Medicine*. 1999;340:1221-1227.
7. Lawes CMM, Bennett DA, Feigin VL, Rodgers A. Blood Pressure and Stroke: An Overview of Published Reviews. *Stroke*. 2004;35:1024-1033.
8. Kannel W. New perspectives on cardiovascular risk factors. *American Heart Journal*. 1987;114:213-219.
9. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, Levy D. Impact of High-Normal Blood Pressure on the Risk of Cardiovascular Disease. *N Engl J Med*. 2001;345:1291-1297.
10. Staessen JA, Wang J, Bianchi G, Birkenhager WH. Essential hypertension. *The Lancet*. 2003;361:1629-1641.
11. Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense H-W, Joffres M, Kastarinen M, Poulter N, Primatesta P, Rodriguez-Artalejo F, Stegmayr B, Thamm M, Tuomilehto J, Vanuzzo D, Vescio F. Hypertension Prevalence and Blood Pressure Levels in 6 European Countries, Canada, and the United States. *JAMA*. 2003;289:2363-2369.
12. Franklin SS, Larson MG, Khan SA, Wong ND, Leip EP, Kannel WB, Levy D. Does the Relation of Blood Pressure to Coronary Heart Disease Risk Change With Aging? : The Framingham Heart Study. *Circulation*. 2001;103:1245-1249.
13. Gillum RF. The Epidemiology of Cardiovascular Disease in Black Americans. *N Engl J Med*. 1996;335:1597-1599.
14. Lorenzo C, Serrano-Rios M, Martinez-Larrad MT, Gabriel R, Williams K, Gonzalez-Villalpando C, Stern MP, Hazuda HP, Haffner S. Prevalence of Hypertension in Hispanic and non-Hispanic White Populations. *Hypertension*. 2002;39:203-208.
15. Hajjar I, Kotchen TA. Trends in Prevalence, Awareness, Treatment, and Control of Hypertension in the United States, 1988-2000. *JAMA*. 2003;290:199-206.
16. Wolf-Maier K, Cooper RS, Kramer H, Banegas JR, Giampaoli S, Joffres MR, Poulter N, Primatesta P, Stegmayr B, Thamm M. Hypertension Treatment and Control in Five European Countries, Canada, and the United States. *Hypertension*. 2004;43:10-17.

17. Kiefe CI, Williams OD, Bild DE, Lewis CE, Hilner JE, Oberman A. Regional Disparities in the Incidence of Elevated Blood Pressure Among Young Adults : The CARDIA Study. *Circulation*. 1997;96:1082-1088.
18. Strogatz D, Croft J, James S, Keenan N, Browning S, Garrett J, Curtis A. Social support, stress, and blood pressure in black adults. *Epidemiology*. 1997;8:482-487.
19. Vargas CM, Ingram DD, Gillum RF. Incidence of Hypertension and Educational Attainment The NHANES I Epidemiologic Followup Study. *Am. J. Epidemiol.* 2000;152:272-278.
20. Feinleib M, Garrison RJ, Fabsitz R, Christian JC, Hrubec Z, Borhani NO, Kannel WB, Rosenman R, Schwartz JT, Wagner JO. The NHLBI twin study of cardiovascular disease risk factors: methodology and summary of results. *Am J Epidemiol*. 1977;106:284-5.
21. Longini IM, Jr., Higgins MW, Hinton PC, Moll PP, Keller JB. Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. *Am J Epidemiol*. 1984;120:131-44.
22. Biron P, Mongeau JG, Bertrand D. Familial aggregation of blood pressure in 558 adopted children. *Can Med Assoc J*. 1976;115:773-4.
23. Rice T, Vogler GP, Perusse L, Bouchard C, Rao DC. Cardiovascular risk factors in a French Canadian population: resolution of genetic and familial environmental effects on blood pressure using twins, adoptees, and extensive information on environmental correlates. *Genet Epidemiol*. 1989;6:571-88.
24. Hunt SC, Williams RR, Barlow GK. A comparison of positive family history definitions for defining risk of future disease [published erratum appears in J Chronic Dis 1987;40(4):369]. *J Chronic Dis*. 1986;39:809-21.
25. Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, Lalouel JM. A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature*. 1992;355:262-5.
26. Mune T, Rogerson FM, Nikkila H, Agarwal AK, White PC. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nat Genet*. 1995;10:394-9.
27. Shimkets RA, Warnock DG, Bositis CM, Nelson Williams C, Hansson JH, Schambelan M, Gill JR, Jr., Ulick S, Milora RV, Findling JW, et al. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell*. 1994;79:407-14.
28. The Human Genome. *Science*. 2001;291:1145-1434.
29. Kruglyak L, Daly M, Reeve-Daly MP, Lander E. Parametric and non-parametric linkage analyses: A unified multipoint approach. *Am J Hum Genet*. 1996;58:1347-1363.
30. Kruglyak L, Lander ES. Complete multipoint sib-pair analysis of qualitative and quantitative traits [see comments]. *Am J Hum Genet*. 1995;57:439-54.
31. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science*. 1996;273:1516-7.
32. Colletto GM, Cardon LR, Fulker DW. A genetic and environmental time series analysis of blood pressure in male twins. *Genet Epidemiol*. 1993;10:533-8.
33. Ditto B. Familial influences on heart rate, blood pressure, and self-report anxiety responses to stress: results from 100 twin pairs. *Psychophysiology*. 1993;30:635-45.
34. Fagard R, Brguljan J, Staessen J, Thijs L, Derom C, Thomis M, Vlietinck R. Heritability of conventional and ambulatory blood pressures. A study in twins. *Hypertension*. 1995;26:919-24.

35. Hunt SC, Hasstedt SJ, Kuida H, Stults BM, Hopkins PN, Williams RR. Genetic heritability and common environmental components of resting and stressed blood pressures, lipids, and body mass index in Utah pedigrees and twins. *Am J Epidemiol*. 1989;129:625-38.
36. Havlik RJ, Garrison RJ, Katz SH, Ellison RC, Feinleib M, Myrianthopoulos NC. Detection of genetic variance in blood pressure of seven-year-old twins. *Am J Epidemiol*. 1979;109:512-6.
37. Slattery ML, Bishop DT, French TK, Hunt SC, Meikle AW, Williams RR. Lifestyle and blood pressure levels in male twins in Utah. *Genet Epidemiol*. 1988;5:277-87.
38. Annest JL, Sing CF, Biron P, Mongeau JG. Familial aggregation of blood pressure and weight in adoptive families. II. Estimation of the relative contributions of genetic and common environmental factors to blood pressure correlations between family members. *Am J Epidemiol*. 1979;110:492-503.
39. Biron P, Mongeau JG. Familial aggregation of blood pressure and its components. *Pediatr Clin North Am*. 1978;25:29-33.
40. Ward R. Familial aggregation and genetic epidemiology of blood pressure. *Hypertension: Pathophysiology, Diagnosis and Management*. 1990;Laragh, J.H. and Brenner, B.M. New York:Raven Press:81-100.
41. Fava C, Burri P, Almgren P, Groop L, Hulthen UL, Melander O. Heritability of ambulatory and office blood pressure phenotypes in Swedish families. *J Hypertens*. 2004;IN PRESS.
42. Corvol P, Persu A, Gimenez-Roqueplo AP, Jeunemaitre X. Seven lessons from two candidate genes in human essential hypertension: angiotensinogen and epithelial sodium channel. *Hypertension*. 1999;33:1324-31.
43. Niu T, Xu X, Rogus J, Zhou Y, Chen C, Yang J, Fang Z, Schmitz C, Zhao J, Rao VS, Lindpaintner K. Angiotensinogen gene and hypertension in Chinese. *J Clin Invest*. 1998;101:188-94.
44. Luft FC. Molecular genetics of human hypertension. *Curr Opin Nephrol Hypertens*. 2000;9:259-66.
45. Province MA, Kardia SL, Ranade K, Rao DC, Thiel BA, Cooper RS, Risch N, Turner ST, Cox DR, Hunt SC, Weder AB, Boerwinkle E. A meta-analysis of genome-wide linkage scans for hypertension: the National Heart, Lung and Blood Institute Family Blood Pressure Program. *Am J Hypertens*. 2003;16:144-7.
46. Guyton A. Blood pressure control--special role of the kidneys and body fluids. *Science*. 1991;252:1813-1816.
47. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, 3rd, Simons-Morton DG, Karanja N, Lin PH. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med*. 2001;344:3-10.
48. Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, Klag MJ. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. *JAMA*. 1997;277:1624-1632.
49. Allender PS, Cutler JA, Follmann D, Cappuccio FP, Pryer J, Elliott P. Dietary Calcium and Blood Pressure: A Meta-Analysis of Randomized Clinical Trials. *Ann Intern Med*. 1996;124:825-831.
50. Cushman WC, Cutler JA, Hanna E, Bingham SF, Follmann D, Harford T, Dubbert P, Allender PS, Dufour M, Collins JF, Walsh SM, Kirk GF, Burg M, Felicetta JV, Hamilton BP, Katz LA, Perry HM, Jr, Willenbring ML, Lakshman R, Hamburger RJ, for the PATHS Group. Prevention and Treatment of Hypertension Study (PATHS):

Effects of an Alcohol Treatment Program on Blood Pressure. *Arch Intern Med*. 1998;158:1197-1207.

51. Kelley GA, Kelley KS. Progressive Resistance Exercise and Resting Blood Pressure : A Meta-Analysis of Randomized Controlled Trials. *Hypertension*. 2000;35:838-843.
52. Weinberger MH, Miller JZ, Luft FC, Grim CE, Fineberg NS. Definitions and characteristics of sodium sensitivity and blood pressure resistance. *Hypertension*. 1986;8:Ii127-34.
53. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S. Insulin resistance in essential hypertension. *N Engl J Med*. 1987;317:350-7.
54. Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P. Hyperaldosteronism Among Black and White Subjects With Resistant Hypertension. *Hypertension*. 2002;40:892-896.
55. Ergul S, Parish DC, Puett D, Ergul A. Racial Differences in Plasma Endothelin-1 Concentrations in Individuals With Essential Hypertension. *Hypertension*. 1996;28:652-655.
56. Nasjletti A. The Role of Eicosanoids in Angiotensin-Dependent Hypertension. *Hypertension*. 1998;31:194-200.
57. Cai H, Harrison DG. Endothelial Dysfunction in Cardiovascular Diseases: The Role of Oxidant Stress. *Circ Res*. 2000;87:840-844.
58. Campese VM, Tawadrous M, Bigazzi R, Bianchi S, Mann AS, Oparil S, Raij L. Salt Intake and Plasma Atrial Natriuretic Peptide and Nitric Oxide in Hypertension. *Hypertension*. 1996;28:335-340.
59. Rhaleb N-E, Yang X-P, Nanba M, Shesely EG, Carretero OA. Effect of Chronic Blockade of the Kallikrein-Kinin System on the Development of Hypertension in Rats. *Hypertension*. 2001;37:121-128.
60. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The Disease Burden Associated With Overweight and Obesity. *JAMA*. 1999;282:1523-1529.
61. Berk BC. Vascular Smooth Muscle Growth: Autocrine Growth Mechanisms. *Physiol. Rev*. 2001;81:999-1030.
62. Zhan C, Sindhu R, Pang J, Ehdaie A, Vaziri N. Superoxide dismutase, catalase and glutathione peroxidase in the spontaneously hypertensive rat kidney: effect of antioxidant-rich diet. *Journal of Hypertension*. 2004;22:2925-2033.
63. Thybo NK, Stephens N, Cooper A, Aalkjaer C, Heagerty AM, Mulvany MJ. Effect of Antihypertensive Treatment on Small Arteries of Patients With Previously Untreated Essential Hypertension. *Hypertension*. 1995;25:474-481.
64. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system. *N Engl J Med*. 1996;334:374-81.
65. Alberti KGMM, Zimmet PZ, Consultation W. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus. Provsional Report of a WHO Consultation. *Diabetic Medicine*. 1998;15:539-53.
66. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med*. 1999;16:442-3.
67. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. *Circulation*. 2002;106:3143-.

68. Ford ES, Giles WH. A comparison of the prevalence of the metabolic syndrome using two proposed definitions. *Diabetes Care*. 2003;26:575-81.
69. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1595-607.
70. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001;24:683-9.
71. Juhan-Vague I, Thompson SG, Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome. A study of 1500 patients with angina pectoris. The ECAT Angina Pectoris Study Group. *Arterioscler Thromb*. 1993;13:1865-73.
72. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. *Ann Intern Med*. 1993;118:956-63.
73. Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*. 2000;102:42-7.
74. Han TS, Sattar N, Williams K, Gonzalez-Villalpando C, Lean ME, Haffner SM. Prospective study of C-reactive protein in relation to the development of diabetes and metabolic syndrome in the Mexico City Diabetes Study. *Diabetes Care*. 2002;25:2016-21.
75. Shen DC, Shieh SM, Fuh MM, Wu DA, Chen YD, Reaven GM. Resistance to insulin-stimulated-glucose uptake in patients with hypertension. *J Clin Endocrinol Metab*. 1988;66:580-583.
76. Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Jarvinen H. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European Group for the Study of Insulin Resistance (EGIR). *Hypertension*. 1997;30:1144-9.
77. Skarfors ET, Selinus KI, Lithell HO. Risk factors for developing non-insulin dependent diabetes: a 10 year follow up of men in Uppsala. *Bmj*. 1991;303:755-60.
78. Lissner L, Bengtsson C, Lapidus L, Kristjansson K, Wedel H. Fasting insulin in relation to subsequent blood pressure changes and hypertension in women. *Hypertension*. 1992;20:797-801.
79. Pollare T, Lithell H, Berne C. Insulin resistance is a characteristic feature of primary hypertension independent of obesity. *Metabolism*. 1990;39:167-74.
80. Zavaroni I, Mazza S, Dall'Aglio E, Gasparini P, Passeri M, Reaven GM. Prevalence of hyperinsulinaemia in patients with high blood pressure. *J Intern Med*. 1992;231:235-40.
81. Pearce D. The role of SGK1 in hormone-regulated sodium transport. *Trends Endocrinol Metab*. 2001;12:341-7.
82. Tarnow L, Rossing P, Gall MA, Nielsen FS, Parving HH. Prevalence of arterial hypertension in diabetic patients before and after the JNC-V. *Diabetes Care*. 1994;17:1247-51.
83. Blackburn H, Prineas R. Diet and hypertension: anthropology, epidemiology, and public health implications. *Prog Biochem Pharmacol*. 1983;19:31-79.
84. Remission of Essential Hypertension After Renal Transplantation: [Curtis JJ, Luke RG, Dustan HP, Kashgarian M, Whelchel JD, Jones P, Diethelm AG: N. Engl. J. Med. 309:1009-1015, 1983 (with comments by John J. Curtis, Robert G. Luke, and Eberhard Ritz)]. *J Am Soc Nephrol*. 2000;11:2404-2412.
85. Mullins MM. Body fluid volumes in prehypertensive spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol*. 1983;244:H652-655.
86. Toal C, Leenen F. Body fluid volumes during development of hypertension in the spontaneously hypertensive rat. *Journal of Hypertension*. 1983;1:345-350.

87. Bianchi G, Baer P, Fox U, Duzzi L, Pagetti D, Giovannetti A. Changes in renin, water balance, and sodium balance during development of high blood pressure in genetically hypertensive rats. *Circulation Research*. 1975;36:153-161.
88. Harrap S. Genetic analysis of blood pressure and sodium balance in spontaneously hypertensive rats. *Hypertension*. 1986;8:572-582.
89. Grim C, Luft F, Miller J, Rose R, Christian J, Weinberger M. An approach to the evaluation of genetic influences on factors that regulate arterial blood pressure in man. *Hypertension*. 1980;2:I34-42.
90. Grim CE, Luft FC, Miller JZ, Brown PL, Gannon MA, Weinberger MH. Effects of sodium loading and depletion in normotensive first-degree relatives of essential hypertensives. *J Lab Clin Med*. 1979;94:764-71.
91. Grim CE, Miller JZ, Luft FC, Christian JC, Weinberger MH. Genetic influences on renin, aldosterone, and the renal excretion of sodium and potassium following volume expansion and contraction in normal man. *Hypertension*. 1979;1:583-90.
92. Widgren BR, Herlitz H, Hedner T, Berglund G, Wikstrand J, Jonsson O, Andersson OK. Blunted renal sodium excretion during acute saline loading in normotensive men with positive family histories of hypertension. *Am J Hypertens*. 1991;4:570-8.
93. Lindeman RD, Tobin JD, Shock NW. Association between blood pressure and the rate of decline in renal function with age. *Kidney Int*. 1984;26:861-8.
94. Rowe JW, Andres R, Tobin JD, Norris AH, Shock NW. The effect of age on creatinine clearance in men: a cross-sectional and longitudinal study. *J Gerontol*. 1976;31:155-63.
95. Luft FC, Weinberger MH, Fineberg NS, Miller JZ, Grim CE. Effects of age on renal sodium homeostasis and its relevance to sodium sensitivity. *Am J Med*. 1987;82:9-15.
96. Berglund G. The role of salt in hypertension. *Acta Med Scand Suppl*. 1983;672:117-120.
97. Beretta-Piccoli C, Weidmann P, Brown JJ, Davies DL, Lever AF, Robertson JI. Body sodium blood volume state in essential hypertension: abnormal relation of exchangeable sodium to age and blood pressure in male patients. *J Cardiovasc Pharmacol*. 1984;6 Suppl 1:S134-42.
98. Beretta-Piccoli C, Davies DL, Boddy K, Brown JJ, Cumming AM, East WB, Fraser R, Lever AF, Padfield P, Robertson JI, Weidmann P, Williams ED. Relation of arterial pressure with exchangeable and total body sodium and with plasma exchangeable and total body potassium in essential hypertension. *Clin Sci (Lond)*. 1981;61 Suppl 7:81s-84s.
99. Toal CB, Leenen FH. Body fluid volumes during development of hypertension in the spontaneously hypertensive rat. *J Hypertens*. 1983;1:345-50.
100. Andersson B, Eriksson L, Fernandez O, Kolmodin CG, Oltner R. Centrally mediated effects of sodium and angiotensin II on arterial blood pressure and fluid balance. *Acta Physiol Scand*. 1972;85:398-407.
101. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res*. 2002;91:406-13.
102. Gotoh E. [Cerebrospinal fluid sodium concentrations and blood pressure in essential hypertension: a comparison between the salt-sensitive and salt-resistant groups]. *Nippon Naika Gakkai Zasshi*. 1982;71:1528-3.
103. Kawano Y, Yoshida K, Kawamura M, Yoshimi H, Ashida T, Abe H, Imanishi M, Kimura G, Kojima S, Kuramochi M, et al. Sodium and noradrenaline in cerebrospinal fluid and blood in salt-sensitive and non-salt-sensitive essential hypertension. *Clin Exp Pharmacol Physiol*. 1992;19:235-41.

104. Komiya I, Yamada T, Takasu N, Asawa T, Akamine H, Yagi N, Nagasawa Y, Ohtsuka H, Miyahara Y, Sakai H, Sato A, Aizawa T. An abnormal sodium metabolism in Japanese patients with essential hypertension, judged by serum sodium distribution, renal function and the renin-aldosterone system. *J Hypertens*. 1997;15:65-72.
105. Wannamethee G, Whincup PH, Shaper AG, Lever AF. Serum sodium concentration and risk of stroke in middle-aged males. *J Hypertens*. 1994;12:971-9.
106. Oliver W, Cohen E, Neel J. Blood pressure, sodium intake, and sodium related hormones in the Yanomamo Indians, a "no-salt" culture. *Circulation*. 1975;52:146-151.
107. Mancilha-Carvalho JJ, de Oliveira R, Esposito RJ. Blood pressure and electrolyte excretion in the Yanomamo Indians, an isolated population. *J Hum Hypertens*. 1989;3:309-14.
108. Sasaki N. High blood pressure and the salt intake of the Japanese. *Japanese Heart Journal*. 1962;3:313-324.
109. Sasaki N. THE RELATIONSHIP OF SALT INTAKE TO HYPERTENSION IN THE JAPANESE. *Geriatrics*. 1964;19:735-744.
110. Siani A, Iacoviello L, Giorgione N, Iacone R, Strazzullo P. Comparison of variability of urinary sodium, potassium, and calcium in free-living men. *Hypertension*. 1989;13:38-42.
111. Luft F, Fineberg N, Sloan R. Estimating dietary sodium intake in individuals receiving a randomly fluctuating intake. *Hypertension*. 1982;4:805-808.
112. Rose G, Stamler J. The INTERSALT study: background, methods and main results. INTERSALT Co-operative Research Group. *J Hum Hypertens*. 1989;3:283-288.
113. Law M, Frost C, Wald N. By how much does dietary salt reduction lower blood pressure? *BMJ*. 1991;302:811-825.
114. Group ICR. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group. *BMJ*. 1988;297:319-328.
115. Yamori Y, Nara Y, Mizushima S, Mano M, Sawamura M, Kihara M, Horie R. International cooperative study on the relationship between dietary factors and blood pressure: a report from the Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study. *J Cardiovasc Pharmacol*. 1990;16:S43-47.
116. Yamori Y, Liu L, Ikeda K, Mizushima S, Nara Y, Simpson F. Different associations of blood pressure with 24-hour urinary sodium excretion among pre- and post-menopausal women. WHO Cardiovascular Diseases and Alimentary Comparison (WHO-CARDIAC) Study. *J Hypertens*. 2001;19:535-538.
117. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group. *Bmj*. 1988;297:319-28.
118. MacGregor GA, Markandu ND, Best FE, Elder DM, Cam JM, Sagnella GA, Squires M. Double-blind randomised crossover trial of moderate sodium restriction in essential hypertension. *Lancet*. 1982;1:351-5.
119. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. *J Hum Hypertens*. 2002;16:761-70.
120. Poulter N, Khaw KT, Hopwood BE, Mugambi M, Peart WS, Rose G, Sever PS. Blood pressure and associated factors in a rural Kenyan community. *Hypertension*. 1984;6:810-3.

121. Poulter NR, Khaw KT, Mugambi M, Peart WS, Sever PS. Migration-induced changes in blood pressure: a controlled longitudinal study. *Clin Exp Pharmacol Physiol*. 1985;12:211-6.
122. He J, Tell GS, Tang YC, Mo PS, He GQ. Relation of electrolytes to blood pressure in men. The Yi people study. *Hypertension*. 1991;17:378-85.
123. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med*. 1997;336:1117-24.
124. Forte JG, Miguel JM, Miguel MJ, de Padua F, Rose G. Salt and blood pressure: a community trial. *J Hum Hypertens*. 1989;3:179-84.
125. Tian HG, Guo ZY, Hu G, Yu SJ, Sun W, Pietinen P, Nissinen A. Changes in sodium intake and blood pressure in a community-based intervention project in China. *J Hum Hypertens*. 1995;9:959-68.
126. Staessen J, Bulpitt CJ, Fagard R, Joossens JV, Lijnen P, Amery A. Salt intake and blood pressure in the general population: a controlled intervention trial in two towns. *J Hypertens*. 1988;6:965-73.
127. Sharma AM, Schattenfroh S, Kribben A, Distler A. Reliability of salt-sensitivity testing in normotensive subjects. *Klin Wochenschr*. 1989;67:632-4.
128. Weinberger MH, Stegner JE, Fineberg NS. A comparison of two tests for the assessment of blood pressure responses to sodium. *Am J Hypertens*. 1993;6:179-84.
129. Forte LR. A novel role for uroguanylin in the regulation of sodium balance. *J Clin Invest*. 2003;112:1138-41.
130. John SW, Krege JH, Oliver PM, Hagaman JR, Hodgin JB, Pang SC, Flynn TG, Smithies O. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science*. 1995;267:679-81.
131. Melo LG, Veress AT, Chong CK, Pang SC, Flynn TG, Sonnenberg H. Salt-sensitive hypertension in ANP knockout mice: potential role of abnormal plasma renin activity. *Am J Physiol*. 1998;274:R255-61.
132. Melander O, Frandsen E, Groop L, Hulthen UL. Plasma ProANP(1-30) reflects salt sensitivity in subjects with heredity for hypertension. *Hypertension*. 2002;39:996-9.
133. Melander O, Frandsen E, Groop L, Hulthen UL. No evidence of a relation between 11beta-hydroxysteroid dehydrogenase type 2 activity and salt sensitivity. *Am J Hypertens*. 2003;16:729-33.
134. Blaustein MP. Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. *Am J Physiol*. 1977;232:C165-73.
135. de Wardener H, MacGregor G. The relation of a circulating sodium transport inhibitor (the natriuretic hormone?) to hypertension. *Medicine*. 1983;62:310-326.
136. Bagrov AY, Fedorova OV, Dmitrieva RI, French AW, Anderson DE. Plasma marinobufagenin-like and ouabain-like immunoreactivity during saline volume expansion in anesthetized dogs. *Cardiovasc Res*. 1996;31:296-305.
137. Gonick HC, Ding Y, Vaziri ND, Bagrov AY, Fedorova OV. Simultaneous measurement of marinobufagenin, ouabain, and hypertension-associated protein in various disease states. *Clin Exp Hypertens*. 1998;20:617-27.
138. Manunta P, Messaggio E, Ballabeni C, Sciarrone MT, Lanzani C, Ferrandi M, Hamlyn JM, Cusi D, Galletti F, Bianchi G. Plasma ouabain-like factor during acute and chronic changes in sodium balance in essential hypertension. *Hypertension*. 2001;38:198-203.

139. Lorenz JN, Nieman M, Sabo J, Sanford LP, Hawkins JA, Elitsur N, Gawenis LR, Clarke LL, Cohen MB. Uroguanylin knockout mice have increased blood pressure and impaired natriuretic response to enteral NaCl load. *J Clin Invest*. 2003;112:1244-54.
140. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science*. 1994;265:2037-48.
141. Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. *Cell*. 1996;85:311-8.
142. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes*. 1993;42:359-62.
143. Frayling TM, Walker M, McCarthy MI, Evans JC, Allen LI, Lynn S, Ayres S, Millauer B, Turner C, Turner RC, Sampson MJ, Hitman GA, Ellard S, Hattersley AT. Parent-offspring trios: a resource to facilitate the identification of type 2 diabetes genes. *Diabetes*. 1999;48:2475-9.
144. Neel JV. The "thrifty genotype" in 1998. *Nutr Rev*. 1999;57:S2-9.
145. Cardon LR, Bell JI. ASSOCIATION STUDY DESIGNS FOR COMPLEX DISEASES. *Nat Rev Genet*. 2001;2:91-99.
146. Tabor HK, Risch NJ, Myers RM. CANDIDATE-GENE APPROACHES FOR STUDYING COMPLEX GENETIC TRAITS: PRACTICAL CONSIDERATIONS. *Nat Rev Genet*. 2002;3:391-397.
147. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. 2003;33:177-182.
148. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med*. 2002;4:45-61.
149. Altshuler D, Daly M, Kruglyak L. Guilt by association. *Nat Genet*. 2000;26:135-7.
150. Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet*. 1996;59:983-9.
151. Hoh J, Ott J. Complex Inheritance and Localizing Disease Genes. *Human Heredity*. 2000;50:85-89.
152. Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SCL, Jenkins SC, Palmer SM, Balfour KM, Rowe BR, Farrall M, Barnett AH, Bain SC, Todd JA. A genome-wide search for human type 1 diabetes susceptibility genes. 1994;371:130-136.
153. Wong ZY, Stebbing M, Ellis JA, Lamantia A, Harrap SB. Genetic linkage of beta and gamma subunits of epithelial sodium channel to systolic blood pressure [see comments]. *Lancet*. 1999;353:1222-5.
154. Wang WY, Barratt BJ, Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet*. 2005;6:109-18.
155. Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS. Replication of linkage studies of complex traits: an examination of variation in location estimates. *Am J Hum Genet*. 1999;65:876-84.
156. Altmuller J, Palmer LJ, Fischer G, Scherb H, Wjst M. Genomewide scans of complex human diseases: true linkage is hard to find. *Am J Hum Genet*. 2001;69:936-50.
157. Evans DM, Cardon LR. Guidelines for genotyping in genomewide linkage studies: single-nucleotide-polymorphism maps versus microsatellite maps. *Am J Hum Genet*. 2004;75:687-92.
158. John S, Shephard N, Liu G, Zeggini E, Cao M, Chen W, Vasavda N, Mills T, Barton A, Hinks A, Eyre S, Jones KW, Ollier W, Silman A, Gibson N, Worthington J, Kennedy GC. Whole-genome scan, in a complex disease, using 11,245 single-

nucleotide polymorphisms: comparison with microsatellites. *Am J Hum Genet*. 2004;75:54-64.

159. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, Cupples LA, Myers RH. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. *Hypertension*. 2000;36:477-83.
160. Blangero J. Localization and identification of human quantitative trait loci: king harvest has surely come. *Curr Opin Genet Dev*. 2004;14:233-40.
161. Levinson DF, Levinson MD, Segurado R, Lewis CM. Genome scan meta-analysis of schizophrenia and bipolar disorder, part I: Methods and power analysis. *Am J Hum Genet*. 2003;73:17-33.
162. Wise LH, Lanchbury JS, Lewis CM. Meta-analysis of genome searches. *Ann Hum Genet*. 1999;63 (Pt 3):263-72.
163. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC. Identification of the cystic fibrosis gene: genetic analysis. *Science*. 1989;245:1073-80.
164. Kruglyak L. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nat Genet*. 1999;22:139-44.
165. Pritchard JK, Przeworski M. Linkage disequilibrium in humans: models and data. *Am J Hum Genet*. 2001;69:1-14.
166. Ardlie KG, Kruglyak L, Seielstad M. Patterns of linkage disequilibrium in the human genome. *Nat Rev Genet*. 2002;3:299-309.
167. Tishkoff SA, Varkonyi R, Cahinhinan N, Abbes S, Argyropoulos G, Destro-Bisol G, Drousiotou A, Dangerfield B, Lefranc G, Loiselet J, Piro A, Stoneking M, Tagarelli A, Tagarelli G, Touma EH, Williams SM, Clark AG. Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. *Science*. 2001;293:455-62.
168. Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES. Linkage disequilibrium in the human genome. 2001;411:199-204.
169. Abecasis GR, Noguchi E, Heinzmann A, Traherne JA, Bhattacharyya S, Leaves NI, Anderson GG, Zhang Y, Lench NJ, Carey A, Cardon LR, Moffatt MF, Cookson WO. Extent and distribution of linkage disequilibrium in three genomic regions. *Am J Hum Genet*. 2001;68:191-197.
170. Jeffreys AJ, Kauppi L, Neumann R. Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. 2001;29:217-222.
171. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The Structure of Haplotype Blocks in the Human Genome. *Science*. 2002;296:2225-2229.
172. Jorde L. Linkage disequilibrium as a gene-mapping tool. *Am J Hum Genet*. 1995;56:11-14.
173. Jorde LB. Linkage Disequilibrium and the Search for Complex Disease Genes. *Genome Res.* 2000;10:1435-1444.
174. Lonjou C, Collins A, Morton NE. Allelic association between marker loci. *Proc Natl Acad Sci U S A*. 1999;96:1621-6.
175. Kruglyak L, Nickerson DA. Variation is the spice of life. *Nat Genet*. 2001;27:234-6.
176. The International HapMap Project. 2003;426:789-796.
177. Collins FS, Guyer MS, Charkravarti A. Variations on a theme: cataloging human DNA sequence variation. *Science*. 1997;278:1580-1.

178. de Bakker P. Tagger. *http://www.broad.mit.edu/mpg/tagger/*. 2005.
179. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *Journal of the National Cancer Institute*. 2004;96:434-442.
180. Risch NJ. Searching for genetic determinants in the new millennium. *Nature*. 2000;405:847-856.
181. Roses AD. Pharmacogenetics and the practice of medicine. *Nature*. 2000;405:857-865.
182. Weiss KM, Terwilliger JD. How many diseases does it take to map a gene with SNPs? *Nat Genet*. 2000;26:151-7.
183. Bennett ST, Todd JA. Human type 1 diabetes and the insulin gene: principles of mapping polygenes. *Annu Rev Genet*. 1996;30:343-70.
184. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*. 2001;411:599-603.
185. Platt R. Heredity in hyperetension. *Q J Med*. 1947;16:111-132.
186. Weitz W. Zur atiologie der genuinen oder vascularen hypertension. *Z Klin Med*. 1923;96:151.
187. Hamilton M, Pickering GW, Roberts JAF, Sowry GSC. The etiology of essential hypertension. 4. The role of inheritance. *Clin Sci*. 1954;13:273-304.
188. Hamilton M, Pickering GW, Roberts JAF, Sowry GSC. The etiology of essential hypertension. 1. The arterial pressure in the general population. *Clin Sci*. 1954;13:11-35.
189. Hamilton M, Pickering GW, Roberts JAF, Sowry GSC. The etiology of essential hypertension. 2. Scores for arterial blood pressure adjusted for differences in age and sex. *Clin Sci*. 1954;13:37-49.
190. Initial sequencing and analysis of the human genome. 2001;409:860-921.
191. Chakravarti A. Population genetics--making sense out of sequence. *Nat Genet*. 1999;21:56-60.
192. Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Shaw N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES. Characterization of single-nucleotide polymorphisms in coding regions of human genes. *Nat Genet*. 1999;22:231-8.
193. Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, Chakravarti A. Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. *Nat Genet*. 1999;22:239-47.
194. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet*. 2001;29:306-9.
195. Takahashi N, Smithies O. Gene targeting approaches to analyzing hypertension. *Journal of the American Society of Nephrology*. 1999;10:1598-1605.
196. Gavras I, Manolis AJ, Gavras H. The alpha2 -adrenergic receptors in hypertension and heart failure: experimental and clinical studies. *J Hypertens*. 2001;19:2115-24.
197. Wulff P, Vallon V, Huang DY, Volkl H, Yu F, Richter K, Jansen M, Schlunz M, Klingel K, Loffing J, Kauselmann G, Bosl MR, Lang F, Kuhl D. Impaired renal Na+ retention in the sgk1-knockout mouse. *J. Clin. Invest.* 2002;110:1263-1268.
198. Rohrer DK, Desai KH, Jasper JR, Stevens ME, Regula DP, Jr., Barsh GS, Bernstein D, Kobilka BK. Targeted disruption of the mouse beta1-adrenergic receptor gene:

developmental and cardiovascular effects. *Proc Natl Acad Sci U S A*. 1996;93:7375-80.

199. Shesely EG, Maeda N, Kim H-S, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, Smithies O. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;93:13176-13181.
200. Gariepy CE, Ohuchi T, Williams SC, Richardson JA, Yanagisawa M. Salt-sensitive hypertension in endothelin-B receptor-deficient rats. *Journal of Clinical Investigation*. 2000;105:925-933.
201. Plato CF, Shesely EG, Garvin JL. eNOS mediates L-arginine-induced inhibition of thick ascending limb chloride flux. *Hypertension*. 2000;35:319-323.
202. Liddle GW, Bledsoe T, Coppage WS. A familial renal disorder simulating primary aldosteronism but with negligible aldosterone secretion. *Trans. Am. Assoc. Phys.* 1963;76:199-213.
203. Goulet CC, Volk KA, Adams CM, Prince LS, Stokes JB, Snyder PM. Inhibition of the epithelial Na+ channel by interaction of Nedd4 with a PY motif deleted in Liddle's syndrome. *J Biol Chem*. 1998;273:30012-7.
204. Chang SS, Grunder S, Hanukoglu A, Rosler A, Mathew PM, Hanukoglu I, Schild L, Lu Y, Shimkets RA, Nelson Williams C, Rossier BC, Lifton RP. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nat Genet*. 1996;12:248-53.
205. Strautnieks SS, Thompson RJ, Gardiner RM, Chung E. A novel splice-site mutation in the gamma subunit of the epithelial sodium channel gene in three pseudohypoaldosteronism type 1 families. *Nat Genet*. 1996;13:248-50.
206. Cheek D, Perry JW. A salt wasting syndrome in infancy. *Arch Dis Childh*. 1958;33:252-256.
207. Kerem E, Bistritzer T, Hanukoglu A, Hofmann T, Zhou Z, Bennett W, MacLaughlin E, Barker P, Nash M, Quittell L, Boucher R, Knowles MR, Homolya V, Keenan B. Pulmonary Epithelial Sodium-Channel Dysfunction and Excess Airway Liquid in Pseudohypoaldosteronism. *N Engl J Med*. 1999;341:156-162.
208. DeFronzo RA, Goldberg M, Agus ZS. The effects of glucose and insulin on renal electrolyte transport. *J Clin Invest*. 1976;58:83-90.
209. Webster MK, Webster MK, Goya L, Ge Y, Maiyar AC, Firestone GL. Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. *Mol Cell Biol.* 1993;13:2031-40.
210. Wang J, Barbry P, Maiyar AC, Rozansky DJ, Bhargava A, Leong M, Firestone GL, Pearce D. SGK integrates insulin and mineralocorticoid regulation of epithelial sodium transport. *Am J Physiol Renal Physiol*. 2001;280:F303-313.
211. Debonneville C, Flores SY, Kamynina E, Plant PJ, Tauxe C, Thomas MA, Munster C, Chraibi A, Pratt JH, Horisberger JD, Pearce D, Loffing J, Staub O. Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na(+) channel cell surface expression. *EMBO*. 2001;20:7052-9.
212. Bhargava A, Pearce D. Mechanisms of mineralocorticoid action: determinants of receptor specificity and actions of regulated gene products. *Trends in Endocrinology and Metabolism*. 2004;15:147-153.
213. Busjahn A, Aydin A, Uhlmann R, Krasko C, Bahring S, Szelestei T, Feng Y, Dahm S, Sharma AM, Luft FC, Lang F. Serum- and Glucocorticoid-Regulated Kinase (SGK1) Gene and Blood Pressure. *Hypertension*. 2002;40:256-260.

214. Pankow J, Dunn D, Hunt S, Leppert M, Miller M, Rao D, Heiss G, Oberman A, Lalouel J, Weiss R. Further evidence of a quantitative trait locus on chromosome 18 influencing postural change in systolic blood pressure: the Hypertension Genetic Epidemiology Network (HyperGEN) Study. *American Journal of Hypertension*. 2005;18:672-678.
215. Russo CJ, Melista E, Cui J, DeStefano AL, Bakris GL, Manolis AJ, Gavras H, Baldwin CT. Association of NEDD4L Ubiquitin Ligase With Essential Hypertension. *Hypertension*. 2005;46:488-491.
216. Dunn D, Ishigami T, Pankow J, von Niederhausern A, Alder J, Hunt S, Leppert M, Lalouel J, Weiss R. Common variant of human NEDD4L activates a cryptic splice site to form a frameshifted transcript. *Journal of Human Genetics*. 2002;47:665-76.
217. Leineweber K, BÃ¼scher R, Bruck H, Brodde O-E. ÃŸ-Adrenoceptor polymorphisms. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2004;369:1-22.
218. Frielle T, Kobilka B, Lefkowitz RJ, Caron MG. Human beta 1- and beta 2-adrenergic receptors: structurally and functionally related receptors derived from distinct genes. *Trends Neurosci*. 1988;11:321-4.
219. Bengtsson K, Melander O, Orho-Melander M, Lindblad U, Ranstam J, Rastam L, Groop L. Polymorphism in the beta(1)-Adrenergic Receptor Gene and Hypertension. *Circulation*. 2001;104:187-90.
220. Iwai C, Akita H, Kanazawa K, Shiga N, Terashima M, Matsuda Y, Takai E, Miyamoto Y, Shimizu M, Kajiya T. Arg389Gly polymorphism of the human [beta]1-adrenergic receptor in patients with nonfatal acute myocardial infarction. *American Heart Journal*. 2003;146:106-109.
221. Mason DA, Moore JD, Green SA, Liggett SB. A Gain-of-function Polymorphism in a G-protein Coupling Domain of the Human beta 1-Adrenergic Receptor. *J. Biol. Chem.* 1999;274:12670-12674.
222. White HL, Maqbool A, McMahon AD, Yates L, Ball SG, Hall AS, Balmforth AJ. An evaluation of the beta-1 adrenergic receptor Arg389Gly polymorphism in individuals at risk of coronary events. A WOSCOPS substudy. *Eur Heart J*. 2002;23:1087-1092.
223. Bengtsson K, Orho-Melander M, Melander O, Lindblad U, Ranstam J, Rastam L, Groop L. Beta(2)-adrenergic receptor gene variation and hypertension in subjects with type 2 diabetes. *Hypertension*. 2001;37:1303-8.
224. Timmermann B, Mo R, Luft FC, Gerdts E, Busjahn A, Omvik P, Li G-H, Schuster H, Wienker TF, Hoehe MR, Lund-Johansen P. [bgr]-2 Adrenoceptor genetic variation is associated with genetic predisposition to essential hypertension: The Bergen Blood Pressure Study. 1998;53:1455-1460.
225. Busjahn A, Li GH, Faulhaber HD, Rosenthal M, Becker A, Jeschke E, Schuster H, Timmermann B, Hoehe MR, Luft FC. beta-2 adrenergic receptor gene variations, blood pressure, and heart size in normal twins. *Hypertension*. 2000;35:555-60.
226. Hawrylyshyn KA, Michelotti GA, Coge F, Guenin SP, Schwinn DA. Update on human alpha1-adrenoceptor subtype signaling and genomic organization. *Trends Pharmacol Sci*. 2004;25:449-55.
227. Flordellis C, Manolis A, Scheinin M, Paris H. Clinical and pharmacological significance of [alpha]2-adrenoceptor polymorphisms in cardiovascular diseases. *International Journal of Cardiology*. 2004;97:367-372.
228. Small KM, Brown KM, Forbes SL, Liggett SB. Polymorphic deletion of three intracellular acidic residues of the alpha 2B-adrenergic receptor decreases G protein-coupled receptor kinase-mediated phosphorylation and desensitization. *J Biol Chem*. 2001;276:4917-22.

229. Zhang H, Li X, Huang J, Li Y, Thijs L, Wang Z, Lu X, Cao K, Xie S, Staessen J, Wang J. Cardiovascular and metabolic phenotypes in relation to the ADRA2B insertion/deletion polymorphism in a Chinese population. *Journal of Hypertension*. 2005;23:2201-2207.
230. Mogensen CE, Viberti GC, Peheim E, Kutter D, Hasslacher C, Hofmann W, Renner R, Bojestig M, Poulsen PL, Scott G, Thoma J, Kuefer J, Nilsson B, Gambke B, Mueller P, Steinbiss J, Willamowski KD. Multicenter evaluation of the Micral-Test II test strip, an immunologic rapid test for the detection of microalbuminuria. *Diabetes Care*. 1997;20:1642-6.
231. Goring HH, Ott J. Relationship estimation in affected sib pair analysis of late-onset diseases. *Eur J Hum Genet*. 1997;5:69-77.
232. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results [see comments]. *Nat Genet*. 1995;11:241-7.
233. Hunt SC, Ellison RC, Atwood LD, Pankow JS, Province MA, Leppert MF. Genome scans for blood pressure and hypertension: the National Heart, Lung, and Blood Institute Family Heart Study. *Hypertension*. 2002;40:1-6.
234. Kardia SL, Rozek LS, Krushkal J, Ferrell RE, Turner ST, Hutchinson R, Brown A, Sing CF, Boerwinkle E. Genome-wide linkage analyses for hypertension genes in two ethnically and geographically diverse populations. *Am J Hypertens*. 2003;16:154-7.
235. Perola M, Kainulainen K, Pajukanta P, Terwilliger JD, Hiekkalinna T, Ellonen P, Kaprio J, Koskenvuo M, Kontula K, Peltonen L. Genome-wide scan of predisposing loci for increased diastolic blood pressure in Finnish siblings. *J Hypertens*. 2000;18:1579-85.
236. Rao DC, Province MA, Leppert MF, Oberman A, Heiss G, Ellison RC, Arnett DK, Eckfeldt JH, Schwander K, Mockrin SC, Hunt SC. A genome-wide affected sibpair linkage analysis of hypertension: the HyperGEN network. *Am J Hypertens*. 2003;16:148-50.
237. Rice T, Rankinen T, Chagnon YC, Province MA, Perusse L, Leon AS, Skinner JS, Wilmore JH, Bouchard C, Rao DC. Genomewide linkage scan of resting blood pressure: HERITAGE Family Study. Health, Risk Factors, Exercise Training, and Genetics. *Hypertension*. 2002;39:1037-43.
238. Rice T, Rankinen T, Province MA, Chagnon YC, Perusse L, Borecki IB, Bouchard C, Rao DC. Genome-wide linkage analysis of systolic and diastolic blood pressure: the Quebec Family Study. *Circulation*. 2000;102:1956-63.
239. Thiel BA, Chakravarti A, Cooper RS, Luke A, Lewis S, Lynn A, Tiwari H, Schork NJ, Weder AB. A genome-wide linkage analysis investigating the determinants of blood pressure in whites and African Americans. *Am J Hypertens*. 2003;16:151-3.
240. von Wowern F, Bengtsson K, Lindgren C, Orho-Melander M, Fyhrqiust F, Lindblad U, Råstam L, Forsblom C, Kanninen T, Almgren P, Burri P, Katzman P, Groop L, Hulthén UL, Melander O. A Genome Wide Scan For Early Onset Primary Hypertension in Scandinavian Families. *Hum Mol Genet*. 2003;12:2077-2081.
241. Demenais F, Kanninen T, Lindgren CM, Wiltshire S, Gaget S, Dandrieux C, Almgren P, Sjogren M, Hattersley A, Dina C, Tuomi T, McCarthy MI, Froguel P, Groop LC. A meta-analysis of four European genome screens (GIFT Consortium) shows evidence for a novel region on chromosome 17p11.2-q22 linked to type 2 diabetes. *Hum Mol Genet*. 2003;12:1865-73.
242. Bengtsson K, Orho-Melander M, Lindblad U, Melander O, Bog-Hansen E, Ranstam J, Rastam L, Groop L. Polymorphism in the angiotensin converting enzyme but not in the angiotensinogen gene is associated with hypertension and type 2 diabetes: the Skaraborg Hypertension and diabetes project. *J Hypertens*. 1999;17:1569-75.

243. Berglund G, Eriksson KF, Israelsson B, Kjellstrom T, Lindgarde F, Mattiasson I, Nilsson JA, Stavenow L. Cardiovascular risk groups and mortality in an urban swedish male population: the Malmo Preventive Project. *J Intern Med*. 1996;239:489-97.
244. Berglund G, Elmstahl S, Janzon L, Larsson S. The Malmo Diet and Cancer Study. Design and feasibility. *J Intern Med*. 1993;233:45-51.
245. Hedblad B, Nilsson P, Engstrom G, Berglund G, Janzon L. Insulin resistance in non-diabetic subjects is associated with increased incidence of myocardial infarction and death. *Diabet Med*. 2002;19:470-5.
246. McAlister FA, Straus SE. Evidence based treatment of hypertension. Measurement of blood pressure: an evidence based review. *Bmj*. 2001;322:908-11.
247. Atwood LD, Samollow PB, Hixson JE, Stern MP, MacCluer JW. Genome-wide linkage analysis of blood pressure in Mexican Americans. *Genet Epidemiol*. 2001;20:373-82.
248. Cooper RS, Luke A, Zhu X, Kan D, Adeyemo A, Rorimi C, Bouzekri N, Ward R. Genome Scan Among Nigerians Linking Blood Pressure to Chromosomes 2, 3, and 19. *Hypertension*. 2002;40:629-633.
249. Kristjansson K, Manolescu A, Kristinsson A, Hardarson T, Knudsen H, Ingason S, Thorleifsson G, Frigge ML, Kong A, Gulcher JR, Stefansson K. Linkage of essential hypertension to chromosome 18q. *Hypertension*. 2002;39:1044-9.
250. Cordell HJ. Sample size requirements to control for stochastic variation in magnitude and location of allele-sharing linkage statistics in affected sibling pairs. *Ann Hum Genet*. 2001;65:491-502.
251. Province MA. The significance of not finding a gene. *Am J Hum Genet*. 2001;69:660-3.
252. Fisher SA, Lanchbury JS, Lewis CM. Meta-analysis of four rheumatoid arthritis genome-wide linkage studies: confirmation of a susceptibility locus on chromosome 16. *Arthritis Rheum*. 2003;48:1200-6.
253. Caulfield M, Munroe P, Pembroke J, Samani N, Dominiczak A, Brown M, Benjamin N, Webster J, Ratcliffe P, O'Shea S, Papp J, Taylor E, Dobson R, Knight J, Newhouse S, Hooper J, Lee W, Brain N, Clayton D, Lathrop GM, Farrall M, Connell J. Genome-wide mapping of human loci for essential hypertension. *Lancet*. 2003;361:2118-23.
254. Koivuoski L, Fisher SA, Kanninen T, Lewis CM, von Wowern F, Hunt SC, Kardia SL, Levy D, Perola M, Rankinen T, Rao DC, Rice T, Thiel BA, Melander O. Meta-analysis of genome-wide scans for hypertension and blood pressure in Caucasians shows evidence of susceptibility regions on chromosomes 2 and 3. *Submitted*.
255. Snapir A, Scheinin M, Groop LC, Orho-Melander M. The insertion/deletion variation in the alpha2B-adrenoceptor does not seem to modify the risk for acute myocardial infarction, but may modify the risk for hypertension in sib-pairs from families with type 2 diabetes. *Cardiovasc Diabetol*. 2003;2:15.
256. Snapir A, Heinonen P, Tuomainen TP, Alhopuro P, Karvonen MK, Lakka TA, Nyyssonen K, Salonen R, Kauhanen J, Valkonen VP, Pesonen U, Koulu M, Scheinin M, Salonen JT. An insertion/deletion polymorphism in the alpha2B-adrenergic receptor gene is a novel genetic risk factor for acute coronary events. *J Am Coll Cardiol*. 2001;37:1516-22.
257. Talke P, Stapelfeldt C, Lobo E, Brown R, Scheinin M, Snapir A. Alpha-2B adrenoceptor polymorphism and peripheral vasoconstriction. *Pharmacogenet Genomics*. 2005;15:357-63.
258. Baldwin CT, Schwartz F, Baima J, Burzstyn M, DeStefano AL, Gavras I, Handy DE, Joost O, Martel T, Manolis A, Nicolaou M, Bresnahan M, Farrer L, Gavras H.

Identification of a polymorphic glutamic acid stretch in the alpha2B-adrenergic receptor and lack of linkage with essential hypertension. *Am J Hypertens*. 1999;12:853-7.

259. Kintsurashvili E, Johns C, Ignjacev I, Gavras I, Gavras H. Central alpha2B-adrenergic receptor antisense in plasmid vector prolongs reversal of salt-dependent hypertension. *J Hypertens*. 2003;21:961-7.
260. Makaritsis KP, Handy DE, Johns C, Kobilka B, Gavras I, Gavras H. Role of the alpha2B-adrenergic receptor in the development of salt-induced hypertension. *Hypertension*. 1999;33:14-7.
261. Link RE, Desai K, Hein L, Stevens ME, Chruscinski A, Bernstein D, Barsh GS, Kobilka BK. Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science*. 1996;273:803-5.
262. Sharma AM, Schorr U. Salt sensitivity and insulin resistance: Is there a link? *Blood Press Suppl*. 1996;5:59-63.
263. Lemmon S, Traub L. Sorting in the endosomal system in yeast and animal cells. *Current Opinion in Cell Biology*. 2000;12:457-466.
264. Pankow JS, Rose KM, Oberman A, Hunt SC, Atwood LD, Djousse L, Province MA, Rao DC. Possible locus on chromosome 18q influencing postural systolic blood pressure changes. *Hypertension*. 2000;36:471-6.
265. DeStefano A, Baldwin C, Burzstyn M, Gavras I, Handy D, Joost O, Martel T, Nicolaou M, Schwartz F, Streeten D, Farrer L, Gavras H. Autosomal dominant orthostatic hypotensive disorder maps to chromosome 18q. *American Journal of Human Genetics*. 1998;63:1425-1430.
266. Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense HW, Joffres M, Kastarinen M, Poulter N, Primatesta P, Rodriguez-Artalejo F, Stegmayr B, Thamm M, Tuomilehto J, Vanuzzo D, Vescio F. Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. *Jama*. 2003;289:2363-9.
267. Staub O, Abriel H, Plant P, Ishikawa T, Kanelis V, Saleki R, Horisberger J-D, Schild L, Rotin D. Regulation of the epithelial Na+ channel by Nedd4 and ubiquitination. *Kidney Int*. 2000;57:809-815.
268. Naray-Fejes-Toth A, Fejes-Toth G. The sgk, an aldosterone-induced gene in mineralocorticoid target cells, regulates the epithelial sodium channel. *Kidney Int*. 2000;57:1290-1294.
269. Naray-Fejes-Toth A, Helms MN, Stokes JB, Fejes-Toth G. Regulation of sodium transport in mammalian collecting duct cells by aldosterone-induced kinase, SGK1: structure/function studies. *Molecular and Cellular Endocrinology*. 2004;217:197-202.
270. Farjah M, Roxas BP, Geenen DL, Danziger RS. Dietary Salt Regulates Renal SGK1 Abundance: Relevance to Salt Sensitivity in the Dahl Rat. *Hypertension*. 2003;41:874-878.
271. Kamynina E, Tauxe C, Staub O. Distinct characteristics of two human Nedd4 proteins with respect to epithelial Na+ channel regulation. *Am J Physiol Renal Physiol*. 2001;281:F469-477.
272. Kamynina E, DeBonneville C, Bens M, Vanderwalle A, Staub O. A novel mouse Nedd4 protein suppresses the activity of the epithelial Na+ channel. *FASEB J*. 2001;15:204-214.
273. Wagner CA, Ott M, Klingel K, Beck S, Friedrich B, Wild K-N, Bröer S, Moschen I, Albers A, Waldegger S, Tümmler B, Egan ME, Geibel JP, Kandolf R, Lang F. Effects of the Serine/Threonine Kinase SGK1 on the Epithelial Na + Channel (ENaC) and

CFTR: Implications for Cystic Fibrosis. *Cellular Physiology and Biochemistry*. 2001;11:209-218.

274. Chen S-y, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, Firestone GL, Verrey F, Pearce D. Epithelial sodium channel regulated by aldosterone-induced protein sgk. *PNAS*. 1999;96:2514-2519.
275. Naray-Fejes-Toth A, Canessa C, Cleaveland ES, Aldrich G, Fejes-Toth G. sgk Is an Aldosterone-induced Kinase in the Renal Collecting Duct. EFFECTS ON EPITHELIAL Na+ CHANNELS. *J. Biol. Chem.* 1999;274:16973-16978.
276. Shigaev A, Asher C, Latter H, Garty H, Reuveny E. Regulation of sgk by aldosterone and its effects on the epithelial Na+ channel. *Am J Physiol Renal Physiol*. 2000;278:F613-619.
277. Park J, Leong ML, Buse P, Maiyar AC, Firestone GL, Hemmings BA. Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. *EMBO J.* 1999;18:3024-33.
278. Kobayashi T, Deak M, Morrice N, Cohen P. Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. *Biochem J*. 1999;344:189-97.
279. Snyder PM, Olson DR, Thomas BC. Serum and Glucocorticoid-regulated Kinase Modulates Nedd4-2-mediated Inhibition of the Epithelial Na+ Channel. *J. Biol. Chem.* 2002;277:5-8.
280. Manjer J, Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M, Mattisson I, Berglund G. The Malmo diet and cancer study: representativity, cancer incidence and mortality in participants and non-participants. *Eur J Cancer Prev*. 2001;10:489-99.
281. Graudal NA, Galloe AM, Garred P. Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterols, and triglyceride: a meta-analysis. *Jama*. 1998;279:1383-91.
282. Neter JE, Stam BE, Kok FJ, Grobbee DE, Geleijnse JM. Influence of weight reduction on blood pressure: a meta-analysis of randomized controlled trials. *Hypertension*. 2003;42:878-84.
283. Fagerberg B, Andersson OK, Isaksson B, Bjorntorp P. Blood pressure control during weight reduction in obese hypertensive men: separate effects of sodium and energy restriction. *Br Med J (Clin Res Ed)*. 1984;288:11-4.
284. Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). SHEP Cooperative Research Group. *Jama*. 1991;265:3255-64.
285. Mattes RD, Donnelly D. Relative contributions of dietary sodium sources. *J Am Coll Nutr*. 1991;10:383-93.