CHAPTER 01

General introduction
Introduction

Hypertension is characterized by a persistently elevated high blood pressure (BP) in the systemic arteries. BP is the pressure of circulating blood against the walls of blood vessels, which is usually expressed as the systolic blood pressure (SBP, the pressure when the heart contracts) over the diastolic blood pressure (DBP, the pressure when the heart relaxes) in units of mmHg (e.g. 120/80 mmHg). BP levels tend to increase continuously with age in both men and women\(^1\), and hypertension becomes more common with advancing age, with a prevalence of more than 50% in people aged 50 years and older\(^2\). Over the life course, hypertension is caused by multifactorial determinants including genetic predisposition, an adverse intrauterine environment, numerous other environmental factors and their mutual interactions. The complicated etiology of hypertension is still not fully understood. Especially genetic studies of hypertension at an early age are, therefore, warranted.\(^3\) This thesis is an effort to improve our understanding of both environmental and genetic origins of hypertension from a life course perspective. In particular, I explore the role of genetic factors in BP development at an early age.

Hypertension definition, prevalence and consequence

In adults

Although the definition of hypertension in adults differs between the guidelines, most major guidelines recommend that hypertension be diagnosed when SBP\(\geq 140\) mmHg or DBP\(\geq 90\) mmHg in the office or clinic following repeated examination (Table 1).\(^4-6\) These numbers were chosen because BP levels above this threshold significantly increase the risks for morbid events and the benefits of antihypertensive treatment are established. The majority (90–95%) of hypertensive cases are termed as essential or primary hypertension with a multifactorial gene-environment etiology, and the remaining cases are referred to as secondary hypertension which is caused by a specific, remediable condition (e.g. primary aldosteronism).\(^1,4\)

Hypertension has a high prevalence across the world. In a large global screening campaign conducted during May 2019, 34% of 1.5 million screenees had hypertension.\(^7\) Another study showed that the global age-standardized prevalence of hypertension was 24.1% in men and 20.1% in women in 2015, with an increase in the number of adults with hypertension (prevalence) from 594 million (12.8%) in 1975 to 1.13 billion (15.5%) in 2015.\(^8\) The prevalence of hypertension worldwide is expected to continue to rise because of ageing populations and increased body weight.
As a major risk factor for coronary artery disease, stroke and chronic kidney disease, in combination with its high prevalence, hypertension becomes the leading risk for global disease burden accounting for the largest number of deaths (~10 million) each year compared with other risk exposures.\textsuperscript{9,10} It is also recognized that a continuous association exists between higher BP and increased risk of cardiovascular events beginning at BP levels below 140/90 mmHg.\textsuperscript{11,12} Taken together, understanding mechanisms of BP development and improving BP control is a key point of public health.

### In children

Given the lack of data to identify a specific level of BP in childhood that increases the risk for cardiovascular events in adulthood, a statistical definition is used for hypertension in children and adolescents. As BP levels rise with age and growth, children are categorized as having hypertension if their SBP or DBP $\geq$95th percentile for sex, age, and height based on normative distribution of BP in healthy children.\textsuperscript{13,14}

<table>
<thead>
<tr>
<th>BP category</th>
<th>Systolic and diastolic blood pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The 2017 American College of Cardiology–American Heart Association guidelines\textsuperscript{4}</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;120 and &lt;80</td>
</tr>
<tr>
<td>Elevated</td>
<td>120–129 and &lt;80</td>
</tr>
<tr>
<td>Hypertension*</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>130–139 and/or 80–89</td>
</tr>
<tr>
<td>Stage 2</td>
<td>$\geq$140 and/or $\geq$90</td>
</tr>
<tr>
<td><strong>The 2018 European Society of Cardiology–European Society of Hypertension guidelines\textsuperscript{5}</strong></td>
<td></td>
</tr>
<tr>
<td>Optimal</td>
<td>&lt;120 and &lt;80</td>
</tr>
<tr>
<td>Normal</td>
<td>120–129 and/or 80–84</td>
</tr>
<tr>
<td>High normal</td>
<td>130–139 and/or 85–89</td>
</tr>
<tr>
<td>Grade 1 hypertension</td>
<td>140–159 and/or 90–99</td>
</tr>
<tr>
<td>Grade 2 hypertension</td>
<td>160–179 and/or 100–109</td>
</tr>
<tr>
<td>Grade 3 hypertension</td>
<td>$\geq$180 and/or $\geq$110</td>
</tr>
<tr>
<td>Isolated systolic hypertension</td>
<td>$\geq$140 and $&lt;90$</td>
</tr>
<tr>
<td><strong>The 2020 International Society of Hypertension guidelines\textsuperscript{6}</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;130 and $&lt;85$</td>
</tr>
<tr>
<td>High-normal</td>
<td>130–139 and/or 85–89</td>
</tr>
<tr>
<td>Grade 1 hypertension</td>
<td>140–159 and/or 90–99</td>
</tr>
<tr>
<td>Grade 2 hypertension</td>
<td>$\geq$160 and/or $\geq$100</td>
</tr>
</tbody>
</table>

*The rationale for this categorization is that an increasing number of studies have reported a gradient of progressively higher CVD risk going from normal BP to stage 1 hypertension.
A meta-analysis in 2019 including 47 studies that had BP measurements from at least 3 separate occasions estimated that the global prevalence of hypertension was 4% in children 19 years and younger. An upward trend in childhood BP levels and hypertension prevalence has been observed during the past 2 decades, which was largely attributed to the global childhood obesity epidemic.

Substantial evidence shows that BP in childhood predicts future BP. A systematic review and analysis of 50 prospective cohort studies demonstrated that BP track from childhood into adulthood with an average tracking correlation coefficient of 0.38 for SBP. In addition, elevated BP in childhood can also lead to target organ damage early in life, such as left ventricular hypertrophy. Therefore, recognizing hypertension at an early stage provides the potential of timely prevention to reduce the risk of hypertension and adverse cardiovascular events in later life.

**Life course trajectories of BP**

BP levels change with age across the whole lifespan. Understanding age-related changes and trajectories of BP increases our knowledge on BP development and potentially benefits prevention of hypertension and related outcomes. One study investigated life course trajectories of SBP using longitudinal data from eight UK cohorts each covering different but overlapping periods spanning from age 7 to >80 years, and they observed four chronological phases over life: (1) a rapid increase in SBP coinciding with peak adolescent growth; (2) a more gentle increase in early adulthood; (3) a midlife acceleration beginning in the fourth decade; and (4) a period of deceleration in late adulthood where increases in SBP slowed and SBP appeared to decline at very old age. These findings also show that SBP increases monotonically across most of life. In another longitudinal cohort, SBP also showed a linear rise from age 30 to 84 years, but DBP was observed to increase only until age 50 to 60, and thereafter declines.

In contrast to the substantial age-related increases in BP levels seen in industrialized populations, little age-related change in BP or a considerably lower rate of increase was found in some non-industrialized remote populations. This difference suggests that western lifestyles are driving the age-related increases in BP. Besides the differences between populations, evidence also shows considerable variation within populations as subgroups with differential BP trajectories were identified in several studies. For instance, one study identified 4 distinct trajectory groups from age 7 to 38 years using group-based trajectory modeling: normal (21.8%), high-normal (43.3%), prehypertensive (31.6%), and hypertensive (4.2%) (Figure 1). The hypertensive group had the highest mean
BP at age 7 and displayed the fastest rate of SBP increase with mean BP in the hypertensive range (≥140 mmHg) at age 38. The results indicate the possibility of estimating risk for future hypertension in childhood, long before BP rises to clinically detectable levels of prehypertension or hypertension in adulthood. Overall, this heterogeneity in BP trajectories over time both within and between populations suggests that life course BP trajectories may be influenced by individuals’ genetic predisposition and exposure to environmental risk factors such as unhealthy lifestyles of BP across the lifespan.

![Figure 1. Plot of predicted trajectory lines with 95% confidence intervals for the four blood pressure trajectory groups identified in a general population longitudinal birth cohort. Adapted from Theodore et al. Hypertension 2015;66:1108-15.](image)

**Life course determinants of BP**

Over the life course from as early as the prenatal period, in childhood and adolescence and in early adulthood and middle-to-old age, multifactorial determinants contribute to BP development. Some factors such as an adverse intrauterine environment may operate at a specific time period but with long-term effects on BP. Determinants such as genetic predisposition do not change but may act differently over the life course. Other factors like lifestyle habits are modifiable and may have accumulative effects on BP. Here I briefly summarize the evidence of factors influencing BP over the lifespan and highlight those that I have investigated in this thesis.
Developmental origins of BP

In the womb, the fetus can already be exposed to adverse intrauterine circumstances which can impact health in later life. This hypothesis was initially proposed in the 1980s by David Barker, who observed the inverse relationship between birth weight and BP. He thus proposed that hypertension was a link between an adverse intrauterine environment and the risk of cardiovascular disease in adult life. The concept has subsequently expanded to the so-called “Developmental origins of Health and Disease” (DOHaD) hypothesis that health and diseases have their origins in prenatal life and early childhood. During the past decades, the developmental origins of diverse diseases has been studied extensively and hypertension is one of the spotlights in this field. Numerous studies have investigated the influence of specific early factors on blood pressure in later life, such as maternal nutritional factors, intrauterine smoke-exposure, birth weight, gestational age, breastfeeding, and postnatal growth. However, results for some factors are controversial and inconclusive. Furthermore, these factors have complex interrelationships which makes it difficult to disentangle their respective roles in epidemiological studies. Therefore, there is still much unknown in the field of developmental origins of BP and more studies are required.

Lifestyle factors influencing BP

During the transition from childhood to early adulthood, people tend to be prone to lifestyle risk factors of BP, such as obesity, excessive salt intake, alcohol drinking, physical inactivity, and poor diet. Lifestyle modifications have been shown to be effective in hypertension prevention and treatment in adults and therefore are recommended in all major BP guidelines. These include weight loss, salt restriction, smoking cessation, moderation of alcohol consumption, regular physical activity, diets like the Dietary Approaches to Stop Hypertension (DASH) diet rich in fruits, vegetables, whole grains, and low-fat dairy products, with reduced content of saturated and total fat. Any type of physical activity such as aerobic or resistance exercise and high-intensity interval training appears to be beneficial in lowering BP. The DASH diet and moderate to vigorous physical activity of at least 3 to 5 days per week (30–60 minutes per session) are also recommended to help reduce BP in children.

Spousal similarity of BP

In late adolescence and early adulthood, people start developing partner relationships. Similarities between such partners or spouses were observed for many coronary risk factors, such as BP, BMI, and lipid traits. A meta-analysis showed that spouses of individuals
with hypertension had a 41% increased odds of having hypertension themselves. This spousal similarity of BP can be explained by assortative mating and cohabitation effects. Assortative mating is the tendency of people to select mates with similar characteristics, such as discernible traits, lifestyle patterns, or socio-economic status, which causes an initial similarity between spouses. Over the course of the relationship, spouses share their environments and may influence each other’s behavior. These “cohabitation effects” may lead to increasing spousal concordance with the duration of the partnership. There is no conclusive evidence showing which of those two factors plays a more important role. In fact, assortative mating and cohabitation effects may operate jointly and both contribute to spousal resemblance of BP. The concordance of hypertension between couples suggests that spouse-based prevention and intervention strategies may be more effective than those aimed at individuals, which constitutes a useful public health message.

**Genetics of BP**

Besides the abovementioned environmental factors, genetic factors also contribute to BP regulation (Box 1). Twin and family studies suggest a sizable genetic component to BP, with most heritability estimates around 50%-60% in both adults and children, i.e. around 50-60% of the variance in BP can be attributed to genetics.

The sequence of the human genome and efforts to discover single nucleotide polymorphisms (SNPs) have greatly facilitated the development of genetic association analyses. With rapid progress and lower cost of high-throughput genotyping arrays scanning the entire human genome, genome-wide association study (GWAS) became possible playing a key role in detecting genetic regions associated with BP since 2007 when the first GWAS was published. Several consortia combining different studies powered the GWAS on BP. In 2009, the Global BPgen (Global BP Genetics) and CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortia identified 13 novel BP loci in 63,569 adults. Two years later, the ICBP (International Consortium for BP GWAS) analyzed data from 200,000 individuals of European descent and identified another 16 novel loci. However, these identified variants in total explained only 1–2% of SBP and DBP variance. After that, the list of identified genetic markers for BP has expanded dramatically, with the increasing sample size, the development of denser imputation reference panels and exome chip as well as inclusion of other ethnicities (Figure 2). In 2018, a GWAS meta-analysis in over one million European individuals identified 535 novel BP loci and confirmed 366 previous reported loci. These 901 BP-associated loci explained in total 5-6% of the variance in SBP and DBP. A recent study in 2020 using exome chip data of up to ~1.3 million individuals
discovered an additional 106 novel loci and 87 of them were rare variants. To date, over 1000 BP-associated loci have been identified indicating the polygenicity of BP. However, they only explain a small proportion of the total heritability estimated from twin and family studies, this phenomenon is called missing heritability. New loci are expected to be discovered with an ever-increasing sample size and advances in methodology, which will further improve our understanding of the genetic architecture of BP.

Figure 2. Advancing discovery in blood pressure genetics studies over the past 10 years. From selected major blood pressure genetic studies from the past 10 years, the number of novel discoveries reported from primary analyses are shown, distinguishing between novel common loci (with replication from 2-stage designs or nonreplicated from 1-stage designs) and rare variants. The printed numbers state the total sample size including discovery and replication stage. *indicates studies focusing on exome-chip content. Adapted from Magavern et al. 2021.

Genetic information can potentially benefit early risk prediction as an individual’s genome is stable from birth. However, effect sizes of single genetic markers are generally small, thus genetic risk scores (GRSs) have been developed by combining significantly associated SNPs identified from GWAS. Later, these were improved by generating polygenic risk scores (PRSs) including a broader range of SNPs that may not reach genome-wide significance but could still be predictive of phenotypes. One recent BP-GRS combining 901 loci found 12.9 mmHg higher SBP along with a more than threefold higher risk of hypertension comparing the highest GRS decile with the lowest decile, which indicates the utility of GRSs for early prevention.

There is strong evidence showing age-dependent genetic effects on BP, i.e. different genes may act on BP at different ages or the magnitude of effects of the same genes may change over time. However, most GWAS of BP have been conducted in adults and only one GWAS was performed in children. Using genome-wide data from ~10,000 children of European
ancestry, it identified only two novel loci associated with SBP across specific age epochs: one during 4-7 years and the other during 8-12 years. Therefore, GWASs focusing on childhood BP with a larger sample size are urgently needed. In addition, some studies showed BP-GRSs based on SNPs identified in adults can also predict BP in youth. As the number of BP-related markers has recently expanded dramatically, the effect of updated allelic scores using the latest GWAS findings requires evaluation at an early age.

**Interaction of genetic and environmental factors**

Interactions between genetic and environmental factors complicate the etiology of hypertension. Previous GWASs identified novel loci associated with BP traits dependent on alcohol intake and smoking exposure. Furthermore, interactions were found between GRSs of BP and lifestyles such as diet and physical activity. These studies suggest that people with a genetic predisposition for high BP may have amplified risk when exposed to adverse lifestyles, consequently their risk exceeds the sum of each risk factor separately and such knowledge may directly benefit personalized prevention and intervention efforts.

**Box 1. Glossary of genetic terms used in this thesis**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Heritability</td>
<td>The proportion of phenotypic variation attributable to genetic variation, which is an index of the importance of heredity in explaining the distribution of a trait or disease in a population.</td>
</tr>
<tr>
<td>Single nucleotide polymorphism (SNP)</td>
<td>A DNA variant that represents variation in a single base with a population frequency &gt;1%. Across the 3.3 billion base pairs of the human genome there could be 10 million common SNPs.</td>
</tr>
<tr>
<td>Genome-wide association study (GWAS)</td>
<td>Hypothesis-free methods to detect associations between millions of genetic variations across the DNA and particular traits or diseases. To correct for multiple testing, a stringent threshold of $5 \times 10^{-8}$ is commonly used for genome-wide significance.</td>
</tr>
<tr>
<td>Genetic risk score (GRS)</td>
<td>A score calculated as a sum of risk allele dosage of identified trait-associated genetic markers weighted by corresponding effect size estimates derived from GWAS results.</td>
</tr>
<tr>
<td>Polygenic risk score (PRS)</td>
<td>PRS is similar to GRS but includes a larger number of independent SNPs by using more lenient significance thresholds.</td>
</tr>
<tr>
<td>SNP array</td>
<td>A chip that allows rapid scanning of hundreds of thousands of SNPs across the whole genome.</td>
</tr>
<tr>
<td>Imputation panel</td>
<td>Imputation panels enable the imputation of SNPs not featured on arrays as SNP arrays include only a small part of the genetic variations in the genome. Common imputation panels include the Haplootype Map (HapMap) with information on 1.6 million variants in 1,184 individuals, 1000 Genome with 81.7 million variants in 2504 samples, and the Haployte Reference Consortium (HRC) with ~40 million variants in 32,470 samples.</td>
</tr>
<tr>
<td>Exome chip</td>
<td>An array of predominantly low frequency and rare variants mostly located in exonic (i.e. coding) regions of genes.</td>
</tr>
</tbody>
</table>
Chapter 1

Thesis outline

Aim of thesis
This thesis aims to understand environmental and genetic origins of hypertension from a life course perspective. In particular, I explore the role of genetic factors in BP development from an early age.

General design
The studies included in this thesis were based on multiple data sources from the Netherlands and other countries (Table 2). The three most important are the Groningen Expert Center for Kids with Obesity (GECKO) Drenthe study\textsuperscript{73}, the Tracking Adolescents’ Individual Lives Survey (TRAILS) cohort\textsuperscript{74}, and the Lifelines Cohort Study and Biobank\textsuperscript{75}. Furthermore, we collected GWAS summary results of BP from other cohorts and performed meta-analyses in both adults and children. Leveraging all the data, we investigated the influence of environmental and genetic factors on BP from the perinatal period, to childhood and adolescence and into adulthood. Information on data sources and study design is presented in Table 2 and further details on these data sources can be found in the referred chapters.

Structure of thesis
The chapters in the thesis are arranged chronologically from childhood to adolescence and adulthood including both environmental and genetic aspects.

Part 1 Childhood
Chapter 2 explores the association between early life determinants and BP at age 6 years in 2 Dutch birth cohorts. Studied early determinants include maternal smoking, maternal prepregnancy BMI, maternal hypertension, standardized birth weight, gestational age, duration of breastfeeding, and early BMI gain.

Chapter 3 reports on a meta-GWAS of BP and heart rate in over 28,000 European children from more than 20 cohorts with the aim to identify genetic markers related to childhood BP and heart rate.

Part 2 Adolescence
Chapter 4 evaluates the variance explained by adult-based GRSs on a wide variety of disease-related traits (e.g. BP, BMI, and lipid traits) in adolescents from the Netherlands.
Chapter 5 explores the effects of PRSs and physical activity and their interactions on BP trajectories in childhood, adolescence and young adulthood.

Part 3 Adulthood

Chapter 6 quantifies and compares the spousal similarities of multiple cardiometabolic risk factors (e.g. BP, lipid traits, lifestyle habits, and hypertension) in European and Asian populations, collectively including over 30,000 spousal pairs.

Chapter 7 performs a single-stage discovery GWAS meta-analysis of BP in over 1 million adults of European descent to identify additional novel variants and improve the understanding of the genetic architecture of BP.
Table 2. Overview of thesis chapters: data sources, design, sample size, age period, determinants, and outcomes

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Data sources</th>
<th>Design</th>
<th>total N</th>
<th>Age</th>
<th>Determinants</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 2  Early Determinants of Childhood Blood Pressure at the Age of 6 Years</td>
<td>Gecko (N=1613) ABCD (N=2052)(^a)</td>
<td>Cohort study</td>
<td>3,665</td>
<td>5-6 years</td>
<td>Maternal smoking, maternal pre-pregnancy BMI, maternal hypertension, birth weight, gestational age, early BMI gain, breastfeeding</td>
<td>SBP, DBP</td>
</tr>
<tr>
<td>Chapter 3  Genome-wide association meta-analyses provide insights into the genetic architecture of childhood blood pressure and heart rate</td>
<td>22 studies of European ancestry (N=28,425) African American (N=835) Japanese (N=1114)</td>
<td>Meta-GWAS</td>
<td>30,374</td>
<td>4-17 years</td>
<td>Hypothesis free: &gt;8 million SNPs</td>
<td>SBP, DBP, MAP, PP, heart rate</td>
</tr>
<tr>
<td>Chapter 4  Genetic Risk Scores for Complex Disease Traits in Youth</td>
<td>TRAILS</td>
<td>Cohort study</td>
<td>1,354</td>
<td>16 years</td>
<td>Genetic risk scores based on published trait-associated SNPs</td>
<td>Twenty continuous disease-related traits including SBP and DBP</td>
</tr>
<tr>
<td>Chapter 5  Effects of polygenic risk scores and physical activity on blood pressure trajectories</td>
<td>Gecko (prepuberty, N=1063) Lifelines (puberty N=2494; postpuberty, N=2549)</td>
<td>Longitudinal study (3-5 years follow-up duration)</td>
<td>6,106</td>
<td>baseline: prepuberty (5-6 yrs); puberty (8-14 yrs) postpuberty (15-20 yrs)</td>
<td>Polygenic risk scores of BP, Physical activity</td>
<td>SBP and DBP trajectory</td>
</tr>
<tr>
<td>Chapter 6  Spousal similarities in cardiometabolic risk factors</td>
<td>Lifelines (Dutch, N=28,265 couples) ToMMo (Japan, N=5391 couples)(^b)</td>
<td>Cross-sectional design</td>
<td>33,656</td>
<td>≥20 years</td>
<td>Spousal relationship</td>
<td>Multiple traits, lifestyles and diseases including BP and hypertension</td>
</tr>
<tr>
<td>Chapter 7  Genome-wide analysis in over 1 million individuals reveals over 2,000 independent genetic signals for blood pressure</td>
<td>UKB (N=458,577) ICBP (N=299,024) MVP (N=220,501) BioVU (N=50,649)</td>
<td>Meta-GWAS</td>
<td>1,028,980</td>
<td>≥18 years</td>
<td>Hypothesis free: ~7.5 million SNPs</td>
<td>SBP, DBP, PP</td>
</tr>
</tbody>
</table>

\(^a\)ABCD indicates Amsterdam Born Children and Their Development study; BioVU, Vanderbilt University's biorepository of DNA linked to de-identified medical records; BMI, body mass index; DBP, diastolic blood pressure; Gecko, the Groningen Expert Center for Kids with Obesity Drenthe study; GWAS, genome-wide association study; ICBP, the International Consortium for Blood Pressure; MAP, mean arterial pressure; MVP, the Million Veteran Program; PP, pulse pressure; SBP, systolic blood pressure; SNP, Single nucleotide polymorphisms; TRAILS, the Tracking Adolescents’ Individual Lives Survey; ToMMo, Tohoku Medical Megabank Organization Cohort; UKB, UK Biobank; yrs, years.
References


