A comparative study on genetic and environmental influences on metabolic phenotypes in Eastern (Chinese) and Western (Danish) populations

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A comparative study on genetic and environmental influences on metabolic phenotypes in Eastern (Chinese) and Western (Danish) populations

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Preface and acknowledgements

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I also wish to express my gratitude to all the participants from both Danish and Chinese twins and their families.

Finally, I would like to thank my family members and my friends who are always supportive and giving me confidence to finish my PhD study.
This thesis is based on three papers

**Paper I**


*Obesity* 2013; 21(9): 1908-1914.

**Paper II**


**Paper III**


Genetic and environmental regulation on longitudinal change of metabolic phenotypes in Danish and Chinese adult twins.

Submitted.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AIC</td>
<td>Akaike’s Information Criterion</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control and Prevention</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CTCTC</td>
<td>Cross-twin cross-trait correlation coefficient</td>
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<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
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<tr>
<td>DTR</td>
<td>Danish Twin Registry</td>
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<td>DZ</td>
<td>Dizygotic twins</td>
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<tr>
<td>GLU</td>
<td>Glucose</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein cholesterol</td>
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<tr>
<td>ICC</td>
<td>Intra-pair correlation coefficient</td>
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<td>LDL</td>
<td>Low density lipoprotein cholesterol</td>
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<tr>
<td>LRT</td>
<td>Likelihood ratio test</td>
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<td>MZ</td>
<td>Monozygotic twins</td>
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<tr>
<td>NCD</td>
<td>Non-communicable diseases</td>
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</table>
QTR  Qingdao Twin Registry
SBP  Systolic blood pressure
SNP  Single Nucleotide Polymorphism
SEM  Structural equation model
TC   Total cholesterol
TG   Triglycerides
WHR  Waist to hip ratio
WT   Weight
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1. Introduction

1.1 Metabolic disorder: A global challenge of public health

The human metabolic phenotypes cover a broad range of measurements including anthropometric measures collectively referred as the body mass traits, blood biochemical measurements on lipids and glucose levels, and blood pressure, etc. Metabolic phenotypes are the products of interactions among a variety of factors — genetic (individual genetic variation on susceptibility), environmental (e.g. social-economic status, geography, culture, life style, behavior) factors and their interaction. During the last decades the abnormality of metabolic phenotypes (metabolic disorders) such as elevated blood pressure (hypertension), high blood glucose (hyperglycemia), imbalance of blood lipids (dyslipidemia), overweight and obesity are among the leading global risks for mortality and morbidity (Lim et al. 2012). Those risks are mostly responsible for the raising epidemic of non-communicable diseases (NCD) e.g. type 2 diabetes, cardiovascular disease, hypertension and stroke.

The combined manifestation of increased blood pressure, a high blood sugar level, excess body fat around the waist and abnormal cholesterol levels is defined as metabolic syndrome, a condition that increases the risk of heart disease, stroke and type 2 diabetes mellitus. The prevalence of metabolic syndrome, in years 2003-2006, was approximately 34% of U.S. adults and the number keeps increasing over time (Ford et al. 2010). In a recent study, the prevalence of metabolic syndrome in Europe was estimated as 24.3% (Scuteri et al. 2015). In the developing countries, changes in dietary (high fat, high caloric food) and physical activity (e.g. sedentary lifestyle) patterns associated with economic development and lack of supportive policy interventions are driving the escalation of metabolic disorders and associated metabolic diseases (such as diabetes, hypertension and stroke). Taking China
for example, the economic boom over the past three decades has been accompanied by a dramatic increase in NCD characterized by metabolic disorders such as type 2 diabetes and hypertension. A meta-analysis of nationally representative data by Wang et al. (2006) estimated that the prevalence of overweight and obesity rose 49.5 percent in 10 years, between 1992 and 2002, from 20.0 to 29.9 percent. The rapid increase in the rates of obesity and a reduction in physical activity over the recent decades have led to raising prevalence of type 2 diabetes with more than 100 million people estimated to be affected accounting for about one in ten people in China — more than any other country in the world (Ma & Chan, 2013; Xu et al. 2013; Ma et al. 2014). In the China National Diabetes and Metabolic Disorders Study conducted in 2007–2008, about 26.6% of the 46239 participants aged over 20 years had hypertension (Gao et al. 2013). The escalating epidemic of metabolic disorder in both developed and developing countries is changing the disease pattern globally. According to a WHO report in 2011 (http://www.who.int/ageing/publications/global_health.pdf), NCD accounted for, in 2008, an estimated 86 percent of the burden of disease in high-income countries, 65 percent in middle-income countries, and a surprising 37 percent in low-income countries. By 2030, non-communicable diseases are projected to account for more than one-half of the disease burden in low-income countries and more than three-fourths in middle-income countries. Combating the epidemic of NCD such as metabolic diseases imposes a new challenge for public health in both developed and developing countries.

1.2 The etiology and development of metabolic disorders: nature versus nurture

The correlated pattern between social-economic development (accompanied by sedentary lifestyle, physical inactivity, stress, fast-foods, nutritional imbalance, increased consumption of alcohol and tobacco, etc.) and increased incidence of metabolic disorders/diseases is a good example for the
interplay between the changing environment (nurture) and our genome (nature). Meanwhile, epidemiologic studies have provided strong evidences for ethnic differences in the susceptibility to metabolic diseases. For example, previous studies have revealed that Asian Indians have a higher predisposition to type 2 diabetes than other ethnic groups (Abate & Chandalia, 2001; Mohan et al. 2005; Rampal et al. 2012). In another study, American black males were found to be highly susceptible to hypertension (Grundy et al. 2014). It has been postulated that the population difference in susceptibility to metabolic diseases is a result of adaptation to different environmental conditions including both physical environment (Li et al. 2013; Sellayah et al. 2014) and social-economic conditions. Studying the genetic and environmental basis of the development of metabolic disorders/diseases can help us gaining a better understanding of the disease etiology and developing more efficient treatment, intervention and prevention strategies.

1.3 The intermediate metabolic phenotypes

By definition, metabolic syndrome is a cluster of abnormal manifestations in multiple metabolic phenotypes including central obesity (excess body fat around the waist), elevated blood pressure, a high blood sugar level and blood lipid disturbance. Because the levels of metabolic phenotypes are highly indicative of metabolic health status and are involved in the development of metabolic diseases (the clinical endpoint), the metabolic phenotypes are also referred to as the intermediate metabolic phenotypes. Kronenberg (2012) pointed out the multiple advantages in genetic studies on intermediate phenotypes as compared to the endpoint diseases. As shown in Figure 1.1, the intermediate phenotype lies within the pathway between segregated genes and disease, and is more specific in reflecting one aspect of the pathogenesis. Intermediate phenotypes can often be measured more exactly, easily, objectively and reproducible than the endpoint disease phenotype. Moreover, the genetic and non-
genetic factors (e.g. lifestyle factors) that influence the intermediate phenotype are easier to identify than those which influence the final pathogenetically heterogeneous phenotype (endpoint disease). Finally, statistical analyses on the continuous intermediate phenotypes are more powerful than the analysis of binary disease outcome. Therefore, instead of metabolic diseases (diabetes, cardiovascular diseases), this PhD project focuses on the genetic and environmental aspects in the development of intermediate metabolic phenotypes (subsequently referred to as metabolic phenotypes).

Figure 1.1 The relationships of gene, environment with intermediate phenotypes and with clinical endpoint. The genetic and environmental factors are more closely related with intermediate metabolic phenotypes than with the endpoint metabolic diseases. Modified from: Florian Kronenberg in “Genetics Meets Metabolomics: from Experiment to Systems Biology” (DOI 10.1007/978-1-4614-1689-0_15).

1.4 Twin studies on metabolic phenotypes

Metabolic phenotypes e.g. the levels of blood glucose and lipids, measurements of blood pressure and body mass index are, similar to most complex traits, regulated by both genetic and environmental factors with their interplay as central to the development of metabolic abnormality and diseases.
(Andreassi 2009; Phillips 2013). In the literature, the genetic and environmental contributions to metabolic phenotypes and metabolic diseases have been intensively studied using family (Henneman et al. 2008; Zabaneh et al. 2009; Svati et al. 2009; Herbeth et al. 2010) and twin (Benyamin et al. 2007; Almgren et al. 2011; Duan et al. 2011; Zarkesh et al. 2012; van Dongen et al. 2013; Silventoinen et al. 2015) data with interesting results pointing to significant genetic and environmental regulation on the level of intermediate metabolic phenotypes. It is necessary to mention that, over the past decade, studies on Danish twins have made a considerable contribution to the literature of genetics on metabolic phenotypes and diabetes (Poulsen et al. 1999; Poulsen et al. 2001; Poulsen & Vaag 2003; Schousboe et al. 2003; Schousboe et al. 2004; Benyamin et al. 2007; Fenger et al. 2007). Although interesting, the early twin studies of metabolic phenotypes were conducted in developed countries and mainly in Caucasians. In contrast, only sporadic studies were done in the world largest population in China and with limited phenotypes (Zhang et al. 2009; Lee et al. 2010). In addition, most of the published studies focused on the levels of metabolic phenotype using the cross-sectional design and only a few studies were interested in the longitudinal change of metabolic phenotypes. Although the intra-individual change of metabolic phenotypes can be more indicative of disorder-related modifications and disease onset (Yousri et al. 2014), published longitudinal studies were either limited to body mass traits (weight, height and BMI) (Austin et al. 1997; Franz et al. 2007; Silventoinen et al. 2007; Hjelmborg et al. 2008; Dubois et al. 2012) or focused on phenotype stability or correlation over ages instead of longitudinal change in metabolic phenotypes (Middelberg et al. 2006; Zhang et al. 2010; Koenis et al. 2013).

Supported by research grants from Novo Nordisk Foundation in 2006 and the European Foundation for the Study of Diabetes in 2008, a collaboration project on twin study of metabolic
phenotypes has been set up between Danish researchers at University of Southern Denmark (SDU) and Chinese scientists from Qingdao Center for Disease Control and Prevention (Qingdao CDC) and Qingdao University Medical College. Since the establishment, the collaboration has resulted in a number of studies on metabolic phenotypes in Chinese twins (Pang et al. 2010; Duan et al. 2011; Zhang et al. 2012a; Zhang et al. 2012b; Zhang et al. 2012c; Jiang et al. 2012; Duan et al. 2013; Wu et al. 2014). Most importantly, the collaboration has, over time, accumulated twin data on metabolic phenotypes from both cross-sectional and longitudinal surveys enabling the first relative large and highly comprehensive cross-population comparison of genetic and environmental influences on the level as well as the longitudinal change of multiple metabolic phenotypes (Figure 1.2).

Figure 1.2 The two types of data for a metabolic phenotype, (1) levels of metabolic phenotype measured in a cross-sectional study conducted at time x (marked red) and (2) change in the level of phenotype over time measured in a longitudinal follow-up study from time t1 to t2 (marked green).
2. Objectives

Although both genetic and environmental factors are involved in the development of metabolic disorders, the role of environment should be emphasized as the expression or function of gene can be regulated to adapt to existing environmental circumstance. In other words, adaptive evolution in populations under distinct environmental and cultural circumstances could have resulted in varying genetic basis of metabolic factors and development of metabolic disorders or diseases. It is well known that ethnic disparities in the pathophysiology and pathogenesis of metabolic diseases (Abate & Chandalia, 2003) can have an important impact in prevention and management (Dagogo-Jack 2003) given the complex nature in disease development which involves both genetic and environmental factors (e.g. different food structure and lifestyles, lower baseline BMI in Chinese). Actually, previous studies have shown ethnic difference in the prevalence of metabolic disorders with, for example, markedly high prevalence of metabolic syndrome in ethnic Indians in a population-based survey in Malaysia (Rampal et al. 2012); high susceptibility to type 2 diabetes in east Asian populations including the Chinese population (Kodama et al. 2013; Ma et al. 2014). It can therefore be interesting to conduct cross-population analysis of the relative importance of genetic and environmental contributions to metabolic phenotypes. Such studies can help us not only with a better understanding of the disease etiology but also with the development of more efficient and population specific treatment, intervention and prevention strategies for metabolic disorders. Furthermore, such studies could be particularly important for promoting public health in China, a country that is experiencing an alarmingly rapid increase in the prevalence of obesity and related problems in the past decade. This PhD project takes advantage of the Chinese-Danish collaboration to conduct twin analysis and cross-population comparison of genetic and environmental components in the variations of level as well as longitudinal change of multiple metabolic phenotypes.
3. Materials and Methods

3.1 The metabolic phenotypes

The metabolic phenotypes in this study refer to the multiple intermediate phenotypes to metabolic
disorders and diseases (Figure 1.1) covering anthropometric variables including body mass index
(BMI), body weight (WT), waist and hip circumference (WAIST, HIP), and ratio (WHR); blood
biochemical measurements including fasting blood glucose (GLU), triglyceride (TG), total cholesterol
(TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL); and blood pressure including
systolic (SBP) and diastolic (DBP) blood pressure.

3.2 The Danish twin samples

3.2.1 The Danish Twin Registry

Established in the 1950s, the population based Danish Twin Registry (DTR) is one of the oldest twin
registries in the world. Over time, the DTR has gradually been expanded. The DTR has now collected
information about almost all twins born in Denmark (Figure 3.1) since 1870. By January 1st, 2012, the
DTR comprised 86,398 twin pairs born from 1870 to 2009 making DTR one of the largest twin
registers in the world (Skytthe et al. 2013). Data on the twins have been collected mainly through a
number of large questionnaire surveys, longitudinal interview surveys, and a large number of clinical
investigations of different subgroups of the twin cohorts. This is supplemented with data on health and
vital status collected by linkage to national registers of cancer, hospitalization, birth characteristics, and
mortality using the unique personal identification number assigned to every Danish resident (Skytthe et
al. 2013).
3.2.2 Cross-sectional data on metabolic phenotypes

The Danish twins in this study were recruited from two cohorts of the population-based Danish Twin Registry during 1997-2000 to examine the impact of genes and environment on insulin resistance, obesity and cardiovascular disease risk factors i.e. the GEMINAKAR study as described previously (Benyamin et al. 2007; Schousboe et al. 2003, 2004; Fenger et al. 2007). Cohort I covers the birth cohorts 1931-1952, whereas cohort II covers the years 1953-1982. The cross sectional data used in studies I and II originated from the first of two rounds of clinical investigations of these twins.

From the randomly selected twins who consented to participate, an exclusion criterion was applied to remove those who had known diabetes or cardiovascular diseases, pregnancy, breast feeding, physical inability, and incomplete twin pairs. The co-twins in a pair underwent an extensive full day clinical examination of a variety of phenotypes. The final sample contained 756 twin pairs (309 monozygotic or MZ pairs, 447 dizygotic or DZ pairs, among them, 299 pairs of male and 326 pairs of female like-sex twins, 131 pairs of opposite-sex twins). The mean age of twins was 38 years, range 18-67 years. The data collection was running from 1997 to 2000 in two clinical investigation sites (Odense and Copenhagen, Denmark). Twin zygosity was determined by nine polymorphic DNA microsatellite markers (Benyamin et al. 2007).

3.2.3 Longitudinal data on metabolic phenotypes

The longitudinal data originated from both rounds of clinical investigations of the GEMINAKAR twin study, which was followed up during 2010 to 2012. The same procedures as the baseline were applied. At follow-up, a total of 502 complete pairs (545 females, 459 males), hereof 226 monozygotic (MZ)
pairs, 189 same-sex dizygotic (DZ) pairs, and 87 opposite-sex DZ pairs were available. The mean age of participants at follow-up was 50 years, range 30-75 years.

All participants gave their written informed consent to participate and the local scientific committee of the Region of Southern Denmark (baseline, S-VF-19970271; follow-up, S-20090065) and Danish Data protection Board (baseline, 1999-1200-441; follow-up, 2009-41-2990) approved the study protocol.

3.2.4 Phenotype measurements

Body weight was measured to the nearest 0.1 kg using a standing beam scale. Height was measured to the nearest centimeter using a vertical scale with a horizontal moving headboard. BMI was calculated as weight (kg) divided by the square of height (m). Waist and hip circumferences were taken in standing position. Waist circumference was measured midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteal region (Schousboe et al. 2004). Systolic and diastolic blood pressure was measured after at least 5 minutes of rest following a standard procedure using a conventional mercurial sphygmomanometer. Three measurements were taken from each subject, with at least 1 min between each measurement. The mean of these three was calculated and used in subsequent analyses. Blood glucose concentration was analyzed by the glucose dehydrogenase oxidation method (Schousboe et al. 2003). Triglycerides, total cholesterol and HDL were measured using standard methods by Vitros 950 analyzer (Johnson & Johnson, USA). LDL was calculated (Friedewald formula) by subtracting HDL and (0.45 x TG) from total cholesterol (Fenger et al. 2007). All the biochemical measurements were done following routine laboratory protocols. Twins in a pair were examined on the same day. Twin zygosity was determined using microsatellite markers.
3.3 The Chinese twin samples

3.3.1 The Qingdao Twin Registry

Qingdao is a major costal city in eastern Shandong Province, geographically belonging to the northern part of China (Figure 3.1) with a population of about 7 million. The Qingdao Twin Registry (QTR), initially established by Qingdao Centers for Disease Control and Prevention (CDC) in 1998, is now the largest twin registry in China with over ten thousands twin pairs collected across all age groups in the area of Qingdao municipality (Figure 3.1).

![Figure 3.1 The location of DTR and QTR.](image)

The purpose of the QTR was primarily to recruit twins born in the Qingdao region to study the genetic and environmental influence on non-communicable diseases (NCD) (Pang et al., 2006).
Today the research coverage of QTR has been extended to include NCD, metabolic health, aging as well as international and cross-cultural collaborations with Denmark, Finland and United States (Pang et al. 2010; Duan et al. 2011; Zhang et al. 2012a; Zhang et al. 2012b; Zhang et al. 2012c; Jiang et al. 2012; Duan et al. 2013; Wu et al. 2014; Xu et al. 2015a; Xu et al. 2015b; Silventoinen et al. 2015).

Twins were recruited through medical records, school, and media coverage (Pang et al. 2006). The tertiary prevention and health system (at village, township, and county level) in Qingdao has provided assistance for the registries with high efficiency, coverage, and accuracy of information (Li et al., 2006).

### 3.3.2 Cross-sectional data on metabolic phenotypes

The Chinese twin sampling for this study was based on the Qingdao Twin Registry with twins sampled through the local disease control network and residence registry. The exclusion criteria removed those who were pregnant, breastfeeding, had known diabetes and/or cardiovascular disease, or were taking weight-reducing medicaments within one month, and incomplete twin pairs. Co-twins in a pair were invited to a clinical investigation and had a clinical examination in the same day after a 10 to 12 hours overnight fasting. Body measures and biochemical variables were collected in a total of 325 pairs of twins (183 MZ twin pairs and 142 DZ twin pairs, among them, 104 pairs of male and 158 pairs of female like-sex twins, 63 pairs of opposite-sex twins) with a mean age of 40.5 years (range 18-69 years, birth cohorts 1937-1988) (Duan et al. 2011). Twin zygosity was determined by DNA testing using 16 short tandem repeat DNA markers at the central laboratory of Qingdao Blood Bank (Duan et al. 2011). This study was carried out during 2006 to 2007 in Qingdao, China.

### 3.3.3 Longitudinal data on metabolic phenotypes
Similar to the cross-sectional data, the longitudinal Chinese twin samples in this study were collected by the Qingdao Twin Registry through residence registry and the local disease control network of Qingdao CDC. The same procedures as the cross-sectional data collection were applied. Co-twins in a pair were invited to a clinical investigation conducted in the same day. Finally, a total of 181 completed twin pairs (101 MZ and 80 DZ pairs) were identified with longitudinal measurements taken about 7 years apart with a mean baseline age of 39.5 years and an age range of 23-64 years. Among them 245 were females and 117 were males.

The Chinese study was approved by the local ethics committee at Qingdao CDC, Qingdao, China.

### 3.3.4 Phenotype measurements

BMI was calculated as weight (kilogram, kg) divided by the square of height (meter, m) with body weight measured using a standing beam scale and to the nearest 0.1 kg and height measured using a vertical scale with a horizontal moving headboard and to the nearest centimeter. Waist and hip circumferences were taken in standing position with waist circumference measured midway between the lowest rib and the iliac crest, and hip circumference measured over the widest part of the gluteal region (Duan et al. 2011). Systolic and diastolic blood pressure measurements were taken after at least 5 minutes of break following a standard procedure using a standard mercurial sphygmomanometer. The mean of three measurements (taken at least 1 minute apart) was calculated and used in subsequent analyses. Co-twins were examined on the same day after a 10–12 hours overnight fasting. Serum and plasma were separated from blood cells in the field within 30 min and kept at low temperature before sending to routine laboratory test. Blood glucose concentration was analyzed by the glucose
dehydrogenase oxidation method (Duan et al. 2011) similar to the Danish study (Schousboe et al. 2003). Triglycerides, total cholesterol, HDL and LDL were measured on the semi-automatic analyzer (Hitachi 7600, Japan) (Duan et al. 2011) using standard clinical biochemical methods as for the Danish twins except LDL (Fenger et al. 2007).

3.4 Twin correlation on metabolic phenotypes

3.4.1 Intra-pair correlation coefficient

Twin correlation on each phenotype was estimated by calculating the intra twin pair correlation coefficient (ICC) as \[ \rho = \frac{\sigma_s^2}{\sigma_s^2 + \sigma_e^2} \] with \( \sigma_s^2 \) defined as the between pair variance and \( \sigma_e^2 \) as the within pair variance in the phenotype. A higher ICC in MZ twins as compared with DZ twins provides an indication of genetic influence on phenotype level. For the longitudinal data with two time points (time 1 or baseline to time 2 or follow-up), ICC was estimated for intra-individual variation for each phenotype, i.e. \( \Delta\text{phenotype} = \text{phenotype}_{\text{time2}} - \text{phenotype}_{\text{time1}} \). Likewise, a higher ICC for \( \Delta\text{phenotype} \) in MZ twins than in DZ twins suggests genetic regulation on change of the phenotype over time (\( \Delta\text{phenotype} \)).

The effects of age and sex were adjusted by including them as covariates in the regression models and using the residuals from the regression model for calculation of intra-pair correlation coefficient or ICC. Correlation coefficients were estimated using the free R software (http://www.r-project.org/) and R package mets (http://cran.r-project.org/web/packages/mets/mets).

3.4.2 Cross-twin cross-trait correlation
The intra pair correlation coefficient represents similarity or correlation on one phenotype between two twins in a pair. Different from ICC, the cross-twin cross-trait correlation (CTCTC) is the correlation between one phenotype in twin 1 and a different phenotype in twin 2, for example, the correlation between SBP in twin 1 and DBP in twin 2. The magnitude of CTCTC in MZ and DZ twins provides indication of genetic influences on the covariance between two phenotypes. Before fitting the bivariate twin model, we estimated CTCTC for MZ and DZ twin pairs separately for comparison between MZ and DZ twins. When CTCTC_{MZ} > CTCTC_{DZ}, we could assume that the genetic factors contribute to the correlation between two phenotypes, e.g. between SBP and DBP. Likewise, age and sex were adjusted using regression to control for their effects when CTCTC was calculated.

### 3.5 The univariate twin models

As the most popular design in twin studies, the classical twin method disentangles the influences of genetic and environmental factors on a trait by estimating and comparing the phenotype correlation in MZ or identical twin pairs who share 100% of their genetic materials and in DZ or fraternal twin pairs who share, on average, 50% of their genes. Based on the classical twin study design, univariate twin modeling were done by fitting structural equation models (SEM) to each metabolic trait with adjustment for sex and age. For each phenotype, the total phenotypic variance was decomposed into different “latent” factors (or model parameters) representing additive genetic (A), non-additive genetic (D), common or shared environmental (C), and unique environmental (E) components. Figure 3.2 illustrates a univariate twin model that includes A, C, D, and E components. In this model, A represents the sum of the effects of the individual alleles at all loci that influence the phenotype, or cumulative effect of individual loci, therefore the overall effect is the summed contribution of all the loci; D represents interaction between alleles at the same locus (dominance), or different loci (epistasis). If a
trait is controlled by a dominant allele, then both homozygous and heterozygous will display the same phenotypic value; C represents the events that happen to both twins, affecting them in the same way such like family socio-economic status, parenting style, childhood diet etc.; E represents the environment which co-twins do not shared such as individual lifestyle, education, economic status in adulthood. The E component also includes random measurement error. In the twin model, the correlations for both A and D are 1 for MZ twin pairs whereas for DZ twins 0.5 for A and 0.25 for D respectively. As shown in Figure 3.2, the classical twin design assumes equal environmental sharing by both MZ and DZ pairs, i.e. correlation on C equals 1 for both MZ and DZ twins who were reared in the same family, and E is uncorrelated for both types of twins (Rijstijl & Sham 2002).

Figure 3.2 The univariate ACDE twin model decomposes variance of a metabolic phenotype into additive genetic (A), dominant genetic (D), common environmental (C) and unique environmental (E) components and assumes equal sharing of the common environment in MZ and DZ twins but differential additive and dominant genetic correlation in MZ (1.0) and DZ (0.5 for additive and 0.25 for dominant effects) twin pairs.
In the model, referred to as ACDE model, C and D cannot be estimated simultaneously in the classical twin study of MZ and DZ twins reared together. Two separate models containing the A, C and E components (the ACE model) and the A, D and E components (the ADE model) were fitted with the latter usually preferred when the MZ correlation is more than double the DZ correlation for a given phenotype, i.e. ICC_{MZ}>2ICC_{DZ} (Rijswijk & Sham 2002; Ozaki et al. 2011).

Based on the full ACE model, nested models were also fitted by dropping the C (AE model), the A (CE model), or both (E model) components for best model selection. Likewise two nested models (AE and E) were fitted for comparison with the full ADE model. The DE model was excluded because it is biologically implausible considering that the dominant genetic effects alone are not enough to explain the very low DZ correlation when compared with MZ correlation (Eaves 1988). The performances of different models were compared using Akaike’s Information Criterion (AIC) (Akaike 1974), such that the model with the lowest AIC reflected the best balance between goodness-of-fit and parsimony, with AIC calculated as

\[ AIC = -2 \text{ (ln (likelihood))} + 2 \text{ (the number of free parameters in the model)} \]

Comparison on performances between the full model and its nested models was done using the likelihood ratio test (LRT). The LRT calculates twice the difference in the log likelihood for the full and the nested models which can be approximated by a chi-squared distribution with degree of freedom equaling the difference in the number of parameters in the two models. In model comparison, the parsimonious model was preferred when no statistical significance was observed in the likelihood ratio (chi-squared) test between the two models. Robustness of parameter estimates was assessed using bootstrap re-sampling for empirically calculating the 95% confidence intervals (CIs).
For the longitudinal data with two time points, the same univariate model and best model selection procedure were applied to the change in each metabolic phenotype, i.e. Δphenotype instead of the level of phenotype to estimate the genetic and environmental contributions to the variation in the change of phenotype level over time.

Univariate twin models were fitted using the free software package Mx (http://www.vcu.edu/mx) for cross-sectional data, and the free R package mets (http://cran.r-project.org/web/packages/mets/index.html) for longitudinal data.

3.6 The bivariate twin models

We introduced a bivariate model for the joint analysis of SBP and DBP using the structural equation modeling (SEM) approach (Rijssdijk & Sham 2002). In SEM, parameter estimates are obtained by using a fitting function that minimizes the difference between the observed covariance matrix and the expected covariance matrix implied by the model under the assumption of bivariate normality. In this approach, variances in the observed traits (SBP and DBP) and their covariance are decomposed into latent additive genetic (A), non-additive genetic (D), shared environmental (C), and unique environmental (E) components.

Considering the fact that data on twins reared together do not contain enough information to tease out the contrasting effects of common environmental (C) and dominant genetic (D) components (Rijssdijk & Sham 2002), our model fitting started with consideration of both the bivariate ACE and the bivariate ADE models. Similar to the univariate model, CTCTC_{MZ}>2CTCTC_{DZ} is an indication that a bivariate ADE model should be preferred rather than a bivariate ACE model. In case that the estimated CTCTC in DZ is more than half of that in MZ twins, a phenomenon that does not
support the presence of dominant genetic effect, the ADE model is then dropped. Consequently, the full bivariate ACE (Figure 3.3) and its nested bivariate models (AE, CE, E) were fitted.

Based on variance estimates, the additive genetic correlation ($r_g$) between SBP and DBP can be calculated as 

$$ r_g = \frac{\text{cov}_g(SBP, DBP)}{\sqrt{\text{var}_g(SBP) \text{var}_g(DBP)}} $$

where $\text{var}_g(SBP)$, $\text{var}_g(DBP)$ are the additive genetic variance of SBP and DBP respectively, and $\text{cov}_g(SBP,DBP)$ is their genetic covariance.
Likewise, correlations for common environmental ($r_c$), and unique environmental ($r_e$) factors can be calculated.

Similar to the univariate twin models, the performances of the different bivariate models were compared using Akaike’s Information Criterion (AIC) (Akaike 1974) in that the model with the lowest AIC reflects the best balance between goodness-of-fit and parsimony. Meanwhile, the fitting of nested models enabled assessment of statistical significance of the A, C and E component using the likelihood ratio ($\chi^2$) test at a significance level of $p<0.05$. This led to the most parsimonious (or the “best fitting”) model in which the pattern of variances and covariances is explained by as few parameters as possible.

Model fitting was carried out separately for Danish and Chinese twins with age, sex and BMI included as covariates. Bivariate twin models were fitted using the free Mx software package (http://mxscripts.ctglab.nl/index.php?page=mx_tree) (Neale et al. 2003).

### 3.7 Comparison strategies

In all model fitting, bootstrap resampling with 100 replicates was used to obtain the 95% confidence intervals (CIs) for the estimated model parameters. The estimated 95% CI was used (1) to assess the statistical significance of the parameter estimate ($p<0.05$ when the 95% CI does not contain zero); and (2) to infer the statistical significance in the difference between parameter estimates from the Danish and the Chinese twin samples ($p<0.05$ when there is no overlap between the 95% CI for the Danish twins and the 95% CI for the Chinese twins). As the free Mx package used for twin modeling in this study did not estimate standard errors for parameter estimates, the bootstrap resampling method had to
be used for inference of statistical significance. In the following, it is shown that the bootstrap-based method was more stringent than the statistical testing using, for example the t-test.

In a t-test for comparing two means $x_1$ and $x_2$ (assuming $x_1 > x_2$), statistical significance is reached ($\alpha=0.05$, 2 sided test) when
\[
\frac{x_1 - x_2}{\sqrt{SE_1^2 + SE_2^2}} > 1.96, \text{ equivalent to } x_1 - x_2 > 1.96\sqrt{SE_1^2 + SE_2^2}.
\]

When the two confidence intervals for $x_1$ and $x_2$ do not overlap, i.e. the lower bound of the CI for the greater mean (here $x_1$) is greater than the upper bound of the CI for smaller mean (here $x_2$), we have
\[
x_1 - 1.96SE_1 > x_2 + 1.96SE_2, \text{ equivalent to } x_1 - x_2 > 1.96(SE_1 + SE_2).
\]
Since $SE_1 + SE_2 > \sqrt{SE_1^2 + SE_2^2}$, the decision on difference based on comparison of CIs requires a larger difference between $x_1$ and $x_2$ than that required by a t-test (Figure 3.4). If the two CIs overlap, we cannot determine if there is a significant difference or not. However, if there is no overlap between the two CIs, we can be sure that there is a significant difference.

Figure 3.4 Comparison of t-test with bootstrap CIs. Different from the t-test, decision based on confident intervals requires a larger difference in the two means.
4. Results

This section summarizes main findings from the 3 studies in the PhD project. Detailed data can be found in the 3 enclosed papers at end of the thesis.

4.1 Study I

This is a twin-based comparative study on the genetic influence on multiple metabolic phenotypes in Danish and Chinese twins. Data on eleven metabolic phenotypes including anthropometric measures, blood glucose and lipids levels as well as blood pressure were available from 756 pairs of Danish twins (309 monozygotic and 447 dizygotic twin pairs) with a mean age of 38 years (range: 18-67) and from 325 pairs of Chinese twins (183 monozygotic and 142 dizygotic twin pairs) with a mean age of 40.5 years (range: 18-69). Univariate twin modeling was performed on full and nested models with the best fitting models selected. Heritability estimates were compared between Danish and Chinese samples to identify differential genetic influences on each of the phenotypes. Except for hip circumference, all other body measures exhibited comparable heritability patterns in the two samples with body weight showing only a slight difference. Higher genetic influences were estimated for fasting blood glucose level in Chinese twins while the Danish twins showed more genetic control over lipids phenotypes (triglycerides and high density lipoprotein cholesterol). Systolic blood pressure was more genetically controlled in Danish than in Chinese twins. In conclusion, metabolic phenotypes showed disparity patterns in their genetic determinants in the two samples representing populations under distinct ethnicity and environmental conditions.

This study has been published in *Obesity* with the following reference information and can be found as paper I in the enclosed papers of the thesis:

4.2 Study II

Although the phenotypic correlation between systolic blood pressure (SBP) and diastolic blood pressure (DBP) is well-observed, the genetic basis for the correlation has been rarely investigated. This study aims at examining the genetic overlap between SBP and DBP by fitting bivariate models to Danish and Chinese twins and comparing ethnic difference between the two samples. Our estimates revealed a high proportion of additive genetic component shared by both SBP and DBP in Danish (0.71, 95% CI: 0.65-0.75) and in Chinese (0.62, 95% CI: 0.50-0.71) twins with no statistical significance for ethnic difference. The estimated genetic component in phenotypic correlation could serve to guide molecular genetic studies aiming at looking for genetic variants that affect both SBP and DBP. The bivariate model also estimated genetic and environmental contributions to SBP and DBP separately with an overall pattern of higher genetic regulation or heritability in Danish (0.72, 95% CI: 0.67-0.76 for SBP; 0.70, 95% CI: 0.65-0.75 for DBP) than in Chinese (0.54, 95% CI: 0.44-0.63 for SBP; 0.57, 95% CI: 0.47-0.65 for DBP) twins while higher contribution by unique environment in Chinese (0.46, 95% CI: 0.37-0.56 for SBP; 0.43, 95% CI: 0.35-0.53 for DBP) than in Danish (0.28, 95% CI: 0.24-0.33 for SBP; 0.30, 95% CI: 0.25-0.35 for DBP) twins. The estimated contribution of unique environment to blood pressure suggests that promoting healthy lifestyle could provide an efficient way for the control of high blood pressure especially in the Chinese population.

This study has been published in *Hypertension Research* with the following reference information and can be found as paper II in the enclosed papers of the thesis:

### 4.3 Study III

This study focused on the longitudinal patterns of the metabolic phenotypes because the rate of change in metabolic phenotypes can be highly indicative of metabolic disorders and disorder-related modifications. We analyzed data from longitudinal twin studies on multiple metabolic phenotypes in Danish and Chinese twins representing two populations of distinct ethnic, cultural, social-economic backgrounds and geographical environments. The study covered a relatively large sample of 502 pairs of Danish adult twins followed up for a long period of 12 years with a mean age at intake of 38 years (range: 18 - 65) and a total of 181 Chinese adult twin pairs traced for about 7 years with a mean baseline age of 39.5 years (range: 23 - 64). The univariate twin models were fitted to the longitudinal change in each phenotype (Δphenotype = phenotype_{time2} – phenotype_{time1}) to estimate the genetic and environmental contributions to the variation in Δphenotype. Moderate to high contributions by the unique environment were estimated for all phenotypes in both Danish (from 0.51 for low density lipoprotein up to 0.72 for triglycerides) and Chinese (from 0.41 for triglycerides up to 0.73 for diastolic blood pressure) twins; low to moderate genetic components were estimated for long-term change in most of the phenotypes in Danish twins except for triglycerides and hip circumference. Compared with Danish twins, the Chinese twins tended to have higher genetic control over the longitudinal changes in lipids (total cholesterol, triglycerides and low density lipoprotein cholesterol) and glucose; higher unique environmental contribution to blood pressure but no genetic contribution to longitudinal change in body mass traits. Our results emphasized the major contribution of unique environment to the
observed intra-individual longitudinal variation in all metabolic phenotypes in both samples, and meanwhile revealed differential patterns of genetic and common environmental regulation on changes over time in metabolic phenotypes across the two samples.

This study has been submitted for consideration of publication and can be found as paper III in the enclosed papers of the thesis:

5. Discussion

We have conducted a comparative study on genetic influences on the level as well as on the longitudinal change in multiple metabolic phenotypes covering anthropometric measures, blood biochemical variables (lipids and glucose levels) and blood pressure in two populations of distinct ethnic, environmental and cultural differences. Results from our cross-population comparison revealed disparity patterns in the genetic and environmental contribution to both the level and the rate of change in different clusters of metabolic phenotypes.

5.1 Population differences in the genetic control over the level of metabolic phenotypes

In study I, striking patterns were found for fasting blood glucose and triglyceride with the former displaying higher genetic control in Chinese twins while the latter with higher genetic contribution in Danish twins in both the best fitting and the full ACE models (Figures 3, 4 in paper I). The significant difference in the genetic influences on these phenotypes could imply more genetic involvement in the regulation of blood glucose level in the Chinese while a higher genetic component in the regulation of blood triglyceride in the Danish population. This hypothesis seems also to be supported by results for other lipid phenotypes. For example, both Danish and Chinese samples have the AE model as the best fitting model for HDL but with higher additive genetic component in Danish (0.68) than in Chinese (0.59) twins with only slightly overlapping 95% CIs (Danish: 0.62-0.72; Chinese: 0.50-0.67) (Table 3, Figure 3 in paper I). Another example, a higher heritability for total cholesterol is estimated in Danish (0.58) than in Chinese (0.51) twins although their 95% CIs overlapped considerably. The contrasting genetic effects on glucose and lipids phenotypes in Chinese and Danish twins lead us to postulate a varying genetic basis of metabolic phenotypes in Eastern and Western populations adapted to different
environmental conditions e.g. diets (Chinese food dominated by cereals but Danish food by animal products). As a molecular evidence, Spielman et al. (2007) reported differential gene expression patterns among ethnic groups and pointed out that population differences in prevalence of complex diseases such as diabetes could be partly accounted for by the differential gene expression levels. Tan and Tai (2004) studied the relationship between food and genetic influences on biomarkers of metabolic disorders among ethnic groups in Singapore and reported association between HDL concentration and polymorphisms in several regulatory genes. In order to reconfirm our hypothesis, more work including molecular genetic studies needs to be done to elucidate the differential regulatory network of metabolic pathway genes in the two populations.

In paper I, the differential additive genetic effects in fasting blood glucose in Danish and Chinese twins were observed from two different best fitting models, i.e. the ACE model in Danish and the AE model in Chinese twins. We point out that the differential patterns remained even when the same full ACE model was fitted (Figure 4 in paper I) with 0.58 (95% CI: 0.28-0.71) for additive genetic, 0.06 (95% CI: 0-0.32) for common environmental, and 0.36 (95% CI: 0.29-0.46) for unique environmental effects in the Chinese twins while with 0.26 (95% CI: 0.23-0.29) for A, 0.27 (95% CI: 0.23-0.42) for C and 0.47 (95% CI: 0.41-0.55) for E components in Danish twins. In addition to the differential genetic effects, results from our model fitting also revealed significant differential contributions to fasting glucose level by the common environment (Table 3 in paper I). Similar patterns were also observed in hip circumference, WHR, total cholesterol and diastolic pressure, all with ACE in Danish and AE in Chinese twins as their best fitting models. Although our limited data cannot provide a clear explanation to the phenomenon, perhaps one could link it with the poor rearing
environments that were more or less similar across families shared by the Chinese twins about 20 to 30 years ago, which may lead to lower estimates on the C components.

Of the two blood pressure variables, the one that exhibited differential genetic control between Danish and Chinese twins was the systolic blood pressure, one of the important intermediate phenotypes of metabolic disorders. Our estimates in the best fitting models (the AE model for both samples) indicated that more variation in systolic blood pressure could be ascribed to genetic regulation in the Danish than in the Chinese twins (Table 3, Figure 3 in paper I). In other words, more unique environmental influences were responsible for the systolic blood pressure variation in Chinese than in Danish twins. As one possible explanation, we emphasize the increasing proportion of Chinese under stress from both work and family life in a currently fast transiting society particularly during the last 20 years, an important condition that has been associated with hypertension (Markovitz et al. 1993). One more explanation could be the dietary salt intake which is usually high in the northern part of China (Yan et al. 2011) where Qingdao is located (Figure 3.1). In fact, studies have already shown the significant association between high dietary salt intake and inflated blood pressure especially systolic blood pressure (He & MacGregor 2002). If this was the case, our results could suggest the high importance in promoting healthy individual behavior, life style and diet habits in hypertension control and prevention in the Chinese population.

Overall, the results of study I revealed disparity patterns of genetic and environmental regulations of important intermediate metabolic phenotypes between two ethnic populations under the distinct culture, social, economic and geographic environmental conditions. This finding could serve to guide molecular genetic studies on genetic predisposition to metabolic disease in different ethnic
populations and could help with development of more efficient prevention and intervention schemes for metabolic disorders/diseases and eventually promote public health.

5.2 The genetic overlap between systolic and diastolic blood pressure in Danish and Chinese twins

The results from bivariate modeling of blood pressure data on Danish and Chinese twins both indicated a significant genetic component in the phenotypic correlation between SBP and DBP in the two populations. In an early study, Schieken et al. (1992) reported a genetic proportion of 0.74 in SBP-DBP covariance in Caucasian boys and girls with an average age of 11 years. This estimate is very close to our estimate of 0.71 in middle aged Danish twins. The shared genetic regulation on SBP and DBP was also reported by a recent study in Brazilian nuclear families with a genetic correlation of 0.67 (Forjaz et al. 2012). In fact, previous genetic association studies have already reported common single nucleotide polymorphisms (SNPs) that significantly affect both SBP and DBP providing molecular evidence for shared genetic mechanism on SBP and DBP co-regulation in Caucasians and in East Asians (Ehret et al. 2011; Kato et al. 2011). The estimated proportion of genetic components in blood pressure correlation could serve to assess the potential of heritable genetic variations not yet identified by linkage and association studies. Based on the estimates of high genetic regulation to the correlation between SBP and DBP, more functional and static genetic variants that affect both SBP and DBP are expected to be discovered using functional genomics, next generation sequencing and epigenetic approaches (Tan et al. 2013).

Besides the high genetic correlation between SBP and DBP in both Danish and Chinese twins, results from our bivariate model also revealed that the Danish sample tended to exhibit higher
genetic control over blood pressure as well as their correlation, while in the Chinese sample more unique environmental influences (Tables 3-4 in paper II). As displayed by Figures 2 and 3 of paper II, although the same pattern held for SBP, DBP and their correlation, statistical significance was only obtained for SBP with no overlapping 95% CIs of A and E between Danish and Chinese twins. Interestingly, the identified pattern for genetic and environmental contribution to SBP and DBP from the bivariate model is consistent with results from the univariate model in study I which reported significantly higher A component in SBP in Danish than in Chinese twins but overlapping CIs of A for DBP between Danish and Chinese twins. Considering the limited sample sizes especially for the Chinese twins, large scale studies are required to elucidate the clear pattern of ethnic difference in blood pressure.

In Table 4 of paper II, the estimated additive genetic component in SBP-DBP covariance was higher in Danish (0.71, 95% CI: 0.65-0.75) than in Chinese (0.62, 95% CI: 0.50-0.71) twins, however, the pattern on genetic correlation ($r_g$) was just opposite with higher genetic correlation in Chinese (0.81, 95% CI: 0.73-0.88) than in Danish (0.71, 95% CI: 0.67-0.75) twins. Even though these differences were not statistically significant considering their overlapping confidence intervals, the reversed pattern deserves some discussions. Note that the estimated proportion of additive genetic component is a measurement for the relative importance of genetic factors taking into account of environmental effects, while genetic correlation ($r_g$) measures the absolute co-variation between the additive genetic variances of SBP and DBP. In fact, a high genetic correlation could serve as an indication of more shared genetic basis for SBP and DBP in a number of genetic variants, i.e. pleiotropic genes. However, the strong common genetic basis can be diluted by the interference of
environmental effects when the proportion of genetic contribution is calculated, which might be the case for the Chinese twins. More molecular genetic studies are required to verify our hypothesis.

In both the full and the nested models, the unique environmental effects on blood pressure and blood pressures correlation were significant in Danish as well as in Chinese twins suggesting the crucial importance of individual environment in explaining blood pressure variation and covariation. Despite the consistency in the importance of unique environment, we point out the noticeable ethnic difference in the two samples with higher proportion of E components in Chinese twins than in Danish twins. This is clearly demonstrated by Figure 3 of paper II where the 95% CIs of the E component do not overlap for SBP (p<0.05) and only slightly overlap for DBP (borderline significance). Consistent with our findings from univariate model in study I, these results again indicated the special importance of individual environmental factors, including social and behavioral factors, e.g. life style (hectic and stressful life, lack of physical exercise), occupation (sedentary work), and dietary habits (high dietary sodium) (Markovitz et al. 1993; Beunza et al. 2007; Yan et al. 2011) in controlling blood pressure in the Chinese population.

In summary, we have identified and mutually validated a relatively high genetic overlap between SBP and DBP providing strong evidence for common genetic mechanisms in the regulation of SBP and DBP in both Chinese (Eastern) and Danish (Western) populations which could serve as epidemiological basis for the search of pleiotropic genes shared by different populations. Meanwhile our results also point to the high importance of individual environment to blood pressure variation suggesting that implementation of measures aimed at promoting personal healthy lifestyle should help to efficiently reduce the risk of hypertension and cardiovascular problems especially in the Chinese population.
5.3 Population differences in the genetic control over longitudinal change in metabolic phenotypes

Instead of the level of metabolic phenotypes, study III focused on the intra-individual change over time (i.e. Δphenotype) as the phenotype of interest. One important finding from study III was the moderate to high estimates of the unique environmental component in the longitudinal change of all 12 phenotypes in both samples (Table 5 of paper III). In contrast, the estimates of genetic component had only low to moderate contribution to Δphenotypes. The results emphasized the high importance of unique environmental factors in controlling intra-individual variation in metabolic phenotypes over time, both in Danish and in Chinese twins. In addition to the unique environmental factors, the shared environments were also involved in regulating the longitudinal change of all body mass traits in Chinese twins which was in contrast to the Danish twins. The phenomenon could indicate, in addition to the unique environment, early-life shared environment could also play an important role in determining the individual trajectory of body mass traits in the Chinese adult twins.

In the best fitting models, the genetic estimates to longitudinal changes for lipids (except HDL) and glucose tended to be higher in Chinese than in Danish twins with only a slight overlap in the 95% CIs for GLU (0.58, 95% CI: 0.46-0.70 in Chinese versus 0.39, 95% CI: 0.28-0.49 in Danish twins) but with considerable overlaps for TC and LDL (Table 5 of paper III). Although the difference lacked strong statistical support for each phenotype considered individually, the same trend of difference (i.e. A for Chinese > A for Danish) in biochemical measurements could reflect interesting population differences in the genetic and environmental control over longitudinal patterns of lipids and glucose. In view of the fact that Chinese twins were sampled from the countryside (the suburban area of Qingdao) where staple food is characterized by high cereal and vegetable content, we assume that the Chinese
samples might be more restricted in their dietary pattern being much more plant based than the Danish twins who had more sufficient food supply and in general had a dietary pattern that included high intakes of animal-based food (Yang & Zhang 2010; Knudsen et al. 2012). As a result, the difference in dietary habits between the two samples could lead to low unique environmental and high genetic components in the variation of Δphenotype for blood lipids and glucose in the Chinese twins, while high unique environmental and low genetic components in the Danish twins. Future cross-population studies should help to validate our hypothesis.

Among the lipid phenotypes, no genetic component was estimated in the best fitting models (i.e. the CE model) for ΔTG in Danish twins and for ΔHDL in the Chinese twins. The absence of genetic control over ΔTG is consistent with Friedlander et al (1997) who reported no genetic influence on the change in TG over a 10-year follow-up in an adult cohort of American twins. In another longitudinal study conducted in adult Caucasian twins, Goode et al. (2007) reported no significant proportion of genetic contribution to the variation in age-related change of blood lipids. Different from the results in adult twins, Middelberg et al. (2007) and Zhang et al. (2010) estimated significant genetic component in age-related change on the level of blood lipids in adolescent Caucasian and Chinese twins respectively. Comparing the results for adolescent and adult twins, one could conclude that the genes are important in regulating the developmental changes of blood lipids in adolescent twins in both Eastern and Western populations while in adult twins, the genetic effects on long-term change for some lipids (here TG in Danish twins and HDL in Chinese twins) could have been weakened, and perhaps with population-specific patterns.

Different from the lipids and glucose phenotypes, longitudinal change in blood pressure was highly attributable to unique environment in Chinese twins (0.72 for SBP, 95% CI: 0.50-0.93; 0.73
for DBP, 95% CI: 0.56-0.90). The estimates of E components for the change of blood pressures in Danish twins (0.64 for SBP, 95% CI: 0.53-0.74; 0.53 for DBP, 95% CI: 0.44-0.63) tended to be lower than that for the Chinese twins although their 95% CIs overlapped. On the other hand, the Danish twins had moderate genetic influence on change in blood pressure (0.36, 95% CI: 0.26-0.47 for SBP; 0.47, 95% CI: 0.37-0.56 for DBP) which was in contrast to the lower or no genetic control in the Chinese twins (0.28 for SBP, 95% CI: 0.07-0.50; 0 for DBP). Although the different patterns could be ascribed to the different ethnic (genetic) backgrounds, we point out again the importance of salt consumption in China especially in the rural areas. According to a global epidemiological study, China was on the top rank in dietary salt intake (Brown et al. 2009) and the intake level changed with age (Bi et al. 2014). We think that the high contribution by unique environment to change in blood pressure can be, at least, partly explained by the high level of salt intake in China considering the significantly positive association of salt intake with blood pressure (Brown et al. 2009). If this was the case, the high salt intake affects not only the variation in the level (Yan et al. 2011; Qin et al. 2014; Peng et al. 2014) but also the variation in the rate of change of blood pressure in the Chinese population.

The findings from this study could serve to motivate epigenetic epidemiology studies (1) to identify gene regulatory networks modulated by early life common environmental exposure and/or by individual unique environmental factors during the life course; (2) to look for the associated environmental risk factors for the development of more efficient individualized prevention strategies to promote metabolic health.

5.4 Limitations of these studies
It is necessary to mention that firstly, these studies were based on limited samples available, especially for the twins from northern China, which could confine the power and generalization of our results. Secondly, in this study the statistical significance in comparison between two samples were determined by comparing the 95% CIs. Although two non-overlapping intervals provided strong evidence of statistical difference with a type I error rate of less than 5%, slightly overlapping intervals might not rule out the possibility of statistical difference which means that our conclusions were conservative. Finally, the cross-population comparison on genetic influence on longitudinal change in metabolic phenotypes was made on different years of follow-up with 12 years in Danish but only 7 years in Chinese twins. Although the ages at intake for twins in the two samples were comparable, the shorter follow-up time and relatively small sample size in the Chinese twins could limit the comparability.
6. Conclusions and Future Research Perspectives

Based on the Danish and Chinese twin samples, this PhD study identified both consistent and differential patterns in the genetic and environmental regulation over multiple metabolic phenotypes in two populations of substantial ethnic, environmental, and cultural differences. The consistent patterns of genetic (e.g. high genetic correlation between systolic and diastolic blood pressure) and environmental (e.g. high unique environmental components in longitudinal change of metabolic phenotypes) influences on metabolic phenotypes could reflect endogenous biological (genetic) and exogenous (environmental) mechanisms in regulating metabolism independent of ethnicity. More importantly, the disparity patterns of genetic and environmental control over glucose (significantly higher genetic contribution in Chinese than in Danish twins), lipids (triglycerides and HDL), and blood pressure (significantly higher genetic components in Danish than in Chinese twins) could suggest adaptation to environmental conditions during evolution by the Danish and Chinese populations living in different geographical, social and cultural circumstances. Furthermore, the differential pattern of genetic and common environmental involvements in the intra-individual or longitudinal change of body-mass traits (shared environment in the Chinese versus additive genetic effect in the Danish twins) could imply population-specific genetic and epigenetic control over body shape development. The differential pattern in genetic regulation over metabolic phenotypes across the two samples could have high impact on the development of more efficient strategies for prevention, intervention and treatment of metabolic disorders/diseases.

With the expansion of twin research from both sides, (1) more and more data especially for Chinese twins are going to be collected which will enable more powerful cross-population comparison
and validation of the current findings; (2) meanwhile, results from this PhD study could motivate new genetic and epigenetic research directions. Previous molecular studies have reported genetic (Spielman et al. 2007), epigenetic (Zhang et al. 2011; Liu et al. 2010) and gene expression (Spielman et al. 2007) markers for ethnicity and population structure which can be linked to disease susceptibility (Li et al. 2010). To this end, the observed population-specific patterns in genetic and environmental control over metabolic phenotypes call for functional genomic studies aimed at identifying the molecular adaptation mechanisms (e.g. epigenetics) in the genetic regulation on metabolic phenotypes induced by different environmental conditions. Taking advantage of the high-throughput techniques for genomic analysis such as microarray and next-generation sequencing, the Danish-Chinese collaboration on twin studies of metabolic phenotypes is extending from phenotype-based twin analysis to functional genomic studies using twins. Such an approach could provide new opportunities for identifying the genetic, epigenetic and transcriptomic biomarkers underlying the observed population differences in genetic control over metabolic phenotypes, and eventually help with promoting precision medicine (Collins & Varmus 2015).
7. References


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8. Summary

The genetic and environmental contributions to the variation in metabolic phenotypes have been revealed by family and twin studies performed on phenotypes pertaining to both anthropometric and biochemical measurements. The complex nature of metabolic phenotypes could suggest that there can be clusters of sub-phenotypes differentially affected by genetic and environmental factors under given environmental conditions as the expression or function of a gene can be regulated to adapt to existing environmental conditions. This project conducted an inter-population comparative study to examine the relative importance of genetic and environmental factors in multiple metabolic phenotypes in Chinese and Danish twins representing two populations of substantial ethnic, environmental, and cultural differences. The classical twin methods have been applied to adult twin samples collected using cross-sectional (756 pairs of Danish twins; 325 pairs of Chinese twins) and longitudinal (502 pairs of Danish twins followed up for 12 years; 181 pairs of Chinese twins traced for 7 years) designs. Analysis on cross-sectional twin samples estimated moderate to high genetic control over almost all metabolic phenotypes but with higher genetic influence on fasting blood glucose level in Chinese than in Danish twins, whereas the Danish twins showed more genetic control over most lipids phenotypes as compared with Chinese twins. Bivariate twin modeling on cross-sectional twin data on systolic and diastolic blood pressure revealed a high proportion of additive genetic components shared by systolic and diastolic blood pressure in both Danish and Chinese twins with no significant ethnic differences. The bivariate model also estimated an overall pattern of higher genetic regulation or heritability for blood pressure in Danish than in Chinese twins. Twin modeling on longitudinal twin data estimated moderate to high unique environmental contributions to intra-individual longitudinal change in all metabolic phenotypes in both Danish and Chinese twins. Compared with Danish twins, the Chinese twins tended
to have higher genetic control over intra-individual variation over time in lipids (except high density lipoprotein) and glucose; higher unique environmental contribution to longitudinal changes in blood pressure; no genetic contribution to longitudinal change in body mass traits.

Overall, our comparative study identified both specific and common patterns of genetic and environmental control over metabolic phenotypes in twin samples from two distinct populations. The differential patterns are characterized with by (1) significantly higher genetic contribution to the level of glucose in Chinese than in Danish twins, and to the levels of lipids and blood pressure in Danish than in Chinese twins; (2) relatively higher genetic components in the regulation of longitudinal changes in glucose and lipids in Chinese than in Danish twins, and in blood pressure in Danish than in Chinese twins; (3) absence of genetic control over body mass traits in Chinese twins. The common patterns include consistently high genetic components in the correlation of blood pressure and high unique environmental components in longitudinal change of all metabolic phenotypes. The consistent patterns could reflect endogenous biological (genetic) and exogenous (environmental) mechanisms in regulating metabolic phenotypes independent of ethnicity. The differential patterns could suggest adaptation to environmental conditions during evolution by the Danish and Chinese populations living in different geographical, social and cultural circumstances and could impact the development of more efficient strategies for prevention, intervention and treatment of metabolic disorders. Both the consistent and the differential patterns could provide useful information for guiding genetic and epigenetic studies to look for molecular markers of metabolic disorders, all with aim at promoting metabolic health.
9. Summary in Danish

Sammenlignet med danske tvillinger udvistede kinesiske tvillinger tendens til at have højere genetisk kontrol over de longitudinelle ændringer i blodlipider (undtagen lipoprotein med høj densitet) og blodsukker, højere enestående miljømæssige bidrag til blodtryk, men ingen genetiske bidrag til ændringer i kropsmasse over tid.

Samlet set påviste vores sammenlignende undersøgelse både specifikke og fælles mønstre af genetisk og miljømæssig kontrol over metaboliske fænityper i to prøver fra to forskellige populationer med (1) signifikant højere genetisk bidrag til niveauet af glukose i kinesiske end i danske tvillinger, og til niveauerne af lipider og blodtryk i dansk end i kinesiske tvillinger; (2) relativt højere genetisk regulering i longitudinelle ændringer i glukose og lipider i kinesiske end i danske tvillinger, og i blodtrykket i danske end i kinesiske tvillinger; og (3) manglende genetisk kontrol over træk relateret til kropsmassei kinesiske tvillinger. De fælles mønstre omfatter konsekvent høje genetiske komponenter i kontrol af blodtryk og høje unikke miljømæssige komponenter i ændring over tid af alle metaboliske fænityper. De-konsistente mønstre kunne afspejle endogene biologiske (genetiske) og eksogene (miljø) mekanismer i reguleringen af metaboliske fænityper uafhængigt af etnicitet. De differentielle mønstre kunne pege på tilpasning til miljøforholdene i evolutionen af de danske og kinesiske befolkningsgrupper, der bor under forskellige geografiske, sociale og kulturelle forhold, og kunne have en stor betydning ved udviklingen af mere effektive strategier for forebyggelse, intervention og behandling af metaboliske sygdomme. Både de konsistente og den differentierede mønstre kunne give nyttige oplysninger til genetiske og epigenetiske undersøgelser med henblik på at fremme den metaboliske sundhed.
10. Enclosed Papers I - III
Paper I


Heritability of Eleven Metabolic Phenotypes in Danish and Chinese Twins: A Cross-Population Comparison

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Objectives: A twin-based comparative study on the genetic influences in metabolic endophenotypes in two populations of substantial ethnic, environmental, and cultural differences was performed.

Design and Methods: Data on 11 metabolic phenotypes including anthropometric measures, blood glucose, and lipids levels as well as blood pressure were available from 756 pairs of Danish twins (309 monozygotic and 447 dizygotic twin pairs) with a mean age of 38 years (range: 18-67) and from 325 pairs of Chinese twins (183 monozygotic and 142 dizygotic twin pairs) with a mean age of 40.5 years (range: 18-69). Twin modeling was performed on full and nested models with the best fitting models selected.

Results: Heritability estimates were compared between Danish and Chinese samples to identify differential genetic influences on each of the phenotypes. Except for hip circumference, all other body measures exhibited similar heritability patterns in the two samples with body weight showing only a slight difference. Higher genetic influences were estimated for fasting blood glucose level in Chinese twins, whereas the Danish twins showed more genetic regulation over most lipids phenotypes. Systolic blood pressure was more genetically controlled in Danish than in Chinese twins.

Conclusions: Metabolic endophenotypes show disparity in their genetic determinants in populations under distinct environmental conditions.

Introduction

Metabolic phenotypes, similar to most complex traits, are influenced by multiple genetic and environmental factors together with their interplay. Abnormality in metabolic phenotypes can be associated with cardiometabolic risks leading to, e.g., type 2 diabetes, hypertension, atherosclerosis, stroke, and cardiovascular diseases. In the past decades, the epidemic of metabolic disorders has become a major public health issue in both developed and developing countries due to high prevalence. The genetic and environmental contributions to variation in metabolic phenotypes have been revealed by family (1-4) and twin (5-10) studies performed on phenotypes pertaining to both morphological and biochemical measurements. The complex nature of metabolic phenotypes could suggest that there can be clusters of sub-phenotypes differentially affected by genetic and environmental factors (11) under given environmental conditions.

Although both genetic and environmental factors are involved in the development of metabolic disorders, the rule of environment should be emphasized as the expression or function of a gene can be regulated to adapt to existing environmental conditions (12). In other words, adaptive evolution in populations under distinct environmental and cultural circumstances could have resulted in varying genetic basis of disease etiology. Thus, it can be interesting to conduct cross-population analysis of the relative importance of genetic and environmental contributions to metabolic phenotypes. Such studies can help us not only with a better understanding of the disease etiology but also with the development of more efficient treatment and prevention strategies. In the literature, although many heritability studies have been done within populations, inter-population studies have been rare and limited to anthropometric phenotypes. For example, Hur et al. (13) compared heritability differences in body measures including height, weight, and body mass index (BMI).
between Caucasian and East Asian adolescent twins aged from 13 to 15 years. Their study reported no significant difference in the genetic contribution to variations in the selected variables between the two samples. Another study by Ordoniana et al. (14) compared heritability for height, weight, and BMI in middle aged Dutch and Spanish twins and found no difference in the two populations.

To take advantage of an existing Sino-Danish collaboration on twin studies, we have conducted an inter-population comparison on heritability of 11 metabolic phenotypes covering anthropometric (BMI, weight, waist and hip circumference, and ratio), biochemical [fasting blood glucose, triglyceride, total cholesterol, and high-density lipoprotein (HDL)] and blood pressure (systolic and diastolic) variables. Twin modeling was done using structural equation models for dissecting the total variation of a phenotype into genetic and environmental components with a focus on inter-population comparison in two populations of substantial ethnic, environmental, and cultural differences.

Methods

The Danish twins

The Danish twins were recruited from two cohorts of the population-based Danish Twin Registry, a nation-wide registration system established in the 1950s and now located at the Institute of Public Health, University of Southern Denmark. Cohort I covers the birth cohorts 1931-1952, whereas cohort II covers the years 1953-1982. From the randomly selected twins who consented to participate, an exclusion criterion was applied to remove those who had known diabetes or cardiovascular diseases, pregnancy, breast feeding, physical inability, and incomplete twin pairs. The final sample contained 756 twin pairs (309 monozygotic or MZ pairs, 447 dizygotic or DZ pairs, among them, 299 pairs of male and 326 pairs of female like-sex twins, 131 pairs of opposite-sex twins) with a mean age of 38 years (range 18-67 years). The data collection was running from 1997 to 2000 in two clinical investigation sites (Odense and Copenhagen, Denmark). Twin zygosity was determined by nine polymorphic DNA microsatellite markers (8).

Body weight was measured to the nearest 0.1 kg using a standing beam scale. Height was measured to the nearest centimeter using a vertical scale with a horizontal moving headboard. BMI was calculated as weight (kg) divided by the square of height (m). Waist and hip circumferences were taken in standing position. Waist circumference was measured midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteal region (7). Systolic and diastolic blood pressure was measured following a standard procedure using a conventional mercurial sphygmomanometer. Three measurements were taken from each subject, with at least 1 min between each measurement. The mean of these three was calculated and used in subsequent analyses. Blood glucose concentration was analyzed by the glucose dehydrogenase oxidation method (6). Triglycerides, total cholesterol, and HDL levels were measured using standard methods by Vitros 950 analyzer (Johnson & Johnson, USA) (15). All the biochemical measurements were done following routine laboratory protocols. Twins in a pair were examined on the same day. Phenotype data were transformed using the Box-Cox transformation for normality (16).

The Danish part of the study was approved by the Danish regional ethical committees and the Danish Data Protection Agency. Journal number: S-VF-19970271.

The Chinese twins

The Chinese twin sampling for this study was based on the Qingdao Twin Registry established in 2001 at the Qingdao Center for Disease Control and Prevention (Qingdao CDC). Twins were sampled through the local disease control network and residence registry. The exclusion criterion removed those who were pregnant, breastfeeding, had known diabetes and/or cardiovascular disease, or were taking weight-reducing medications within one month, and incomplete twin pairs. Body measures and biochemical variables were collected in a total of 325 pairs of twins (183 MZ twin pairs and 142 DZ twin pairs, among them, 104 pairs of male and 158 pairs of female like-sex twins, 63 pairs of opposite-sex twins) with a mean age of 40.5 years (range 18-69 years, birth cohorts 1937-1988) (10). Zygosity of like-sex twin pairs was determined by DNA testing using 16 short tandem repeat DNA markers at the central laboratory of Qingdao Blood Bank.

All anthropometric measures and blood pressure were taken using standard procedures as described for the Danish twins. All twins underwent a 10-12 h overnight fast before blood sampling. Serum and plasma were separated from blood cells within 30 min after blood collection and kept at low temperature before sending to routine laboratory testing. Triglycerides, total cholesterol, and HDL were measured on the Semi-automatic Analyzer (Hitachi 7600, Japan). Plasma glucose concentrations were analyzed by the glucose dehydrogenase oxidation method similar to that in the Danish study. Co-twins were examined on the same day. The examination of twin pairs for this study was carried in 2006-2007 in Qingdao, China. Phenotypes with skewed distribution were log transformed to ensure approximate normality.

The Chinese part of the study was approved by the local ethics committee at Qingdao CDC, Qingdao, China.

Data analysis

The Pearson’s product-moment correlation coefficients were calculated for intra-pair phenotype correlation in MZ and DZ twins. To adjust for the effects of age and sex, a linear regression model was fitted for each phenotype with age and sex as covariates and residuals recorded. The recorded residuals were then used for calculation of correlation coefficients.

Twin modeling and model comparison were performed using the free software package Mx (http://www.vcu.edu/mx). The modeling process started with introducing the full ACE model with A standing for the additive genetic variance, C the common, and E the unique environmental variances. Based on the full ACE model, nested models were also fitted by dropping the C (AE model), the A (CE model), or both (E model) components for best model selection. For a phenotype with MZ correlation more than twice of the DZ correlation, a full ADE model was also fitted with D representing the dominant genetic variance, and likewise compared with its nested models (AE, DE, and E). Comparisons on performances between the full models and its nested models were done using the likelihood ratio test. The likelihood ratio test calculates twice the difference in the log likelihoods for the full and the nested models which can be approximated by a chi-squared distribution with degree of freedom equaling the difference in the number of parameters in the two corresponding models. When no statistical significance is observed between two models, the parsimonious one was preferred.
The Akaike Information Criterion (17) was used as a measurement for goodness of fit. In all model fitting, age and sex were included as covariates to adjust for their effects on each of the phenotypes.

Robustness of analysis was assessed using bootstrap resampling to calculate empirical 95% confidence intervals (CIs) for the estimated parameters. Statistical significance for the differences in heritability estimates in the Danish and the Chinese twins were determined by comparing the empirical 95% CIs for each phenotype with $P < 0.05$ for those phenotypes showing no overlap.

### Results

The basic statistics of the two study samples are shown in Table 1. The mean ages are close (38 for Danish twins; 41 for Chinese twins) and their 5-95 percentiles overlap nearly fully (Danish twins 20-56 years; Chinese twins 24-56 years). An obvious difference in body weight is observed with the Danes weighing about 9 kg more than the Chinese on average, although there is no difference in BMI (both samples are 24). The means for waist and hip circumferences and waist-to-hip ratio (WHR) as well as HDL are very close in the two samples. The Chinese tend to have a higher fasting glucose level than the Danish twins (5.2 versus 4.8). Contrary to this, the Danish twins have slightly higher levels of triglyceride and total cholesterol (means of 1.25 and 5.37 in Danish but 1.05 and 4.53 in Chinese twins, respectively). However, their distributions as indicated by corresponding 5-95 percentiles overlap largely. In general, blood pressure in Chinese twins is higher than in Danish twins with a moderate difference in diastolic blood pressure (122 versus 116) and a slight difference in systolic blood pressure (122 versus 116) both with broad overlaps.

In Table 2, we show the phenotype correlation in MZ and DZ twin pairs in the two samples. The correlation coefficients were calculated from residuals in regression models with age and sex as covariates. Except for triglyceride and systolic blood pressure in the Chinese twins, all other phenotypes showed significantly higher correlation in MZ than in DZ twins in both samples suggesting potential genetic controls over these phenotypes. The results are also shown by the star plots in Figure 1 displaying the high correlations in MZ twins (thick outer curve) and low correlations in DZ twins (thin inner curve) both in Danish (left panel) and Chinese (right panel) samples. The different curve shapes between the two samples suggest differential genetic and environmental contributions in the observed phenotypes. To further illustrate this point, Figure 2 plots the correlation coefficients for the Danish against the Chinese twins with the estimates in MZ and DZ twins linked by a solid line for each phenotype (MZ on top of DZ twins). Except for hip circumference, the lines for all other anthropometric phenotypes go in parallel with the diagonal meaning similar MZ to DZ correlation patterns in Danish and Chinese twins. Whereas, the lines for fasting glucose, HDL, and systolic blood pressure diverge from the diagonal indicating different correlation patterns due perhaps to distinct genetic and environmental influences in the two populations.

Next, we fitted the full ACE model as well as its nested AE, CE, and E models and selected the best fitting model for each phenotype in both Danish and Chinese samples. For the four phenotypes with MZ correlation more than two times DZ correlation (Table 2, systolic blood pressure in Danish twins, waist and hip circumferences in Chinese twins, body weight in both samples), the full ADE model was additionally fitted. Our results showed that the ADE models performed only equally well as the ACE models for the four phenotypes which all had the AE model as their best fitting models after model comparison. The ACE models were, hence, preferred in the subsequent analysis. In Table 3, we show parameter estimates with 95% CIs in the best fitting models for the two samples. Heritability estimates for each phenotype in both samples are also plotted in Figure 3. For body measures, weight and hip circumference showed partially or slightly overlapping 95% CIs for their additive genetic effects between Danish and Chinese twins. The rests (BMI, WHR, and waist circumference) have large overlaps on their CIs for additive genetic effects in the two samples. For biochemical variables, the estimated additive genetic effect for fasting blood glucose is notably higher in Chinese (0.64, 95% CI: 0.55-0.71) than in Danish (0.26, 95% CI: 0.23-0.29) twins. On the opposite, triglyceride has different models
that best fitted to Danish (AE model) and Chinese (CE model) data suggesting a moderate genetic control (0.49, 95% CI: 0.41-0.56) in Danish but no genetic influence in Chinese twins. Likewise, the lipids phenotype HDL displayed more genetic control in the Danish (0.68, 95% CI: 0.62-0.72) than in the Chinese (0.59, 95% CI: 0.50-0.67) twins with partially overlapping 95% CIs. Higher genetic effect in Danish twins is also observed for systolic blood pressure with a heritability estimate of 0.71 (95% CI: 0.66-0.75) as compared with that in the Chinese twins 0.53 (95% CI: 0.43-0.62).

In addition to the best fitting models, comparison on heritability estimates was also done on the full ACE models fitted to each dataset. The results showed more or less similar patterns of difference. In Figure 4, the heritability estimates in the Danish twins are plotted against that in the Chinese twins for the full ACE models (Figure 4a) and the best fitting models (Figure 4b) with similar patterns retained.

**Discussion**

We have conducted a comparative study on genetic influences on multiple metabolic phenotypes covering anthropometric measures, biochemical variables (lipids and glucose levels), and blood pressure in two populations of distinct ethnic, environmental, and cultural

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**TABLE 2 Phenotype correlation in Danish and Chinese twin pairs**

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Danish twins</th>
<th>Chinese twins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>n</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.76</td>
<td>616</td>
</tr>
<tr>
<td>Weight</td>
<td>0.84</td>
<td>618</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.85</td>
<td>617</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.78</td>
<td>617</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>0.77</td>
<td>616</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>0.55</td>
<td>607</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.46</td>
<td>599</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.81</td>
<td>603</td>
</tr>
<tr>
<td>High density lipoprotein</td>
<td>0.69</td>
<td>600</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.73</td>
<td>615</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.71</td>
<td>618</td>
</tr>
</tbody>
</table>

DZ, dizygotic twins; MZ, monozygotic twins; n, number of subjects.
Heritability of 11 Metabolic Phenotypes

Different from body measures, our heritability estimates on other phenotypes vary between the two populations. Striking differences were found for fasting blood glucose and triglycerides with the former displaying higher genetic control in Chinese twins while the latter with higher genetic contribution in Danish twins in both the best fitting and the full ACE models (Figures 3 and 4). The significant disparity in the genetic influences on these phenotypes could imply that the Chinese may have developed a more efficient genetic mechanism on glucose while the Danes a higher genetic regulation in lipids metabolism. Our hypothesis seems also to be supported by results for other phenotypes. For example, both Danish and Chinese samples have the AE model as the best fitting model for HDL but with higher additive genetic component in Danish (0.68) than in Chinese (0.59) twins with only slightly overlapping percentiles (Danish: 0.62-0.72; Chinese: 0.50-0.67) (Table 3, Figure 3). Another example, a higher heritability for total cholesterol is estimated in Danish (0.58) than in Chinese (0.51) twins, although their percentiles overlap considerably. The contrasting genetic effects on glucose and lipids phenotypes in Chinese and Danish twins lead us to postulate a varying genetic basis of metabolic phenotypes in Eastern and Western populations adapted to different environmental conditions, e.g., diets (the Chinese food dominated by cereals but Danish food by animal products). As molecular evidence, Spielman et al. (18) reported differential gene expression patterns among ethnic groups and pointed out that population differences in prevalence of complex diseases such as diabetes could be partly accounted for by the differential gene expression levels. Tan and Tai (19) studied the relationship between food and genetic influences on biomarkers of metabolic disorders among ethnic groups in Singapore and reported association between HDL concentration and polymorphisms in several regulatory genes. To reconfirm our hypothesis, more work including molecular genetic studies needs to be done to elucidate the differential regulatory network in metabolic pathway genes in the two populations.

Table 3: Parameter estimates with 95% confidence intervals in the best fitting models for Danish and Chinese twins

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Danish twins</th>
<th>Chinese twins</th>
<th>Overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a^2</td>
<td>c^2</td>
<td>e^2</td>
</tr>
<tr>
<td>BMI</td>
<td>0.78 (0.74–0.81)</td>
<td>0.22 (0.19–0.26)</td>
<td>0.75 (0.69–0.80)</td>
</tr>
<tr>
<td>WT</td>
<td>0.83 (0.80–0.86)</td>
<td>0.17 (0.14–0.20)</td>
<td>0.77 (0.71–0.82)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.69 (0.56–0.84)</td>
<td>0.16 (0.10–0.28)</td>
<td>0.64 (0.55–0.72)</td>
</tr>
<tr>
<td>WC</td>
<td>0.78 (0.74–0.81)</td>
<td>0.22 (0.19–0.26)</td>
<td>0.77 (0.70–0.82)</td>
</tr>
<tr>
<td>HIP</td>
<td>0.59 (0.45–0.74)</td>
<td>0.19 (0.00–0.32)</td>
<td>0.78 (0.72–0.83)</td>
</tr>
<tr>
<td>FG</td>
<td>0.26 (0.23–0.29)</td>
<td>0.27 (0.23–0.42)</td>
<td>0.47 (0.41–0.55)</td>
</tr>
<tr>
<td>TG</td>
<td>0.49 (0.41–0.56)</td>
<td>0.51 (0.44–0.59)</td>
<td>0.51 (0.43–0.59)</td>
</tr>
<tr>
<td>TC</td>
<td>0.58 (0.44–0.73)</td>
<td>0.23 (0.09–0.35)</td>
<td>0.19 (0.16–0.23)</td>
</tr>
<tr>
<td>HDL</td>
<td>0.68 (0.62–0.72)</td>
<td>0.32 (0.28–0.38)</td>
<td>0.58 (0.50–0.67)</td>
</tr>
<tr>
<td>SP</td>
<td>0.71 (0.66–0.75)</td>
<td>0.29 (0.25–0.34)</td>
<td>0.53 (0.43–0.62)</td>
</tr>
<tr>
<td>DP</td>
<td>0.52 (0.35–0.69)</td>
<td>0.18 (0.02–0.33)</td>
<td>0.30 (0.26–0.36)</td>
</tr>
</tbody>
</table>

a^2, c^2, and e^2: standardized genetic, common environmental, and unique environmental variances; BMI, body mass index; DP, diastolic blood pressure; FG, fasting blood glucose; HIP, hip circumference; SP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHR, waist-to-hip ratio; WT, weight.
FIGURE 3 Error bar plot for heritability estimates and their 95% CIs with a bold bar for Danish and a thin bar for Chinese twins plotted adjacently for each of the 11 phenotypes. BMI, body mass index; WT, weight; WHR, waist-to-hip ratio; WC, waist circumference; HIP, hip circumference; FG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; SP, systolic blood pressure; DP, diastolic blood pressure.

FIGURE 4 Additive heritability estimates from the ACE model (a) and the best fitting model (b), Danish plotted against Chinese twins. Phenotypes deviate from the diagonal show differential heritability patterns in the two populations. BMI, body mass index; WT, weight; WHR, waist-to-hip ratio; WC, waist circumference; HIP, hip circumference; FG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; SP, systolic blood pressure; DP, diastolic blood pressure.
Note that the differential additive genetic effects in fasting blood glucose in Danish and Chinese twins were observed from two different best-fitting models, i.e., the ACE model in Danish and the AE model in Chinese twins. It is necessary to point out that the differential patterns remained even when the same ACE model was fitted (Figure 4) to each sample with 0.58 (95% CI: 0.28-0.71) for additive genetic, 0.06 (95% CI: 0-0.32) for common environmental, and 0.36 (95% CI: 0.29-0.46) for unique environmental effects in the Chinese twins. In addition to the differential genetic effects, results from our model fitting also revealed significant differential contributions to fasting glucose level by the common environment (Table 3). Similar patterns were also observed in hip circumference, WHR, total cholesterol, and diastolic pressure, all with ACE in Danish and AE in Chinese twins as their best-fitting models. Although our limited data cannot provide a clear explanation to the phenomenon, perhaps one could link it with the poor rearing environments that were more or less similar across families shared by the Chinese twins about 20-30 years ago.

Of the two blood pressure variables, the one that exhibits differential genetic control between Danish and Chinese twins is the systolic blood pressure, one of the important intermediate phenotypes of metabolic disorders. Our estimates in the best-fitting models (the AE model for both samples) indicate that more variation in systolic blood pressure can be ascribed to genetic regulation in the Danish than in the Chinese twins (Table 3, Figure 3). In other words, more unique environmental influences are responsible for the systolic blood pressure variation in Chinese than in Danish twins. As one possible explanation, we emphasize the increasing proportion of Chinese under stress from both work and family life in a currently fast-transitioning society particularly during the last 20 years, an important condition that has been associated with hypertension (20). One more explanation could be the dietary salt intake which is usually high in the northern part of China (21) where Qingdao is located. In fact, studies have already shown the significant association between high dietary salt intake and inflated blood pressure especially systolic blood pressure (22). If this was the case, our results could suggest the high importance in promoting healthy individual behavior, life style and diet habits in hypertension control, and prevention in the Chinese population.

In this study, statistical significances in heritability comparison were determined by comparing the 95% CIs, the only output for inferring errors in heritability estimates produced empirically by the Mx software package. Although two nonoverlapping intervals provide strong evidence of statistical difference with a type I error rate of less than 5%, slightly overlapping intervals may not rule out the possibility of statistical difference which means that our conclusions are conservative due to method used. Moreover, our study is based on relatively small sample sizes available especially for the Chinese samples from northern China which limits generalization of our results. We expect that a clearer pattern of differences can be revealed in larger studies to come.

In summary, results from our study show differential genetic and environmental contributions to important phenotypes related to metabolic disorders in populations under distinct cultural and environmental conditions.

Acknowledgments

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References


Probing genetic overlap in the regulation of systolic and diastolic blood pressure in Danish and Chinese twins

Shuxia Li1, Zengchang Pang2, Dongfeng Zhang3, Haiping Duan2, Jacob von Bornemann Hjelmborg4, Qihua Tan1,4,5, Torben Arvid Kruse1,5 and Kirsten Ohm Kyvik6

Although the phenotypic correlation between systolic blood pressure (SBP) and diastolic blood pressure (DBP) is well known, the genetic basis for the correlation has rarely been investigated. The aim of this paper is to examine the genetic overlap between SBP and DBP by fitting bivariate models to Danish and Chinese twins and comparing ethnic differences between the two samples. Our estimates revealed a high proportion of additive genetic components shared by both SBP and DBP in Danish (0.71, 95% confidence interval (CI): 0.65–0.75) and Chinese (0.62, 95% CI: 0.50–0.71) twins with no statistically significant ethnic differences. The estimated genetic component in phenotypic correlation could serve to guide molecular genetic studies searching for genetic variants that affect both SBP and DBP. The bivariate model also estimated genetic and environmental contributions to SBP and DBP separately, with an overall pattern of higher genetic regulation or heritability in Danish (0.72, 95% CI: 0.67–0.76 for SBP; 0.70, 95% CI: 0.65–0.75 for DBP) than in Chinese (0.54, 95% CI: 0.44–0.63 for SBP; 0.57, 95% CI: 0.47–0.65 for DBP) twins and a higher contribution from unique environmental factors in Chinese compared with Danish twins. The estimated contribution from unique environmental factors suggests that promoting healthy lifestyles may provide an efficient way of controlling high blood pressure, particularly in the Chinese population.

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Keywords: blood pressure; Chinese; Danish; genetic overlap; twins

INTRODUCTION
Cardiovascular disease is the leading cause of mortality and morbidity worldwide. High blood pressure is known as the most important modifiable risk factor for cardiovascular disease.1 Efficient control and adequate management of high blood pressure can therefore have a significant impact on public health. Blood pressure is a complex phenotype involving many control systems operated by physiological mechanisms under multiple genetic and environmental regulations2 that may have a diverse pattern of regulation across different ethnic populations. For example, genetic factors have been estimated to account for as much as 71% of the total variation in systolic blood pressure (SBP) in Danish twins3 and 58% in Chinese twins.4 The moderate to high influence genetic factors have on blood pressure has led to a considerable amount of research aimed at identifying the molecular genetic basis of blood pressure regulation; recent research activity has intensified, driven by the rapid development of high-throughput analyses at the genome level.

SBP represents the maximum exerted pressure on the vessels when the heart contracts (when the heart is beating); diastolic blood pressure (DBP) is the minimum pressure in the vessels when the heart relaxes (when the heart is resting between beats and refilling with blood). Often more attention is given to SBP as a major risk factor for cardiovascular disease.5 Although SBP and DBP represent the maximum and minimum blood pressure in the arteries, respectively, results from recent genome-wide association studies (GWAS) indicate that there are genetic variants for SBP and DBP shared in both eastern and western populations.6,7 For example, of the 29 genetic variants associated with SBP at a genome-wide significance level (P<2.5e−08) reported in the study by Ehret et al.,7 23 variants were also significantly associated with DBP. Genome-wide association study is based on common surrogate variants with limited depth of genomic coverage,8 and as a result more research is needed to provide an overall picture of the extent of genetic relatedness between SBP and DBP. Based on an existing Danish–Chinese collaboration network on twin research, the aim of this study is to determine the genetic factors responsible for blood pressure and to evaluate the phenotypic correlation between SBP and DBP across populations using bivariate models9 to compare Danish and Chinese twins (representing western populations).
and eastern populations, respectively). The results of analyses of Danish and Chinese twins may provide mutual validation and will be compared to determine whether there are ethnic differences in the role that genetics and environment have in blood pressure variation and correlation.

**METHODS**

**The Danish twin samples**

The collection of Danish twins used in the present study has been described previously.

**The Chinese twin samples**

The sampling of Chinese twins was based on the Qingdao Twin Registry established in 2001 at the Qingdao Center for Disease Control and Prevention (Qingdao CDC). Twins were identified through the local disease control network and residence registry. Exclusion criteria removed those who were pregnant, breastfeeding, those with diabetes and/or cardiovascular disease or who had been taking blood pressure or weight-reducing medications within the previous month. Data were collected from a total of 325 pairs of twins (183 MZ twin pairs and 142 DZ twin pairs). Zygosity of same-sex twin pairs was determined by DNA testing using 16 short tandem repeat DNA markers at the local laboratory of the Qingdao Blood Bank.

Blood pressure was measured following a standard procedure using a conventional mercurial sphygmomanometer. Three measurements were taken from each subject, with at least 1 min between each measurement. The mean of these three values was calculated and used in subsequent analyses. Body weight was measured to the nearest 0.1 kg using a standing beam scale. Height was measured to the nearest centimeter using a vertical scale with a horizontal moving headboard. Body mass index was calculated as weight (kg) divided by the square of height (m). The Danish part of the study was approved by the Danish regional ethics committees and the Danish Data Protection Agency with journal number: S-VF-19970271.

**Bivariate modeling on twins**

We introduced a bivariate model jointly to analyze SBP and DBP (Figure 1) using the typical structural equation modeling (s.e.m.) approach. In this approach, variances in the observed traits (SBP and DBP) and their covariance are decomposed into latent additive genetic (A), shared environmental (C), and unique environmental (E) components. Based on variance estimates, the genetic correlation \( r_g \) can be calculated as \( r_g = \sqrt{\text{cov}_{\text{SBP}}(\text{SBP}) \times \text{cov}_{\text{DBP}}(\text{DBP})} \) where \( \text{var}_{\text{SBP}}(\text{SBP}) \) and \( \text{var}_{\text{DBP}}(\text{DBP}) \) are the additive genetic variance of SBP and DBP, respectively, and \( \text{cov}_{\text{SBP}}(\text{SBP}, \text{DBP}) \) is their genetic covariance. Likewise, correlations for common environmental \( r_c \) and unique environmental \( r_u \) factors can be calculated. In s.e.m., parameter estimates are obtained using a fitting function that minimizes the difference between the observed covariance matrix and the expected covariance matrix implied by the model, assuming bivariate normality.

Model fitting was carried out separately for Danish and Chinese twins using the Mx software package, with age, sex and body mass index included as covariates. Considering that data on twins reared together does not contain enough information to tease out the contrasting effects of common environmental (C) and dominant genetic (D) components, our model fitting started with consideration of both the bivariate ACE and the bivariate ADE models, with the latter including additive genetic, dominant genetic and unique environmental effects. As a rule of thumb, the presence of dominance can be recognized when DZ correlation is much lower than half of MZ correlation. Based on twin correlation in the results section, the ADE model can be recognized when DZ correlation is more than half of MZ correlation. Based on variance estimates, the model was rejected because each DZ correlation was more than half of MZ correlation. Furthermore, the presence of dominance can be recognized when DZ correlation is much lower than half of MZ correlation. Based on twin correlation in the results section, the ADE model was rejected because each DZ correlation was more than half of the corresponding MZ correlation (Table 2), which does not support the presence of a dominant genetic effect. The full bivariate ACE and its nested bivariate

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**Table 1 Descriptive statistics for Danish and Chinese twins**

<table>
<thead>
<tr>
<th>Basic statistics</th>
<th>Median</th>
<th>2.5–97.5% Quantiles</th>
<th>Median</th>
<th>2.5–97.5% Quantiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38</td>
<td>19–57</td>
<td>40</td>
<td>20–60</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>24</td>
<td>18.8–32</td>
<td>24</td>
<td>18.6–32</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>116</td>
<td>94–146</td>
<td>120</td>
<td>100–160</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>68</td>
<td>50–90</td>
<td>80</td>
<td>60–106</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

**Table 2 Within- and cross-trait correlation in MZ and DZ twin pairs**

<table>
<thead>
<tr>
<th></th>
<th>Danish twins</th>
<th>Chinese twins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP¹–DBP¹</td>
<td>SBP²–DBP²</td>
</tr>
<tr>
<td>MZ 309</td>
<td>0.71</td>
<td>0.71</td>
</tr>
<tr>
<td>DZ 447</td>
<td>0.49</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Abbreviations: DZ, dizygotic; MZ, monozygotic.

¹Cross twin within-trait correlation.
²Cross twin cross-trait correlation.
models (AE, CE, E) were fitted. The performances of the different bivariate models were compared using Akaike’s information criterion,\textsuperscript{14} such that the model with the lowest Akaike’s information criterion reflected the best balance between goodness-of-fit and parsimony. Meanwhile, the fitting of nested models enabled assessment of statistical significance of the A, C and E component using the likelihood ratio ($\chi^2$) test at a significance level of $P<0.05$. This led to the most parsimonious (or ‘best fitting’) model in which the pattern of variances and covariances was explained by as few parameters as possible. In all model fitting, bootstrap resampling with 100 replicates was used to obtain 95% confidence intervals (CIs) for the estimated model parameters.

RESULTS
Table 1 presents the basic characteristics of the Danish and Chinese twins. The age structures of the two samples are very similar, with the median age of the Chinese twins approximately 2 years older than that of the Danish twins. Both the median and the 2.5–97.5 percentiles for body mass index are very similar in the two samples. Both SBP and DBP tend to be higher in the Chinese twins than in the Danish twins; however, there is large overlap in the 2.5–97.5 percentiles. Table 2 shows the correlation within and cross traits in MZ and DZ twins. All correlation coefficients are highly significant with $P<0.001$. The phenotypic correlation coefficients are all higher in MZ twins than DZ twins, which suggest genetic control over the variation (within-trait) in SBP, DBP as well as over the covariation (cross-trait) between SBP and DBP. In all cases, DZ correlation is greater than half of the MZ correlation, suggesting that the ACE models can be appropriately applied to the data.

We first fitted the full bivariate ACE models to Danish and Chinese twins (Table 3). The Danish twins displayed higher genetic but lower unique environmental impact on SBP and DBP covariance when compared with the Chinese twins. The proportion of genetic contribution to SBP–DBP correlation in Danish twins was 0.56 (95% CI: 0.34–0.76); the value in the Chinese twins was 0.39 (95% CI: 0.02–0.71). The difference is not statistically significant because of the overlapping 95% CIs. In Table 4, we also show the estimated genetic and unique environmental components to SBP–DBP correlation. The figure shows that the correlation between SBP and DBP is mainly the result of genetic factors in both samples; these genetic factors are more pronounced in Danish than in Chinese twins. However, unique environment has a larger role in the SBP–DBP correlation in Chinese twins than Danish twins. Neither result is statistically significant with respect to ethnic difference with overlapping 95% CIs. In Table 4, we also show the estimated $r_g$ and $r_e$ from the best models fitted to Danish and Chinese twins. In contrast to the proportion of genetic and unique environmental influences on the correlation between SBP and DBP, a higher $r_e$ was estimated in Chinese twins (0.81, 95% CI: 0.73–0.88) compared with the Danish twins (0.71, 95% CI: 0.67–0.75), whereas a higher $r_g$ was observed in Danish twins (0.70, 95% CI: 0.65–0.75) than in Chinese twins (0.63, 95% CI: 0.54–0.71). None of these results was statistically significant between the two samples.

In addition, the best fitting model estimated significant differences in the additive genetic and unique environmental influences on SBP with no overlapping 95% CIs for the genetic (0.72, 95% CI: 0.67–0.76 in Danish; 0.54, 95% CI: 0.44–0.63 in Chinese twins) and unique environmental (0.28, 95% CI: 0.24–0.33 in Danish; 0.46, 95% CI: 0.38–0.51 in Chinese twins).

Table 3 Variance components and correlation coefficients for A, C and E from bivariate models fitted to blood pressure in Danish and Chinese twins

<table>
<thead>
<tr>
<th></th>
<th>Danish twins</th>
<th></th>
<th>Chinese twins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$A$</td>
<td>$C$</td>
<td>$E$</td>
<td>$A$</td>
</tr>
<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>8.3e–03</td>
<td>1.1e–03</td>
<td>3.9e–03</td>
<td>4.2e–03</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.62 (0.45, 0.75)</td>
<td>0.09 (0.00, 0.24)</td>
<td>0.29 (0.25, 0.34)</td>
<td>0.28 (0.01, 0.60)</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>1.1e–02</td>
<td>4.2e–03</td>
<td>6.8e–03</td>
<td>8.4e–03</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.50 (0.32, 0.66)</td>
<td>0.19 (0.03, 0.34)</td>
<td>0.31 (0.26, 0.37)</td>
<td>0.45 (0.17, 0.62)</td>
</tr>
<tr>
<td><strong>SBP–DBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>6.8e–03</td>
<td>1.7e–03</td>
<td>3.6e–03</td>
<td>4.8e–03</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.56 (0.34, 0.76)</td>
<td>0.14 (−0.04, 0.33)</td>
<td>0.30 (0.24, 0.37)</td>
<td>0.39 (0.02, 0.71)</td>
</tr>
<tr>
<td>$r_g$, $r_c$, $r_e$</td>
<td>0.71 (0.57, 0.81)</td>
<td>0.79 (−1.00, 1.00)</td>
<td>0.71 (0.65, 0.76)</td>
<td>0.80 (0.80, 1.00)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>26.76</td>
<td>1.99</td>
<td>262.25</td>
<td>3.72</td>
</tr>
<tr>
<td>$P$-value</td>
<td>2.3e–07</td>
<td>0.16</td>
<td>5.58e–09</td>
<td>5.38e–02</td>
</tr>
</tbody>
</table>

Abbreviations: DBP, diastolic blood pressure; $r_c$, common environmental correlation; $r_e$, unique environmental correlation; $r_g$, genetic correlation; SBP, systolic blood pressure.
0.37–0.56 in Chinese twins) components. The same pattern of genetic and environmental influences holds for DBP with slightly overlapping 95% CIs of the A and E components (Table 4 and Figure 3).

**DISCUSSION**

The results of bivariate modeling of blood pressure data taken from Danish and Chinese twins both indicated that there was a significant genetic component in the phenotypic correlation between SBP and DBP, which exists in two populations of distinct ethnic, cultural and environmental circumstances. The consistent findings in our separate analyses of two independent samples serve as mutual validation of our conclusion. In an earlier study, Schieken et al. reported a genetic proportion of 74% in SBP–DBP covariance in Caucasian boys and girls with an average age of 11 years. The result of that study is very close to our estimate of 0.71 in Danish twins, which represent middle-aged Caucasians. The existence of shared genetic regulation of SBP and DBP was also reported in a very recent study of Brazilian nuclear families; in that study, the genetic correlation coefficient was 0.67. Recent genetic association studies have reported common single-nucleotide polymorphisms that significantly affect both SBP and DBP, and provide molecular evidence for shared genetic mechanisms that

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**Table 4 Variance and covariance components in the best fitting models for SBP and DBP in Danish and Chinese twins**

<table>
<thead>
<tr>
<th></th>
<th>Danish twins</th>
<th></th>
<th>Chinese twins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>E</td>
<td>A</td>
<td>E</td>
</tr>
<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>9.4e-03</td>
<td>3.8e-03</td>
<td>8.0e-03</td>
<td>6.7e-03</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.72 (0.67, 0.76)</td>
<td>0.28 (0.24, 0.33)</td>
<td>0.54 (0.44, 0.63)</td>
<td>0.46 (0.37, 0.56)</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>1.53e-02</td>
<td>6.5e-03</td>
<td>1.06e-02</td>
<td>8.0e-03</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.70 (0.65, 0.75)</td>
<td>0.30 (0.25, 0.35)</td>
<td>0.57 (0.47, 0.65)</td>
<td>0.43 (0.35, 0.53)</td>
</tr>
<tr>
<td><strong>SBP–DBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariance</td>
<td>8.6e-03</td>
<td>3.5e-03</td>
<td>7.5e-03</td>
<td>4.6e-03</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.71 (0.65, 0.75)</td>
<td>0.29 (0.24, 0.35)</td>
<td>0.62 (0.50, 0.71)</td>
<td>0.38 (0.29, 0.50)</td>
</tr>
<tr>
<td>r_e</td>
<td>0.71 (0.67, 0.75)</td>
<td>0.70 (0.65, 0.75)</td>
<td>0.81 (0.73, 0.88)</td>
<td>0.63 (0.54, 0.71)</td>
</tr>
</tbody>
</table>

Abbreviations: DBP, diastolic blood pressure; r_e, common environmental correlation; r_u, unique environmental correlation; r_g, genetic correlation; SBP, systolic blood pressure.

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**Figure 2** Estimated proportions with 95% confidence intervals (CIs) of A and E components for the covariance of systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the best fitting model. The thick error bars are for Danish twins and the thin error bars are for Chinese twins.

**Figure 3** Estimated proportion of variance components for systolic blood pressure (SBP) (left panel) and diastolic blood pressure (DBP) (right panel) in the best fitting model with 95% confidence intervals (CIs) empirically obtained using bootstrap resampling. The thick error bars are for Danish twins and the thin error bars are for Chinese twins.
are responsible for SBP and DBP coregulation in both Caucasians and East Asians.\textsuperscript{6,7} The estimated proportion of genetic components that are responsible for blood pressure correlation may help determine potential heritable genetic variations that have not yet been identified by linkage and association studies. Considering that genetic regulation may have a large role in the correlation between SBP and DBP, additional functional and static genetic variants that affect both SBP and DBP are expected to be discovered using functional genomics, next-generation sequencing and epigenetic approaches.\textsuperscript{12,13}

A general pattern of ethnic differences in genetic and environmental contributions to blood pressure is revealed by the results of the present study. Genetic control over blood pressure and the correlation between SBP and DBP are higher in the Danish sample, whereas more unique environmental influences were observed in the Chinese sample (Tables 3 and 4). As displayed in Figures 2 and 3, although the same pattern holds for SBP, DBP and their correlation, the results are only significant for SBP, with no overlapping 95% CIs of the A and E components between Danish and Chinese twins. In view of the limited sample sizes, particularly in the case of Chinese twins, large-scale studies and more detailed genetic investigations are required to determine whether there is a clear pattern of ethnic differences in factors influencing blood pressure.

As indicated in Table 4, the estimated additive genetic component responsible for SBP–DBP covariance is higher in Danish twins (0.71, 95% CI: 0.65–0.75) than in Chinese twins (0.62, 95% CI: 0.50–0.71). However, the results of genetic correlation show the opposite result, with higher genetic correlation in Chinese twins (0.81, 95% CI: 0.73–0.88) than Danish twins (0.71, 95% CI: 0.67–0.75). Although these differences are not statistically significant when considering their overlapping confidence intervals, the reversed pattern does deserve some discussion. Note that the estimated proportion of the additive genetic component is a measure of the relative importance of genetic factors taking into account environmental effects, whereas genetic correlation measures the absolute covariation between the additive genetic variances of SBP and DBP. A high genetic correlation could indicate a shared genetic basis for SBP and DBP in a number of genetic variants, that is, pleiotropic genes. A strong common genetic basis can be diluted when large environmental effects interfere at the time the level of genetic contribution is calculated, which might be the case for the Chinese twins. More molecular genetic studies are required to verify our hypothesis.

In both full and nested models, the unique environmental effects on blood pressure and the correlation between SBP and DBP are significant in both Danish and Chinese twins, which suggests that the effects of the individual environment is crucial for explaining blood pressure variation and covariance. Although the importance of unique environmental factors was consistent between the sample groups, we note that there is a noticeable ethnic difference in the two samples, with a higher proportion of environmental factors in Chinese than Danish twins. This is clearly demonstrated in Figure 3, which indicates that the 95% CIs of the E component do not overlap for SBP (P<0.05) and only slightly overlap for DBP (borderline significance). These results indicate the special importance of individual environmental factors, including social and behavioral factors such as lifestyle (hazardous stress and stressful life), occupation (sedentary work) and dietary habits (high dietary sodium)\textsuperscript{10,11} in controlling blood pressure in the Chinese population.

Overall, we have identified relatively high genetic overlap between SBP and DBP, providing strong evidence that there are common genetic mechanisms responsible for regulating SBP and DBP in both Chinese (eastern) and Danish (western) populations. Our results also suggest that individual environment has a large role in blood pressure variation; measures that promote healthy lifestyles should efficiently reduce the risk of hypertension and cardiovascular problems, particularly in the Chinese population.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGEMENTS
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Paper III


Submitted.
Genetic and environmental regulation on longitudinal change of metabolic phenotypes in Danish and Chinese adult twins

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Abstract

The rate of change in metabolic phenotypes can be highly indicative of metabolic disorders and disorder-related modifications. We analyzed data from longitudinal twin studies on multiple metabolic phenotypes in Danish and Chinese twins representing two populations of distinct ethnic, cultural, social-economic backgrounds and geographical environments. The study covered a relatively large sample of 502 pairs of Danish adult twins followed up for a long period of 12 years with a mean age at intake of 38 years (range: 18 - 65) and a total of 181 Chinese adult twin pairs traced for about 7 years with a mean baseline age of 39.5 years (range: 23 - 64). The classical twin models were fitted to the longitudinal change in each phenotype (Δphenotype) to estimate the genetic and environmental contributions to the variation in Δphenotype. Moderate to high contributions by the unique environment were estimated for all phenotypes in both Danish (from 0.51 for low density lipoprotein up to 0.72 for triglycerides) and Chinese (from 0.41 for triglycerides up to 0.73 for diastolic blood pressure) twins; low to moderate genetic components were estimated for long-term change in most of the phenotypes in Danish twins except for triglycerides and hip circumference. Compared with Danish twins, the Chinese twins tended to have higher genetic control over the longitudinal changes in lipids (except high density lipoprotein) and glucose, higher unique environmental contribution to blood pressure but no genetic contribution to longitudinal change in body mass traits. Overall, our results emphasize the major contribution of unique environment to the observed intra-individual variation in all metabolic phenotypes in both samples, and meanwhile suggest differential patterns of genetic and common environmental regulation on changes over time in blood lipids and glucose, body mass traits and blood pressure across the two samples.

Key words: longitudinal change, metabolic phenotypes, Danish, Chinese, twins
Introduction

Metabolic disorders including obesity, impaired glucose regulation, dyslipidemia, and hypertension are among the top preventable risk factors in association with the development of type 2 diabetes and atherosclerotic cardiovascular disease (CVD) (1-3). Metabolic phenotypes e.g. blood glucose, blood lipids, blood pressure, and body mass index are, similar to most complex traits, regulated by both genetic and environmental factors with the interaction between them as central to the development of metabolic abnormality and diseases (4-5). In the literature, the genetic and environmental contributions to metabolic phenotypes and metabolic diseases have been intensively studied using family (6-8) and twin (9-14) data with interesting results pointing to significant genetic and environmental regulation on the level of metabolic phenotypes.

Although the levels of metabolic traits are good indicators of an individual’s health status and provide the basis for defining and diagnosis of metabolic abnormality, the rate of change of metabolic phenotypes in adults may be more indicative of disorder-related modifications and disease onset (15) given the fact that metabolic profiles are age dependent (16,17). This is true not only for metabolism but also for human health in general. For example, based on 10 years follow-up data, Turiano et al. (18) reported that longitudinal change in personality traits are associated with self-reported health outcomes. From a public health point of view, studying the individual progression of metabolic traits may contribute to personalized approaches in health care and for disease control. Likewise, dissecting the genetic and environmental regulation of the intra-individual change over time in metabolic traits can help with development of more effective strategies for intervention and prevention. Although the genetic and environmental influences on the level of metabolic phenotypes have been intensively studied using twin methods, twin studies on longitudinal change in metabolic phenotypes have been
are due to high expense, loss of follow up, and long waiting time in prospective investigations. Nevertheless, there have been several longitudinal twin studies on metabolic phenotypes (19-26). However, these studies were either limited to body mass traits (weight, height and BMI) (19-23) or focused on phenotype stability or correlation over ages instead of longitudinal change in metabolic phenotypes (24-26).

Based on a Danish-Chinese collaboration on twin studies, we collected longitudinal data on multiple metabolic phenotypes in Danish and Chinese adult twins. The two samples of twins represent western (Danish) and eastern (Chinese) populations of distinct ethnic, cultural, socio-economic background, and geographical environment, providing unique data for twin modeling on longitudinal patterns of multiple metabolic phenotypes within and for comparison across the two samples.

**Materials and methods**

*The Danish cohort*

The Danish cohort consisted of twins originally recruited from the nationwide, population-based Danish Twin Registry during 1997-2000 to examine the importance of genes, family environment and individual environment for the development of insulin resistance, abdominal obesity and cardiovascular risk factors, i.e. the GEMINAKAR study as described previously (10, 27-29). This cohort was followed up during 2010 to 2012. At baseline, only twins without self-reported cardiovascular disease and diabetes could participate. The cohort consisted of 756 twin pairs who underwent an extensive full day clinical examination of a variety of phenotypes. The mean age of the participants at baseline was 38 years, range 18-67 years. At follow-up, a total of 502 complete pairs (545 females, 459 males), hereof
226 monozygotic (MZ) pairs, 189 same-sex dizygotic (DZ) pairs, and 87 opposite-sex DZ pairs were available.

Twin zygosity was determined using microsatellite markers. All participants gave their written informed consent to participate and the local scientific committee of the Region of Southern Denmark (baseline, S-VF-19970271; follow-up, S-20090065) and Danish Data protection Board (baseline, 1999-1200-441; follow-up, 2009-41-2990) approved the study protocol.

**The Chinese cohort**

The Chinese twin samples were collected by the Qingdao Twin Registry at the Qingdao Center for Disease Control and Prevention (Qingdao CDC). Twins were recruited through residence registry and the local disease control network of Qingdao CDC. Twins were excluded from the study due to pregnancy, breastfeeding, known diabetes and/or cardiovascular disease and use of weight-reducing medicaments within one month. Only complete twin pairs were included. A total of 181 twin pairs (101 MZ and 80 DZ pairs) were identified with longitudinal measurements taken about 7 years apart with a mean baseline age of 39.5 years and an age range of 23-64 years. Among them 245 were females and 117 were males. Twin zygosity was determined by DNA testing using 16 short tandem repeat DNA markers at the central laboratory of Qingdao Blood Bank. The Chinese study was approved by the local ethics committee at Qingdao CDC, Qingdao, China.

**Phenotypes studied**

Both studies covered 12 metabolic phenotypes, i.e. total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting blood glucose (GLU), body weight (WT), body mass index (BMI), waist (WAIST), hip (HIP) circumference, waist-hip ratio (WHR),
systolic (SBP) and diastolic (DBP) blood pressure. BMI was calculated as weight (kilogram, kg) divided by the square of height (meter, m) with body weight measured using a standing beam scale and to the nearest 0.1 kg and height measured using a vertical scale with a horizontal moving headboard and to the nearest centimeter. Waist and hip circumferences were taken in standing position with waist circumference measured midway between the lowest rib and the iliac crest, and hip circumference measured over the widest part of the gluteal region (12, 28). Systolic and diastolic blood pressure measurements were taken after at least 5 minutes of rest following a standard procedure using a conventional mercurial sphygmomanometer. The mean of three measurements (taken at least 1 minute apart) was calculated and used in subsequent analyses. Blood glucose concentration was analyzed by the glucose dehydrogenase oxidation method (27) both for Danish and Chinese blood samples. TG, TC, HDL and LDL were measured using standard clinical biochemical methods for Danish twins (29) and for Chinese twins (12).

Statistical analysis and twin modeling

Statistical significance of longitudinal change for each phenotype was assessed by fitting the mixed effect kinship model (30-31) as $y = \beta_0 + \beta_1 \text{age} + \beta_2 \text{sex} + \beta_3 \text{time} + \text{random effects}$, with fixed effects for time (0 for baseline or time 1, 1 for time 2), baseline age at intake and sex, and random effect for twin pairing to account for the intra-pair genetic correlation in MZ and DZ twins.

Twin correlation on longitudinal change in each phenotype, i.e. $\Delta \text{phenotype} = \text{Phenotype}_{\text{time}2} - \text{Phenotype}_{\text{time}1}$ was estimated by calculating the intra twin pair correlation coefficient (ICC) as

$$\rho = \frac{\sigma_s^2}{\sigma_s^2 + \sigma_e^2}$$

with $\sigma_s^2$ defined as the between pair variance and $\sigma_e^2$ as the within pair variance in
Δphenotype. A higher ICC in MZ twins as compared with DZ twins provides an indication of genetic influence on Δphenotype.

Univariate twin models were fitted to Δphenotype for each of the 12 metabolic traits with sex, age and baseline phenotype level at intake adjusted. For each phenotype, the variance for Δphenotype was decomposed into additive genetic (A), dominant genetic (D), common or shared environmental (C), and unique environmental (E) components. In the model, referred to as ACDE model, C and D cannot be estimated simultaneously in the classical twin study of MZ and DZ twins reared together (32-33). Two separate models containing the A, C and E components (the ACE model) and the A, D and E components (the ADE model) were fitted with the latter usually preferred when the MZ correlation is more than double the DZ correlation for a given phenotype. Based on the full ACE model, nested models were also fitted by dropping the C (AE model), the A (CE model), or both (E model) components for best model selection. Likewise two nested models (AE and E) were fitted for comparison with the full ADE model. The DE model was excluded because it is biologically implausible considering that the dominant genetic effects alone are not enough to explain the very low DZ correlation when compared with MZ correlation (34). The likelihood ratio test (LRT) was applied for comparisons on performances between the full models and their nested models. In model comparison, the parsimonious model was preferred when no statistical significance was observed between the two models. Goodness of fit was assessed by calculating the Akaike Information Criterion (AIC) (35). Robustness of parameter estimates was assessed using bootstrap re-sampling for empirically calculating the 95% confidence intervals (CIs).

In all the analysis, each phenotype value was log transformed to minimize possible skewed phenotype distribution. Phenotype values 3 standard deviations above or below the phenotype mean
were set to missing (36). The mixed effect kinship model was fitted using the free R package *kinship* (http://cran.r-project.org/src/contrib/Archive/kinship/). The calculation of ICC and twin modeling were done by using the free R package *mets* (http://cran.r-project.org/web/packages/mets/index.html).

**Results**

*Longitudinal change in metabolic phenotypes*

Table 1 shows the basic statistics (mean, 95% CI) for all the metabolic phenotypes at each time point together with the statistical testing on their rate of change with age and sex adjusted. For the Danish twins, statistically significant increases in phenotype values over the follow-up were observed for most phenotypes except for TG and WHR which decreased over time (Table 1). The patterns of longitudinal change in Danish twins are further illustrated in Figure 1 by plotting, for each phenotype, the residuals of phenotype measurement from the mixed effect model at time 1 (horizontal axis) against that at time 2 (vertical axis). Samples with no longitudinal change in the phenotype would fall along the diagonal line from bottom left-hand to top right-hand corner. Patterns that deviate from the diagonal line would indicate increase (above the diagonal) or decrease (below the diagonal) in the phenotype values over the follow-up time. The longitudinal patterns observed in Figure 1 correspond well to the test results from the mixed effects model in Table 1 for the Danish twins. Moreover, Figure 1 reveals no obvious difference in the longitudinal change between females (red dots) and males (black dots) after adjustment for sex in the regression model.

For the Chinese twins, 8 phenotypes increased and 2 (TC, LDL) decreased significantly while the mean level of TG and HDL remained unchanged (Table 1). Figure 2 is the scatter plot for time
points 1 and 2 plotted in the same way as Figure 1. The figure visualizes the results from Table 1 with no obvious sex difference in the longitudinal trends after adjustment for age and sex.

Twin correlation on longitudinal change of phenotypes

Table 2 presents the ICCs on Δphenotype in MZ and DZ twins for both samples. In the Danish twins, all phenotypes showed higher ICC in MZ than in DZ twins (mostly more than double). In the Chinese twins, ICCs for the body mass phenotypes showed only slight differences between MZ and DZ twins with BMI, WAIST and HIP displaying even higher ICC in DZ than in MZ twins, an indication of limited or lack of genetic control over the longitudinal change in anthropometrical traits in adult Chinese. The blood biochemical measurements and blood pressure all had higher ICCs in MZ than in DZ Chinese twins. Overall, ICCs on Δphenotype provided evidence of genetic contributions to longitudinal changes in most phenotypes.

Twin modelling on longitudinal change of phenotype

Considering the ICCs for many of the phenotypes in MZ were more than double in DZ twins, both ACE and ADE models were subsequently fitted to each Δphenotype with the model of lower AIC chosen as the full model. Tables 3 and 4 show the parameter estimates in the full model and statistics for the best fitting model for each of the 12 phenotypes in Danish and Chinese twins respectively.

For the Danish twins, 9 phenotypes were fitted by the ADE model and only three by the ACE model (TG, HIP, SBP) as expected from the ICCs in Table 2. The full models (both ACE and ADE) estimated moderate to high E component in Δphenotype from 0.49 for LDL to 0.71 for TG. In contrast, only low to moderate effects were estimated for the A, C or D components. For most estimates of A, C or D components, the 95% CIs included zero suggesting the need for fitting nested models and for best
model selection. In Table 3, the best performance models were also selected for each Δphenotype with the AE model best fitted to 10 phenotypes and the CE model to TG and HIP only. According to AICs in Table 3, all the best fitting models outperformed their full models except for HDL and WHR but none showed statistically significant difference to its full model. Note that the best fitting models again estimated moderate A (from 0.36 for SBP to 0.49 for LDL) and low to moderate C (from 0.28 for TG to 0.43 for HIP) components but, in contrast, high estimates for the E component (from 0.51 for LDL to 0.72 for TG) (Table 5).

For the Chinese twins, 11 phenotypes were fitted by the ACE model with only SBP by ADE model (Table 4). Similar to the Danish twins, all full models estimated moderate to high E component (from 0.32 for HDL to 0.70 for SBP); very low to moderate A, C or D components. As shown in Table 4, all the selected sub-models outperformed their corresponding full models with lower AICs and none displayed significant statistical difference in the goodness of fit as compared to the full models.

Different from the Danish twins that predominantly had the AE model as the best, the various categories of metabolic phenotypes for the Chinese twins were best fitted by different sub-models with the AE model fitted to biochemical measurements (i.e. lipids and glucose except HDL) and the CE model fitted to all body mass traits (Tables 4-5). Moreover, the estimated A components for lipids and glucose traits tended to be higher in Chinese twins (from 0.54 for TC and LDL to 0.59 for TG) than in Danish twins (from 0.39 for GLU to 0.49 for LDL) except for HDL (CE model in Chinese twins). Note that, although most of the body mass traits in the two samples were best fitted by different models (AE for Danish and CE for Chinese), one trait, i.e. HIP had consistently the CE model as the best in both Danish and Chinese twins with comparable estimates (Table 5). The blood pressure traits in the Chinese twins were best fitted by the AE model for SBP and CE model for DBP, both with very high E
estimates (SBP: 0.72; DBP: 0.73) in comparison with other phenotypes in Chinese twins and also with Danish twins (SBP: 0.64; DBP: 0.53).

**Discussion**

By treating the longitudinal change in a phenotype, i.e. Δphenotype as the phenotype of interest, we have conducted a longitudinal twin study on multiple metabolic phenotypes in samples from two populations of distinct ethnic background and social environmental circumstances. One important finding in the study is the moderate to high contribution by the unique environment to intra-individual longitudinal change (Δphenotype) for all 12 phenotypes (Table 5). In contrast, the genetic component has only low to moderate contribution to Δphenotype. In summary, the results emphasize the high importance of unique environmental factors in controlling intra-individual variation in metabolic phenotypes over time, both in Danish and in Chinese twins.

In addition to the unique environmental factors, the shared environments were also involved in regulating the longitudinal change of all body mass traits in Chinese twins which is in contrast to the Danish twins. The phenomenon could indicate, in addition to the unique environment, early-life shared environment could also play an important role in determining the individual trajectory of body mass traits in the Chinese adult twins.

In the best fitting models, the genetic estimates to longitudinal changes for lipids (except HDL) and glucose tended to be higher in Chinese than in Danish twins with only a slight overlap in the 95% CIs for GLU (0.58, 95% CI: 0.46-0.70 in Chinese versus 0.39, 95% CI: 0.28-0.49 in Danish twins) but with considerable overlaps for TC and LDL (Table 5). Although the difference lacks strong statistical support for each phenotype considered individually, the same trend of difference (i.e. A for Chinese >
A for Danish) in biochemical measurements could reflect interesting population differences in the genetic and environmental control over longitudinal patterns of lipids and glucose. In view of the fact that Chinese twins were sampled from the countryside (the suburban area of Qingdao) where staple food is characterized by high cereal and vegetable content, we assume that the Chinese samples might be more restricted in their dietary pattern being much more plant based than the Danish twins who had more sufficient food supply and in general have a dietary pattern that includes high intakes of animal-based food (37-38). As a result, the difference in dietary habits between the two samples could lead to low unique environmental and high genetic components in the variation of Δphenotype for blood lipids and glucose in the Chinese twins, while high unique environmental and low genetic components in the Danish twins. Future cross-population studies should help to validate our hypothesis.

Among the lipid phenotypes, no genetic component was estimated in the best fitting models (i.e. the CE model) for ΔTG in Danish twins and for ΔHDL in the Chinese twins. The absence of genetic control over ΔTG is consistent with Friedlander et al (39) who reported no genetic influence on the change in TG over a 10-year follow-up in an adult cohort of American twins. In another longitudinal study conducted in adult Caucasian twins, Goode et al. (40) reported no significant proportion of genetic contribution to the variation in age-related change of blood lipids. Different from the results in adult twins, Middelberg et al. (41) and Zhang et al. (25) estimated significant genetic component in age-related change on the level of blood lipids in adolescent Caucasian and Chinese twins respectively. Comparing the results for adolescent and adult twins, one could conclude that the genes are important in regulating the developmental changes of blood lipids in adolescent twins in both Eastern and Western populations while in adult twins, the genetic effects on long-term change for some lipids (here
TG in Danish twins and HDL in Chinese twins) could have been weakened and perhaps with population-specific patterns.

Different from the lipids and glucose phenotypes, longitudinal change in blood pressure was highly attributable to unique environment in Chinese twins (0.72 for SBP, 95% CI: 0.50-0.93; 0.73 for DBP, 95% CI: 0.56-0.90). The estimates of E components for the change of blood pressures in Danish twins (0.64 for SBP, 95% CI: 0.53-0.74; 0.53 for DBP, 95% CI: 0.44-0.63) tended to be lower than that for the Chinese twins although their 95% CIs overlapped. On the other hand, the Danish twins had moderate genetic influence on change in blood pressure (0.36, 95% CI: 0.26-0.47 for SBP; 0.47, 95%CI: 0.37-0.56 for DBP) which is in contrast to the lower or no genetic control in the Chinese twins (0.28 for SBP, 95% CI: 0.07-0.50; 0 for DBP). Although the different patterns could be ascribed to the different ethnic (genetic) backgrounds, we emphasize the importance of salt consumption in China especially in the rural areas. According to a global epidemiological study, China was on the top rank in dietary salt intake (42) and the intake level changed with age (43). We think that the high contribution by unique environment to change in blood pressure can be, at least, partly explained by the high level of salt intake in China considering the significantly positive association of salt intake with blood pressure (42). If this was the case, the high salt intake affects not only the variation in the level (44-47) but also in the variation in the rate of change of blood pressure in the Chinese population.

This longitudinal twin analysis was based on intra-pair correlation (ICC) on Δphenotype over time, which did not necessarily imply significant longitudinal change at the mean level of the phenotype. For example, the mean level of HDL in the Chinese twins had no significant change over time (p=0.72) (Table 1) but high ICCs on ΔHDL were estimated for both MZ (0.68, 95% CI: 0.40-0.84) and DZ (0.63, 95% CI: 0.35-0.81) (Table 2) twins which led to a best fitting CE model (Table 5). In
another example, the mean level for TG had no significant change over the follow-up period in the Chinese twins (p=0.38) (Table 1). However, the estimated ICCs for TG were higher in MZ (0.58, 95% CI: 0.40-0.72) than in DZ (0.37, 95% CI: 0.16-0.55) twins (Table 2) suggesting genetic involvement in the intra-individual change over time. This was confirmed by the best fitted AE model for TG (Table 4) with A counting for 59% of the total variance in ΔTG (Table 5). If our results on TG are validated, we can assume that there could have been genetic polymorphisms inherited by different twin pairs that up- or down-regulated TG with comparable effect size in each direction and eventually resulted in no change at the overall mean level of TG. The two examples demonstrate the need to differentiate the genetic and environmental control over intra-individual longitudinal change from that over the level of a phenotype. By applying the growth curve model to multi-wave measurements on BMI, Hjelmborg et al. (22) were able to show that the genetic variants for longitudinal change in BMI were likely to be different from those affecting the level of BMI. The estimated genetic regulation of intra-individual phenotype variation could help to guide genetic association studies to look specifically for genes that influence the rate of change in multiple metabolic phenotypes.

It is necessary to point out the limitations of our comparative study. First, the Danish and Chinese twins were followed up for different length of time which could possibly result in different degrees of accumulation for the random environmental effects on Δphenotype. Although the estimated E components in Table 5 do not seem to support the speculation, we cannot rule out the existence of differential accumulation effects in the two samples. Second, in both samples, phenotypes were measured at only two time points. Because of that, it was impossible to fit the growth curve model and thus it was unable to estimate the genetic and environmental effects in the correlation between rate of change and baseline level of the phenotypes. Third, the small sample size of Chinese twins could be
responsible for the insignificant results on longitudinal change in TG and HDL and likewise higher uncertainty in the parameter estimates for the twin models.

To summarize, our study has identified differential genetic and common environmental regulation of different clusters of metabolic phenotypes between Danish and Chinese twins, and at the same time emphasizes the major role of individual unique environment in controlling the long-term intra-individual variation in metabolic phenotypes in both Danish and Chinese twins.

Acknowledgements

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References


Figure 1. Star plots showing the residuals of phenotype measurement from the mixed effect model at time 1 (horizontal axis) against that at time 2 (vertical axis) for 12 phenotypes in Danish twins (females in red; males in black). TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GLU: fasting blood glucose; BMI: body mass index; Waist: waist circumference; Hip: hip circumference; WHR: waist-hip-ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Figure 2. Star plots showing the residuals of phenotype measurement from the mixed effect model at time 1 (horizontal axis) against that at time 2 (vertical axis) for 12 phenotypes in Chinese twins (females in red; males in black). TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GLU: fasting blood glucose; BMI: body mass index; Waist: waist circumference; Hip: hip circumference; WHR: waist-hip-ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Table 1. Basic statistics for baseline/wave 1 and follow up/wave 2 in Danish and Chinese twins

<table>
<thead>
<tr>
<th>Traits</th>
<th>Danish Twins (n=1004)</th>
<th>Chinese Twins (n=362)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, 1</td>
<td>2.5-97.5% Quatiles</td>
</tr>
<tr>
<td>TC</td>
<td>5.36</td>
<td>3.30 - 8.00</td>
</tr>
<tr>
<td>TG</td>
<td>1.27</td>
<td>0.60 - 2.90</td>
</tr>
<tr>
<td>HDL</td>
<td>1.52</td>
<td>0.86 - 2.50</td>
</tr>
<tr>
<td>LDL</td>
<td>3.29</td>
<td>1.50 - 5.56</td>
</tr>
<tr>
<td>GLU</td>
<td>4.76</td>
<td>3.90 - 6.00</td>
</tr>
<tr>
<td>WT</td>
<td>73.18</td>
<td>50.30 - 100.29</td>
</tr>
<tr>
<td>BMI</td>
<td>24.43</td>
<td>19.03 - 32.61</td>
</tr>
<tr>
<td>WAIST</td>
<td>83.77</td>
<td>66.00 - 108.00</td>
</tr>
<tr>
<td>HIP</td>
<td>96.40</td>
<td>81.00 - 115.00</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87</td>
<td>0.72 - 1.04</td>
</tr>
<tr>
<td>SBP</td>
<td>116.36</td>
<td>93.33 - 145.33</td>
</tr>
<tr>
<td>DBP</td>
<td>68.16</td>
<td>50.67 - 90.00</td>
</tr>
</tbody>
</table>

TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GLU: fasting blood glucose; WT: body weight; BMI: body mass index; WAIST: waist circumference; HIP: hip circumference; WHR: waist-hip-ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.
### Table 2. ICC for longitudinal change of each phenotype in Danish and Chinese twins

<table>
<thead>
<tr>
<th>Trait</th>
<th>ICC(_{MZ}) (n=452)</th>
<th>95% CI</th>
<th>ICC(_{DZ}) (n=552)</th>
<th>95% CI</th>
<th>ICC(_{MZ}) (n=202)</th>
<th>95% CI</th>
<th>ICC(_{DZ}) (n=160)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.50*</td>
<td>0.38 - 0.60</td>
<td>0.18</td>
<td>0.06 - 0.29</td>
<td>0.54</td>
<td>0.37 - 0.67</td>
<td>0.29</td>
<td>0.06 - 0.50</td>
</tr>
<tr>
<td>TG</td>
<td>0.29</td>
<td>0.17 - 0.41</td>
<td>0.26</td>
<td>0.14 - 0.38</td>
<td>0.58</td>
<td>0.40 - 0.72</td>
<td>0.37</td>
<td>0.16 - 0.55</td>
</tr>
<tr>
<td>HDL</td>
<td>0.47*</td>
<td>0.36 - 0.57</td>
<td>0.12</td>
<td>0.00 - 0.24</td>
<td>0.68</td>
<td>0.40 - 0.84</td>
<td>0.63</td>
<td>0.35 - 0.81</td>
</tr>
<tr>
<td>LDL</td>
<td>0.51*</td>
<td>0.39 - 0.61</td>
<td>0.20</td>
<td>0.08 - 0.31</td>
<td>0.53</td>
<td>0.35 - 0.68</td>
<td>0.34</td>
<td>0.12 - 0.53</td>
</tr>
<tr>
<td>GLU</td>
<td>0.42*</td>
<td>0.29 - 0.53</td>
<td>0.12</td>
<td>0.00 - 0.24</td>
<td>0.56</td>
<td>0.38 - 0.71</td>
<td>0.41</td>
<td>0.19 - 0.59</td>
</tr>
<tr>
<td>WT</td>
<td>0.40*</td>
<td>0.27 - 0.51</td>
<td>0.17</td>
<td>0.06 - 0.28</td>
<td>0.38</td>
<td>0.18 - 0.54</td>
<td>0.35</td>
<td>0.15 - 0.53</td>
</tr>
<tr>
<td>BMI</td>
<td>0.41*</td>
<td>0.29 - 0.52</td>
<td>0.16</td>
<td>0.04 - 0.27</td>
<td>0.27(\Delta)</td>
<td>0.05 - 0.46</td>
<td>0.35</td>
<td>0.15 - 0.51</td>
</tr>
<tr>
<td>WAIST</td>
<td>0.41*</td>
<td>0.31 - 0.51</td>
<td>0.13</td>
<td>-0.02 - 0.27</td>
<td>0.37(\Delta)</td>
<td>0.10 - 0.59</td>
<td>0.46</td>
<td>0.23 - 0.64</td>
</tr>
<tr>
<td>HIP</td>
<td>0.44</td>
<td>0.33 - 0.54</td>
<td>0.41</td>
<td>0.29 - 0.52</td>
<td>0.36(\Delta)</td>
<td>0.11 - 0.57</td>
<td>0.42</td>
<td>0.18 - 0.62</td>
</tr>
<tr>
<td>WHR</td>
<td>0.48*</td>
<td>0.37 - 0.57</td>
<td>0.13</td>
<td>-0.01 - 0.26</td>
<td>0.49</td>
<td>0.22 - 0.68</td>
<td>0.43</td>
<td>0.21 - 0.61</td>
</tr>
<tr>
<td>SBP</td>
<td>0.36</td>
<td>0.22 - 0.48</td>
<td>0.20</td>
<td>0.08 - 0.30</td>
<td>0.30*</td>
<td>0.05 - 0.51</td>
<td>0.10</td>
<td>-0.17 - 0.36</td>
</tr>
<tr>
<td>DBP</td>
<td>0.49*</td>
<td>0.37 - 0.59</td>
<td>0.17</td>
<td>0.06 - 0.28</td>
<td>0.32</td>
<td>0.05 - 0.55</td>
<td>0.23</td>
<td>-0.01 - 0.45</td>
</tr>
</tbody>
</table>

* ICC\(_{MZ}\)>2 times ICC\(_{DZ}\); \(\Delta\) ICC\(_{MZ}\)<ICC\(_{DZ}\). TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GLU: fasting blood glucose; WT: body weight; BMI: body mass index; WAIST: waist circumference; HIP: hip circumference; WHR: waist-hip-ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.
<table>
<thead>
<tr>
<th>Traits</th>
<th>Full models</th>
<th>Parameter estimates</th>
<th>AIC</th>
<th>Best models</th>
<th>AIC</th>
<th>Likelihood Ratio Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>ADE</td>
<td>0.21 (0.00-0.66)</td>
<td>0.29 (0.00-0.77)</td>
<td>0.50 (0.40-0.60)</td>
<td>-786.50</td>
<td>AE -787.10</td>
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<tr>
<td>TG</td>
<td>ACE</td>
<td>0.06 (0.00-0.38)</td>
<td>0.23 (0.00-0.49)</td>
<td>0.71 (0.59-0.82)</td>
<td>771.89</td>
<td>CE 770.02</td>
</tr>
<tr>
<td>HDL</td>
<td>ADE</td>
<td>0.02 (0.00-0.48)</td>
<td>0.45 (0.00-0.94)</td>
<td>0.53 (0.43-0.63)</td>
<td>-495.54</td>
<td>AE -494.54</td>
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<tr>
<td>LDL</td>
<td>ADE</td>
<td>0.29 (0.00-0.74)</td>
<td>0.22 (0.00-0.70)</td>
<td>0.49 (0.39-0.59)</td>
<td>-121.07</td>
<td>AE -122.24</td>
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<tr>
<td>GLU</td>
<td>ADE</td>
<td>0.07 (0.00-0.51)</td>
<td>0.35 (0.00-0.82)</td>
<td>0.58 (0.47-0.69)</td>
<td>-2078.00</td>
<td>AE -2078.23</td>
</tr>
<tr>
<td>WT</td>
<td>ADE</td>
<td>0.29 (0.00-0.72)</td>
<td>0.10 (0.00-0.56)</td>
<td>0.60 (0.50-0.71)</td>
<td>-2154.63</td>
<td>AE -2156.46</td>
</tr>
<tr>
<td>BMI</td>
<td>ADE</td>
<td>0.22 (0.00-0.65)</td>
<td>0.19 (0.00-0.65)</td>
<td>0.59 (0.48-0.70)</td>
<td>-2140.59</td>
<td>AE -2142.01</td>
</tr>
<tr>
<td>WAIST</td>
<td>ADE</td>
<td>0.10 (0.00-0.60)</td>
<td>0.31 (0.00-0.83)</td>
<td>0.59 (0.50-0.68)</td>
<td>-2115.32</td>
<td>AE -2116.18</td>
</tr>
<tr>
<td>HIP</td>
<td>ACE</td>
<td>0.06 (0.00-0.32)</td>
<td>0.38 (0.17-0.60)</td>
<td>0.56 (0.47-0.66)</td>
<td>-2843.69</td>
<td>CE -2845.53</td>
</tr>
<tr>
<td>WHR</td>
<td>ADE</td>
<td>0.03 (0.00-0.49)</td>
<td>0.45 (0.00-0.93)</td>
<td>0.52 (0.43-0.61)</td>
<td>-2336.23</td>
<td>AE -2335.66</td>
</tr>
<tr>
<td>SBP</td>
<td>ACE</td>
<td>0.33 (0.02-0.63)</td>
<td>0.03 (0.00-0.27)</td>
<td>0.64 (0.52-0.76)</td>
<td>-2071.94</td>
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<td>DBP</td>
<td>ADE</td>
<td>0.20 (0.00-0.63)</td>
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<td>0.51 (0.41-0.61)</td>
<td>-1940.72</td>
<td>AE -1940.98</td>
</tr>
</tbody>
</table>

TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GLU: fasting blood glucose; WT: body weight; BMI: body mass index; WAIST: waist circumference; HIP: hip circumference; WHR: waist-hip-ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Table 4: Full models for longitudinal change in the Chinese twins and statistics for best fitting models

<table>
<thead>
<tr>
<th>Traits</th>
<th>Full models</th>
<th>Parameter estimates</th>
<th>AIC</th>
<th>Best models</th>
<th>AIC</th>
<th>Likelihood Ratio Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>ACE</td>
<td>A 0.49 (0.00-0.97)</td>
<td>0.05 (0.00-0.49)</td>
<td>0.46 (0.33-0.59)</td>
<td>-362.90</td>
<td>AE -364.85</td>
</tr>
<tr>
<td>TG</td>
<td>ACE</td>
<td>A 0.42 (0.00-0.84)</td>
<td>0.16 (0.00-0.53)</td>
<td>0.42 (0.29-0.54)</td>
<td>-364.85</td>
<td>AE -362.90</td>
</tr>
<tr>
<td>HDL</td>
<td>ACE</td>
<td>A 0.09 (0.00-0.38)</td>
<td>0.58 (0.33-0.84)</td>
<td>0.32 (0.22-0.42)</td>
<td>-131.95</td>
<td>CE -133.53</td>
</tr>
<tr>
<td>LDL</td>
<td>ACE</td>
<td>A 0.38 (0.00-0.84)</td>
<td>0.15 (0.00-0.56)</td>
<td>0.47 (0.33-0.61)</td>
<td>-154.34</td>
<td>AE -155.86</td>
</tr>
<tr>
<td>GLU</td>
<td>ACE</td>
<td>A 0.31 (0.00-0.75)</td>
<td>0.25 (0.00-0.63)</td>
<td>0.44 (0.30-0.57)</td>
<td>-495.38</td>
<td>AE -495.96</td>
</tr>
<tr>
<td>WT</td>
<td>ACE</td>
<td>A 0.05 (0.00-0.51)</td>
<td>0.33 (0.00-0.70)</td>
<td>0.62 (0.46-0.79)</td>
<td>-965.73</td>
<td>CE -967.69</td>
</tr>
<tr>
<td>BMI</td>
<td>ACE</td>
<td>A 0.00 (0.00-0.00)</td>
<td>0.31 (0.18-0.44)</td>
<td>0.69 (0.56-0.82)</td>
<td>-969.89</td>
<td>CE -971.89</td>
</tr>
<tr>
<td>WAIST</td>
<td>ACE</td>
<td>A 0.00 (0.00-0.00)</td>
<td>0.42 (0.27-0.57)</td>
<td>0.58 (0.43-0.73)</td>
<td>-546.17</td>
<td>CE -548.17</td>
</tr>
<tr>
<td>HIP</td>
<td>ACE</td>
<td>A 0.00 (0.00-0.00)</td>
<td>0.39 (0.24-0.55)</td>
<td>0.61 (0.45-0.76)</td>
<td>-841.78</td>
<td>CE -843.78</td>
</tr>
<tr>
<td>WHR</td>
<td>ACE</td>
<td>A 0.11 (0.00-0.62)</td>
<td>0.38 (0.00-0.77)</td>
<td>0.51 (0.31-0.72)</td>
<td>-677.13</td>
<td>CE -678.97</td>
</tr>
<tr>
<td>SBP</td>
<td>ADE</td>
<td>A 0.10 (0.00-1.00)</td>
<td>0.20 (0.00-1.00)</td>
<td>0.70 (0.48-0.93)</td>
<td>-437.99</td>
<td>AE -439.87</td>
</tr>
<tr>
<td>DBP</td>
<td>ACE</td>
<td>A 0.17 (0.00-0.81)</td>
<td>0.15 (0.00-0.64)</td>
<td>0.68 (0.44-0.92)</td>
<td>-360.47</td>
<td>CE -362.19</td>
</tr>
</tbody>
</table>

TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GLU: fasting blood glucose; WT: body weight; BMI: body mass index; WAIST: waist circumference; HIP: hip circumference; WHR: waist-hip-ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Table 5: Parameter estimates in best fitting models in the Danish and Chinese twins

<table>
<thead>
<tr>
<th>Traits</th>
<th>Danish twins</th>
<th></th>
<th></th>
<th>Chinese twins</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Best model</td>
<td>A</td>
<td>C/D</td>
<td>E</td>
<td>A</td>
<td>C/D</td>
</tr>
<tr>
<td>TC</td>
<td>AE</td>
<td>0.48 (0.38-0.57)</td>
<td>0.52 (0.43-0.62)</td>
<td>AE</td>
<td>0.54 (0.42-0.66)</td>
<td>0.46 (0.34-0.58)</td>
</tr>
<tr>
<td>TG</td>
<td>CE</td>
<td>0.28 (0.19-0.36)</td>
<td>0.72 (0.64-0.81)</td>
<td>AE</td>
<td>0.59 (0.48-0.71)</td>
<td>0.41 (0.29-0.52)</td>
</tr>
<tr>
<td>HDL</td>
<td>AE</td>
<td>0.44 (0.33-0.54)</td>
<td>0.56 (0.46-0.67)</td>
<td>CE</td>
<td>0.66 (0.57-0.74)</td>
<td>0.34 (0.26-0.43)</td>
</tr>
<tr>
<td>LDL</td>
<td>AE</td>
<td>0.49 (0.39-0.59)</td>
<td>0.51 (0.41-0.61)</td>
<td>AE</td>
<td>0.54 (0.42-0.67)</td>
<td>0.46 (0.33-0.58)</td>
</tr>
<tr>
<td>GLU</td>
<td>AE</td>
<td>0.39 (0.28-0.49)</td>
<td>0.61 (0.51-0.72)</td>
<td>AE</td>
<td>0.58 (0.46-0.70)</td>
<td>0.42 (0.30-0.54)</td>
</tr>
<tr>
<td>WT</td>
<td>AE</td>
<td>0.39 (0.28-0.49)</td>
<td>0.61 (0.51-0.72)</td>
<td>CE</td>
<td>0.36 (0.24-0.49)</td>
<td>0.64 (0.51-0.76)</td>
</tr>
<tr>
<td>BMI</td>
<td>AE</td>
<td>0.39 (0.29-0.49)</td>
<td>0.61 (0.51-0.71)</td>
<td>CE</td>
<td>0.31 (0.18-0.44)</td>
<td>0.69 (0.56-0.82)</td>
</tr>
<tr>
<td>WAIST</td>
<td>AE</td>
<td>0.40 (0.30-0.49)</td>
<td>0.60 (0.51-0.70)</td>
<td>CE</td>
<td>0.42 (0.27-0.57)</td>
<td>0.58 (0.43-0.73)</td>
</tr>
<tr>
<td>HIP</td>
<td>CE</td>
<td>0.43 (0.35-0.50)</td>
<td>0.57 (0.50-0.65)</td>
<td>CE</td>
<td>0.39 (0.24-0.55)</td>
<td>0.61 (0.45-0.76)</td>
</tr>
<tr>
<td>WHR</td>
<td>AE</td>
<td>0.45 (0.36-0.54)</td>
<td>0.55 (0.46-0.64)</td>
<td>CE</td>
<td>0.45 (0.31-0.60)</td>
<td>0.55 (0.40-0.69)</td>
</tr>
<tr>
<td>SBP</td>
<td>AE</td>
<td>0.36 (0.26-0.47)</td>
<td>0.64 (0.53-0.74)</td>
<td>AE</td>
<td>0.28 (0.07-0.50)</td>
<td>0.72 (0.50-0.93)</td>
</tr>
<tr>
<td>DBP</td>
<td>AE</td>
<td>0.47 (0.37-0.56)</td>
<td>0.53 (0.44-0.63)</td>
<td>CE</td>
<td>0.27 (0.10-0.44)</td>
<td>0.73 (0.56-0.90)</td>
</tr>
</tbody>
</table>

TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GLU: fasting blood glucose; WT: body weight; BMI: body mass index; WAIST: waist circumference; HIP: hip circumference; WHR: waist-hip-ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.