ESTIMATING THE EFFECTS OF OVERWEIGHT DURATION, SODIUM INTAKE AND GENETIC VARIANTS ON HYPERTENSION RISK AMONG FILIPINO WOMEN IN CEBU, PHILIPPINES

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Abstract

Nanette R. Lee: Estimating the effects of overweight duration, sodium intake and genetic variants on hypertension risk among Filipino women in Cebu, Philippines
(Under the direction of Dr. Linda S. Adair)

Hypertension makes the largest contribution to the worldwide burden of cardiovascular diseases. It is a multi-factorial disease that develops from the complex interplay of environmental and genetic factors. Asians are a special concern because they tend to develop hypertension at a lower body mass index (BMI) compared to Caucasian populations and have been reported to consume high amounts of sodium. This research examined the roles of overweight duration, variants of the angiotensinogen (*AGT* Met235Thr), angiotensin converting enzyme (*ACE* insertion/deletion (I/D) in intron 16) and alpha adducin (*ADD1* Gly460Trp) genes, and high sodium consumption on hypertension risk among adult Filipino women in Cebu, Philippines. Additionally, this research explored potential heterogeneity of effects according to different genetic and environmental characteristics. We used data gathered by the Cebu Longitudinal Health and Nutrition Survey (CLHNS). Aside from detailed individual, household and community level socio-demographic characteristics, this data contained genetic information, repeated anthropometric measures that span over two decades, dietary measures including sodium intake, and repeated blood pressure measurements. Using poisson regression with robust error variance, we found that overweight duration influenced the 5-year cumulative incidence (2002-2007) of hypertension independent of 2002 BMI. Results of logistic regression analyses showed that *AGT* Met235Thr appeared to influence hypertension risk

regardless of age, BMI and presence of the other variants. We found possible age-dependent effects for the *ACE* and *ADD1* variants. Our findings also suggest that the effect of high sodium intake on hypertension is: (1) enhanced in women with the *ADD1* TrpTrp genotype but not evident in women with the GlyGly or GlyTrp genotypes; (2) increased with increasing age, in women who had never been overweight and those who were smokers; and (3) decreased with increasing BMI. Overall, this research found that the duration of being overweight, selected genetic variants and sodium intake may influence hypertension risk in adult Filipino women. We observed potential heterogeneity of effects and support the importance of conducting context-dependent analyses. Results of this research may be used to design a more comprehensive hypertension prevention program in the Philippines and possibly in other Asian and developing countries.

To my family.

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List of Abbreviations

ACE	angiotensin converting enzyme
ADD1	alpha adducin
AGT	angiotensinogen
BP	blood pressure
CI	confidence interval
CLHNS	Cebu Longitudinal Health and Nutrition Survey
DALY	disability adjusted life years
DASH	Dietary Approaches to Stop Hypertension
I/D	insertion/deletion
FNRI	Food and Nutrition Research Institute
Gly/Trp	glycine/tryptophan
IR	incidence ratio
kcal	kilocalorie
kg	kilogram
LDL-C	low density lipoprotein cholesterol
m	meter
MAP	mean arterial pressure
mEq	milli-equivalent
Met/Thr	methionine/threonine
mg	milligram
MSG	monosodium glutamate
OR	odds ratio

OW	overweight
RAAS	renin-antiontensin-aldosterone system
RAS	renin angiotensin system
WHO	World Health Organization

I. Introduction

A. Background

Cardiovascular diseases (CVD) are the leading causes of morbidity and mortality in the world. They account for 10% of the world's disability adjusted life years (DALYs) and 30% of all deaths (WHO 2002; Mackay and Mensah 2004; Lopez, Mathers et al. 2006). Among the known CVD risk factors, hypertension makes the largest contribution to the CVD burden accounting for almost half of all CVD-related deaths (Mackay and Mensah 2004; Lawes, Vander Hoorn et al. 2006; Lopez, Mathers et al. 2006). Alarmingly, the prevalence of hypertension continues to rise, especially in lower income countries that are overburdened by both communicable and chronic diseases (WHO 2002; WHO 2005; Lopez, Mathers et al. 2006; Kengne, Patel et al. 2007). The World Health Organization (WHO) projects the proportion of CVD-related DALYs for high income countries to remain steady while that for low- and middle-income countries to rise sharply, further widening health disparities (WHO 2002; WHO 2005).

Hypertension is a complex multifactorial disease caused by the combined actions of genetic and environmental factors (Lifton 1996; Arnett 2007). Few studies have examined the interaction of these factors and fewer have looked at their effects in populations of developing Asian countries that are plagued with increasing levels of obesity and rapidly changing food environments (Popkin, Horton et al. 2001; Adair 2004). The information gap may be due to the lack of population-based studies with adequate depth and detail. There is a

paucity of information on adiposity trends derived from longitudinal studies and there are inadequate dietary and genetic data, especially among Asians who tend to develop CVD risk factors at lower body mass index (BMI) thresholds (Colin Bell, Adair et al. 2002; WHO 2004; Nguyen, Adair et al. 2008). In addition, sodium consumption, which can increase hypertension risk, has been found to be high in this population (INTERSALT 1988; FNRI-DOST 2000; WHO 2007). The demographic and health trends in the Philippines exemplify those of other developing Asian countries undergoing the nutrition transition: (a) heart and vascular diseases are the leading causes of death in the country (DOH 2007); and (b) there is a high prevalence (with an increasing trend) in CVD risk factors including excess weight and hypertension (FNRI 2006). Thus, examining the influence of overweight (OW) history, sodium intake and genetic variants in an Asian population from a transitional country such as the Philippines can provide critical information necessary for a better understanding of the mechanisms leading to the development of a complex and deadly disease such as hypertension and can potentially guide more tailored hypertension prevention efforts.

B. Overall objective and specific research aims

The study aimed to understand how OW duration, sodium intake, and selected genetic variants independently and jointly affect hypertension risk among adult Filipino women (ages 35 to 68 yr in 2005) using the Cebu Longitudinal Health and Nutrition Survey (CLHNS), an ongoing community-based study which began in 1983. Specific aims and hypotheses were as follows:

1. **Determine the effect of OW duration on the 5-year cumulative incidence of hypertension.** We examined patterns of OW from 1983/84 to 2002 to determine

whether a more recent change or a longer history of being OW might be more influential in developing hypertension from 2002 to 2007. We hypothesized that duration of OW is positively associated with having hypertension independent of current weight status. To assess this relationship, we used poisson regression with the robust error variance.

2. **Determine the effects of selected genetic variants on hypertension risk.** We aimed to examine the effects of *AGT* Met235Thr, *ACE* insertion/deletion (I/D) in intron 16, and *ADD1* Gly460Trp polymorphisms on the risk of hypertension in Filipino women. Additionally, we aimed to explore the heterogeneity of the effects of these three genetic variants according to different genetic and environmental contexts by looking at potential gene-gene, gene-age and gene-BMI interactions. We hypothesized that these variants influence hypertension risk and that their effects vary according to age, BMI and the presence of the other variants. To achieve our aims we used logistic regression analysis and tested heterogeneity of effects through likelihood ratio tests.

3. **Estimate the effect of high sodium intake on hypertension risk.** We determined the association between high sodium intake (≥4600 mg/day) and hypertension in Filipino women and examined whether there is heterogeneity in the effect of sodium intake according to different genetic and environmental characteristics. For the genetic characteristics, we specifically sought to assess effect measure modification by the genetic variants that we investigated in the second aim: *AGT* Met235Thr, *ACE* I/D, and *ADD1* Gly460Trp. For the environmental characteristics, we aimed to examine the potential modifying role of age, BMI, OW duration, and smoking status. We hypothesized that high sodium intake is positively associated with hypertension

risk in Filipino women and further, that its effect is not homogenous across women with different characteristics. To answer our research questions, we used logistic regression analysis and tested heterogeneity of effects through likelihood ratio tests.

II. Literature review

This section presents the scope of the problem, reviews the literature on specific topics covered by the aims of the study, and summarizes the limitations of previous studies and strengths of the current study.

A. Scope of the problem

Currently, cardiovascular diseases (CVD) are the leading causes of morbidity and mortality not only in developed countries but also in developing countries (WHO 2002; Mackay and Mensah 2004; WHO 2005; Lopez, Mathers et al. 2006; Arnett, Baird et al. 2007; WHO 2007; Lawes, Vander Hoorn et al. 2008). CVDs are responsible for 30% of all deaths (Mackay and Mensah 2004; WHO 2005; Lopez, Mathers et al. 2006) and are major contributors to the global burden of disease accounting for: (a) 10% of disability adjusted life years (DALYs) globally; (b) 20% of DALYs in high income countries; and, (c) 8% of DALYs in low and middle income countries (WHO 2002; WHO 2005).

Among the known CVD risk factors, hypertension makes the largest contribution to the CVD burden accounting for almost half of all CVD-related deaths (Mackay and Mensah 2004; Lawes, Vander Hoorn et al. 2006; Lopez, Mathers et al. 2006) and a third of CVD-related DALYs (WHO 2002). Of the estimated 16.7 million CVD-related deaths per year, about 8 million are attributed to hypertension (WHO 2002; Mackay and Mensah 2004). In

2001, about 54% of stroke and 47% of ischaemic heart disease worldwide were attributable to high blood pressure (BP) (Lawes, Vander Hoorn et al. 2008).

Further aggravating this issue is the seemingly parallel increase in obesity rates worldwide. In the US, age-adjusted obesity prevalence tripled among adolescents (from 5 to 16%) and doubled among children (from 7 to 16%) and adults (from 15 to 31%) in the last 2 decades (CDC/NCHS 2002). These increases are observed regardless of gender, age or race/ethnicity (Flegal, Carroll et al. 2002). In developing countries, the proportion of overweight (OW) individuals has exceeded the proportion of underweight (Mendez, Monteiro et al. 2005). This scenario poses a major public health concern since OW and/or obesity can lead to grave consequences – (a) it is significantly associated with increased incidence of hypertension, diabetes mellitus, hypercholesterolemia, asthma, arthritis, poor health status, and some forms of cancer; and, (b) it is one of the leading causes of death (Mokdad, Ford et al. 2003; Mackay and Mensah 2004). According to WHO (2002), approximately 58% of diabetes, 21% of ischaemic heart disease, and 8 to 42% of certain cancers globally were attributable to a body mass index (BMI) above 21 kg/m^2 (WHO 2002).

Developing countries bear most of the burden caused by CVD-related mortality and morbidities. Approximately 80% of the burden attributed to high BP is borne by low- and middle-income countries (Lawes, Vander Hoorn et al. 2008). Moreover, the proportion of DALY attributed to CVDs for high income countries is projected to remain steady while those for low- and middle-income countries are projected to rise sharply (WHO 2002). Asian population is of special concern because they have the most rapid increases in obesity and obesity-related diseases worldwide (Popkin, Horton et al. 2001; Mendez, Monteiro et al. 2005), and they tend to develop CVD risk factors at lower BMI thresholds (Colin Bell, Adair

et al. 2002; WHO 2004; Nguyen, Adair et al. 2008). For example, it was shown that Filipino women had higher prevalence of hypertension compared with non-Hispanic Whites and Mexican Americans at all levels of BMI and that the BMI-hypertension association among Chinese men was stronger compared with non-Hispanic Whites at BMI levels less than 25 kg/m^2 (Colin Bell, Adair et al. 2002). This may be attributed to the observation that Asians tend to have higher percent body fat and central adiposity, given the same BMI (Deurenberg-Yap, Chew et al. 2002; Deurenberg, Deurenberg-Yap et al. 2002). In addition, sodium consumption, which can increase hypertension risk, has been found to be high in this population (INTERSALT 1988; FNRI-DOST 2000; WHO 2007). These alarming scenarios contribute to the widening global health disparities as they additionally stretch the already limited health resources of these countries.

Along with other developing countries, the Philippines with a population of over 88 million has been undergoing rapid socio-economic changes. Accompanying these developments are the changing food environment (shift from traditional to western diets) as well as the increasing prevalence of non-communicable diseases, particularly CVDs. In 2003, heart diseases, vascular system diseases and diabetes were among the leading causes of death in the country (DOH 2007). Philippine national survey data showed high prevalence with increasing trend of CVD risk factors: from 1998 to 2003, the proportion of adult Filipinos with total cholesterol >200 mg/dL increased from 16 to 28%, those with low density lipoprotein cholesterol (LDL-C) >130 mg/dL increased from 28 to 32%, and hypertension prevalence slightly increased from 21 to 23%. The proportion of adult women having a waist-to-hip ratio >0.85, a level associated with increased risk of hypertension and diabetes, increased from 40 to 55% and the prevalence of OW increased from 23 to 27% (FNRI 2001;

FNRI 2006). In the US, CVD was reported as the leading cause of death among Filipino Americans, the largest subset of Asian Americans (NHLBI 2003). Examining CVD-related morbidities in societies like the Philippines that are transitioning toward developed country morbidity profiles can provide essential information necessary for designing interventions that can potentially narrow within-US and global health disparities.

In summary, CVDs and obesity are serious health problems worldwide. The alarming levels and trends of CVD risk factors, including hypertension, suggest continuing increases in CVDs, especially in developing countries. These conditions underscore the need to examine factors associated with hypertension in a transitional country such as the Philippines that can potentially guide more targeted public health interventions.

B. Factors associated with hypertension

Hypertension is a multi-factorial, polygenic disease. The homeostatic control of BP is complex affecting a wide variety of physiologic systems – baroreceptors that sense acute changes in blood vessel pressure, natriuretic peptides produced in response to increased pressure in the brain and heart, renin-angiotensin-aldosterone system (RAAS) that influence vascular volume homeostasis and tone, kinin-kallikrein system that affects vascular tone and renal salt handling, adrenergic receptor system that influences heart rate, cardiac contraction and vascular tone, and those that are produced by blood vessels causing vasolidation or contraction (Lifton, Gharavi et al. 2001; Kaplan, Lieberman et al. 2002). Hypertension results from abnormalities in any of these factors or in the interactions of these factors that

can increase cardiac output and/or peripheral resistance (Krause, Winston et al. 1998; Kaplan, Lieberman et al. 2002).

Factors associated with the development of hypertension involve a combination of genetic predisposition, and modifiable factors such as increasing weight with central distribution, unhealthy diet, and physical inactivity (Brown, Higgins et al. 2000; Janssen, Katzmarzyk et al. 2002; Mackay and Mensah 2004; WHO 2005; Narkiewicz 2006). This study covered key risk factors focusing on the influence of the duration of excess weight, genetic variants and high sodium intake, and explored potential interactions of their effects on hypertension risk.

B.1. BMI, overweight duration and hypertension

It is well documented that excess weight/fat increases the risk of CVDs. Adiposity measured through BMI or abdominal fat patterning reflected by waist-hip ratio or waist circumference has been associated with BP in both genders and across ethnicities (Must, Spadano et al. 1999; Brown, Higgins et al. 2000; Janssen, Katzmarzyk et al. 2002; Adair 2004; Davy and Hall 2004). In fact, a Framingham Heart Study data suggested that 65 to 75% of the risk of hypertension may be attributed to excess weight (Garrison, Kannel et al. 1987). Possible mechanisms involved in the excess weight-increased BP relationship include insulin resistance and hyperinsulinemia (Reaven, Lithell et al. 1996; Davy and Hall 2004), leptin resistance (Hall, Jones et al. 2003; Davy and Hall 2004; Berg and Scherer 2005; Mathew, Patel et al. 2007), activation of the sympathetic nervous and RAAS (Hall, Jones et al. 2003; Segura and Ruilope 2007), increased procoagulatory activity and endothelial dysfunction (Berg and Scherer 2005; Shankar and Steinberg 2005), subclinical inflammation

(Berg and Scherer 2005), and increased renal sodium re-absorption resulting in volume expansion (Hall, Jones et al. 2003; Wofford and Hall 2004; Mathew, Patel et al. 2007).

Despite studies that suggest significant associations between obesity/OW duration and obesity-related morbidities such as insulin resistance (Ivandic, Prpic-Krizevac et al. 1998; Muscelli, Camastra et al. 1998; Janssen, Katzmarzyk et al. 2004), type 2 diabetes mellitus (Everhart, Pettitt et al. 1992; Pontiroli and Galli 1998; Wannamethee and Shaper 1999; Sakurai 2000; Janssen, Katzmarzyk et al. 2004; Wannamethee, Shaper et al. 2005), and left ventricular muscle hypertrophy which can accompany increased BP (Nakajima, Fujioka et al. 1985; Licata, Scaglione et al. 1992; Urbina, Gidding et al. 1995), few studies have investigated OW duration as a risk factor for hypertension. To our knowledge, only three studies have looked at the OW or obesity duration-hypertension association (Pontiroli and Galli 1998; Hekimsoy and Oktem 2003; Janssen, Katzmarzyk et al. 2004) and the results have been inconsistent. Janssen et al showed that the odds of having hypertension among women who were OW $\geq$ 10 years were double that of normal weight women (these results were not observed in men). Alternatively, the other two studies focused on obese patients and did not observe a significant association between obesity duration and hypertension (Pontiroli and Galli 1998; Hekimsoy and Oktem 2003). No studies have looked at this association in developing countries.

The inconsistency in the findings of previous studies dealing with duration of OW and hypertension may be due to methodological differences. These studies were cross-sectional and relied on self-reported or recalled weight, some relying on recalls as far back as more than 20-30 years (Pontiroli and Galli 1998; Hekimsoy and Oktem 2003; Janssen, Katzmarzyk et al. 2004). To define OW or obesity duration, none of these studies used

repeatedly measured weights. Effect estimates could have been biased since recalled weight is not independent of current BMI status, especially for OW/obese women who are more likely to underestimate self-reported or recalled weight (Perry, Byers et al. 1995; Troy, Hunter et al. 1995; Gillum and Sempos 2005; Brunner Huber 2007; Nyholm, Gullberg et al. 2007; Shields, Gorber et al. 2008). Our study overcame this limitation by defining OW history and duration based on measured weights collected using the same protocols for about two decades of adult life. In addition, having measured BP in more than one time point allowed us to examine hypertension incidence. Longitudinal analysis of hypertension incidence can ensure that the entire duration of excess weight happened before the development of hypertension, thus lending more support for causation.

B.2. Genetic variants and hypertension

Looking at heritability estimates across studies and across populations, about 30% of the population variability in BP may be attributed to genetic factors (Ward 1995; Arnett, Baird et al. 2007). Candidate gene studies have shown evidence for association between specific genes and hypertension, albeit inconsistently (Luft 2004; Arnett, Baird et al. 2007). The scientific statement of the American Heart Association Council assessing the relevance of genetics and genomics for the prevention and treatment of CVD listed at least 30 candidate genes associated with high BP and/or essential hypertension (Arnett, Baird et al. 2007). However, recent genome wide association studies aiming to elucidate the genetic basis of hypertension have not been successful – no associations exceeded the criteria of statistical significance (p-value 5 X 10-7) (Levy, Larson et al. 2007; WellcomeTrust 2007).

Of the genes associated with BP, we focused on polymorphisms suggested to be involved in the development of high BP or hypertension. The renin-angiotensin system (RAS) is known to regulate BP by influencing vascular volume homeostasis and tone (Lifton, Gharavi et al. 2001). Considerable efforts have been made in investigating polymorphisms in genes encoding the main components of this system. Of the *AGT* gene polymorphisms linked to hypertension, the Met235Thr variant is one of the most widely examined (Binder 2007) and showed possible effect differences by race. Jeunemaitre et al were the first to suggest the role of the *AGT* Met235Thr polymorphism in the development of hypertension (Jeunemaitre, Soubrier et al. 1992). Using data from 267 men and women of Northern and Western European descent, they showed that the ThrThr genotype was associated with an increase in plasma angiotensinogen and a 95% increase in the odds of hypertension compared to the MetMet genotype. Increased BP may be explained by the role of *AGT* in encoding the precursor molecule for angiotensin II which causes intense vasoconstriction and sodium re-absorption in the kidney. However, results of the numerous studies that followed this pioneering work were not consistent. A case-control study involving 1476 subjects reported a null association between Met235Thr and hypertension (Kato, Sugiyama et al. 2000) but a meta-analyses involving 25055 individuals from 3 ethnicities (Whites, Blacks, Asians) concluded that *AGT* Met235Thr was associated with increased risk of hypertension in Whites (OR: MetThr 1.08, ThrThr 1.19) and Asians (OR: MetThr 1.29, ThrThr 1.60) (Sethi, Nordestgaard et al. 2003).

The I/D polymorphism of the *ACE* gene has also been suggested in the pathophysiology of hypertension, with the D allele associated with higher ACE levels and endothelial dysfunction and therefore increased BP (Luft 2004; Penesova, Cizmarova et al.

2006). This variant may be one of the most consistently linked polymorphisms to CVD in the general population; but, its association with hypertension has been less convincing (Niu, Chen et al. 2002; Luft 2004). *ACE* DD has been shown to influence hypertension risk with possible gender-specific effects (O'Donnell, Lindpaintner et al. 1998; Higaki, Baba et al. 2000; Lynch, Arnett et al. 2007). For example, using a population-based sample of mostly Caucasian men and women (N=3095, Framingham Study), O'Donnell et al reported adjusted odds ratios (ORs (95% confidence interval)) for hypertension in 1445 men for the DD and DI genotypes as 1.59 (1.13-2.23) and 1.18 (0.87-1.62), respectively, versus II (O'Donnell, Lindpaintner et al. 1998). The corresponding ORs in 1650 women were 1.00 (0.70-1.44) and 0.78 (0.56-1.09), respectively.

The *ADD1* Gly460Trp variant may cause hypertension by enhancing tubular sodium re-absorption in the kidney (Bianchi, Ferrari et al. 2005). The relationship between *ADD1* Gly460Trp polymorphism and hypertension in humans was first demonstrated by Cusi et al (Cusi, Barlassina et al. 1997). As with the other polymorphisms, results of studies that followed were inconsistent. For example, among Asians, Kato et al did not find a significant association between the 460Trp allele and hypertension in 507 Japanese (Kato, Sugiyama et al. 1998), Shin et al corroborated the negative finding examining 903 Koreans (Shin, Chung et al. 2004), but Huang et al found a significant association (OR: 1.43, $p = 0.029$) among 751 Han Chinese (Huang, Sun et al. 2007).

High risk genetic environments may be necessary for hypertension to develop. Studies have shown that the effects of the selected polymorphisms may only be evident when other genetic and environmental characteristics were taken into account (Barlassina, Schork et al. 2000; Svetkey, Moore et al. 2001; van der Kleij, de Jong et al. 2002; Katsuya, Ishikawa

et al. 2003; Tamaki, Nakamura et al. 2005; Wang, Zhu et al. 2006; Fava, Montagnana et al. 2007; Franks 2008; Jiang, Sheng et al. 2008). In a review focusing on the effect of *ADD1* on hypertension, Bianchi et al concluded that the impact of this gene variant was clear when context was taken into consideration (Bianchi, Ferrari et al. 2005). Joint effects of *AGT*, *ACE* and *ADD1*on hypertension have been reported (Barlassina, Schork et al. 2000; Staessen, Wang et al. 2001; Tamaki, Nakamura et al. 2005). For example, *ACE* DD homozygozity was associated with a 31% increase in the incidence of hypertension in a single gene analysis and with a 59% increase in carriers of the *ADD1* Trp allele in 1461 randomly selected Caucasians (Staessen, Wang et al. 2001). The separate effects of *ACE* D homozygosity or *ADD* Trp homozygosity were not significant in a sample of 1647 Japanese but when these genotypes were combined (*ACE* DD and *ADD* TrpTrp), a significant association became apparent (OR: 1.37 (1.03-1.82)) (Tamaki, Nakamura et al. 2005). As regards to *AGT-ACE* interaction, Kato et al did not find significant synergy for hypertension (Kato, Sugiyama et al. 2000).

BMI and age are among the strongest predictors of hypertension and have been reported to modify the effects of gene variants. Adiposity positively correlates with the adipose AGT mRNA expression and may influence the expression of other RAS components (Van Harmelen, Ariapart et al. 2000). The *ACE* gene may be upregulated in obese individuals (Gorzelniak, Engeli et al. 2002). It may also be possible that effects of gene variants are stronger in individuals considered to be low risk for conventional factors such as BMI (i.e. <26 kg/m^2) and age (i.e. <55 years) (Cambien, Costerousse et al. 1994). Unfortunately, studies examining potential effect measure modification by these factors have been lacking and results of the few studies conducted have not been consistent. Fava et al showed that among 6103 Swedes, age did not modify the effect of carrying the *ADD1*

460Trp allele but showed that its hypertensive effect were significant only in women belonging to the highest tertile of BMI ($\geq$ 27 kg/m^2). Zaman et al did not observe age modification in the effect of *ACE* I/D in a study involving 1340 Japanese (Zaman, Yoshiike et al. 2001). Yoshida et al, examining 263 Japanese reported that having at least one I allele was associated with hypertension in the elderly group (>70 years) but not in the middle aged group (30-60 years (Yoshida, Ishigami et al. 2000). On the contrary, Sugimoto et al observed that among 1490 Japanese, the effect of *ADD1* Trp allele was significant only in younger subjects (< 60 yrs) with low plasma renin activity (Sugimoto, Hozawa et al. 2002).

B.3. Sodium intake and hypertension

Dietary sodium influences BP. Although not all authors agree, most deduce that "there is conclusive evidence that dietary salt is positively associated with BP" (Chobanian, Bakris et al. 2003; WHO 2007). Increased sodium intake can raise BP by increasing fluid volume and preload (extracellular volume expansion) and by mechanisms that are volume-independent (i.e. increase sympathetic nervous system activity, angiotensin-mediated central nervous system effects) (Rodriguez-Iturbe, Romero et al. 2007).

Numerous epidemiologic studies and clinical trials support the positive salt-BP relationship. The INTERSALT study, a cross-sectional study involving 32 countries with more than 10,000 respondents, showed a positive correlation between sodium excretion and BP: a 100 mEq difference in urinary sodium was associated with a 3-6 mmHg difference in systolic BP (INTERSALT 1988; Stamler, Rose et al. 1989). Another population-based study of 24 communities confirmed this association (Law, Frost et al. 1991).

However, there is varying BP response to salt intake suggestive of salt-sensitivity. Most individuals consume high-sodium diets but not all of these individuals develop hypertension. This suggests the involvement of genetic factors and interactions with other exposures. For example, obesity has been found to increase the hypertensive effect of salt. The excretion of excess sodium is decreased in the presence of obesity, therefore elevation of BP is needed to excrete salt loads (Karppanen and Mervaala 2006). However, a positive interaction of the effects of BMI and sodium intake on hypertension has not been consistently shown. In a study involving 60 obese and 18 non-obese adolescents, a change from a high salt to a low salt diet resulted to a larger mean change in mean arterial pressure (MAP) in the obese group suggesting salt sensitivity (Rocchini, Key et al. 1989). A 24-week, placebo-controlled, two-period, crossover trial of sodium supplementation in 112 African Americans, aged 25 to 64 years, showed that variability-adjusted BP change correlated with BMI (Flack, Grimm et al. 2002). However, a double-blind randomized study of 46 White non-obese subjects aged 25 to 80 years showed that salt-sensitive subjects had lower body weight than salt-resistant individuals and that subjects with a BMI <26 kg/m^2 showed a rise in MAP when moving from a low sodium to a high sodium diet, whereas subjects with BMI >26 kg/m^2 did not (Overlack, Ruppert et al. 1995).

Older age is associated with increased sodium sensitivity, a steeper BP and exchangeable body sodium relationship, decreased gain of the baroreceptor reflex, reduced renal perfusion, and a compromised buffering effect of large arteries on systolic and diastolic pressure (Staessen, Wang et al. 2003). Law et al analyzed published reports of BP and sodium intake (47000 people from 24 communities around the world) and found that a difference of 2300 mg/day was associated with an average difference in systolic BP that

ranged from 5 mmHg at age 15-19 to 10 mmHg at age 60-69 years (Law, Frost et al. 1991). The Dietary Approaches to Stop Hypertension (DASH)-sodium trial where participants were given 3 levels of sodium (1150, 2300, 3450 mg/2100 kcal) for 30 days while consuming the DASH diet or a more typical American diet showed that age had a strong and graded influence on the effect of sodium on BP: sodium intake reduction from 3450 to 2300mg/2100 kcal and reduction from 2300 to 1150 mg/2100 kcal resulted in reduction of systolic BP by 4.8 and 1.0 mmHg for 23 to 41 years, 5.9 and 1.8 mm Hg for 42 to 47 years, 7.5 and 4.3 mm Hg for 48 to 54 years, and 8.1 and 6.0 mm Hg for 55 to 76 years (Bray, Vollmer et al. 2004). Results of a recent dietary feeding (GenSalt) study in China with 1906 participants corroborated these findings (He, Gu et al. 2009).

C. Summary and significance

Developing countries and/or transitional countries experiencing rapid socio-economic changes with consequent changes in food environment and lifestyle are critical settings in studying the emergence of obesity and the development of CVD risk factors. However, large population-based longitudinal studies of sufficient detail and duration are largely lacking. Most developing country and/or Asian studies are cross-sectional and cannot establish temporality therefore weak in determining causality. We are not aware of any longitudinal study in developing and transitional countries that have the time depth and number of follow-ups that the CLHNS has. Studies in developed countries, mostly from the US, fail to examine ethnic differences other than the usual classifications (i.e. African Americans versus Whites), neglecting the growing Asian population, including the Filipino American population that represents almost 20% of Asian Americans. Understanding factors that influence the

development of hypertension among Filipinos can potentially provide insights in narrowing health disparities in the US. Furthermore, research in developing and transitional countries such as the Philippines are essential in the context of global health, especially when the prevalence of obesity and CVDs are increasing in more and more resource-poor countries around the world. The study may provide critical information necessary for the prevention efforts in these settings and potentially narrow global health disparities.

Our study, using CLHNS data with genetic information, repeated anthropometric measures that span over 20 years, dietary sodium intake and recent BP measurements, offers a unique opportunity to address information gaps by improving our understanding of the effects of long-term exposures on hypertension and looking at gene-gene and gene-environment interactions. To our knowledge, no other study with sufficient detail and duration has explored the interactions between gene, sodium intake and longitudinal measures of weight in predicting hypertension risk.

III. Overweight duration and incidence of hypertension in Filipino women

A. Abstract

Background: It is well known that excess weight increases the risk of hypertension. Less is known about whether the risk of hypertension differs in those who have recently become overweight (OW) from those who have been OW for a longer period. **Methods and Results:** We examined the effect of duration of OW (BMI>=25 kg/m^2) independent of current BMI on the five-year (2002 to 2007) cumulative incidence of hypertension in a sample of 1607 women participants of the Cebu Longitudinal Health and Nutrition Survey. OW duration was determined by using repeatedly measured weight and height gathered from 1983/84 to 2002. We identified 6 patterns representing OW history and duration: never OW (56.8%), not currently OW but had been OW (4.5%), OW < 4 years (9.1%), OW 4-10 years (13.1%), OW 11-17 years (12.5%), and OW ≥ 18 years (4.0%) with corresponding 5-year cumulative incidence of hypertension of 20.8%, 27.4%, 29.5%, 32.4%, 40.3% and 56.9%, respectively. After adjusting for 2002 age and BMI using poisson regression, we observed an increasing trend in the five-year cumulative incidence of hypertension according to OW history and duration. Compared to never OW women, the adjusted incidence ratio for hypertension was 1.40 (95% confidence interval (CI), 1.01-1.94) for women OW between 11-17 years and 1.68 (95% CI: 1.09-2.59) for women OW for ≥ 18 years. **Conclusions:** Since OW duration regardless of current weight appear to increase hypertension incidence, efforts to prevent earlier weight gain should be stressed.

B. Introduction

Large shifts in obesity, hypertension, and many other non-communicable diseases are occurring across the globe (Kearney, Whelton et al. 2005; Popkin 2008). The Philippines, a developing country with a population of over 88 million (NSO 2008), has not been immune from these changes.(Adair 2004; Mackay and Mensah 2004; FNRI 2006; DOH 2007) Heart and vascular diseases are ranked first and second leading causes of death in the country accounting for 17% and 13% of deaths (in 2002), respectively (DOH 2007). Estimated disability adjusted life years lost per 1000 population from heart disease are higher in the Philippines than in the U.S. or China (Mackay and Mensah 2004). Overweight (OW) or obesity prevalence in the country has increased from 16.6% in 1993 to 24% in 2003 (FNRI 2006).

Adiposity measured through the body mass index (BMI) or abdominal fat patterning reflected by waist-hip ratio or waist circumference has been associated with increased blood pressure (BP) in both genders and across ethnicities (Must, Spadano et al. 1999; Brown, Higgins et al. 2000; Janssen, Katzmarzyk et al. 2002; Adair 2004; Davy and Hall 2004). A Framingham Heart Study data suggested that 65-75% of the risk of hypertension can be attributed to excess weight (Garrison, Kannel et al. 1987). Possible mechanisms involved in the excess weight-increased BP relationship include insulin resistance and hyperinsulinemia (Reaven, Lithell et al. 1996; Davy and Hall 2004), leptin resistance (Hall, Jones et al. 2003; Davy and Hall 2004; Berg and Scherer 2005; Mathew, Patel et al. 2007), activation of the sympathetic nervous and renin-angiotensin-aldosterone systems (Hall, Jones et al. 2003; Segura and Ruilope 2007), increased procoagulatory activity and endothelial dysfunction

(Berg and Scherer 2005; Shankar and Steinberg 2005), subclinical inflammation (Berg and Scherer 2005), and increased renal sodium re-absorption resulting in volume expansion (Hall, Jones et al. 2003; Wofford and Hall 2004; Mathew, Patel et al. 2007).

However, less is known about whether the risk of hypertension differs in those who have recently become OW compared to those who have been OW for a longer period, especially among Asians who tend to develop hypertension at lower BMI thresholds (Colin Bell, Adair et al. 2002; Lear, Toma et al. 2003; Nguyen, Adair et al. 2008). Although OW duration has been associated with other obesity-related morbidities such as insulin resistance (Muscelli, Camastra et al. 1998; Janssen, Katzmarzyk et al. 2004), type 2 diabetes mellitus (Pontiroli and Galli 1998; Wannamethee and Shaper 1999; Sakurai 2000), and left ventricular muscle hypertrophy which can accompany increased BP (Nakajima, Fujioka et al. 1985; Licata, Scaglione et al. 1992; Urbina, Gidding et al. 1995), few studies have investigated OW duration as a risk factor for hypertension. Results of studies examining the association between OW duration and hypertension were inconsistent: two studies found no association (Pontiroli and Galli 1998; Hekimsoy and Oktem 2003) while one found a positive association among women (Janssen, Katzmarzyk et al. 2004). Moreover, these studies were cross-sectional, and relied on recall of OW duration (Pontiroli and Galli 1998; Hekimsoy and Oktem 2003; Janssen, Katzmarzyk et al. 2004). The present study examined this relationship by using population-based longitudinal anthropometric data measured over a substantial proportion of adult life of Filipino women. Unlike previous studies, we used OW duration before the onset of hypertension as the exposure and 5-year cumulative incidence of hypertension (2002-2007) as the outcome to establish that the entire duration of excess

weight happened before the development of hypertension. We hypothesized a positive relationship between OW history and incident hypertension, independent of 2002 BMI.

C. Methods

C.1. Data source and sample

We used data from the ongoing Cebu Longitudinal Health and Nutrition Survey (CLHNS) (Adair and Popkin 2001). The CLHNS was conducted in Metropolitan Cebu, one of the metropolitan areas in the Philippines, with a population of over 2 million (NSO 2008). The study area is ecologically diverse, ranging from densely populated barangays (communities) in the cities, less dense peri-urban areas and rural towns, to the more isolated mountain and island rural areas. It is one of the most rapidly developing regions of the country. In 1983, a stratified, single stage sampling design was used to randomly select 33 barangays and all pregnant women in the selected barangays were initially invited to participate and were included in the survey if they gave birth between May 1983 and April 1984 (n=3327). Subsequent surveys took place immediately after birth, then every 2 months for 24 months for all women with singleton, live births. Follow-up surveys were conducted in 1991, 1994, 1998, 2002, 2005 and 2007. The sample for this study is limited to women who remained in the 2002 and 2007 surveys (n=1962). In 2002, their ages ranged from 32 to 65 years. We excluded women who were pregnant in either survey (n=30), those missing BP measurements (n=3) and those who were hypertensive in 2002 (n=322) leaving an analysis sample of 1607. None had missing data on any of the 2002 survey anthropometric measurements and other covariates.

C.2. Analysis variables

BP was measured using a mercury column sphygmomanometer after a 10 minute rest when the woman was seated. The mean of three measurements was used to represent systolic BP and diastolic BP. Hypertension was defined as systolic BP $\geq$ 140 mmHg or diastolic BP $\geq$ 90 mmHg (Chobanian, Bakris et al. 2003). Data on the use of anti-hypertensive medications was not available in the 2007 survey, therefore we did not include this in the definition to be consistent in both surveys. Analyses excluding women who reported taking anti-hypertensive medications in 2002 (n=45) yielded similar results. We calculated 5-year cumulative incidence by dividing new cases of hypertension occurring in the study interval 2002-2007 by the total at risk population (non-hypertensive in 2002).

Anthropometric measurements were taken at home by trained research personnel while participants were wearing light clothing. Weight was measured using portable scales to the nearest 0.1 kg while height was measured using portable stadiometers to the nearest 0.1 cm. BMI was calculated as weight in kg divided by squared height in meters. For the first non-pregnant BMI, we used weight collected 4 months postpartum. About 76% of women had non-pregnant BMI for all survey rounds (from 1983/84 to 2002); only 3% had missing data in more than 1 survey. If non-pregnant BMI was missing, we imputed using the mean BMI of adjacent surveys. For each survey round (1983/84, 1991, 1994, 1998, 2002), we created a binary variable indicating whether the non-pregnant woman was OW (BMI $\geq$ 25 kg/m^2) (WHO 2000). These binary OW variables were examined for patterns over time and were used to group women into 6 OW duration categories. Women who were not OW in 2002 were classified as: never OW or with OW history (had been OW in at least 1 survey). Women who were OW in 2002 were classified according to the number of consecutive

preceding surveys that they were OW and grouped as: < 4 yrs OW, 4-10 yrs OW, 11-17 yrs OW, and ≥ 18 yrs OW. Waist circumference was measured using standard measuring tapes and deemed high risk if ≥ 88 cm (WHO 2000).

Community, household and individual level covariates were collected through in-home face-to-face interviews using structured questionnaires. Age, occupational physical activity, smoking habits, alcohol consumption, income, education, reproductive history (number of pregnancies and menopausal status) and community urbanicity level at the time of the 2002 survey were examined as potential effect measure modifiers or confounders. Age, log of household income, education (number of years of formal schooling), and number of pregnancies were used as continuous variables. Indicator variables were created to represent levels of occupational physical activity (not working, sedentary, light, moderate, or heavy) based on detailed occupation code, posture at work and physical demands of jobs. Occupational physical activity is an important component of energy expenditure in developing countries such as the Philippines (Forrest, Bunker et al. 2001). Binary indicator variables were created for smoking status (current smoker or not), alcohol consumption (current drinker or not) and menopausal status (postmenopausal or not). We used a continuous urbanicity index variable (0-70 scale) based on population size and density, community infrastructure, economic and environment characteristics. This index has been validated and found to be a better measure of urbanicity than the urban-rural dichotomy (Dahly and Adair 2007).

We obtained informed consents from all participants. The study protocol was approved by the University of North Carolina Institutional Review Board for the Protection of Human Subjects.

C.3. Statistical analysis

Differences in proportions and means of selected characteristics of women in 2002 according to OW duration categories were compared using chi-square test and analysis of variance, respectively. Poisson regression with the robust error variance (Zou 2004) was used to estimate the effect of OW duration on hypertension risk. For the main exposure variable, we used indicator variable coding to represent the OW duration groups, with the never OW group as reference. Considering the well-known effect of BMI on BP, we purposely controlled for 2002 BMI (continuous) in the model to isolate the effect of OW duration. To determine which other covariates to retain in the final model, we evaluated each variable's potential as an effect measure modifier and/or confounder. First, we assessed for effect measure modification by including interaction terms with OW duration in the model. We deemed the covariate as an effect measure modifier if the p-value of the interaction term (with OW duration) was < 0.10. We found no significant effect measure modifiers. Next, potential confounders were assessed based on the backward elimination, change-in-estimate approach (Rothman and Greenland 1998). If the exclusion of the covariate substantially (at least 10%) changed the exposure-outcome effect estimates, then the covariate was identified as a confounder. Only age was found to be a significant confounder. Thus, the final model controlled for BMI and age. We evaluated attrition bias by weighting the observations by the reciprocal of the probability of being in the study sample (Fitzgerald, Gottschalk et al. 1998). Since the weighted analysis was not substantially different from the un-weighted analysis, we present un-weighted results. STATA/SE version 10.1 was used in all analyses.

D. Results

The largest difference in average age among the OW duration groups was < 4 years (Table 3.1). BP, BMI and waist circumference were positively associated with OW duration. Women who were not OW at any survey had the lowest systolic and diastolic BP, BMI and waist circumference while those who had been OW for ≥18 years had the highest values. Compared to women who were OW in 2002, those who were not OW were more likely to smoke and work in jobs requiring moderate to heavy exertion. Never OW women were the least educated.

OW prevalence increased 9-fold, from 4.3% to 38.7% (Figure 3.1) from 1983/84 to 2002. The greatest annual increase in OW prevalence occurred between the first three survey rounds. Almost all OW women in 1983/84 and about 88% of OW women in 1991 remained OW in succeeding surveys to 2002.

Never OW women comprised 56.8% of the sample. Among OW women, the most frequent duration categories were 4-10 and 11-17 years. Five-year cumulative incidence (per 100 persons at risk) of hypertension differs by OW duration group (Figure 3.2). From 2002 to 2007, 27.3% of women developed hypertension and crude analysis suggests hypertension incidence was positively associated with OW duration (p for trend <0.01). Cumulative incidence of hypertension for women OW for ≥18 years was 2.7 times more than that of the never OW women (56.9% and 20.8%, respectively).

After adjusting for 2002 age and BMI using poisson regression, we still observed an increasing trend in the five-year risk of hypertension according to OW history and duration (Table 3.2). The average risk for hypertension among women who had been OW for more than 10 years was significantly higher than never OW women (reference). Specifically,

compared to never OW women, the adjusted incidence ratios (IR) for hypertension were 1.40 (95% confidence interval (CI), 1.01-1.94) for women OW between 11-17 years and 1.68 (95% CI: 1.09-2.59) for women OW for at least 18 years (Model 1).

To underscore the importance of controlling for BMI when examining the association between duration of OW and hypertension incidence, we examined the effect estimates with BMI excluded from the model. Taking out 2002 BMI from the model substantially increased the cumulative IR for all OW duration categories when compared to the never OW women, especially that of the OW $\geq$ 18 years group where IR changed from 1.68 to 2.64 (Model 2 versus Model 1). In addition, inclusion of the binary waist circumference variable in the age- and BMI-adjusted model did not change the OW duration effect estimates (Model 3 versus Model 1).

Using coefficients from our regression model, the likelihood of developing hypertension can be estimated under different scenarios, holding age constant. Compared to never OW women with a BMI of 23 kg/m^2, women with a BMI of 26 kg/m^2 who had been OW for <4 years were 36% more likely to develop hypertension, while women with a BMI of 25 kg/m^2 who had been OW for at least 18 years were 83% more likely to develop hypertension.

E. Discussion

Excess weight is an established risk factor for hypertension but not all OW or obese individuals have high BP. This may be partly explained by the variations in the length of exposure to excess weight, a potentially important factor in the development of hypertension. As suggested, a constant elevation in body weight may be necessary for obesity-related

morbidities to develop (Janssen, Katzmarzyk et al. 2004). This study showed that duration of OW, independent of magnitude of BMI, increases the 5-year risk of developing hypertension among adult women in Cebu, Philippines. Women who had been OW for more than 10 years had significantly higher propensity to develop hypertension compared to women who had never been OW. Restricting the analysis to women who were OW in 2002, with the OW <4 years group as reference, yielded similar pattern of results (data not shown).

By showing the significant effect of OW duration independent of BMI, our study underscores the importance of controlling for exposure duration when examining the effect of OW/obesity or BMI on hypertension to prevent mixing of effects. For example, we showed that a longer OW duration increased risk of incident hypertension to a greater extent even at a lower initial BMI. It is therefore useful to assess and separate the effects of current size from weight history.

The biological mechanisms linking prolonged OW and hypertension have not been completely elucidated. Prolonged OW or obesity has been associated with ventricular hypertrophy possibly induced by long-standing volume overload (Nakajima, Fujioka et al. 1985; Scaglione, Dichiara et al. 1992; Alpert, Lambert et al. 1995), insulin resistance and hyperinsulinemia (Muscelli, Camastra et al. 1998; Janssen, Katzmarzyk et al. 2004), and gradual loss of kidney function that worsens with time (Wofford and Hall 2004). These abnormalities in turn may affect BP (Reaven, Lithell et al. 1996; Hall, Jones et al. 2003; Wofford and Hall 2004).

To our knowledge, only three studies have looked at the association of OW/obesity duration with hypertension and their results are inconsistent (Pontiroli and Galli 1998; Hekimsoy and Oktem 2003; Janssen, Katzmarzyk et al. 2004). The earliest studies did not

observe a significant association between obesity duration and hypertension (Pontiroli and Galli 1998; Hekimsoy and Oktem 2003). Examining 200 Turkish women presenting at a clinic because of obesity (mean BMI 38.4 ± 6.1 kg/m^2), Hekimsoy and Oktem did not find the degree, distribution and duration of obesity to be associated with hypertension (Hekimsoy and Oktem 2003). An Italian study of 760 patients (560 women) admitted for obesity-related health problems (mean BMI 36.1 ± 6.1 kg/m^2) found that having hypertension is related to the degree but not the duration of obesity (Pontiroli and Galli 1998). Our findings are in line with a US study involving a representative sample of 2285 men and 2589 women 30-64 years of age participating in NHANES III (Janssen, Katzmarzyk et al. 2004). After controlling for BMI, the odds of having hypertension were 1.97 times higher among women who were OW ≥ 10 years compared to normal weight women; these results were not observed in men. Janssen et al. excluded underweight (BMI < 18.5 kg/m^2) individuals from their sample. Doing the same in our study strengthened the influence of OW duration on hypertension risk – cumulative IR for OW 11-17 yrs and for OW ≥ 18 yrs increased to 1.49 and 1.86, respectively.

Our differing results may be partly attributed to differences in methods. First, our study is population-based and included all women regardless of BMI while the Turkish and Italian studies were clinic-based and limited to obese patients who had relatively high levels of obesity-related metabolic problems including hypertension. For example, 48% of the Turkish sample and 74% of the Italian sample were hypertensive. By design, none of our sample women were hypertensive in 2002 and about 27% were hypertensive by 2007. Second, we used different BP cutpoints in defining hypertension. Hekimsoy and Oktem defined hypertension as BP >135/85 mmHg or being treated for hypertension (including non-

drug treatments such as weight loss), Pontirolli and Galli defined hypertension as BP >160/95 mmHg or taking hypotensive drugs, while our study and that of Janssen et al used the latest recommended cutpoint of BP ≥140/90 mmHg. We calculated duration from the time the woman first had a BMI of at least 25 kg/m^2 while the first two studies assessed time since BMI ≥30 kg/m^2. Also, different duration categories were used.

The present study has a number of unique dimensions, including the use of incident hypertension and repeatedly measured weight and height (using the same protocol). With the use of prospective longitudinal data, we were able to ensure that the entire duration of OW occurred before the development of hypertension. This allows us to better establish temporality and infer causality compared to previous studies which were all cross-sectional. Our use of measured weight and height collected over two decades of adult life reduces measurement error. The previous studies relied on recall, some dating as far back as > 20-30 years. Using self-reported or recalled weight may bias effect estimates since recalled weight is not independent of BMI status, especially for OW/obese women who are more likely to underestimate their weight (Perry, Byers et al. 1995; Nyholm, Gullberg et al. 2007; Shields, Gorber et al. 2008).

Our study has certain limitations. The CLHNS did not continuously monitor the sample women but collected data at certain intervals. We were not able to ascertain the exact time when the women became OW and developed hypertension. As with any prospective study with long follow-up duration, there is sample attrition. Further, to model five-year cumulative incidence of hypertension, we only included women who were not hypertensive in 2002 and were followed-up in 2007. The reduction in sample size may produce bias and limit the generalizability of results. Compared to women who are not in the sample, the

women included in this study had lower baseline BMI (mean BMI: 21.0 vs. 20.4 kg/m^2 (t test $p<.05$), respectively) but similar age (mean age: 26.7 vs. 26.4 years (t test $p>.05$), respectively). BMI is positively associated with OW duration therefore we can posit that women who were excluded from the survey are more likely to have been OW for longer duration and to develop hypertension. For example, compared to the sample women, those who were excluded because they were hypertensive in 2002 had higher baseline BMI and were more likely to have been OW for more than 10 years. Thus, the OW duration-hypertension relationship observed in the study sample is likely attenuated. Finally, results of analyses weighted by the reciprocal of the probability of being in the study sample were not considerably different from those of un-weighted analyses indicating no significant attrition bias.

Using Philippine data allows us to make important contributions. This is a pioneering study on the effect of long term OW on BP involving an Asian population. Asians are of special concern because they have the most rapid increases in obesity and obesity-related diseases worldwide (Popkin, Horton et al. 2001; Mendez, Monteiro et al. 2005) and they tend to develop CVD risk factors at lower BMI thresholds (Colin Bell, Adair et al. 2002; Lear, Toma et al. 2003; Nguyen, Adair et al. 2008). Also, this fills a gap in literature by focusing on a population of a developing country that is undergoing the earlier phases of the nutrition transition. It is in developing countries that we find alarmingly increasing rates of obesity and chronic diseases (WHO 2002; Lawes, Vander Hoorn et al. 2008). Lastly, we add to CVD-related studies focusing on women, a segment of the population who entail special CVD prevention guidelines (Mackay and Mensah 2004; Mosca, Banka et al. 2007). As the Janssen

et al. study indicated, OW duration seems an important factor in having hypertension in women but not in men (Janssen, Katzmarzyk et al. 2004).

In summary, this study suggests that OW duration, independent of the degree of excess weight, increases the risk of developing hypertension in women. OW prevention efforts earlier in life should be stressed, especially since women who become OW tend to remain OW.

Table 3.1. Characteristics of non-pregnant, non-hypertensive Cebu women in the 2002 survey, by overweight (OW) duration*

Characteristics	Never OW n = 912	OW History† n = 73	OW < 4 y n = 146	OW 4-10 y n = 210	OW 11-17 y n = 201	OW ≥18 y n = 65	p-value
Age, y	45.5±0.2	47.2±0.7	43.7±0.4	44.2±0.4	45.3±0.4	47.4±0.6	<0.01
Systolic BP, mmHg	105.6±0.4	110.7±1.2	109.0±1.0	110.4±0.7	114.1±0.7	118.1±1.4	<0.01
Diastolic BP, mmHg	70.3±0.3	73.8±1.0	73.6±0.7	74.7±0.5	76.9±0.5	77.9±0.9	<0.01
BMI, kg/m^2	21.3±0.1	23.8±0.1	26.2±0.1	27.6±0.1	29.1±0.2	31.6±0.5	<0.01
Waist, cm	71.9±0.2	78.1±0.5	82.4±0.4	85.1±0.4	88.5±0.5	92.2±1.1	<0.01
Education, y	6.8±0.1	7.9±0.4	7.2±0.3	7.8±0.2	7.6±0.3	9.0±0.5	<0.01
Smoker, %	19.2	20.5	7.0	12.4	10.4	20.0	<0.01
Alcohol Drinker, %	39.3	43.8	45.5	41.4	37.8	38.5	0.70
Occupational PA, %							<0.01
Not working	14.4	15.1	13.3	15.7	17.9	15.4	
Sedentary	6.6	4.1	7.0	6.2	7.5	9.2	
Light	46.9	54.8	53.8	59.0	56.7	61.5	
Moderate	21.6	19.2	17.5	13.3	11.4	10.8	
Heavy	10.5	6.8	8.4	5.7	6.5	3.1	

* Values are means ± standard error or percentages. † Women were not OW in 2002 survey but had been OW for at least 1 survey. y = year, BP = blood pressure, BMI = body mass index, and PA = physical activity. ‡ P-values for analysis of variance for means and chi-square test for proportions.

TABLE 3.2. Cumulative incidence ratios (95% CI) for hypertension according to overweight (OW) duration in adult Cebu women, 2002-2007

	Model 1[*]	Model 2[†]	Model 3[‡]
Never OW	1.00 (reference)	1.00 (reference)	1.00 (reference)
OW history[§]	1.14 (0.77-1.70)	1.27 (0.86-1.87)	1.13 (0.76-1.69)
OW < 4 y	1.20 (0.87-1.65)	1.47 (1.11-1.95)	1.19 (0.86-1.64)
OW 4-10 y	1.23 (0.90-1.67)	1.60 (1.27-2.02)	1.22 (0.90-1.67)
OW 11-17 y	1.40 (1.01-1.94)	1.95 (1.58-2.40)	1.40 (1.01-1.95)
OW ≥ 18 y	1.68 (1.09-2.59)	2.64 (2.06-3.38)	1.69 (1.10-2.60)

[*] Adjusted for age and body mass index. [†] Adjusted for age. [‡] Adjusted for age, body mass index and waist circumference. [§] Women were not OW in 2002 survey but have been OW for at least 1 survey.

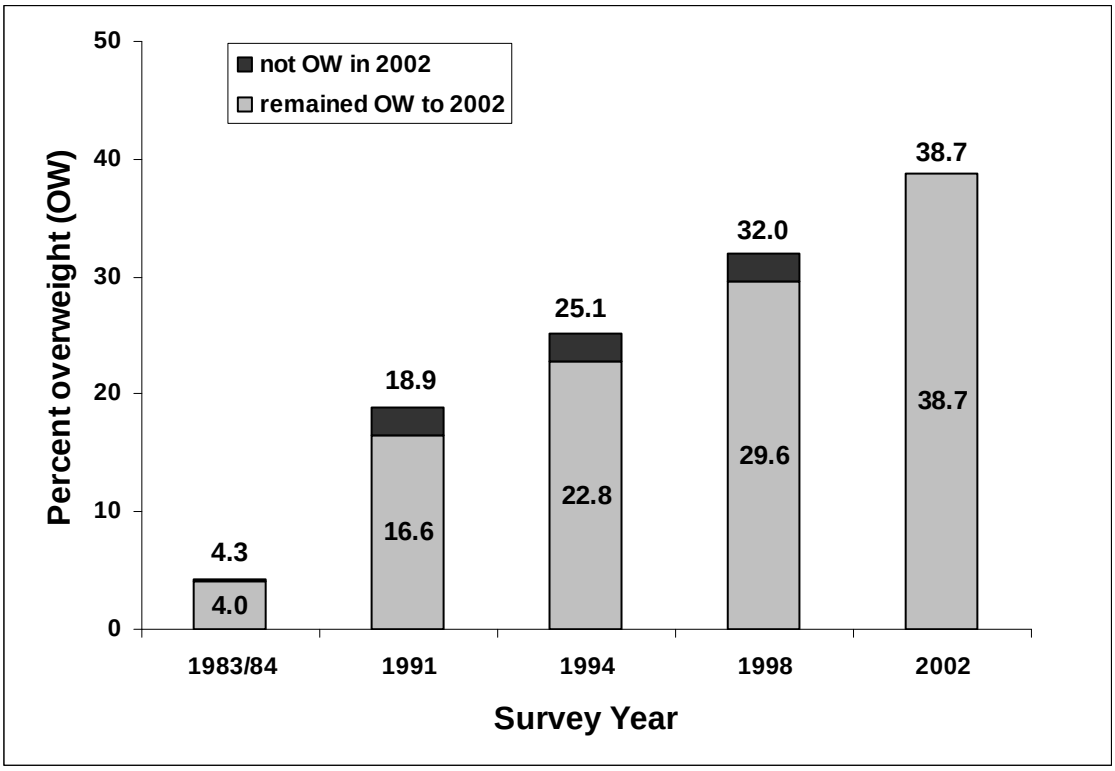

Figure 3.1. Trend in overweight (OW) prevalence from 1983/84 to 2002 among Cebu women who were not hypertensive in 2002. Values in gray area represent the percent of women who remained OW to 2002.

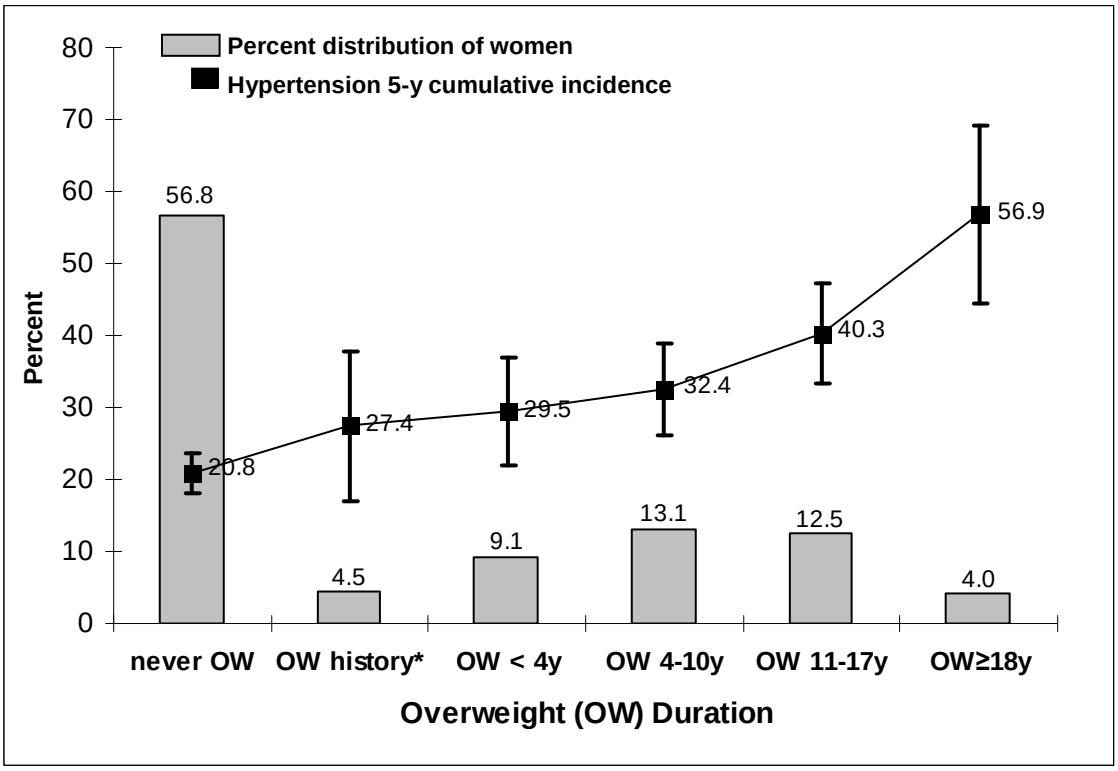

Figure 3.2. Percent distribution of Cebu women by overweight (OW) duration in the 2002 survey (gray bars) and corresponding five-year cumulative incidence (per 100 persons at risk) of hypertension for each OW duration group (dots with error bars representing 95 percent confidence interval). Hypertension cumulative incidence rate P for trend <0.01. *Women were not OW in 2002 survey but had been OW in at least 1 survey. y = years.

IV. Effects of *ACE* and *ADD1* variants on the prevalence of hypertension in Filipino women are modified by age

A. Abstract

Objective: To examine whether effects of the angiotensinogen (*AGT*) Met235Thr, angiotensin converting enzyme (*ACE*) intron 16 insertion/deletion (I/D) and alpha adducin (*ADD1*) Gly460Trp gene variants on hypertension prevalence in Filipino women are modified by age, BMI and presence of the other gene variants. **Methods and Results:** We used data from 1776 participants (age 35-68 years) of the Cebu Longitudinal Health and Nutrition Survey in 2005. We measured blood pressure, weight and height and genotyped *AGT* Met235Thr, *ADD1* Gly460Trp and rs4331 as a proxy for the *ACE* I/D variant. General, dominant, and recessive genetic models were explored. *AGT* Met235Thr influenced hypertension risk in all women. Adjusted OR (95% CI) comparing 1700 women carrying the Thr allele to 51 women homozygous for the Met allele was 2.57 (1.20-5.53). Evidence for association between *ACE* I/D and *ADD1* Gly460Trp and hypertension was only observed considering modification by age. For each year increase in age, the adjusted OR for the effects of *ACE* II (versus DD/DI) and *ADD1* GlyTrp/TrpTrp (versus GlyGly) increased by 6% and 4%, respectively. For women aged ≥ 55 years, OR for *ACE* II (versus DD/DI) was 2.53 (1.43-4.45) and OR for *ADD1* GlyTrp/TrpTrp (versus GlyGly) was 1.83 (1.02-3.27). Corresponding ORs for younger women were not significant. No significant gene-gene and gene-BMI interactions were observed. **Conclusions:** The *AGT* Met235Thr polymorphism

may play an important role in the development of hypertension in Filipino women. There is also some evidence that *ACE* I/D and *ADD1* Gly460Trp may contribute to hypertension risk in older women.

B. Introduction

Among the known cardiovascular disease (CVD) risk factors, hypertension makes the largest contribution to CVD burden, accounting for about 8 million of the estimated 16.7 million CVD-related deaths per year (WHO 2002; Mackay and Mensah 2004). Approximately 80% of this burden is borne by low- and middle- income countries (Lawes, Vander Hoorn et al. 2008). In the Philippines, a country with a population of over 88 million, heart and vascular diseases are the first and second leading causes of death accounting for 17% and 13% of deaths, respectively (DOH 2007).

Hypertension is a complex multifactorial polygenic disease (Pausova, Tremblay et al. 1999). Heritability estimates across studies and populations suggest that ~30% of the population variability in blood pressure (BP) may be attributed to genetic factors (Ward 1995; Arnett, Baird et al. 2007). Candidate gene studies have shown evidence for association between specific genes and hypertension, albeit inconsistently (Arnett, Baird et al. 2007). The scientific statement of the American Heart Association Council assessing the relevance of genetics and genomics for the prevention and treatment of CVD listed at least 30 candidate genes implicated in high BP and/or essential hypertension (Arnett, Baird et al. 2007). However, none of these candidate genes showed significant evidence (p-value $< 5 \times 10^{-7}$) in recent genome wide association studies of hypertension (Levy, Larson et al. 2007; WellcomeTrust 2007).

The renin-angiotensin system (RAS) is known to regulate BP by influencing vascular volume homeostasis and tone (Lifton, Gharavi et al. 2001). Thus, considerable efforts have been made in investigating polymorphisms in genes encoding the main components of this system. Of the angiotensinogen (*AGT*) gene polymorphisms, the Met235Thr polymorphism has been one of the most widely examined (Binder 2007) and has shown possible effect differences between populations. For example, a meta-analysis of 25055 individuals from 40 studies showed that hypertension risk is increased by 29 and 60% in Asians and by 8 and 19% in White subjects (MetThr versus MetMet and ThrThr versus MetMet, respectively). No significant increase was observed in Black subjects (Sethi, Nordestgaard et al. 2003). The insertion/deletion (I/D) polymorphism in intron 16 of the angiotensin converting enzyme (*ACE*) gene has also been suggested in the pathophysiology of hypertension, with the D allele associated with higher ACE levels and endothelial dysfunction and therefore increased BP (Luft 2004; Penesova, Cizmarova et al. 2006). However, there have been inconsistent results regarding the association between these RAS polymorphisms and hypertension, with some studies showing significant associations (O'Donnell, Lindpaintner et al. 1998; Higaki, Baba et al. 2000; Sethi, Nordestgaard et al. 2003) and other studies showing no significant associations (Kato, Sugiyama et al. 2000; Sato, Katsuya et al. 2000; Matsubara, Suzuki et al. 2002).

One of the plausible explanations for the conflicting results is that the risk conveyed by genes in the development of hypertension is context-dependent (Franks 2008). For example, effects of both the *ACE* I/D and *AGT* Met235Thr polymorphisms on BP may be modified by the Gly460Trp variant of the alpha adducin gene (*ADD1*), a polymorphism that impacts BP primarily by enhancing tubular sodium re-absorption through increased cellular

expression and activity of the Na-K pump (Bianchi 2005; Bianchi, Ferrari et al. 2005). Barlassina (2000) showed that the *ACE* and *ADD1* gene variants may act synergistically to increase BP and affect the pressure-natriuresis relationship where the large pressor effect of the *ACE* D allele was only seen in the presence of the *ADD1* 460Trp allele (Barlassina, Schork et al. 2000). Similarly, a study of Japanese individuals showed that *AGT* 235Thr and *ADD1* 460Trp did not increase hypertension odds when analyzed separately but were associated with hypertension when *AGT* ThrThr and *ADD1* TrpTrp were combined (Tamaki, Nakamura et al. 2005). In addition, factors including gender, age, body mass index (BMI), diet, and physical activity have been suggested to modify the effects of candidate genes (Svetkey, Moore et al. 2001; Katsuya, Ishikawa et al. 2003; Wang, Zhu et al. 2006; Fava, Montagnana et al. 2007; Franks 2008; Norat, Bowman et al. 2008). For example, a Swedish study found that the *ADD1* 460Trp variant was associated with higher systolic and diastolic BP only in women belonging to the highest tertile of BMI (Fava, Montagnana et al. 2007).

This study aimed to examine the effects of *AGT* Met235Thr, *ACE* I/D and *ADD1* Gly460Trp polymorphisms on the risk of hypertension in Filipino women and explored the effects of these three polymorphisms in different genetic and environmental contexts by looking at potential gene-age, gene-BMI and gene-gene interactions. Few studies have looked at the effects of genetic polymorphisms on hypertension in developing countries, and fewer have examined potential effect measure modifications. Although there have been studies examining these genes among Asians (mostly Japanese and Chinese), results have not been consistent (Kato, Sugiyama et al. 2000; Sato, Katsuya et al. 2000; Ju, Zhang et al. 2003; Cha, Kim et al. 2007; Huang, Sun et al. 2007). This is the first study investigating the effects of genetic variants on the development of hypertension in Filipinos.

C. Methods

C.1. Data source and sample

The study sample is comprised of participants in the ongoing Cebu Longitudinal Health and Nutrition Survey (CLHNS). Details of the CLHNS study design have been described previously (Adair and Popkin 2001). Briefly, the CLHNS was conducted in Metropolitan Cebu, one of the main metropolitan areas in the Philippines. In 1983, a stratified, single stage sampling design was used to randomly select 33 barangays (villages in rural areas, neighborhoods in urban areas) and all pregnant women in the selected barangays were initially invited to participate. Women were included in the survey if they gave birth between May 1, 1983 and April 30, 1984 (n=3327). Subsequent surveys took place immediately after birth, then every 2 months for 24 months for all women with singleton, live births. Follow-up surveys were conducted in 1991, 1994, 1998, 2002, 2005 and 2007.

For this study, the sample is limited to 1789 women who provided a blood sample in the 2005 survey, who had information on at least one of the polymorphisms, and who did not have an apparent first degree relative in the study based on predicted identity by descent estimates from 95,000 SNPs using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (data not shown) (Purcell, Neale et al. 2007) . We excluded women who were pregnant (n=8) and those missing BP measurements (n=5) for an analysis sample size of 1776.

C.2. Analysis variables

We used data taken during the 2005 survey, except for overweight (OW) duration. BP was measured using a mercury column sphygmomanometer after a 10 minute rest when the

woman was seated. The mean of three measurements was used to represent systolic BP and diastolic BP. Women reported all medications they were taking which were then classified according to their uses by a registered nurse. Hypertension was defined as systolic BP $\geq$ 140 mm Hg or diastolic BP $\geq$ 90 mm Hg or taking anti-hypertensive medications (Chobanian, Bakris et al. 2003).

Anthropometric measurements were taken at home while participants were wearing light clothing. Weight was measured using portable scales while height and waist circumference were measured using portable stadiometers and standard measuring tapes, respectively. BMI was calculated as weight in kg divided by squared height in meters. To determine OW duration, we created a binary variable indicating whether the non-pregnant woman was OW (BMI $\geq$ 25 kg/m^2) (WHO 2000) for each survey round from baseline to 2005. These binary OW variables were examined for patterns over time and were used to group women into five OW duration categories – never OW, with OW history (not OW in 2005 but have been OW in at least 1 survey), OW < 3 yrs, OW 3-10 yrs, and > 10 yrs OW, as determined by the timing of CLHNS surveys. Indicator variables were used to represent each OW duration category. Waist circumference was deemed high risk if $\geq$ 88 cm (WHO 2000).

Other variables of interest were collected by trained personnel through in-home face-to-face interviews using structured questionnaires. Age (years), log of household income, education (number of years of formal schooling), and number of pregnancies were used as continuous variables. Indicator variables were created to represent levels of occupational physical activity (not working, sedentary, light, moderate, or heavy) based on detailed occupation code, posture at work and physical demands of jobs. Binary indicator variables

were created for smoking status (current smoker or not), alcohol consumption (current drinker or not) and menopausal status (postmenopausal or not).

C.3. Genotyping

We selected three genetic polymorphisms suggested to be implicated in the development of high BP or essential hypertension (Jeunemaitre, Soubrier et al. 1992; Niu, Chen et al. 2002; Bianchi, Ferrari et al. 2005; Arnett, Baird et al. 2007) and shown to have minor allele frequency of >0.01 in Asian populations (Higaki, Baba et al. 2000; Ju, Zhang et al. 2003; Tamaki, Nakamura et al. 2005; Cha, Kim et al. 2007). These included the Met235Thr polymorphism of *AGT* (rs699, Met=T, Thr=C), Gly460Trp polymorphism of *ADD1* (rs4961, Gly=G, Trp=T), and I/D polymorphism of *ACE* represented by rs4331 (G/A alleles). Variant rs4331 was reported to be in linkage disequilibrium (D'=1.000, r^2 =0.967) with *ACE* I/D among 511 Japanese and can be more conveniently genotyped than the latter (Rieder, Taylor et al. 1999; Tanaka, Kamide et al. 2003). DNA was isolated from blood using the Gentra Puregene protocol and genotyping was performed using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA). Genotype distributions of the three polymorphisms were consistent with Hardy-Weinberg equilibrium, with Pearson chi-square test p-values >0.05.

We obtained informed consents from all participants. The study protocol was approved by the University of North Carolina Institutional Review Board for the Protection of Human Subjects.

C.4. Statistical analysis

Differences in proportions and means of selected characteristics of women in 2005 according to genotype were compared using chi-square test and t-test, respectively. To estimate the association between the genetic variants and hypertension, we used logistic regression. Since the modes of inheritance of the selected polymorphisms have not been completely elucidated, we initially tested the general or co-dominant model with no assumption of mode of inheritance. Based on the apparent direction of effects observed in the results of the general models, the dominant, recessive or additive genetic modes of inheritance were selected for further exploratory analysis and only significant results are reported. We assessed effect measure modification using likelihood ratio (LR) test where we compared models with and without interaction terms (i.e. gene*age or gene*BMI or gene*gene). Significant effect measure modification was set at LR test chi-square p-value <0.10. Higher alpha was specified because homogeneity tests have lower power to detect differences (Rothman and Greenland 1998). For the effect of the polymorphism on hypertension to be considered significant, alpha was set at p-value <0.05. We did not adjust for the multiple tests of SNPs, models of inheritance and number of interactions, thus results may be considered nominal p-values. Aside from age and BMI, we controlled for waist circumference, OW duration, cigarette smoking, alcohol consumption, menopausal status, years of education, and log of household income. Additionally adjusting for other covariates did not change the magnitude or precision of the estimates. We evaluated attrition bias by weighting the observations by the reciprocal of the probability of being in the study sample (Fitzgerald, Gottschalk et al. 1998). Since the weighted analysis was not substantially

different from the un-weighted analysis, we present un-weighted results. STATA/SE version 10.1 (StataCorp, College Station, TX) was used in all analyses.

D. Results

In 2005, participants' age ranged from 35 to 68 years. Women had an average BMI of 24.3 kg/m^2, and about 42% were OW. Most OW women had been OW for more than 10 years. About 28% were hypertensive (Table 4.1).

Gene-age, gene-BMI and gene-gene interactions were evaluated for the three polymorphisms. The *AGT* Met235Thr polymorphism appeared to be the strongest predictor of hypertension status, with homogenous effects across women with varying age, BMI, and *ACE* or *ADD1* genotypes. The hypertension odds among 1700 women carrying one or more copies of the 235Thr allele were 2.57 times (95% CI: 1.2-5.53; p-value = 0.016) higher than that of 51 women with the MetMet genotype (Table 4.2). No significant gene-BMI or gene-gene interactions were observed for any of the three polymorphisms.

For the effects of *ACE* and *ADD1* polymorphisms, we found age to be a significant effect measure modifier. Figure 4.1 (A) shows that the estimated odds ratio (OR) for hypertension comparing 633 women homozygous for *ACE* II to 1054 women with *ACE* DD/ID (reference) genotypes increased with age. For each year increase in age, the OR for the effect of *ACE* II increased by about 6% (LR test p = 0.006). For example, using coefficients from the logistic regression model, the estimated OR (95% CI) for hypertension comparing women with *ACE* II to women carrying one or more copies of the D allele was 0.69 (0.45-1.06; p-value = 0.089) for 40 year old women but was 2.04 (1.27-3.29; p-value = 0.003) for 60 year old women. We found a similar age modification pattern for the effect of

ADD1 on hypertension (LR test p = 0.079); however the observed gene effects were weaker (Figure 4.1 (B)). For each year increase in age, the OR for hypertension associated with *ADD1* 460Trp increased by about 4% (p for interaction = 0.079). Examining the effect of age on hypertension using spline analysis (not considering genotype), we observed that the effect of age changed at around 55 years. We stratified women into two age groups according to this cut-point and observed the odds of having hypertension among 89 women >=55 years with the *ACE* II genotype were 2.53 times higher (1.43-4.45; p-value = 0.001) compared to 171 women carrying the D allele (Table 2). For *ADD1*, 184 older women with the 460Trp allele had higher odds (OR: 1.83, 95% CI: 1.02-3.27; p-value = 0.041) of hypertension compared to 83 Gly homozygotes.

E. Discussion

Owing to the known influence of the RAS in potentially all aspects of BP regulation, genes in this system are among the most probable and likely have been the most investigated candidates in the quest to unravel the genetics of hypertension (Lifton, Gharavi et al. 2001; Luft 2004; Binder 2007). The current study found that *AGT* Met235Thr polymorphism was associated with the prevalence of hypertension in Filipino women using a model dominant for the common Thr allele. The odds of hypertension were doubled among women with the 235Thr allele. We also observed increased hypertension odds among older women with the *ACE* II genotype using a model recessive for the common I allele. In addition to looking at RAS genes, the current study examined the potential hypertensive effect of the Gly460Trp polymorphism of the *ADD1* gene. We did not find strong evidence supporting this association but the polymorphism may be a contributing factor in the pathophysiology of

hypertension in older women. Gene-BMI and gene-gene interactions were not observed in this study.

Jeunemaitre et al were the first to suggest the role of the *AGT* Met235Thr polymorphism in the development of hypertension (Jeunemaitre, Soubrier et al. 1992). Using data from 267 men and women of Northern and Western European descent, they showed that the ThrThr genotype was associated with an increase in plasma angiotensinogen and a 95% increase in the odds of hypertension compared to the MetMet genotype. Increased BP may be explained by the role of *AGT* in encoding the precursor molecule for angiotensin II which causes intense vasoconstriction and sodium re-absorption in the kidney. However, results of the numerous studies that followed this pioneering work were not consistent. A case-control study involving 1476 subjects reported a null association between Met235Thr and hypertension (Kato, Sugiyama et al. 2000) but a meta-analyses involving 25055 individuals concluded that *AGT* Met235Thr was associated with increased risk of hypertension in white subjects (OR: MetThr 1.08, ThrThr 1.19) and Asians (OR: MetThr 1.29, ThrThr 1.60); Asians were represented by 8 Japanese, 3 Chinese and 1 Emirati studies totaling 5181 individuals (Sethi, Nordestgaard et al. 2003). Results of our study support the findings of latter meta-analysis, showing similar or potentially higher ORs (MetThr 2.53 (1.15-5.56), ThrThr 2.57 (1.19-5.55)) for Filipino women.

The D allele of the *ACE* I/D polymorphism has been linked to higher plasma ACE levels (Tiret, Rigat et al. 1992; Luft 2004) and endothelial dysfunction (Penesova, Cizmarova et al. 2006) providing possible explanations for its role in increased BP. This variant has been reported as one of the most consistently linked polymorphisms to CVD in the general population but evidence of association with hypertension has been less convincing (Niu,

Chen et al. 2002; Luft 2004). *ACE* DD has been shown to influence hypertension risk with possible gender-specific effects (O'Donnell, Lindpaintner et al. 1998; Higaki, Baba et al. 2000; Lynch, Arnett et al. 2007). For example, using a population-based sample of mostly Caucasian men and women (Framingham Study), O'Donnell et al reported adjusted ORs for hypertension in 1445 men for the DD and DI genotypes as 1.59 (1.13-2.23) and 1.18 (0.87-1.62), respectively, versus II (O'Donnell, Lindpaintner et al. 1998). The corresponding ORs in 1650 women were 1.00 (0.70-1.44) and 0.78 (0.56-1.09), respectively. Similarly, Higaki et al analyzing data from a Japanese sample of 2340 men and 2674 women (Suita Study) reported an estimated OR (DD vs II) of 1.75 (1.21-2.53) for men and an OR of 1.17 (0.79-1.72) for women (Higaki, Baba et al. 2000). When stratified by age (≤60, >60 years) they found that the influence of the DD genotype was stronger in the younger age groups and weakest for older women. Zaman et al did not observe age modification in the effect of *ACE* I/D in a study involving 1340 Japanese (Zaman, Yoshiike et al. 2001). Our study found age as a potentially significant effect measure modifier of the relationship between the *ACE* I/D variant and the prevalence of hypertension, where we found significant association only in older women (≥ 55 years). Also, we found an opposite effect of the D allele in the older group – older women with the II genotype had increased risk of hypertension compared to women carrying at least one copy of the D allele. However, this pattern is not unique to our sample. Yoshida et al, examining 263 Japanese reported that having at least one I allele was associated with hypertension in the elderly group (>70 years) but not in the middle aged group (30-60 years) (Yoshida, Ishigami et al. 2000). A potential mechanism may be the higher aortic arterial stiffness observed in II genotypes (versus ID/DD) that has been

observed in different populations (Benetos, Gautier et al. 1996; Taniwaki, Kawagishi et al. 1999; Dima, Vlachopoulos et al. 2008).

The *ADD1* Gly460Trp variant may cause hypertension by enhancing tubular sodium re-absorption in the kidney (Bianchi 2005; Bianchi, Ferrari et al. 2005). The relationship between *ADD1* Gly460Trp polymorphism and hypertension in humans was first demonstrated by Cusi et al (Cusi, Barlassina et al. 1997). As with the other polymorphisms, results of studies that followed were inconsistent. For example, among Asians, Kato et al did not find a significant association between the 460Trp allele and hypertension in 507 Japanese (Kato, Sugiyama et al. 1998), Shin et al corroborated the negative finding examining 903 Koreans (Shin, Chung et al. 2004), but Huang et al found a significant association (OR: 1.43, p = 0.029) among 751 Han Chinese (Huang, Sun et al. 2007). Our study found a nominally significant association between the 460Trp allele and hypertension in older women only. On the contrary, Sugimoto et al observed that among 1490 Japanese, the effect of *ADD1* Trp allele was significant only in younger subjects (< 60 yrs) with low plasma renin activity (Sugimoto, Hozawa et al. 2002). Among 6103 Swedes, age did not modify the effect of *ADD1* 460Trp allele (Fava, Montagnana et al. 2007).

Results of this study should be interpreted with caution. First, the reduction in sample size from baseline (1983/84) to 2005 may produce bias and limit the generalizability of results. Compared to women who were not in the sample, the women included in this study had lower baseline BMI (20.8 versus 20.6 kg/m^2), were older (age at baseline: 26.3 versus 26.8 years) and had less years of education (8.0 versus 7.2 years). However, the main reason for attrition was out-migration (moving outside Metropolitan Cebu) and it is unlikely that this was associated with hypertension status and genotype distribution. Results of analyses

weighted by the reciprocal of the probability of being in the study sample were not considerably different from those of un-weighted analyses indicating no significant attrition bias. Second, the 3 BP measurements that were averaged to represent BP were taken on the same day and cannot account for day to day variations. This may cause regression dilution and result in attenuated results (Rothman and Greenland 1998). Third, different allele frequencies and levels of environmental exposures may make it difficult to compare and transfer the results of one study population to another. We typically observe allele frequencies in this Cebu sample to be similar to Han Chinese and Japanese samples (Marvelle, Lange et al. 2007). The frequencies of the higher risk *AGT* Thr allele (84%) and *ADD1* Trp allele (43%) in our sample are similar to those reported in other East Asians (~68-83% and ~42-66%, respectively) (Kato, Sugiyama et al. 2000; Katsuya, Ishikawa et al. 2003; Sethi, Nordestgaard et al. 2003) and higher than reported in Caucasians (~43-44% and ~18-27%, respectively) (Sethi, Nordestgaard et al. 2003; Bianchi 2005; Goldenberg, Moss et al. 2006). Likewise, the frequency of the *ACE* II genotype in our sample (37%) is similar to other East Asians (~37-43%) (Zaman, Yoshiike et al. 2001; Jiang, Sheng et al. 2008) and higher than reported in Caucasians (~17-23%) (Staessen, Wang et al. 2001; Dima, Vlachopoulos et al. 2008). Considering potential age-dependent effects of the *ACE* I/D and *ADD1* Gly460Trp polymorphisms, the different age distributions and categorizations of participants across studies may contribute to the differing results. Lastly, we performed but did not adjust for multiple tests of SNPs, genetic models, and interactions thus we cannot rule out the possibility of false positive findings. We treated each test for association as a separate hypothesis and did not subscribe to a global null (Rothman 1990).

The present study is the first study that investigated the role of genetic variants in the development of hypertension in Filipinos. By exploring potential effect heterogeneity, we responded to the call for context-dependent analyses (Franks 2008) and contribute to a limited but important body of literature that may help elucidate the complexity of the genetics of hypertension. Higher risk genetic environments may be necessary for hypertension to develop. The effects of the selected polymorphisms may only be evident when other genetic and environmental characteristics are taken into account (Barlassina, Schork et al. 2000; Svetkey, Moore et al. 2001; Katsuya, Ishikawa et al. 2003; Tamaki, Nakamura et al. 2005; Fava, Montagnana et al. 2007; Franks 2008; Jiang, Sheng et al. 2008). We would not have observed the potential role of *ACE* I/D and *ADD1* Gly460Trp polymorphisms in hypertension risk if differential effects by age were not explored.

In summary, the study suggests that AGT Met235Thr polymorphism may play an important role in the development of hypertension in adult Filipino women. There is also some evidence that *ACE* I/D and *ADD1* Gly460Trp may contribute to hypertension risk in older women.

Table 4.1. Selected characteristics of CLHNS women in 2005, N=1776

Characteristic	Mean ± standard deviation or %
Age, year	48.4 ± 6.02
Systolic BP, mmHg	119.8 ± 20.0
Diastolic BP, mmHg	79.8 ± 12.4
Hypertensive, %	27.9
Use of anti-hypertensive medications, %	4.0
BMI, kg/m^2	24.3 ± 4.4
Waist circumference, cm	81.1 ± 10.9
Overweight (OW) duration, %	
Never OW	47.6
History of OW	10.2
OW <3 years	6.2
OW 3-10 years	11.0
OW >10 years	25.0
Education, year	6.8 ± 3.3
Current smoker, %	14.9
Alcohol drinker, %	38.1
Postmenopausal, %	38.4

Table 4.2. Effects of *AGT*, *ACE* and *ADD1* polymorphisms on odds of hypertension, by age

	N (%)				
	Not hypertensive	Hypertensive	Odds Ratio*	95 % CI	p-value
***AGT* rs699**					
All women					
MetMet	42 (82.4)	9 (17.6)	1.00 (ref)		
MetThr/ThrThr	1222 (71.9)	478 (28.1)	2.57	1.20-5.53	0.016
***ACE* rs4331**					
<55 years					
DD/DI	646 (73.2)	237 (26.8)	1.00 (ref)		
II	404 (74.3)	140 (25.7)	0.96	0.75-1.25	0.779
≥55 years					
DD/DI	116 (67.8)	55 (32.2)	1.00 (ref)		
II	44 (49.4)	45 (50.6)	2.53	1.43-4.45	0.001
All women					
DD/DI	762 (72.3)	292 (27.7)	1.00 (ref)		
II	448 (70.8)	185 (29.2)	1.13	0.90-1.43	0.282
***ADD1* rs4961**					
<55 years					
GlyGly	351 (72.7)	132 (27.3)	1.00 (ref)		
GlyTrp/TrpTrp	742 (74.4)	255 (25.6)	0.92	0.71-1.19	0.526
≥55 years					
GlyGly	59 (71.1)	24 (28.9)	1.00 (ref)		
GlyTrp/TrpTrp	106 (57.6)	78 (42.4)	1.83	1.02-3.27	0.041
All women					
GlyGly	410 (72.4)	156 (27.6)	1.00 (ref)		
GlyTrp/TrpTrp	848 (71.8)	333 (28.2)	1.02	0.81-1.29	0.846

*Adjusted for body mass index, waist circumference (high risk or not), overweight duration, cigarette smoking (current smoker or not), alcohol consumption (current drinker or not), menopausal status (postmenopausal or not), years of education and log of household income. Estimates for all women were additionally adjusted for age. ref = reference group

Figure 4.1 (A)

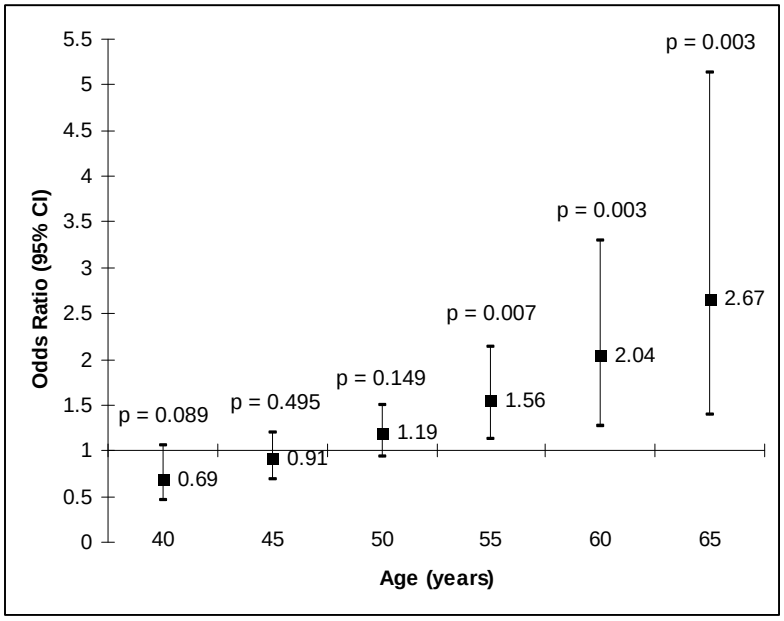

Figure 4.1 (B)

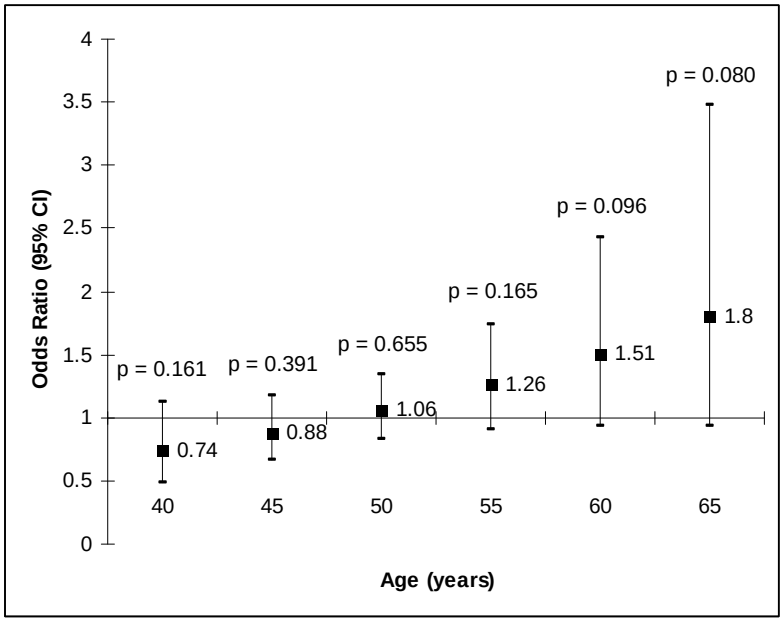

Figure 4.1. Odds of hypertension comparing genotypes at selected ages. (A) *ACE* (rs4331) II vs DD/DI and (B) *ADD1* (rs4961) GlyTrp/TrpTrp vs GlyGly. Adjusted for age, body mass index, waist circumference (high risk or not), overweight duration, cigarette smoking (current smoker or not), alcohol consumption (current drinker or not), menopausal status (postmenopausal or not), years of education and log of household income.

V. The association between high sodium intake and hypertension in Filipino women is influenced by genetic and environmental factors

A. Abstract

Background: Studies investigating the effect of dietary sodium on hypertension in developing Asian countries are largely lacking. Fewer have investigated heterogeneity of the sodium-hypertension association according to different genetic and environmental factors in these settings. **Methods and Results:** We determined the association between high sodium intake (≥4600 mg/day) and hypertension and assessed whether this association was modified by gene variants suggested in salt-sensitivity (angiotensinogen (*AGT*) Met235Thr, angiotensin converting enzyme (*ACE*) insertion/deletion, and alpha adducin, *ADD1* Gly460Trp) and by age, body mass index (BMI), overweight (OW) duration and smoking status. We used data from 1776 women participants (age 35-68 years) of the Cebu Longitudinal Health and Nutrition Survey collected in 2005. Logistic regression analyses showed increased effect of high sodium intake on hypertension in women: (1) with the *ADD1* TrpTrp genotype with adjusted odds ratio (OR, 95% CI: 2.99, 1.44-6.22); (2) who had never been OW (2.27, 1.45-3.55); and (3) who were smokers (3.06, 1.51-6.19). Additionally, OR increased by 6% for each year increase in age and decreased by 7% for each kg/m^2 increase in BMI. Effect measure modification by the *AGT* and *ACE* variants were not observed. **Conclusions:** With relatively high frequency of the *ADD1* 460TrpTrp genotype and as the population gets older, efforts to reduce sodium intake should be intensified. Women who

have lower BMI, those who had never been OW and those who smoke should be especially cautioned.

B. Introduction

Hypertension makes the largest contribution to the worldwide burden of cardiovascular diseases (CVD) accounting for almost half of the estimated 16.7 million CVD-related deaths per year and a third of CVD-related disability adjusted life years (DALYs) (WHO 2002; Mackay and Mensah 2004). Approximately 80% of the burden attributed to high blood pressure (BP) is borne by low- and middle-income countries (Lawes, Vander Hoorn et al. 2008). It is a multi-factorial disease that develops from the complex interplay of environmental and genetic factors (Pausova, Tremblay et al. 1999).

The Philippines, a lower middle income country with a population of more than 88 million, has been undergoing rapid socio-economic changes. The country has not been immune to the large shifts in obesity, hypertension and many other non-communicable diseases that are occurring across the globe (Kearney, Whelton et al. 2005; Popkin 2008). Heart and vascular diseases are ranked as the first and second leading causes of mortality accounting for 17% and 13% of deaths, respectively (DOH 2007). Estimated (DALYs) from heart disease were estimated to be higher in the Philippines than in the U.S. or China (Mackay and Mensah 2004).

The burden of hypertension among Filipinos may reflect a wide range of factors. First, Asians tend to develop hypertension at a lower body mass index (BMI) compared to Caucasian populations (Colin Bell, Adair et al. 2002; WHO 2004). Second, many Asians

consume high amounts of sodium owing to dietary habits that involve extensive use of added salt in the form of high sodium condiments (WHO 2007).

The role of sodium intake in the development of high BP has not been without controversy. Jurgens and Graudal in their systematic review of studies looking at the sodium-BP relationship concluded that the magnitude of the effect of high sodium intake on BP did not warrant a general recommendation to reduce sodium consumption among Caucasians (Jurgens and Graudal 2004). Although there was a higher sodium effect on BP in Blacks and Asians, the number of studies (8 and 1, respectively) was insufficient to make a different recommendation. Nonetheless, the World Health Organization (WHO) declared that the evidence supporting the association between high sodium intake and high BP is conclusive (WHO 2007). One of the largest and most commonly cited studies that investigated the sodium and BP relationship is the INTERSALT. Using data collected from 32 countries, this study reported that sodium excretion was significantly related to the slope of BP with age (INTERSALT 1988).

There is less controversy regarding the heterogeneity of BP response to sodium intake, raising the notion of salt-sensitivity and salt-resistance (INTERSALT 1988; Weinberger 2006). Altered activity of neurohormonal systems and enhanced sodium re-absorption are among the plausible explanations to the differential response to sodium intake (Beeks, Kessels et al. 2004). Thus, genetic factors that affect these processes may play important roles and warrant investigation. Studies have suggested the influence of the alpha adducin (*ADD1*) gene variant Gly460Trp which can potentially influence BP by enhancing tubular sodium re-absorption (Cusi, Barlassina et al. 1997). Similarly, the potential effects of genes encoding the main components of the renin-angiotensin system (RAS) on the BP

response to sodium have been evaluated but results have not been consistent (Beeks, Kessels et al. 2004).

Studies investigating the effects of dietary sodium intake on BP in low and middle income countries are largely lacking, especially among Asians from developing countries such as the Philippines (WHO 2007). This study determined the association between high sodium intake and hypertension in Filipino women and examined whether there is heterogeneity in the effect of sodium according to different genetic and lifestyle characteristics. For the genetic characteristics, we specifically assessed effect measure modification by the following gene variants: angiotensinogen (*AGT*) Met235Thr, angiotensin converting enzyme (*ACE*) insertion/deletion (I/D) in intron 16, and *ADD1* Gly460Trp. These variants have been associated with salt-sensitive hypertension and have been shown to potentially influence hypertension prevalence in our sample (see Chapter IV). We also examined the roles of age, BMI, overweight duration, and smoking status.

C. Methods

C.1. Data source and sample

The study sample is comprised of participants in the ongoing Cebu Longitudinal Health and Nutrition Survey (CLHNS). Details of the CLHNS study design have been described previously (Adair and Popkin 2001). Briefly, the CLHNS was conducted in Metropolitan Cebu, one of the main metropolitan areas in the Philippines. In 1983, a stratified, single stage sampling design was used to randomly select 33 barangays (villages in rural areas, neighborhoods in urban areas) and all pregnant women in the selected barangays were initially invited to participate. Women were included in the survey if they gave birth

between May 1, 1983 and April 30, 1984 (n=3327). Follow-up surveys were conducted in 1991, 1994, 1998, 2002, 2005 and 2007.

For this study, the sample is limited to 1789 women who provided a blood sample in the 2005 survey, who had information on at least one of the polymorphisms, and who did not have an apparent first degree relative in the study based on predicted identity by descent estimates from 95,000 SNPs using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (data not shown) (Purcell, Neale et al. 2007) . We excluded women who were pregnant (n=8) and those missing BP measurements (n=5) for an analysis sample size of 1776.

C.2. Analysis variables

BP. We used data taken during the 2005 survey. BP was measured using a mercury column sphygmomanometer after a 10 minute rest when the woman was seated. The mean of three measurements was used to represent systolic BP and diastolic BP. Women reported all medications they were taking which were then classified according to their uses by a registered nurse. Hypertension was defined as systolic BP $\geq$ 140 mmHg or diastolic BP $\geq$ 90 mmHg or taking anti-hypertensive medications (Chobanian, Bakris et al. 2003).

Sodium Intake. The CLHNS collected two 24-hour dietary recalls that were supplemented by semi-structured questions on the usual intake of salty condiments added during cooking or at the table. Women were asked the frequency, amount and method of use of added salty condiments that included table salt, soy sauce, fish sauce, monosodium glutamate (MSG) and other high sodium flavorings. Dietary data were reviewed by licensed and trained nutritionists and implausible values were immediately identified and verified by revisits. Philippine Food Composition Tables (FCT) produced by the Food and Nutrition

Research Institute (FNRI) of the Philippines and regularly updated by an Office of Population Studies Foundation resident nutritionist were used to calculate energy and nutrient intakes. For foods missing sodium content information in the Philippine FCT, we used values from the China FCT.

Total sodium intake was estimated as the sum from foods and from added condiments. Women who consumed at least 4600 mg of sodium per day were classified as having high sodium intake. This cutpoint is equivalent to 200% of the recommended upper limit for adults (IOM 2004) and has been used in sodium-BP clinical trials to represent high sodium intake (MacGregor, Markandu et al. 1989; van der Kleij, de Jong et al. 2002). This corresponds to the 85^{th} percentile of total sodium intake of our study sample.

Genotyping. We selected three genetic polymorphisms suggested to be implicated in the development of high BP or essential hypertension (Jeunemaitre, Soubrier et al. 1992; Niu, Chen et al. 2002; Bianchi, Ferrari et al. 2005; Arnett, Baird et al. 2007) and shown to have minor allele frequency of >0.01 in Asian populations (Higaki, Baba et al. 2000; Ju, Zhang et al. 2003; Tamaki, Nakamura et al. 2005; Cha, Kim et al. 2007). These included the Met235Thr polymorphism of *AGT* (rs699, Met=T, Thr=C), Gly460Trp polymorphism of *ADD1* (rs4961, Gly=G, Trp=T), and I/D polymorphism of *ACE* represented by rs4331 (G/A alleles). Variant rs4331 was reported to be in linkage disequilibrium (D'=1.000, r^2 =0.967) with *ACE* I/D among 511 Japanese and can be more conveniently genotyped than the latter (Rieder, Taylor et al. 1999; Tanaka, Kamide et al. 2003). DNA was isolated from blood using the Gentra Puregene protocol and genotyping was performed using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA). Genotype distributions of the

three polymorphisms were consistent with Hardy-Weinberg equilibrium, with Pearson chi-square test p-values >0.05.

Covariates. Anthropometric measurements were taken by trained personnel at home while participants were wearing light clothing. Weight was measured using portable scales while height was measured using portable stadiometers. BMI was calculated as weight in kg divided by squared height in meters. To determine overweight duration, we created a binary variable indicating whether the non-pregnant woman was overweight (OW = BMI $\geq$ 25 kg/m^2) (WHO 2000) for each survey round from baseline to 2005. These binary OW variables were examined for patterns over time and were used to group women into five OW duration categories – never OW, with OW history (not OW in 2005 but have been OW in at least 1 survey), OW < 3 yrs, OW 3-10 yrs, and OW > 10 yrs. Indicator variables were used to represent each OW duration category. Waist circumference was measured using standard measuring tapes and was deemed high risk if $\geq$ 88 cm (WHO 2000).

Other variables of interest were collected through in-home face-to-face interviews using structured questionnaires. Age (years), log of household income, education (number of years of formal schooling), and number of pregnancies were used as continuous variables. Indicator variables were created to represent levels of occupational physical activity (not working, sedentary, light, moderate, or heavy) based on detailed occupation codes, posture at work and physical demands of jobs. Binary indicator variables were created for smoking status (current smoker or not), alcohol consumption (current drinker or not) and menopausal status (postmenopausal or not). We used a community level continuous urbanicity index variable (0-70 scale) based on population size and density, community infrastructure, economic and environment characteristics of the woman's barangay of residence in 2005.

This index has been validated and found to be a better measure of urbanicity than the urban-rural dichotomy (Dahly and Adair 2007). Mean total energy intake (kcal) and calcium intake (mg/day) were estimated from two 24-hour recalls.

We obtained informed consent from all participants. The study protocol was approved by the University of North Carolina Institutional Review Board for the Protection of Human Subjects.

C.3. Statistical analysis

Differences in proportions and means of selected characteristics of women in 2005 according to sodium intake (lower versus high) were compared using chi-square test and t-test, respectively. We used logistic regression to estimate the effect of sodium intake on hypertension. To determine whether the association between sodium intake and hypertension was modified by the selected genetic polymorphisms and other covariates, we employed the likelihood ratio (LR) test where models with and without interaction terms (i.e. sodium intake X gene, sodium intake X covariates) were compared. Significant effect measure modification was set at LR test chi-square p-value <0.10. Higher alpha was specified since homogeneity tests have lower power to detect differences (Rothman and Greenland 1998). Among the lifestyle factors, we found age, BMI, OW duration, and smoking status to be significant effect measure modifiers, thus these variables were retained in the model. We also adjusted for total daily energy intake. To estimate the effect of sodium intake on hypertension according to levels or categories of the effect measure modifiers, separate analyses were conducted for each modifier. For example, to estimate the effect of high sodium intake for women at different ages, we used a model with sodium, age and sodium-

age product term and controlled for BMI, OW duration, smoking status and total energy intake. Models examining effect measure modification for each of the genetic variants controlled for age, BMI, OW duration, smoking status and total energy intake. Additionally controlling for other covariates did not substantially (at least 10%) change the exposure-outcome effect estimates. We evaluated attrition bias by weighting the observations by the reciprocal of the probability of being in the study sample (Fitzgerald, Gottschalk et al. 1998). Since the weighted analyses were not substantially different from the un-weighted analyses, we present un-weighted results. STATA/SE version 10.1 (StataCorp, College Station, TX) was used in all analyses.

D. Results

Mean sodium consumption in 2005 was about 2900 mg of sodium per day, with 49.9% of women consuming more than the recommended upper limit (2300 mg/day) and 14.9% consuming at least 4600 mg/day. Salty condiments added during cooking or at the table accounted for 76.3% of sodium intake (Table 5.1). The most significant source of sodium was table salt, contributing 53.3% for women who consumed <4600 mg/day of sodium and 66.5% for women who consumed higher amounts of sodium.

We found no significant differences in women's age, diastolic BP, BMI, waist circumference, occupational physical activity, education, smoking habits, alcohol consumption and genotype distributions between groups defined by sodium intake. Women with higher sodium intake (≥4600 mg) were more likely to be hypertensive and less likely to had never been OW (Table 5.2). Almost 28% of the sample women were hypertensive.

Logistic regression analyses controlling for age, BMI, OW duration, smoking status and energy intake, showed that high sodium intake increased hypertension odds (odds ratio (OR) (95% CI): 1.39 (1.03-1.87). The association between sodium intake and hypertension did not differ by *ACE* I/D and *AGT* Met235Thr polymorphisms but significantly differed by *ADD1* Gly460Trp polymorphism. For women with the *ADD1* GlyGly and GlyTrp genotypes, the OR for hypertension comparing women with high sodium intake to those with lower sodium intake were 1.03 (0.62-1.72) and 1.32 (0.87-2.01), respectively (Table 5.3). However, for women with TrpTrp genotype, high sodium intake was associated with a nearly 3 fold (2.99 (1.44-6.22)) increase in the odds of hypertension compared to the odds for women with lower sodium intake (Table 5.3). The effect estimates for *ADD1* GlyGly and GlyTrp genotypes were not significantly different from each other but were different from the effect estimate for TrpTrp genotype.

Additionally, we found effect measure modification by age, BMI, OW duration and smoking status. The effect of high sodium intake on hypertension increased with age (Figure 5.1). For each year increase in age, the OR increased by 6%. For example, using coefficients from the logistic regression model, the estimated OR for hypertension comparing women with high sodium intake to women with lower sodium intake was 0.81 (0.47-1.39) for 40 year old women but was 2.62 (1.44-4.75) for 60 year old women. The reverse was true for BMI. The association between high sodium intake and hypertension decreased with increasing BMI (7% decrease for each unit increase in BMI); high sodium intake was not associated with hypertension in women with BMI >25 kg/m^2 (Figure 5.2). Similarly, we found that increased odds of hypertension was associated with high sodium intake only for

never OW women (Table 5.3). Smoking magnified the effect of sodium on hypertension from 1.18 (0.85-1.64) for non-smokers to 3.06 (1.51-6.19) for smokers.

We further explored whether the association between high sodium intake and hypertension significantly differed by age, BMI (OW or not), and OW duration categories if we stratify women according *ADD1* Gly460Trp genotype (*ADD1* GlyGly/GlyTrp versus TrpTrp). Women with *ADD1* GlyGly and GlyTrp were combined since the effect of high sodium intake on hypertension in these groups did not significantly differ. We found that the effects of age on the association between high sodium intake and hypertension were similar regardless of *ADD1* genotype – for each year increase in age, the effect of high sodium intake on hypertension prevalence increased by 6%. For BMI, we observed that the weaker effect of sodium on hypertension for OW women was true primarily for women carrying one or more Gly alleles (Table 5.4). The same pattern is seen for OW duration. We did not find significant effect measure modification by OW status or duration among women homozygous for the Trp allele.

E. Discussion

To the best of our knowledge, this population-based study is the first to examine the effect of consuming high amounts of sodium on hypertension in Filipinos. We found that high sodium intake (≥ 4600 mg/day) increased the risk of hypertension among adult women in this population and that the degree of the adverse effect varied according to certain genetic and lifestyle factors. The effect of high sodium intake on hypertension prevalence was greater in women with the *ADD1* TrpTrp genotype (versus GlyGly or GlyTrp), women who

were older, had lower BMI especially those who had never been overweight, and among smokers.

The effect of dietary sodium on hypertension risk may be influenced by the *ADD1* Gly460Trp polymorphism by enhancing renal tubular sodium re-absorption (Bianchi 2005). This mechanism may be driven by an increase in Na-K pump activity and a deficiency of Na-K pump endocytosis that were observed in cells transfected with the *ADD1* Trp allele compared to cells with the Gly allele (Efendiev, Krmar et al. 2004; Bianchi 2005). The TrpTrp genotype was also shown to be associated with reduced renal plasma flow and glomerular filtration rate as compared to the GlyGly genotype (Beeks, van der Klauw et al. 2004). In fact, the different BP response to sodium according to *ADD1* Gly460Trp variant had been previously reported (Cusi, Barlassina et al. 1997; Grant, Romero et al. 2002). In a salt-sensitivity test designed to assess acute BP response to changes in body sodium in 86 hypertensive patients, Cusi et al found that the decrease in mean arterial pressure (MAP) was greater in patients who were heterozygous for the mutant allele (Gly/Trp) than in Gly/Gly homozygotes (mean decrease 15.9 versus 7.4 mmHg; $p = 0.001$). With only 2 subjects having the TrpTrp genotype, this group was not included in the analysis (Cusi, Barlassina et al. 1997). Similarly, Grant et al examined 279 subjects and found greater systolic BP increment in response to the change from low (~230 mg/day) to high sodium diet (~4600 mg/day) in subjects homozygous for the TrpTrp allele (25+/-4 mmHg) compared to heterozygous (12+/-2 mmHg) or homozygous for the Gly allele (14+/-1 mmHg) (Grant, Romero et al. 2002). In line with our results, there was no significant difference in the BP response to sodium intake between the GlyGly and GlyTrp subjects. However, a population-based cross-sectional study of 2823 Japanese men and women found a significant interaction

between sodium excretion and the Gly460Trp polymorphism on systolic BP in men but not in women (Yamagishi, Iso et al. 2004). Mean systolic BP was 5.2 mmHg higher in the TrpTrp group compared to the GlyGly group in men with higher sodium excretion (≥4350 mg/day) but not in men with lower sodium excretion. For men with higher sodium excretion, mean systolic BP of the GlyGly group was similar to the GlyTrp group (133.6 versus 134.6 mmHg).

Older age is associated with increased sodium sensitivity, a steeper BP and exchangeable body sodium relationship, decreased gain of the baroreceptor reflex, reduced renal perfusion, and a compromised buffering effect of large arteries on systolic and diastolic pressure (Staessen, Wang et al. 2003). These mechanisms may explain the increasing hypertensive effect of high sodium intake with increasing age observed in our sample. This finding supports most of the studies that examined potential age-dependent sodium and BP association. The INTERSALT study, one of the largest population based studies that examined the association between sodium excretion and BP with 10079 participants (age 20-59) recruited from 32 countries, showed that in populations with high salt intake, the association between age and BP was steeper than in those with low salt intake suggesting a positive age and salt intake interaction (INTERSALT 1988). Law et al analyzed published reports of BP and sodium intake (47000 people from 24 communities around the world) and found that a difference of 2300 mg/day was associated with an average difference in systolic BP that ranged from 5mmHg at age 15-19 to 10mmHg at age 60-69 years (Law, Frost et al. 1991). The well-known Dietary Approaches to Stop Hypertension (DASH)-sodium trial where participants were given 3 levels of sodium (1150, 2300, 3450 mg/2100 kcal) for 30 days while consuming the DASH diet or a more typical American diet showed

that age had a strong and graded influence on the effect of sodium on BP: sodium intake reduction from 3450 to 2300mg/2100 kcal and reduction from 2300 to 1150 mg/2100 kcal resulted in reduction of systolic BP by 4.8 and 1.0 mmHg for 23 to 41 years, 5.9 and 1.8 mm Hg for 42 to 47 years, 7.5 and 4.3 mm Hg for 48 to 54 years, and 8.1 and 6.0 mm Hg for 55 to 76 years (Bray, Vollmer et al. 2004). Results of a recent dietary feeding (GenSalt) study in China with 1906 participants corroborated these findings (He, Gu et al. 2009).

It has been suggested that excess weight or obesity may enhance the hypertensive effect of sodium (Weinberger 2006). Hyperinsulenemia (Rocchini, Key et al. 1989; Reaven, Lithell et al. 1996; Muscelli, Camastra et al. 1998), increased renal sodium re-absorption and a blunted response to natriutic hormones (Hall, Jones et al. 2003; Chalmers, Kaskel et al. 2006; Mathew, Patel et al. 2007) that can accompany excess weight are possible mechanisms that may lead to different BP responses to sodium intake. However, a positive interaction of the effects of BMI and sodium intake on hypertension has not been consistently shown. In a study involving 60 obese and 18 non-obese adolescents, a change from a high salt to a low salt diet resulted to a larger mean change in mean arterial pressure (MAP) in the obese group suggesting salt sensitivity (Rocchini, Key et al. 1989). A 24-week, placebo-controlled, two-period, crossover trial of sodium supplementation in 112 African Americans, aged 25 to 64 years, showed that variability-adjusted BP change correlated with BMI (Flack, Grimm et al. 2002). However, a double-blind randomized study of 46 white non-obese subjects aged 25 to 80 years showed that salt-sensitive subjects had lower body weight than salt-resistant individuals and that subjects with a BMI <26 kg/m2 showed a rise in MAP when moving from a low sodium to a high sodium diet, whereas subjects with BMI >26 kg/m2 did not (Overlack, Ruppert et al. 1995). Prolonged OW or obesity has also been associated with

insulin resistance and hyperinsulinemia (Muscelli, Camastra et al. 1998; Janssen, Katzmarzyk et al. 2004), and gradual loss of kidney function that worsens with time (Wofford and Hall 2004) but no other study has examined the modifying effect of OW duration on the sodium-BP relationship. Our results showed that the effect of higher sodium intake on hypertension risk was decreased as BMI and OW duration increased. It may be because excess weight (Garrison, Kannel et al. 1987; Must, Spadano et al. 1999; Davy and Hall 2004) and being OW for a longer duration (Janssen, Katzmarzyk et al. 2004) are very strong predictors of hypertension risk such that the effects of other factors including high sodium intake will not add considerable observable risk. Although the increased risk of hypertension in non-OW or never OW women attributed to high sodium consumption has not been shown or investigated in other Asian populations, this may partly explain why Asians tend to develop hypertension at lower BMI.

We showed that the effect of high sodium intake was heightened in smokers compared to non-smokers. We have not found previous studies that investigated whether the effect of high sodium intake on hypertension differ by smoking habits. Nonetheless, smoking has been shown to affect systemic and intrarenal hemodynamics that may lead to renal functional impairment (Orth 2004). It may be possible that these processes may affect sodium re-absorption.

This study has certain limitations. First, sodium intake was estimated using dietary recalls and a food frequency questionnaire which are more prone to measurement errors compared to 24-hour urine collections (WHO 2007). Sodium intake was likely underestimated but we do not believe that the degree of underestimation or error was differential. Also, setting a relatively high cut-point may reduce the probability of

misclassifying women with high sodium intake. Second, the reduction in sample size from baseline (1983/84) to 2005 may introduce selection bias and limit the generalizability of results. Compared to women who were not in the 2005 sample, the women included in this study had lower baseline BMI (20.8 versus 20.6 kg/m^2), were older (age at baseline: 26.3 versus 26.8 years) and had less years of education (8.0 versus 7.2 years). However, the main reason for attrition was migration to areas outside of Metropolitan Cebu and it is unlikely that this was associated with sodium intake and having hypertension. Results of analyses weighted by the reciprocal of the probability of being in the study sample were not different from those of un-weighted analyses indicating no significant attrition bias. Third, we used cross-sectional data and cannot establish that high sodium intake preceded the development of hypertension. But, it is more likely that having hypertension will result in reduced rather than increased sodium intake; therefore reverse causation will more likely bias the estimates to the null. Lastly, the 3 BP measurements that were averaged to represent BP were taken on the same day and cannot account for day-to-day variations. This may cause regression dilution and result in attenuated results (Rothman and Greenland 1998).

This study is the first to determine the effect of high sodium diet on hypertension in Filipinos, a population inflicted with high rates of hypertension and other CVD and who consume relatively high amounts of sodium. We add to the scant literature exploring heterogeneity of the effect of sodium consumption on hypertension according to genetic variants and known environmental hypertension risk factors. With rich dietary, anthropometric, demographic and lifestyle data we were able to assess confounding of other variables. Additionally, we explored and showed potential 3-way interactions of sodium

intake, *ADD1* Gly460Trp and BMI or OW duration. This can guide future studies with larger sample sizes to assess these interactions.

Examining 23 countries that account for 80% of the burden of chronic disease in low- and middle-income regions of the world, Asaria et al estimated that the Philippines, along with China and Egypt, would have the most to gain by salt reduction interventions (in terms of deaths averted by interventions as a proportion of total possible deaths from chronic disease) (Asaria, Chisholm et al. 2007). The findings of this study support the intensification of programs designed to reduce salt intake in the country. First, we found that the frequency of the higher risk *ADD1* Trp allele (43%) or the TrpTrp genotype (19%) in our sample is higher compared to Caucasians (~18-27% and ~2-4%, respectively) (Cusi, Barlassina et al. 1997; Staessen, Wang et al. 2001; Grant, Romero et al. 2002; Fava, Montagnana et al. 2007). Second, our results indicate that greater benefits may be observed in older individuals of whom the rate of CVD can potentially increase at a faster rate (Bray, Vollmer et al. 2004; Mackay and Mensah 2004). Third, in our sample, about 21% of non-OW and 19% of never OW women were hypertensive and our findings suggest that this may be driven by high sodium consumption. Thus, to reduce hypertension in the population, it is imperative that these groups of women, who are most likely presumed to be at low risk for hypertension, should be especially cautioned against high sodium intake.

To effectively design policies for sodium reduction, it is useful to determine the major sources of dietary sodium in the population. In Western industrialized countries such as the UK and US about 75-77% of the sodium intake were estimated to come from restaurant foods or processed foods (James, Ralph et al. 1987; Mattes and Donnelly 1991; WHO 2007). In contrast, the dietary sodium source in our sample was similar to those observed in other

East Asian populations where added salty condiments, especially salt added during cooking or at the table constituted majority of the sodium intake. For example, in China, added salt during cooking contributed 75-78% of dietary sodium; an additional 8% was from soy sauce (WHO 2007). Moreover, the use of added salt appeared to drive the higher sodium consumption in our sample. It should be noted that the use of these sources are well within the control of individuals, thus, programs for reducing salt consumption in the Philippines and other East Asian countries could concentrate on encouraging the public to reduce the use of added salt and other salty condiments in the household more than or in addition to reducing sodium used during food processing (such as advocated in the US). Iodine deficiency remains a public health concern in the country and salt iodization has been used as a primary strategy to eliminate this disorder. However, with the adverse effects of high salt consumption on BP and consequently on CVD, alternate strategies or iodization of other commonly consumed foods should be promoted to avoid potentially conflicting public health messages. As the WHO stated "the promotion of salt consumption overall to prevent iodine deficiencies is unnecessary. Therefore the implementation of a universal salt iodization program should not induce individuals to perceive that increased salt consumption is needed to prevent iodine deficiencies" (WHO 2007).

In summary, the results of this study underscore the importance of heeding the WHO recommendation for population-wide reduction in salt intake to decrease CVD burden attributable to high BP, especially with a relatively high frequency of the *ADD1* Trp460Trp genotype and as the Philippine population gets older. Maximum benefits may be achieved if these programs are accompanied by continued efforts to discourage smoking. For OW

women, it might be more sensible to focus on other hypertension prevention strategies (i.e. for reducing weight) first.

Table 5.1. Percent distribution of sodium sources among women in 2005

Source	All women n = 1776	Lower sodium* n = 1512	High sodium* n = 264	Chi-square p-value
Foods/Diet	23.7	27.6	16.7	<0.01
Salt	58.1	53.3	66.5	
Soy sauce	13.9	14.7	12.4	
Fish sauce	1.4	1.4	1.4	
MSG	2.4	2.5	2.1	
Other flavorings	0.6	0.4	0.8	

* Lower sodium: intake < 4600 mg/day; higher sodium: intake ≥ 4600 mg/day.

Table 5.2. Selected characteristics of women in 2005, by sodium intake*

Characteristic	Lower sodium* n = 1512	High sodium* n = 264	p-value
Sodium intake, mg/day	2173.8 ± 1029.9	7064.2 ± 3805.4	<0.01
Age, years	48.5 ± 5.9	48.4 ± 6.5	0.97
Systolic BP, mmHg	119.4 ± 20.1	121.9 ± 19.3	0.06
Diastolic BP, mmHg	79.8 ± 12.5	79.8 ± 12.0	0.93
Hypertensive, %	26.8	33.7	0.02
BMI, kg/m^2	24.3 ± 4.4	24.7 ± 4.4	0.16
Waist circumference, cm	81.1 ± 11.0	81.3 ± 11.0	0.80
OW duration, %			0.04
Never OW	48.4	43.2	
History of OW†	10.7	7.2	
OW <3 years	5.7	9.1	
OW 3-10 years	10.7	12.9	
OW >10 years	24.5	27.6	
Occupational PA, %			0.36
Not working	21.1	18.2	
Sedentary	6.4	4.5	
Light	47.9	52.3	
Moderate	16.9	18.9	
Heavy	7.7	6.1	
Education, y	6.8 ± 3.3	6.7 ± 3.3	0.55
Current smoker, %	14.5	17.0	0.28
Alcohol drinker, %	38.0	38.6	0.85
ACE (rs4331), N (%)			0.33
DD	215 (14.9)	31 (12.4)	
DI	693 (48.2)	115 (46.2)	
II	530 (36.9)	103 (41.4)	
ADD1 (rs4961), N (%)			0.11
GlyGly	472 (31.8)	94 (35.9)	
GlyTrp	721 (48.5)	130 (49.6)	
TrpTrp	292 (19.7)	38 (14.5)	
AGT (rs699), N (%)			0.32
MetMet	45 (3.0)	6 (2.3)	
MetThr	379 (25.4)	77 (29.6)	
ThrThr	1067 (71.6)	177 (68.1)	

* Values are means ± standard deviation, unless otherwise indicated. Lower sodium: intake < 4600 mg/day; higher sodium: intake ≥ 4600 mg/day. † Women were not OW in 2002 survey but have been OW for at least 1 survey.

BP = blood pressure; BMI = body mass index; OW= overweight; PA = physical activity.

Table 5.3. Odds ratios (95% CI) for hypertension comparing high to low sodium intake (reference) among women, by *ADD1* genotype, OW duration, and smoking status

	n	Odds Ratio[*]	95% CI
ADD1 (rs4961)[†]			
GlyGly	566	1.03	0.62-1.72
GlyTrp	851	1.32	0.87-2.01
TrpTrp	330	2.99	1.44-6.22
OW duration[‡]			
Never OW	845	2.27	1.45-3.55
History of OW	181	0.99	0.34-2.89
OW <3 years	110	2.22	0.79-6.26
OW 3-10 years	196	0.84	0.38-1.88
OW >10 years	444	0.89	0.53-1.52
Current smoking status[§]			
Non-smoker	1512	1.18	0.85-1.64
Smoker	264	3.06	1.51-6.19

[*] Low sodium intake: < 4600 mg/day; high sodium intake: ≥ 4600 mg/day. [†]Adjusted for age, BMI, OW duration, current smoking status and energy intake. Likelihood Ratio (LR) test chi-square p-value = 0.06. [‡]Adjusted for age, BMI, current smoking status and energy intake. LR test chi-square p-value = 0.04. [§]Adjusted for age, BMI, OW duration, and energy intake. LR test chi-square p-value = 0.02.
ADD1 = alpha adducin; BMI= body mass index; OW = overweight.

Table 5.4. Odds ratios (95% CI) for hypertension comparing high to low sodium intake (reference) among women* stratified by BMI and OW duration categories, by *ADD1* genotype

	ADD1 GlyGly/GlyTrp	n	*ADD1* TrpTrp	n
BMI[†], kg/m^2				
< 25	1.83 (1.16-2.88)	815	2.65 (0.94-7.45)	198
≥ 25	0.79 (0.50-1.23)	602	3.13 (1.07-9.16)	132
LR test chi-square p-value	<0.01		0.82	
OW duration[‡]				
Never OW	2.07 (1.26-3.40)	673	3.53 (1.17-10.64)	163
OW < 3 years	2.48 (0.77-8.00)	86	1.20 (0.11-12.92)	20
OW 3-10 years	0.76 (0.32-1.77)	161	1.91 (0.13-26.48)	33
OW ≥ 10 years	0.64 (0.35-1.16)	355	5.10 (1.13-23.10)	79
LR test chi-square p-value	0.02		0.88	

[*] Low sodium intake: < 4600 mg/day; high sodium intake: ≥ 4600 mg/day. [†] Adjusted for age, OW duration, current smoking status, and energy intake. [‡] Adjusted for age, BMI, current smoking status and energy intake. BMI= body mass index; OW = overweight; LR = Likelihood Ratio.

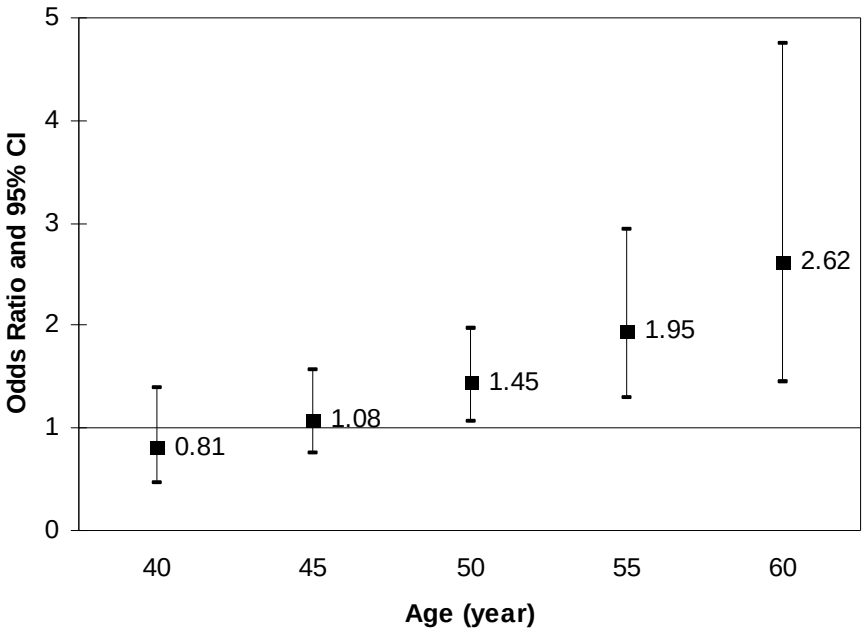

Figure 5.1. Estimated odds ratios with 95% confidence intervals (CI) for hypertension comparing women with high sodium intake to women with low sodium intake (reference) at selected ages. Adjusted for body mass index, overweight duration, current smoking status, and total energy intake. LR test chi-square p-value comparing models with and without the sodium intake (0,1) and age (continuous) interaction term = 0.01.

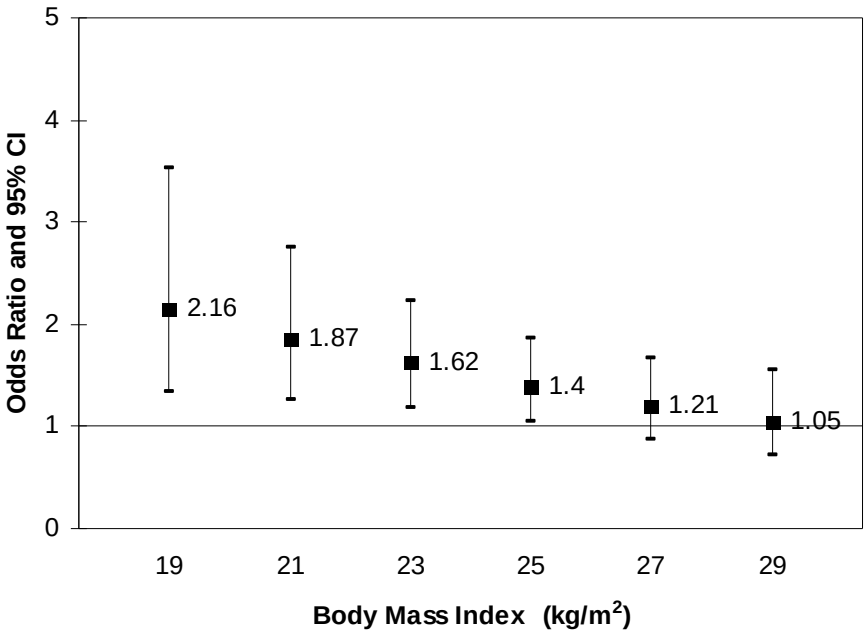

Figure 5.2. Estimated odds ratios with 95% confidence intervals (CI) for hypertension comparing women with high sodium intake to women with low sodium intake (reference) at selected body mass indices (BMI). Adjusted for age, overweight duration, current smoking status, and energy intake. LR test chi-square p-value comparing models with and without the sodium intake (0,1) and BMI (continuous) interaction term = 0.01.

VI. Synthesis

A. Overview of findings

This research examines the roles of overweight duration, genetic variants and sodium consumption on hypertension risk among adult Filipino women in Cebu, Philippines. In addition, this research explored potential heterogeneity of the effects of each of these factors according to different genetic and environmental characteristics. We used data gathered by the Cebu Longitudinal Health and Nutrition Survey (CLHNS). Aside from detailed individual, household and community level socio-demographic characteristics, this survey provided genetic information, repeated anthropometric measures that span more than two decades, dietary measures including sodium intake, and repeated blood pressure (BP) measurements. This offered a unique opportunity to address information gaps by improving our understanding of the effects of key exposures on hypertension risk and looking at gene-gene and gene-environment interactions. First, we examined whether the five-year cumulative incidence of hypertension is influenced by the duration of being overweight (OW) independent of current weight status. Second, we examined the effects of selected genetic variants on the prevalence of hypertension and explored potential gene-gene, gene-age, and gene-BMI interactions. Lastly, we estimated the effect of sodium intake on the prevalence of hypertension and determined whether its effect differed according to important hypertension risk factors including the significant exposures we identified in the first two phases. This section provides a summary and synthesis of our key findings.

A.1. Overweight duration increased the five-year cumulative incidence of hypertension regardless of current weight status in Filipino women

We aimed to examine the relationship between OW duration and five-year cumulative incidence of hypertension by using anthropometric data repeatedly measured before the onset of hypertension (in 1983/84, 1991, 1994, 1998, and 2002) and by using BP measured in 2002 and 2007 to identify incident hypertension. We hypothesized a positive relationship between OW history and incident hypertension, independent of 2002 BMI. We used poisson regression with the robust error variance to estimate the effect of OW history and duration on hypertension risk in 1607 women.

We found that OW prevalence in our sample increased 9-fold from 1983/84 to 2002. Interestingly, for most women, once they became OW, they remained OW. These present an alarming scenario since our analyses confirmed our hypothesis. We observed that the average 5-year risk for hypertension increased with increasing duration of being OW, independent of current BMI. Increased risk became more evident for women who had been OW for more than 10 years. Additionally, we illustrated that a longer OW duration increased the risk of developing hypertension to a greater extent even at a lower initial BMI. Thus, in addition to pathologic processes associated with having excess weight, there may be compounding factors associated with constant, long-term elevation of body weight. These findings underscore the importance of preventing weight gain and accumulation of excess weight earlier in life. We contribute to the elucidation of the pathogenesis of a complex disease such as hypertension. However, it is also worth pointing out that a substantial proportion of hypertensive women are not OW, leading us to explore other key factors suggested to

influence hypertension risk, particularly the influence of genetic variants and sodium consumption.

A.2. Genetic variants influence hypertension risk in Filipino women

Variants of the angiotensinogen (*AGT*, Met235Thr), angiotensin converting enzyme (*ACE*, intron 16 insertion/deletion (I/D)) and alpha adducin (*ADD1* Gly460Trp) genes have been suggested to be associated with the development of hypertension in Caucasians and other East Asians. Mainly using data from the 2005 survey of the CLHNS, we aimed to examine the association between these genetic variants and hypertension risk in a sample of 1776 Filipino women. Acknowledging that the effect of each genetic variant may be dependent on other genes and individual characteristics, we explored potential gene-gene, gene-BMI and gene-age interactions. We hypothesized that these variants influence hypertension risk and that their effects vary according to age, BMI and the presence of the other variants. To achieve our aims we used logistic regression analysis and tested heterogeneity of effects through likelihood ratio tests.

In 2005, more than a quarter of the sample women were hypertensive. Of the three gene variants, it appeared that the *AGT* Met235Thr has the strongest influence on hypertension risk as judged by the magnitude of odds ratios. The odds of having hypertension among women with the 235Thr allele was more than double compared to women with the MetMet genotype. This magnitude of effect was observed regardless of age, BMI, and *ACE* I/D and *ADD1* Gly460Trp genotypes. For the *ACE* and *ADD1* variants, we found age-dependent effects. Specifically, we observed that the effects of the *ACE* II genotype (compared to DD/DI) and the *ADD1* GlyTrp/TrpTrp genotypes (compared to

GlyGly) increased with age and may only be significant for women aged at least 55 years. This study suggests that these genetic variants may contribute to the development of hypertension in adult Filipino women, especially the *AGT* Met235Thr variant. There is also some evidence that *ACE* I/D and *ADD1* Gly460Trp may contribute to hypertension risk in older women. These findings become more important as the population gets older. Adding to the significance of these observations is that the higher risk genotypes are relatively more common in our sample (and other East Asians) than in Caucasians.

Possible increased sodium re-absorption in the kidney appears to be a common mechanism proposed to explain how these variants may influence BP. Thus, dietary sodium intake may be particularly important in the development of hypertension in our sample as well as other East Asians who tend to consume high amounts of sodium. We investigated this proposition in the last section of this research.

A.3. The association between high sodium intake and hypertension in Filipino women is influenced by genetic and environmental factors

Primarily using data from the 2005 survey of the CLHNS, we determined the association between high sodium intake (≥4600 mg/day) and hypertension in Filipino women and examined whether there is heterogeneity in the effect of sodium according to different genetic and environmental characteristics. For the genetic characteristics, we specifically assessed effect measure modification by the genetic variants that we have previously investigated and observed to possibly influence hypertension risk in our sample: *AGT* Met235Thr, *ACE* I/D, and *ADD1* Gly460Trp. For the environmental characteristics, we examined the potential modifying role of age, BMI, OW duration, and smoking status. We

hypothesized that high sodium intake is positively associated with hypertension risk in Filipino women and further, that its effect is not homogenous across women with different characteristics. To answer our research questions, we used logistic regression analysis and tested heterogeneity of effects through likelihood ratio tests.

We observed that the main source of dietary sodium in our sample was salt and salty condiments added during cooking or at the table, and this may drive the high sodium consumption.

Results of the multivariate analyses suggest that the effect of high sodium intake on hypertension is heightened in women with the *ADD1* TrpTrp genotype and is not evident in women with the GlyGly or GlyTrp genotypes. For women with the *ADD1* TrpTrp genotype, the odds of having hypertension in women consuming high amounts of sodium was 3 times higher than women consuming lower amounts of sodium. The *AGT* and *ACE* variants did not seem to modify the effect of high sodium intake on hypertension risk. We also found that the effect of high sodium intake on hypertension is increased with increasing age, in women who had never been OW and those who were smokers. In contrast, the effect of high sodium intake decreased with increasing BMI. Further exploration suggests a potential 3-way interaction between sodium intake, *ADD1* Gly460Trp polymorphism, and BMI and OW duration. It may be possible that the weaker effect of high sodium intake associated with increased BMI is only true for women carrying the Gly allele. We propose that future studies with larger sample sizes explore these 3-way interactions. Based on our findings, we suggest that programs designed to reduce sodium consumption should be intensified and focus on decreasing the use of salty condiments added during cooking or at the table. Women who are older, who are not OW and those who had never been OW, and women who smoke should

be specially cautioned against the adverse effect of high sodium consumption. In addition, these results may partly explain why Asians tend to develop hypertension at lower BMI.

Overall, this research found that duration of being OW, selected genetic variants and sodium intake influenced hypertension risk in Filipino women. Our findings can guide hypertension prevention efforts in the Philippines and potentially in other Asian and developing countries. With limited health infrastructure and resources, it would be particularly beneficial if programs designed to combat hypertension are guided by empirical data. Results of this research should be evaluated in the context of its limitations and strengths.

B. Limitations and strengths

This research has certain limitations. We faced challenges in the measurements of key variables used in the study. The 3 BP measurements averaged to represent BP for each survey round were taken in the same day and cannot account for day to day variations. This may cause regression dilution and result in attenuated results. Pertinent to the investigation looking at OW duration and hypertension incidence, it would have been better if we were able to ascertain the exact time when the women became OW and developed hypertension. However, continuously monitoring the sample women for over two decades would need unlimited resources and would considerably burden the respondents possibly resulting to higher attrition rates. Measurement of sodium intake is challenging. We estimated sodium intake using two 24-hour dietary recalls and a food frequency questionnaire focused on consumption of table salt and other high sodium condiments and sauces. This method is more prone to measurement errors compared to 24-hour urine collections, but much more practical

for a large population-based study. This may have resulted in the underestimation of sodium intake but the degree of underestimation or error was likely non-differential. Also, the probability of misclassifying women with high sodium intake may have been reduced by setting a relatively high cut-point. We cannot establish that high sodium intake preceded the development of hypertension. But, it is more likely that having hypertension will result in reduced rather than increased sodium intake; therefore reverse causation will more likely bias the estimates to the null.

Generalizability of results may be limited by reduction in sample size due to attrition and to the methods used in this research. To model five-year cumulative incidence of hypertension, we only included women who were not hypertensive in 2002 and were followed-up in 2007. For the analyses involving genetic variants, the sample was limited to women who provided blood samples in the 2005 survey and who did not have an apparent first degree relative in the study. However, the main reason for attrition is migration outside Metropolitan Cebu and we have no sufficient reason to believe that this is associated with having hypertension, genotype distribution or amount of sodium intake. Also, estimates weighted by the reciprocal of the probability of being in the study sample were not considerably different from those of un-weighted analyses suggesting no significant attrition bias. Regarding the analyses involving genetic variants, different allele frequencies make it difficult to compare and transfer the results of one study population to another. However, we found that the distributions of the selected gene variants were comparable to other East Asians.

Despite these limitations, this research has numerous strengths anchored on our use of the rich and potentially unique CLHNS dataset. We are not aware of any longitudinal study

in developing and transitional countries that have the time depth and number of follow-ups that the CLHNS has. This is the first study that used incident hypertension and repeatedly measured weight and height in investigating the relationship of OW duration and hypertension. We were able to ensure that the entire duration of OW occurred before the development of hypertension, thus allowing us to better establish temporality and infer causality compared to previous studies which were all cross-sectional. Our use of measured weight and height collected over two decades of adult life using the same protocol reduces measurement error.

We are the first to estimate the magnitude of a sodium effect on hypertension risk among Filipinos who consume high amounts of sodium and have been suggested to be sodium-sensitive. By having genetic information on the selected variants, we add to the scant but important body of literature that examined gene-gene and gene-environment interactions that potentially contribute to our understanding of the complexity of hypertension, especially among Asians in developing countries. We would not have uncovered the potential role of *ACE* I/D and *ADD1* Gly460Trp polymorphisms in hypertension if differential effects by age were not explored. Moreover, we showed heterogeneity of the effect of sodium consumption on hypertension according to *ADD1* Gly460Trp polymorphism and known environmental hypertension risk factors. With rich dietary, anthropometric, demographic and lifestyle data we were able to assess and adjust for confounding of other variables.

C. Public health significance

Our study has strong public health significance. First, CVD is now the leading cause of morbidity and mortality in the world and hypertension accounts for about half of the CVD

burden. Alarmingly, the rate of hypertension continues to rise, especially in lower income countries that are overburdened by both communicable and chronic diseases. Second, the demographic and health trends in the Philippines exemplify those of other developing countries undergoing the nutrition transition: (a) heart and vascular diseases are the leading causes of death in the country; and (b) there is a high prevalence (with an increasing trend) in CVD risk factors. Third, Asians are of special concern because they have the most rapid increases in obesity and obesity-related diseases worldwide, and they tend to develop CVD risk factors at lower BMI thresholds. Moreover, Asians tend to consume high amounts of sodium possibly increasing their likelihood of developing hypertension. We showed that Filipinos have distributions of potentially high risk genotypes and sources of dietary sodium that are comparable to other East Asians. Fourth, we investigated important modifiable and non-modifiable risk factors that have been suggested by previous studies, albeit mostly from developed countries. Thus, our findings can provide critical information necessary for hypertension prevention efforts not only for the Philippines but also for other developing countries, especially for East Asians.

More specifically, our results suggest that for a more comprehensive program aimed at preventing or reducing hypertension prevalence in the population, efforts to prevent weight gain and accumulation of excess weight earlier in life and to reduce sodium intake should be incorporated and intensified. We showed evidence that in addition to presence of OW, the length of exposure to excess weight may increase risk of developing hypertension. However, owing to the prevalence of hypertension in women who are not OW, efforts to reduce hypertension should also focus on the benefits of reducing sodium intake among women who are not OW or who have never been OW. Our results indicate that the effects of high sodium

intake are enhanced in these groups of women who are most likely presumed to be at low risk for hypertension. Moreover, programs for reducing salt consumption in the Philippines and other East Asian countries could concentrate on encouraging the public to reduce the use of added salt and other salty condiments in the household more than or in addition to reducing sodium used during food processing (such as advocated in the US). Since salt has been iodized to prevent iodine deficiency in the Philippines and other countries, alternate strategies or iodization of other commonly consumed foods should be promoted to avoid potentially conflicting public health messages.

D. Direction for future research

Potential mechanisms suggested for the relationship between OW duration and the risk of developing hypertension include hyperinsulinemia and insulin resistance. With CLHNS data on these measures, we can explore this hypothesis.

Another major CVD risk factor raising worldwide concern is diabetes mellitus, particularly type 2 diabetes. Diabetes increases the risk of fatal CVDs two- to three-fold. Thus, we would like to conduct similar analyses using the exposures and methods employed in this research but with diabetes as the main outcome.

The use of dietary patterns rather than individual nutrients in assessing diet and health relationships has been increasing. Advocates of using foods versus nutrients argue that discussing diet in terms of food group intakes are easier to translate into intervention messages. Furthermore, environmental and cultural factors define diet patterns which in turn affect nutrient intakes. Noteworthy is the lack of studies on dietary patterns and health outcomes from developing countries, much less from Asia. We propose to identify dietary

patterns through cluster analysis and to examine their effects on hypertension and type 2 diabetes. We may also identify patterns that cluster with high sodium intake. In addition to contributing to the scant literature on dietary pattern-CVD risk factors in Asia, the study can provide critical information for more tailored disease prevention efforts in the country. We can then assess the appropriateness of dietary public health messages which are patterned mostly from studies involving Western populations.

Hypertension and diabetes frequently coexist, further magnifying CVD-risk. Their coexistence may modify the influence of CVD-risk factors. For example, the effect of dietary salt intake on hypertension is highest on individuals with diabetes, obesity, and renal deficiencies. On the other hand, the use of some hypertensive medications such as beta-blockers may increase the risk of developing diabetes. In addition to identifying weight and dietary patterns associated with hypertension or diabetes, we can examine whether the strength and direction of the weight history and dietary effects are influenced by the independence or coexistence of these morbidities.

We will continue to explore effects of novel gene variants that will be discovered by high-density association studies and genome-wide association studies on the risk of hypertension and diabetes.

In summary, this research found that the duration of being OW, selected genetic variants and sodium intake influence hypertension risk in adult Filipino women. We observed potential gene-gene and gene-environment interactions and support the importance of conducting context-dependent analyses. By examining key modifiable and non-modifiable risk factors, results of this research can be used to design a more comprehensive hypertension prevention program in the Philippines and possibly in other Asian and developing countries.

We propose to employ the methods used in this research to investigate type 2 diabetes, another major CVD risk factor that tend to co-occur with hypertension.

References:

Adair, L. and B. Popkin (2001). "The Cebu Longitudal Health and Nutrition Survey: History and Major Contributions of the Project." Philippine Q Cult Soc **29**: 5-37.

Adair, L. S. (2004). "Dramatic rise in overweight and obesity in adult filipino women and risk of hypertension." Obes Res **12**(8): 1335-41.

Alpert, M. A., C. R. Lambert, et al. (1995). "Relation of duration of morbid obesity to left ventricular mass, systolic function, and diastolic filling, and effect of weight loss." Am J Cardiol **76**(16): 1194-7.

Arnett, D. K. (2007). "Summary of the American Heart Association's scientific statement on the relevance of genetics and genomics for prevention and treatment of cardiovascular disease." Arterioscler Thromb Vasc Biol **27**(8): 1682-6.

Arnett, D. K., A. E. Baird, et al. (2007). "Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: a scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group." Circulation **115**(22): 2878-901.

Asaria, P., D. Chisholm, et al. (2007). "Chronic disease prevention: health effects and financial costs of strategies to reduce salt intake and control tobacco use." The Lancet **370**(9604): 2044-2053.

Barlassina, C., N. J. Schork, et al. (2000). "Synergistic effect of alpha-adducin and ACE genes causes blood pressure changes with body sodium and volume expansion." Kidney Int **57**(3): 1083-90.

Beeks, E., A. G. Kessels, et al. (2004). "Genetic predisposition to salt-sensitivity: a systematic review." J Hypertens **22**(7): 1243-9.

Beeks, E., M. M. van der Klauw, et al. (2004). "Alpha-adducin Gly460Trp polymorphism and renal hemodynamics in essential hypertension." Hypertension **44**(4): 419-23.

Benetos, A., S. Gautier, et al. (1996). "Influence of angiotensin-converting enzyme and angiotensin II type 1 receptor gene polymorphisms on aortic stiffness in normotensive and hypertensive patients." Circulation **94**(4): 698-703.

Berg, A. H. and P. E. Scherer (2005). "Adipose tissue, inflammation, and cardiovascular disease." Circ Res **96**(9): 939-49.

Bianchi, G. (2005). "Genetic variations of tubular sodium reabsorption leading to "primary" hypertension: from gene polymorphism to clinical symptoms." Am J Physiol Regul Integr Comp Physiol **289**(6): R1536-49.

Bianchi, G., P. Ferrari, et al. (2005). "Adducin polymorphism: detection and impact on hypertension and related disorders." Hypertension **45**(3): 331-40.

Binder, A. (2007). "A review of the genetics of essential hypertension." Curr Opin Cardiol **22**(3): 176-84.

Bray, G. A., W. M. Vollmer, et al. (2004). "A further subgroup analysis of the effects of the DASH diet and three dietary sodium levels on blood pressure: results of the DASH-Sodium Trial." Am J Cardiol **94**(2): 222-7.

Brown, C. D., M. Higgins, et al. (2000). "Body mass index and the prevalence of hypertension and dyslipidemia." Obes Res **8**(9): 605-19.

Brunner Huber, L. R. (2007). "Validity of self-reported height and weight in women of reproductive age." Matern Child Health J **11**(2): 137-44.

Cambien, F., O. Costerousse, et al. (1994). "Plasma level and gene polymorphism of angiotensin-converting enzyme in relation to myocardial infarction." Circulation **90**(2): 669-76.

CDC/NCHS (2002). Health E-Stats.

Cha, S. H., H. T. Kim, et al. (2007). "Association of alpha-adducin Gly460Trp polymorphism with coronary artery disease in a Korean population." J Hypertens **25**(12): 2413-20.

Chalmers, L., F. J. Kaskel, et al. (2006). "The role of obesity and its bioclinical correlates in the progression of chronic kidney disease." Adv Chronic Kidney Dis **13**(4): 352-64.

Chobanian, A. V., G. L. Bakris, et al. (2003). "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report." JAMA **289**(19): 2560-2571.

Colin Bell, A., L. S. Adair, et al. (2002). "Ethnic differences in the association between body mass index and hypertension." Am J Epidemiol **155**(4): 346-53.

Cusi, D., C. Barlassina, et al. (1997). "Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension." Lancet **349**(9062): 1353-7.

Dahly, D. L. and L. S. Adair (2007). "Quantifying the urban environment: a scale measure of urbanicity outperforms the urban-rural dichotomy." Soc Sci Med **64**(7): 1407-19.

Davy, K. P. and J. E. Hall (2004). "Obesity and hypertension: two epidemics or one?" Am J Physiol Regul Integr Comp Physiol **286**(5): R803-813.

Deurenberg-Yap, M., S. K. Chew, et al. (2002). "Elevated body fat percentage and cardiovascular risks at low body mass index levels among Singaporean Chinese, Malays and Indians." Obes Rev **3**(3): 209-15.

Deurenberg, P., M. Deurenberg-Yap, et al. (2002). "Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationship." Obes Rev **3**(3): 141-6.

Dima, I., C. Vlachopoulos, et al. (2008). "Association of Arterial Stiffness With the Angiotensin-Converting Enzyme Gene Polymorphism in Healthy Individuals." Am J Hypertens **21**(12): 1354-1358.

DOH (2007). The 2003 Philippine Health Statistics (2007 update). Manila, Department of Health.

Efendiev, R., R. T. Krmar, et al. (2004). "Hypertension-linked mutation in the adducin alpha-subunit leads to higher AP2-mu2 phosphorylation and impaired Na+,K+-ATPase trafficking in response to GPCR signals and intracellular sodium." Circ Res **95**(11): 1100-8.

Everhart, J. E., D. J. Pettitt, et al. (1992). "Duration of obesity increases the incidence of NIDDM." Diabetes **41**(2): 235-40.

Fava, C., M. Montagnana, et al. (2007). "Association between adducin-1 G460W variant and blood pressure in Swedes is dependent on interaction with body mass index and gender." Am J Hypertens **20**(9): 981-9.

Fitzgerald, J., P. Gottschalk, et al. (1998). "An Analysis of Sample Attrition in Panel Data: The Michigan Panel Study of Income Dynamics." The Journal of Human Resources **33**(2): 251-299.

Flack, J. M., R. H. Grimm, Jr., et al. (2002). "New salt-sensitivity metrics: variability-adjusted blood pressure change and the urinary sodium-to-creatinine ratio." Ethn Dis **12**(1): 10-9.

Flegal, K. M., M. D. Carroll, et al. (2002). "Prevalence and trends in obesity among US adults, 1999-2000." Jama **288**(14): 1723-7.

FNRI-DOST (2000). Nutritional Guidelines for Filipinos: Revised Edition, DOST.

FNRI (2001). Philippine Nutrition Facts and Figures. Manila, Unicef.

FNRI (2006). Philippine Nutrition Facts and Figures. Manila, Philippines, Philippine Food and Nutrition Research Institute.

Forrest, K. Y., C. H. Bunker, et al. (2001). "Physical activity and cardiovascular risk factors in a developing population." Med Sci Sports Exerc **33**(9): 1598-604.

Franks, P. W. (2008). "Identifying genes for primary hypertension: methodological limitations and gene-environment interactions." J Hum Hypertens.

Garrison, R. J., W. B. Kannel, et al. (1987). "Incidence and precursors of hypertension in young adults: the Framingham Offspring Study." Prev Med **16**(2): 235-51.

Gillum, R. F. and C. T. Sempos (2005). "Ethnic variation in validity of classification of overweight and obesity using self-reported weight and height in American women and men: the Third National Health and Nutrition Examination Survey." Nutr J **4**: 27.

Goldenberg, I., A. J. Moss, et al. (2006). "Polymorphism in the angiotensinogen gene, hypertension, and ethnic differences in the risk of recurrent coronary events." Hypertension **48**(4): 693-9.

Gorzelniak, K., S. Engeli, et al. (2002). "Hormonal regulation of the human adipose-tissue renin-angiotensin system: relationship to obesity and hypertension." J Hypertens **20**(5): 965-73.

Grant, F. D., J. R. Romero, et al. (2002). "Low-renin hypertension, altered sodium homeostasis, and an alpha-adducin polymorphism." Hypertension **39**(2): 191-6.

Hall, J., D. Jones, et al. (2003). "Impact of the obesity epidemic on hypertension and renal disease." Curr Hypertens Rep **5**(1522-6417 (Print)): 386-392.

He, J., D. Gu, et al. (2009). "Gender difference in blood pressure responses to dietary sodium intervention in the GenSalt study." Journal of Hypertension **27**.

Hekimsoy, Z. and I. K. Oktem (2003). "Duration of obesity is not a risk factor for type 2 diabetes mellitus, arterial hypertension and hyperlipidemia." Diabetes Obesity & Metabolism **5**(6): 432-437.

Higaki, J., S. Baba, et al. (2000). "Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men : the Suita Study." Circulation **101**(17): 2060-5.

Huang, X. H., K. Sun, et al. (2007). "[Association of alpha-adducin gene and G-protein beta3-subuint gene with essential hypertension in Chinese]." Zhonghua Yi Xue Za Zhi **87**(24): 1682-4.

INTERSALT (1988). "Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group." Bmj **297**(6644): 319-28.

IOM (2004). Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Food and Nutrition Board, Institute of Medicine. Washington, DC, National Academies Press.

Ivandic, A., I. Prpic-Krizevac, et al. (1998). "Hyperinsulinemia and sex hormones in healthy premenopausal women: relative contribution of obesity, obesity type, and duration of obesity." Metabolism **47**(1): 13-9.

James, W. P., A. Ralph, et al. (1987). "The dominance of salt in manufactured food in the sodium intake of affluent societies." Lancet **1**(8530): 426-9.

Janssen, I., P. T. Katzmarzyk, et al. (2002). "Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines." Arch Intern Med **162**(18): 2074-9.

Janssen, I., P. T. Katzmarzyk, et al. (2004). "Duration of overweight and metabolic health risk in American men and women." Ann Epidemiol **14**(8): 585-91.

Jeunemaitre, X., F. Soubrier, et al. (1992). "Molecular basis of human hypertension: role of angiotensinogen." Cell **71**(1): 169-80.

Jiang, X., H. Sheng, et al. (2008). "Association between renin-angiotensin system gene polymorphism and essential hypertension: a community-based study." J Hum Hypertens.

Ju, Z., H. Zhang, et al. (2003). "Alpha-adducin gene polymorphism is associated with essential hypertension in Chinese: a case-control and family-based study." J Hypertens **21**(10): 1861-8.

Jurgens, G. and N. A. Graudal (2004). "Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterols, and triglyceride." Cochrane Database Syst Rev(1): CD004022.

Kaplan, N., E. Lieberman, et al. (2002). Kaplan's Clinical Hypertension. Philadelphia, PA, Lippincott, Williams & Wilkins.

Karppanen, H. and E. Mervaala (2006). "Sodium intake and hypertension." Prog Cardiovasc Dis **49**(2): 59-75.

Kato, N., T. Sugiyama, et al. (2000). "Comprehensive analysis of the renin-angiotensin gene polymorphisms with relation to hypertension in the Japanese." J Hypertens **18**(8): 1025-32.

Kato, N., T. Sugiyama, et al. (1998). "Lack of association between the alpha-adducin locus and essential hypertension in the Japanese population." Hypertension **31**(3): 730-3.

Katsuya, T., K. Ishikawa, et al. (2003). "Salt sensitivity of Japanese from the viewpoint of gene polymorphism." Hypertens Res **26**(7): 521-5.

Kearney, P. M., M. Whelton, et al. (2005). "Global burden of hypertension: analysis of worldwide data." Lancet **365**(9455): 217-23.

Kengne, A. P., A. Patel, et al. (2007). "Systolic blood pressure, diabetes and the risk of cardiovascular diseases in the Asia-Pacific region." J Hypertens **25**(6): 1205-13.

Krause, R., M. Winston, et al. (1998). "Obesity. Impact on cardiovascular disease." Circulation **98**: 1472-1476.

Law, M. R., C. D. Frost, et al. (1991). "By how much does dietary salt reduction lower blood pressure? I--Analysis of observational data among populations." Bmj **302**(6780): 811-5.

Lawes, C. M., S. Vander Hoorn, et al. (2006). "Blood pressure and the global burden of disease 2000. Part II: estimates of attributable burden." J Hypertens **24**(3): 423-30.

Lawes, C. M., S. Vander Hoorn, et al. (2008). "Global burden of blood-pressure-related disease, 2001." Lancet **371**(9623): 1513-8.

Lear, S. A., M. Toma, et al. (2003). "Modification of the relationship between simple anthropometric indices and risk factors by ethnic background." Metabolism **52**(10): 1295-301.

Levy, D., M. G. Larson, et al. (2007). "Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness." BMC Med Genet **8 Suppl 1**: S3.

Licata, G., R. Scaglione, et al. (1992). "Left ventricular function response to exercise in normotensive obese subjects: influence of degree and duration of obesity." Int J Cardiol **37**(2): 223-30.

Lifton, R. P. (1996). "Molecular genetics of human blood pressure variation." Science **272**(5262): 676-80.

Lifton, R. P., A. G. Gharavi, et al. (2001). "Molecular mechanisms of human hypertension." Cell **104**(4): 545-56.

Lopez, A. D., C. D. Mathers, et al. (2006). "Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data." Lancet **367**(9524): 1747-57.

Luft, F. C. (2004). "Present status of genetic mechanisms in hypertension." Med Clin North Am **88**(1): 1-18, vii.

Lynch, A. I., D. K. Arnett, et al. (2007). "Sex-specific effects of ACE I/D and AGT-M235T on pulse pressure: the HyperGEN Study." Hum Genet **122**(1): 33-40.

MacGregor, G. A., N. D. Markandu, et al. (1989). "Double-blind study of three sodium intakes and long-term effects of sodium restriction in essential hypertension." Lancet **2**(8674): 1244-7.

Mackay, J. and G. Mensah, Eds. (2004). The Atlas of Heart Disease and Stroke. Geneva, Geneva: World Health Organization.

Marvelle, A. F., L. A. Lange, et al. (2007). "Comparison of ENCODE region SNPs between Cebu Filipino and Asian HapMap samples." J Hum Genet **52**(9): 729-37.

Mathew, B., S. B. Patel, et al. (2007). "Obesity-hypertension: emerging concepts in pathophysiology and treatment." Am J Med Sci **334**(1): 23-30.

Matsubara, M., M. Suzuki, et al. (2002). "Angiotensin-converting enzyme I/D polymorphism and hypertension: the Ohasama study." J Hypertens **20**(6): 1121-6.

Mattes, R. D. and D. Donnelly (1991). "Relative contributions of dietary sodium sources." J Am Coll Nutr **10**(4): 383-93.

Mendez, M. A., C. A. Monteiro, et al. (2005). "Overweight exceeds underweight among women in most developing countries." Am J Clin Nutr **81**(3): 714-21.

Mokdad, A. H., E. S. Ford, et al. (2003). "Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001." Jama **289**(1): 76-9.

Mosca, L., C. L. Banka, et al. (2007). "Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update." Circulation **115**(11): 1481-501.

Muscelli, E., S. Camastra, et al. (1998). "Influence of duration of obesity on the insulin resistance of obese non-diabetic patients." Int J Obes Relat Metab Disord **22**(3): 262-7.

Must, A., J. Spadano, et al. (1999). "The disease burden associated with overweight and obesity." Jama **282**(16): 1523-9.

Nakajima, T., S. Fujioka, et al. (1985). "Noninvasive study of left ventricular performance in obese patients: influence of duration of obesity." Circulation **71**(3): 481-6.

Narkiewicz, K. (2006). "Obesity and hypertension--the issue is more complex than we thought." Nephrol Dial Transplant **21**(2): 264-7.

Nguyen, T. T., L. S. Adair, et al. (2008). "Optimal cutoff values for overweight: using body mass index to predict incidence of hypertension in 18- to 65-year-old Chinese adults." J Nutr **138**(7): 1377-82.

NHLBI (2003). Cardiovascular risk in the Filipino community. Formative research from Daly City and San Francisco, CA., NIH/NHLBI.

Niu, T., X. Chen, et al. (2002). "Angiotensin converting enzyme gene insertion/deletion polymorphism and cardiovascular disease: therapeutic implications." Drugs **62**(7): 977-93.

Norat, T., R. Bowman, et al. (2008). "Blood pressure and interactions between the angiotensin polymorphism AGT M235T and sodium intake: a cross-sectional population study." Am J Clin Nutr **88**(2): 392-7.

NSO (2008). 2007 Census of Population. Manila National Statistics Office.

Nyholm, M., B. Gullberg, et al. (2007). "The validity of obesity based on self-reported weight and height: Implications for population studies." Obesity (Silver Spring) **15**(1): 197-208.

O'Donnell, C. J., K. Lindpaintner, et al. (1998). "Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study." Circulation **97**(18): 1766-72.

Orth, S. R. (2004). "Effects of smoking on systemic and intrarenal hemodynamics: influence on renal function." J Am Soc Nephrol **15 Suppl 1**: S58-63.

Overlack, A., M. Ruppert, et al. (1995). "Age is a major determinant of the divergent blood pressure responses to varying salt intake in essential hypertension." Am J Hypertens **8**(8): 829-36.

Pausova, Z., J. Tremblay, et al. (1999). "Gene-environment interactions in hypertension." Curr Hypertens Rep **1**(1): 42-50.

Penesova, A., E. Cizmarova, et al. (2006). "Insertion/Deletion Polymorphism on ACE Gene is Associated with Endothelial Dysfunction in Young Patients with Hypertension." Horm Metab Res **38**(9): 592-7.

Perry, G. S., T. E. Byers, et al. (1995). "The validity of self-reports of past body weights by U.S. adults." Epidemiology **6**(1): 61-6.

Pontiroli, A. E. and L. Galli (1998). "Duration of obesity is a risk factor for non-insulin-dependent diabetes mellitus, not for arterial hypertension or for hyperlipidaemia." Acta Diabetologica **35**(3): 130-136.

Popkin, B., S. Horton, et al. (2001). The nutritional transition and diet-related chronic diseases in Asia: implications for prevention, IFPRI.

Popkin, B. M. (2008). The World Is Fat--The Fads, Trends, Policies, and Products That Are Fattening the Human Race. New York, Avery-Penguin Group.

Purcell, S., B. Neale, et al. (2007). "PLINK: a tool set for whole-genome association and population-based linkage analyses." Am J Hum Genet **81**(3): 559-75.

Reaven, G. M., H. Lithell, et al. (1996). "Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system." N Engl J Med **334**(6): 374-81.

Rieder, M. J., S. L. Taylor, et al. (1999). "Sequence variation in the human angiotensin converting enzyme." Nat Genet **22**(1): 59-62.

Rocchini, A. P., J. Key, et al. (1989). "The effect of weight loss on the sensitivity of blood pressure to sodium in obese adolescents." N Engl J Med **321**(9): 580-5.

Rodriguez-Iturbe, B., F. Romero, et al. (2007). "Pathophysiological Mechanisms of Salt-Dependent Hypertension." American Journal of Kidney Diseases **50**(4): 655-672.

Rothman, K. and S. Greenland, Eds. (1998). Modern Epidemiology. Philadelphia, PA, Lippincott, Williams & Wilkins.

Rothman, K. J. (1990). "No adjustments are needed for multiple comparisons." Epidemiology **1**(1): 43-6.

Sakurai, Y. (2000). "Duration of obesity and risk of non-insulin-dependent diabetes mellitus." Biomed Pharmacother **54**(2): 80-4.

Sato, N., T. Katsuya, et al. (2000). "Nine polymorphisms of angiotensinogen gene in the susceptibility to essential hypertension." Life Sci **68**(3): 259-72.

Scaglione, R., M. A. Dichiara, et al. (1992). "Left ventricular diastolic and systolic function in normotensive obese subjects: influence of degree and duration of obesity." Eur Heart J **13**(6): 738-42.

Segura, J. and L. M. Ruilope (2007). "Obesity, essential hypertension and renin-angiotensin system." Public Health Nutr **10**(10A): 1151-5.

Sethi, A. A., B. G. Nordestgaard, et al. (2003). "Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis." Arterioscler Thromb Vasc Biol **23**(7): 1269-75.

Shankar, S. S. and H. O. Steinberg (2005). "Obesity and endothelial dysfunction." Semin Vasc Med **5**(1): 56-64.

Shields, M., S. C. Gorber, et al. (2008). "Estimates of obesity based on self-report versus direct measures." Health Rep **19**(2): 61-76.

Shin, M. H., E. K. Chung, et al. (2004). "Alpha-adducin Gly460Trp polymorphism and essential hypertension in Korea." J Korean Med Sci **19**(6): 812-4.

Staessen, J. A., J. Wang, et al. (2003). "Essential hypertension." Lancet **361**(9369): 1629-41.

Staessen, J. A., J. G. Wang, et al. (2001). "Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population." J Hypertens **19**(8): 1349-58.

Stamler, J., G. Rose, et al. (1989). "INTERSALT study findings. Public health and medical care implications." Hypertension **14**(5): 570-7.

Sugimoto, K., A. Hozawa, et al. (2002). "alpha-Adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the Ohasama study." J Hypertens **20**(9): 1779-84.

Svetkey, L. P., T. J. Moore, et al. (2001). "Angiotensinogen genotype and blood pressure response in the Dietary Approaches to Stop Hypertension (DASH) study." J Hypertens **19**(11): 1949-56.

Tamaki, S., Y. Nakamura, et al. (2005). "Combined analysis of polymorphisms in angiotensinogen and adducin genes and their effects on hypertension in a Japanese sample: The Shigaraki Study." Hypertens Res **28**(8): 645-50.

Tanaka, C., K. Kamide, et al. (2003). "An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms." Hypertens Res **26**(4): 301-6.

Taniwaki, H., T. Kawagishi, et al. (1999). "Association of ACE gene polymorphism with arterial stiffness in patients with type 2 diabetes." Diabetes Care **22**(11): 1858-64.

Tiret, L., B. Rigat, et al. (1992). "Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels." Am J Hum Genet **51**(1): 197-205.

Troy, L. M., D. J. Hunter, et al. (1995). "The validity of recalled weight among younger women." Int J Obes Relat Metab Disord **19**(8): 570-2.

Urbina, E. M., S. S. Gidding, et al. (1995). "Effect of body size, ponderosity, and blood pressure on left ventricular growth in children and young adults in the Bogalusa Heart Study." Circulation **91**(9): 2400-6.

van der Kleij, F. G., P. E. de Jong, et al. (2002). "Enhanced responses of blood pressure, renal function, and aldosterone to angiotensin I in the DD genotype are blunted by low sodium intake." J Am Soc Nephrol **13**(4): 1025-33.

Van Harmelen, V., P. Ariapart, et al. (2000). "Increased adipose angiotensinogen gene expression in human obesity." Obes Res **8**(4): 337-41.

Wang, X., H. Zhu, et al. (2006). "Effects of angiotensinogen and angiotensin II type I receptor genes on blood pressure and left ventricular mass trajectories in multiethnic youth." Twin Res Hum Genet **9**(3): 393-402.

Wannamethee, S. G. and A. G. Shaper (1999). "Weight change and duration of overweight and obesity in the incidence of type 2 diabetes." Diabetes Care **22**(8): 1266-72.

Wannamethee, S. G., A. G. Shaper, et al. (2005). "Overweight and obesity and weight change in middle aged men: impact on cardiovascular disease and diabetes." J Epidemiol Community Health **59**(2): 134-9.

Ward, R. (1995). Familial Aggregation and Genetic Epidemiology of Blood Pressure. New York, Raven Press.

Weinberger, M. H. (2006). "Pathogenesis of salt sensitivity of blood pressure." Curr Hypertens Rep **8**(2): 166-70.

WellcomeTrust (2007). "Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls." Nature **447**(7145): 661-78.

WHO (2000). "Obesity: preventing and managing the global epidemic. Report of a WHO consultation." World Health Organ Tech Rep Ser **894**: i-xii, 1-253.

WHO (2002). The World Health Report 2002 - Reducing Risks, Promoting Healthy Life Geneva, World Health Organization.

WHO (2004). "Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies." Lancet **363**(9403): 157-63.

WHO (2005). Preventing chronic diseases: a vital investment. Geneva, World Health Organization.

WHO (2007). Reducing Salt Intake in Populations: Report of a WHO Forum and Technical Meeting. Geneva, Switzerland, World Health Organization.

Wofford, M. R. and J. E. Hall (2004). "Pathophysiology and treatment of obesity hypertension." Curr Pharm Des **10**(29): 3621-37.

Yamagishi, K., H. Iso, et al. (2004). "Alpha-adducin G460W polymorphism, urinary sodium excretion, and blood pressure in community-based samples." Am J Hypertens **17**(5 Pt 1): 385-90.

Yoshida, K., T. Ishigami, et al. (2000). "Association of essential hypertension in elderly Japanese with I/D polymorphism of the angiotensin-converting enzyme (ACE) gene." J Hum Genet **45**(5): 294-8.

Zaman, M. M., N. Yoshiike, et al. (2001). "Angiotensin converting enzyme genetic polymorphism is not associated with hypertension in a cross-sectional sample of a Japanese population: the Shibata Study." J Hypertens **19**(1): 47-53.

Zou, G. (2004). "A modified poisson regression approach to prospective studies with binary data." Am J Epidemiol **159**(7): 702-6.