ASSOCIATIONS BETWEEN TRAITS (BLOOD PRESSURE AND BODY HEIGHT GROWTH) AND REPRODUCTIVE TIMING RELATED GENETIC VARIANTS FROM GENOME-WIDE ASSOCIATION STUDIES

Daojun Mo

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Fairbanks School of Public Health,
Indiana University

September 2017

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Chunyan He, ScD, Chair

Doctoral Committee

Wanzhu Tu, PhD

Yiqing Song, MD, ScD

July 18, 2017

Cynthia S Stone, DrPh, RN

ACKNOWLEDGEMENTS

I would like to thank the technical staff at the Center for Medical Genomics, Indiana University School of Medicine for their collaboration and scientific support in performing the genotyping for the study. I would also like to thank ReproGen Consortium and International Consortium for Blood Pressure GWAS for providing the aggregated data on blood pressure, reproductive timing and SNPs. I appreciate the statistical consulting provided by Department of Biostatistics, School of Public Health, Indiana University. The first study Reproductive Timing Related Genetic Variants from Genome-Wide Association Studies Are Associated with Blood Pressure in Healthy Adolescents was originally supported by the NIH grant R01 HL095086 and a VA Merit Review grant. Lastly, but not the least, I want to express my great gratitude to my PhD research committee for their advice on this study.

Daojun Mo

ASSOCIATIONS BETWEEN TRAITS (BLOOD PRESSURE AND BODY HEIGHT GROWTH) AND REPRODUCTIVE TIMING RELATED GENETIC VARIANTS FROM GENOME-WIDE ASSOCIATION STUDIES

Recent genome-wide association studies (GWAS) have identified many common genetic variants that are associated with women's reproductive timing characteristics including ages at menarche and at natural menopause. However, the associations of these variants with other human health-related phenotypes such as blood pressure, cancer, diabetes, obesity, and body height growth have not been well studied. No published studies to our knowledge have directly assessed the genetic influence of reproductive timing related variants on the aforementioned common traits. A better understanding of pleiotropic effects of these variants is important because it will help elucidate the precise mechanisms of common traits/diseases such as hypertension which have not been fully understood so far, and give clues for developing better solutions for disease prevention and treatment. We, therefore, conducted three studies to explore genetic variant effects on blood pressure and body height growth. In the first study, we analyzed data from a local cohort of 601 healthy adolescents from Indianapolis schools. Mixed effect model analysis revealed that 11 reproductive related single nucleotide polymorphisms (SNPs) were significantly associated with blood pressure in the study subjects. In order to assess if these genetic effects extended to the adult blood pressure, we performed the second study to investigate the genetic effect on blood pressure in adults. We used the summary statistics obtained from the two large international GWAS consortia, the Blood Pressure Consortium and the ReproGen Consortium. Bivariate analyses showed that more than 100 SNPs were associated with both blood pressure and reproductive timing. As the blood pressure development is closely related to somatic growth, we conducted the third study to exam the genetic effect of reproductive-timing related variants on the linear growth from the aforementioned local cohort. We identified 8 genetic variants significantly

associated with the catch-up of linear growth in the study subjects. In conclusion, these three studies collectively provided evidence in support of the pleiotropic effects of the reproductive timing variants, suggesting the common genetic basis underlying the correlated traits. Future research is needed to validate the findings.

Chunyan He, ScD, Chair

TABLE OF CONTENTS

Study 1: Reproductive Timing Related Genetic Variants from Genome-Wide Association Studies Are Associated with Blood Pressure in Healthy Adolescents

Introduction

Blood pressure (BP) increases in adolescents accompanying rapidly linear body growth after reproductive maturity process starts [Ewald, 2016]. Prevalence of prehypertension and hypertension is estimated at between 4% and 15%, and between 2% and 5% among adolescents [Ewald, 2016]. Unfortunately, many hypertensive children are undiagnosed. Increased blood pressure (BP) increases risk of developing long-term health outcomes such as cardiovascular diseases and renal impairment [Ewald, 2016]. Commonly known factors for increased BP in adolescents include obesity, height, sex, age, genetic predisposition, socioeconomic status, cultural influences, physical exercise, and reproductive timing [Ewald, 2016].

With regard to reproductive timing, age at menarche as well as age at natural menopause have been associated with increased BP and risk of cardiovascular diseases [Lakshman, 2009; Hu 1999]. BP increases at an accelerated rate with onset of sexual maturation [Shankar 2005]. Early menarche is associated with elevated BP in adolescent and adulthood [Koziel, 2001; Dianels,1998]. BP is also known to increase more steeply around age at menopause [Lindquist, 1982]. Earlier menopause is associated with higher BP [Staessen, 1989; Izumi, 2007], although it is still unclear whether menopause accelerates BP increase or increased BP leads to earlier menopause. The observed synchronization between reproductive aging and BP increase raises questions about the possibility of common regulating mechanisms shared by these processes.

We hypothesize that common genetic variants have pleiotropic effects on reproductive aging and BP. Although phenotype data suggest an association between reproductive timing and BP increase [Shankar, 2005; Koziel, 2001; Dianels,1998; Lindquist, 1982; Staessen,1989; Izumi,2007], to date, no published data have directly addressed if reproductive timing related genetic variants have a role in hypertension development [Zhang, 2013, Johnson, 2015, Ehret 2011, Spencer 2013]. It is important to identify genetic variants that predispose subgroups to high blood pressure because characterization of genes for hypertension may identify subgroup at hypertension risk in the primary prevention, may develop more effective drugs for clinical intervention, and monitor the target organ damages such as for reducing the clinical complications (e.g., renal failure) [Thiel 2000]. Therefore, we conducted a study to evaluation association between BP and SNPs previously identified from GWAS that were associated with menarche or menopause associated in healthy adolescents.

Patients and Methods

Study population

The study subjects were from a longstanding prospective cohort established in 1986 to study blood pressure development in children and adolescents. The detailed study design and data collection process have been described elsewhere [Pratt, 1989, Tu 2011; Tu 2015]. Briefly, healthy children, aged 4-17 years were enrolled from 33 schools in Indianapolis, IN., and were followed up 5.4 years on average. Their participation was voluntary. Informed consent was obtained from each child as well as from his or her parents or a legal guardian. The study was approved by the institutional review board of Indiana University-Purdue University of Indianapolis. Self-reported race categories were recorded and validated [Tu 2009; Tu 2014]. Children were excluded from the study if they had a history of renal or cardiac disease or diabetes

mellitus, or if they took medications that could affect aldosterone secretion or blood pressure [Pratt, 1989; Manatunga, 1993; Tu, 2011].

Blood pressure assessment

Semiannual assessment of blood pressure was conducted. Blood pressure measurements were carried out at enrollment and semiannually between 08:00 am and 12:00 pm at the subject's school, in some instances in the Indiana University Clinical Research Center, or in the subject's home. BP was measured in the right arm with a random zero sphygmomanometer (Hawksley and Sons, Lancing, West Sussex, UK) while the subject was in a seated position. The first and fifth Korotkoff sounds were used for systolic and diastolic BP measurements, respectively. Three readings were obtained at intervals of at least 2 minutes, and the average of the last two readings was used in the analyses. On average, each subject contributed 11 repeated measurements [Pratt, 1989].

Blood samples, genotyping and SNP selection

DNA was extracted for participants with blood samples stored at -20° C. Genotypes of the candidate SNPs were determined using the Sequenom MassArray iPLEX Platform (Sequenom, San Diego, CA). The genotyping success rate for each SNP was over 95% [Tu 2015]. Samples with missing genotypes higher than 2% were removed from the study. In the retained samples, all SNPs were in Hardy–Weinberg equilibrium (P value > 0.05). The allele frequency for each SNP in our data was consistent with that reported for populations of European and African descent in the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). We selected 46 independent SNPs that have been reported to be associated with reproductive time (age at menarche or with age at menopause) in meta-analyses of GWAS of women of European ancestry [Elks, 2010; Stolk, 2012]. These SNPs were either strongly associated with reproductive time (with reported p-value $\leq 1\times10-8$), or associated with both reproductive time and another adult growth outcome

(weight or height). Based on their associations with age at menarche and other correlated growth traits, we further categorized these SNPs into four groups: Group A included 16 SNPs associated with age at menarche only [Elks, 2010]; Group B included 8 SNPs associated with both age at menarche and adult BMI [Elks, 2010]; Group C included 7 SNPs associated with both age at menarche and adult height [Elks, 2010]; and Group D included 15 SNPs associated with menopause [Stolk, 2012]. Detailed descriptions of the SNPs are listed in Table 1-1.

Other measure assessments

Semiannual assessment of growth in height and weight was also conducted in these subjects [Manatunga, 1993]. BMI, defined as weight (kilograms)/height (meters)^2 was calculated for each follow-up visit.

Data Analysis

Patient characteristics were summarized using sample mean and standard deviation (SD) for continuous variables; and sample proportions for discrete variables. During the study period, the means were calculated by averaging the measurements for the follow-up period, incorporating the repeated measurements on an individual in the standard error. Summary statistics were reported for samples stratified by race. The regression analysis was based on longitudinal data collected from 601 adolescents (263 blacks and 338 whites) (Pratt, 1989; Tu, 2015). Blood pressure data that spanned between ages 5 and 21 years were used in the analyses. Mixed effects models were used to examine the associations of SNPs on systolic and diastolic BP at any given age, in black and white children. The models incorporated genetic effects on blood pressure as fixed effects at the population level; and subject-specific random effects were incorporated into the model to accommodate the potential correlations among measures from the same subject. The association between each SNP and measure of blood pressure (systolic and diastolic blood pressure) was assessed with adjustment for age, race, sex, and BMI. The models included the main effects of

SNP, sex, age, race, BMI, height, and SNP*age interaction. The main effect of SNP was estimated by the intercept of the regression line corresponding to the genotype (shift); the age effect reflected the rate of BP increase of the reference genotype (slope); and the SNP*age interaction assessed whether the increase rate differed by genotype. Because we did not know the real underlying genetic model, we tested dominant, recessive, and additive genetic models. We then tested three-way interactions SNP*age*race in data while adjusting for the variable of sex and SNP* age*sex in data while adjusting for the variable of race. These interaction terms allowed us to determine the heterogeneity of the genotype effect on BP increase rate between males and females and between whites and blacks. In order to control for multiple testing, a Bonferroni correction was used based on the number of SNPs in each group. A statistical significance threshold of 0.0031, 0.0063, 0.0071 and 0.0033 was applied to Group A, B, C and D, respectively. All P values were based on two-sided tests. Genetic associations and interactions that remained significant after adjustment for multiple testing were reported. All statistical analyses were performed using SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of subjects

Participants consisted of 263 blacks (112 boys and 151 girls) and 338 whites (170 boys and 168 girls). At study entry, the average systolic blood pressure (SBP) (mmHg) was higher in black girls (103.0) than that in white girls (98.6) (P=0.002). SBP did not differ significantly between black boys (104.1) and white boys (100.7) or between sex groups for each of the race groups. The average diastolic blood pressure (DBP) (mmHg) did not differ significantly between sex groups or race groups (black girls 63.3; black boys 61.9; white girl 60.5; and white boys 60.4). The average age, weight, height, and BMI did not differ significantly between sex groups for each

of the race groups. However, blacks were on average older, heavier, and taller than whites, and also had a larger average of BMI (P=0.0001).

In the follow-up period, the average SBP continued to be higher in black girls (105.4 mmHg) than white girls (102.4 mmHg, P =0.002). Within each of the race groups, the average SBP was higher in boys than girls (black boys 108.7 versus black girls 105.4, P=0.0238; white boys 106.7 than white girls 102.4, P<0.0001). The average DBP (mmHg) raised marginally significantly amongst black girls (63.1) than white girls (61.7) (P=0.05), and did not differ significant between race groups for each sex group. For either whites or blacks, the mean age and BMI did not differ significantly between males and females. Black children on average remained heavier, taller, and had higher BMI than white children (all P < 0.03).

Genetic associations with blood pressure

Table 1-3 presents the results for SNPs that were statistically significantly associated with blood pressure. Of the 11 SNPs, 10 were statistically associated with increased systolic blood pressure, and only one (rs1042367) with increased diastolic blood pressure.

Of the 16 SNPs previously associated with age at menarche (group A), 6 SNPs were associated with increased blood pressure (Table 1-3). We expected that SNPs associated with early menarche or natural menopause would be associated with an increased level of blood pressure; and found 4 out of the 6 significant SNPs had direction of association with age at menarche as expected: SNP rs10423674 near gene *CRTC1* was associated with increased diastolic blood pressure in black boys (p=0.001); Rs1079866 near *INHBA* was associated with increased systolic blood pressure in white girls (p=0.003), whereas rs13187289 near gene *PHF15* (p=0.001), and rs9635759 near gene *CA10* (p=0.002) were associated with increased systolic blood pressure in black boys. In contrast, the remaining 2 SNPs out of these SNPs were not in the direction

predicted by individual SNP associations with age at menarche: SNPs rs17268785 near gene *CCDC85A*[p=0.001] and rs7642134 near gene *VGLL3*[p=0.003]) were associated with decreased systolic blood pressure in black girls.

Of the 8 SNPs previously reported associated with both age at menarche and BMI (group B), only rs7647305 near gene *ETV5* was associated with increased systolic blood pressure in whites (p=0.003 for additive model and 0.0051 for recessive model, respectively) in the direction of predicted by the association with age at menarche.

Of the 7 SNPs reportedly associated with both age at menarche and height (group C), 3 SNPs were associated with increased blood pressure (Table 1-3). One of the 3 SNPs showed association in the expected direction with age at menarche: SNP rs6440003 near gene *ZBTB38* was associated with increased systolic blood pressure in white girls (p=0.003). The remaining 2 SNPs were not in the direction predicted by individual SNP associations with age at menarche: SNPs (rs10946808 near gene *HIST1H1D* and rs757608 near gene *TBX2*) were associated with decreased systolic blood pressure in white boys (p=0.005) and in black boys (p=0.004), respectively.

Of the 15 SNPs previously associated with natural menopause age (group D), only rs2153157 near gene *SYCP2L* was associated with increased blood pressure (Table 1-3) (p=0.003), in the direction predicted by individual SNP associations with natural menopause age.

Race and sex differences

The statistical modeling demonstrates that the associations between the candidate SNPs and blood pressure appeared to be race and sex specific (Table 1-3). Of the 11 SNPs associated with blood pressure outcomes, 6 were observed in blacks and 5 in whites. The heterogeneity test for

differences in blood pressure by race was significant for all SNPs (all $p<0.05$) except rs1079866. Among blacks, of the 6 SNPs associated with blood pressure outcomes, 4 SNPs were observed in males only and 2 in females only. For whites, of the 5 SNPs associated with blood pressure outcomes, 2 SNPS were in both males and females, 2 SNPs in females and 1 in male only.

Discussion

In this study, we hypothesized that common genetic variants that were previously associated with reproductive timing have pleiotropic effects both on reproductive timing and BP. We had a unique opportunity to examine the effects of reproductive timing related genetic variants on blood pressure in in white and black children and adolescents because both genetic variants and blood pressure were measured in the Indianapolis healthy children cohort. Mixed effect models revealed that 11 reproductive related single nucleotide polymorphisms (SNPs) from previous GWAS were significantly associated with blood pressure.

To date, there are limited data on the association between genetic variation related to reproductive timing and BP development. Epidemiological data on phenotypes suggests that [Lakshman, 2009; Hu 1999; Shankar, 2005; Koziel, 2001; Dianels,1998; Lindquist, 1982; Staessen, 1989; Izumi, 2007] both factors of age at menarche and age at natural menopause are associated with the risk of cardiovascular disease as well as its risk factors including blood pressure (BP). However, no study has directly addressed the association between genetic variation related to reproductive timing and BP development [Zhang, 2013, Johnson, 2015, Ehret 2011, Spencer 2013]. The observed synchronization between reproductive timing and hypertension development raises questions about the possibility of common regulating mechanisms shared by these processes.

Understanding more about the association between the genetic variants and BP development is important for the following reasons. First, more genes that can contribute to blood pressure variation remain to be discovered. The heritability of BP has been estimated to be 30–50%, but the genetic variants identified through GWAS together explain less than 3% of the total variance of BP. Thus, many of the genetic variants that can explain heritability have been missed in the GWAS studies [Munroe, 2013]. Second, any seemly trivial novel gene discovery, even with a small magnitude of association, could have utility for disease prevention and clinical management. Observational data suggests that a slight increase in BP can execute a great impact on health outcome: an increase in SBP by 2 mm Hg increases stroke risk by 10% and coronary risk by 7% (Lewington, 2002). Therefore, any novel genes associated with blood pressure that does not seem impressive at first discovery could have great impact on identifying high risk population for implementing prevention in early life before full-blown presentations of clinical BP and its complications or can be applied in anti-hypertensive medication development. In an analog example, a widely used anti-diabetic medication class, glitazones, has been developed based on original finding on the gene product of PPARG, a gene with an odds ratio only about 1.2 for association with type 2 diabetes mellitus [Lohmueller, 2002].

Our pleiotropic hypothesis was supported by the prospective nature of this longitudinal study because, in time sequence, the modeling suggested that the genetic factors predisposed the study subjects to the different level of BP increase during the adolescence development period. Our hypothesis was also consistent with other studies. A meta-analysis of GWAS showed that genetic variants were associated with both age at menopause and serum urate [Zhang, 2013], a trait correlated with blood pressure [Johnson, 2015]. In addition, genetic variant intronic ARHGAP42 rs633185 was reported associated with a blood pressure in a GWAS study [Ehret 2011], and was also associated with age at menarche and age at natural menopause in another meta-analysis of GWAS [Spencer 2013].

Our results suggested that novel genetic variants associated with both reproductive timing and possible pathways that may help explain more of BP variation. Eleven of the 46 SNPs that had been found associated with reproductive timing in the previous GWAS were associated with blood pressure in our current study. Of note, these 11 SNPs were not included in the recently published SNPs associated with blood pressure [GWAS catalog, 2016; Padmanabhan, 2015; and Cabrera, 2015]. Interestingly, most of the associations (7 out of 11) were in the direction that accelerated increases in BP are associated with a younger age at menarche or age at menopause. This consistency in the direction of association suggests that common genetic variants that were previously associated with reproductive timing can execute pleiotropic effects both on BP and reproductive time in healthy adolescents. A few associations (4 out of 11) were in the opposite direction that accelerated increases in BP are associated with earlier sexual maturation or menopause. The reason behind these opposite directions cannot be explained by our current knowledge.

Note that the observed associations did not appear to be attenuated by BMI in our analysis. This suggested that these genetic variants may influence BP differently from the commonly considered pathways in which BP is mediated by adiposity [Hall, 2003 and 2010]. In some instances, the associations were race-specific and/or gender-specific. Racial differences in genetic effects may reflect differences in underlying BP physiological regulation. A recent review showed that blacks, in comparison to whites, appear to have a more expanded plasma volume and sodium (Na^{2+}) retention due to an increase in Na^{2+} reabsorption by the kidney [Tu,2014]. The mechanism for genetic effect difference between boys and girls is largely unknown in this study. However, we speculated that the difference is attributable to the differential timing of puberty growth spur in boys and girls. It was observed that the peak of increase in BP coincided with the peak in acceleration of height, and girls had earlier puberty onset as well as earlier increase in peak in BP and height than boys [Shankar, 2005; Tu 2009]. The sex difference in genetic effects could be

also attributable to sex hormone role difference between girls and boys. At puberty, increase testosterone production by testes primarily accounts for development of secondary male sex features (SSF) (voice change and pubic hair development) in boys, while the increase in estrogen production by ovary in girls primarily for development of secondary female sex features (age at menarche and breast development). Boys presenting earlier SSF were reported to have a higher fat-free mass likely due to changes in androgen levels [Rogol, 1994], while girls presenting earlier SSF were reported to have a higher fat mass over fat-free mass that coincides with changes in estrogen levels (Bandini, 2008). Thus, difference in body composition that is possibly due to rise of different responsible sex hormones in puberty [Dunger, 2006] will have conceivably different roles in BP development. Sex hormone per se can also affect BP in other pathways than body composition, and the effect of sex hormones vary by sex. BP increases in menopausal women coincides with estrogen decreases but the decrease in estrogen in men after age 50 is not remarkable and the magnitude of BP increase is not as much as women [Remington, 2010]. Estrogen is considered protective against hypertension by activation of the vasodilator pathway mediated by nitric oxide and prostacyclin and inhibition of the vasoconstrictor pathway mediated by the sympathetic nervous system and angiotensin [Ashraf, 2006]. On the other hand, androgen change is in opposite direction for middle aged men and menopausal women: serum androgen level decreases in men after age 50, and increases in postmenopausal women. The changes in androgen that occur in opposite direction in men and women are all thought to promote CVD and hypertension through pathways of oxidative stress, endothelial dysfunction, and inflammation [Reckelhoff, 2011]. Therefore, the race- and sex-specific data above suggested existence of complex genetic pathways that may influence BP during rapid growth period.

The precise mechanisms by which these genetic variants affect BP in various pathways are unknown and are not within the original scope of this investigation. However, with regard to etiology of hypertension, the accumulated data suggest that hypertension in general is likely to be

determined by numerous susceptibility genes and involves multiple environmental determinants. The most prominent gene candidate has been the polymorphisms in the renin–angiotensin–aldosterone system [Singh, 2016]. It was noticed that all these 11 novel genetic variants of interest were relevant to either intronic or intergenic non-coding genes. Therefore, these variants potentially could influence hypertension's pathogenesis by regulating relevant DNA transcription and translation. Regardless, our research points to future investigation direction in which the biological functions of these genetic variants and potential pathways leading to hypertension should be studied more to understand if and how reproductive genetic variants influence hypertension development.

Strengths and limitations

Our study has numerous strengths that can give credence to the observed association between reproductive timing related genetic variants and blood pressure, which suggested that these reproductive timing related genetic variants affect BP during adolescent period, and may be used to further explain BP variation. The study was based on prospective BP assessments with repeated measurements starting in childhood and repeated well into adolescence. With repeated measurement modeling in blood pressure, the weakness of a relative small sample size was offset by increased statistical power. Also the study was able to adjust for potential confounding factors of age, race, sex, and BMI. The study result can be given more credence because the observed association remained significant after adjusting for type 1 error using Bonferroni method, which is the most conservative statistical multiplicity adjustment. A unique strength of our study was that it gave us an opportunity to study the difference in ethnicity effect on blood pressure and enable us to demonstrate the interaction between ethnicity and genetic variants, which suggested the roles of these genetic variants varied across ethnicities in the process of BP development. Including AA subjects in the study provided valuable data that were usually not available in previous GWAS studies.

Even with this new idea of studying the effect of reproductive timing related genetic variants on blood pressure, this study is deemed to be exploratory, and will be limited to scientific assumption generation. Due to a relative small sample size, any discovery of this study will be subject to replication and validation in larger sample sized studies and different study populations with different racial backgrounds. On the other hand, lack of statistical significance for other genetic variants not found statistically significant in this study should not be interpreted as evidence for lack of association because of limited power in this study. This study as an observational study cannot rule out residual confounding completely. To further understand the mechanism how any of these genetic variants play the role in the pathophysiology, it will be important to link any potential discoveries to existing or novel pathways that regulate hypertension. This could be achieved by implementing any or all of these in future studies: sequencing whole genomes, zooming in to deeply sequence target regions, studying the variants expressions, and study microbial diversity in humans or in the environment [Cabrera, 2015; Padmanabhan,2015; Munroe, 2013; Fontana 2015; Arwood,2015].

Conclusion

Genetic variants from previous GWAS that were originally found associated with women's reproductive aging showed significant associations with BP in healthy male and female adolescents. The genetic effects were independent of a subject's sex, race or growth related factors such as weight, height, and BMI. These significant variants were not reported in recently published SNPs association with BP. Thus they may represent novel candidates for BP genes, and give clues for discovering better ways for hypertension prevention and control. The findings need to be validated in different and large-scale populations, and the mechanism how reproductive related genes influence hypertension development needs to be studied.

Table 1-1. Groups of SNPs associated with traits of age at menarche and age of menopause

SNP Group	SNP	Locus	Near Gene	Function	Major/Minor Allele	MAF	Risk Allele *
Group A (n=16): associated with age at menarche	rs466639	1q23.3	RXRG	Intronic	C/T	0.13	T
	rs17268785	2p16.1	CCDC85A	Intronic	A/G	0.17	A
	rs7642134	3p12.1	VGLL3	Intergenic	G/A	0.43	A
	rs6438424	3q13.32	LOC100421670	Intergenic	A/C	0.46	A
	rs13187289	5q31.1	PHF15	Intergenic	C/G	0.23	C
	rs1079866	7p14.1	INHBA	Intergenic	C/G	0.12	C
	rs7821178	8q21.11	PEX2	Intergenic	C/A	0.42	A
	rs2090409	9q31.2	TMEM38B	Intergenic	C/A	0.39	A
	rs10980926	9q31.3	ZNF483	Intronic	G/A	0.40	G
	rs10899489	11q14.1	GAB2	Intronic	C/A	0.12	C
	rs6589964	11q24.1	BSX	Intergenic	C/A	0.48	A
	rs9635759	17q21.33	CA10	Intergenic	G/A	0.30	G
	rs1398217	18q21.1	SKOR2	Intronic	C/G	0.40	G
	rs10423674	19p13.11	CRTC1	Intronic	C/A	0.39	C
Group B (n=8): associated with age at menarche and BMI	rs2815752	1p31.1	NEGR1	Intergenic	A/G	0.36	A, A
	rs10913469	1q25.2	SEC16B	Intronic	T/C	0.25	C, C
	rs6548238	2p25.3	TMEM18	Intergenic	C/T	0.15	C, C
	rs7647305	3q27.2	ETV5	Intergenic	C/T	0.20	C, C
	rs10938397	4p12	GNPDA2	Intergenic	A/G	0.45	G, G
	rs987237	6p12.3	TFAP2B	Intronic	A/G	0.16	G, G
	rs7138803	12q13.12	FAIM2	Intergenic	G/A	0.35	A, A
	rs9939609	16q12.2	FTO	Intronic	T/A	0.46	A, A
Group C (n=7): associated with age at	rs6440003	3q23	ZBTB38	Intronic	A/G	0.43	G,G
	rs10946808	6p22.2	HIST1H1D	Intergenic	A/G	0.30	G, G
	rs7759938	6q16.3	LIN28B	Intergenic	T/C	0.36	T, C

menarche and height	rs4549631	6q22.32	LOC728666	Intergenic	C/T	0.46	C, T
	rs1042725	12q14.3	HMGA2	Intergenic	T/C	0.49	T, T
	rs1659127	16p13.12	MKL2	Intergenic	G/A	0.30	G, G
	rs4794665	17q22	NOG	Intergenic	G/A	0.46	A, G
	rs757608	17q23.2	TBX2	Intergenic	G/A	0.31	A, G
	rs4800148	18q11.2	CABLES1	Intronic	A/G	0.24	A, G
Group D (n=15): associated with age at menopause	rs10183486	2q13.1	TLK1	intronic	C/T	0.37	T
	rs1046089	6p21.33	BAT2	missense	G/A	0.35	A
	rs10852344	16p13.13	--		T/C	0.42	T
	rs11668344	19q13.42	TMEM150B	intronic	A/G	0.36	G
	rs12461110	19q13.43	NLRP11	missense	G/A	0.36	A
	rs1635501	1q43	EXO1	intronic	T/C	0.48	T
	rs16991615	20p12.3	MCM8	missense	G/A	0.07	G
	rs2153157	6p24.4	SYCP2L	intronic	G/A	0.49	G
	rs2277339	12q13.3	PRIM1	missense	T/G	0.10	T
	rs2303369	2p23.3	FNDC4	intronic	C/T	0.39	T
	rs2307449	15q26.1	POLG	intronic	T/G	0.41	G
	rs2517388	8p11.23	ASH2L	intronic	T/G	0.17	T
	rs4246511	1p34.3	RHBDL2	intronic	C/T	0.27	T
	rs4693089	4q21.23	HELQ	intronic	A/G	0.49	G
	rs4886238	13q21.2	TDRD3	intronic	G/A	0.33	G

SNP: single nucleotide polymorphisms
MAF: major allele frequency
* Risk allele refers to the allele that was associated with a younger age at menarche or at natural menopause in previous GWAS [Elks, 2010; Stolk, 2012]

Table 1-2. Characteristics of study subjects

	Boys			Girls		
	Whites (n=170)	Blacks (n=112)	P value	Whites (n=168)	Blacks (n=151)	P value
	Mean ± SD			Mean ± SD		
Study Entry						
Age(yr)	10.2 ± 0.3	12.1 ± 0.3	0.0001	10.8 ± 0.3	12.5 ± 0.3	0.0001
BMI (kg/m2)	19.0 ± 0.4	21.9 ± 0.6	0.0001	18.9 ± 0.3	22.8 ± 0.6	0.0001
Weight(kg)	40.6 ± 1.7	53.1 ± 2.3	0.0001	39.9 ± 1.3	54.3 ± 2.0	0.0001
Height(cm)	141.3 ± 1.5	152.1 ± 1.8	0.0001	141.9 ± 1.3	150.8 ± 1.3	0.0001
SBP (mmHg)	100.7 ± 12.1	104.1 ± 11.9	NS	98.6 ± 11.3	103.0 ± 10.8	0.002
DBP (mmHg)	60.4 ± 10.5	61.9 ± 10.2	NS	60.5 ± 10.2	63.3 ± 9.5	NS
Follow-up period						
Number of visits	12.9 ± 0.6	8.8 ± 6.1	0.0001	12.0 ± 0.5	8.4 ± 0.5	0.0001
Age (yr)	13.6 ± 0.1	14.3 ± 0.2	0.003	13.9 ± 0.1	14.4 ± 0.2	0.02
BMI (kg/m2)	21.6 ± 0.4	23.9 ± 0.5	0.0004	21.4 ± 0.4	25.2 ± 0.4	0.0001
Weight (kg/m2)	57.7 ± 1.3	65.7 ± 1.6	0.0002	52.9 ± 1.3	64.1 ± 1.4	0.0001
Height (cm)	160.4 ± 0.8	163.9 ± 1.0	0.006	155.5 ± 0.7	157.8 ± 0.8	0.03
SBP (mmHg)	106.7 ± 12.7	108.7 ± 12.2	NS	102.4 ± 10.1	105.4 ± 10.4	0.002
DBP (mmHg)	61.9 ± 10.9	63.9 ± 10.8	NS	61.7 ± 9.9	63.1 ± 10.0	0.05

SD: standard deviation; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 1-3. Significant SNP associations with BP

SNP	Group[a]	Genetic Model	Effect allele[e]	BP Outcome	P-value	P-het[b]	P-het[c]
DBP							
rs10423674	BM	dominant	C	more DBP increase (slope)	0.001	0.03	0.003
SBP							
rs1079866	WF	additive	C	more SBP increase (slope)	0.003	NS	0.002
rs13187289	BM	recessive	C	higher SBP (shift)	0.001	0.005	0.08
rs17268785[d]	BF	dominant	A	less SBP increase (slope)	0.001	0.008	0.03
rs7642134[d]	BF	dominant	A	less SBP increase (slope)	0.003	0.02	NS
rs9635759	BM	recessive	G	more SBP increase (slope)	0.002	0.02	0.01
rs7647305	W	additive	C	more SBP increase (slope)	0.003	0.01	NS/NS
rs10946808[d]	WM	dominant	G	lower SBP (shift)	0.005	0.02	0.001
rs6440003	WF	additive	G	more SBP increase (slope)	0.003	0.03	0.007
rs757608[d]	BM	dominant	A	less SBP increase (slope)	0.004	0.03	NS
rs2153157	W	recessive	G	more SBP increase (slope)	0.003	0.04	0.02

SBP: systolic blood pressure; DBP: diastolic blood pressure [a] BF, Black Females; BM, Black Males; WF, White Females; WM, White Males; B, Blacks; W, Whites.

[b] p-value for heterogeneity for racial difference

[c] p-value for heterogeneity for sex difference

[d] the SNP association with BP is not in the direction predicted by its association with age at menarche or menopause

[e] Effect allele refers to the risk allele in the table 1-1

References

Arwood MJ, Cavallari LH, Duarte JD. Pharmacogenomics of hypertension and heart disease. Curr Hypertens Rep. 2015 Sep;17(9):586. doi: 10.1007/s11906-015-0586-5.

Ashraf MS, Vongpatanasin W. Estrogen and hypertension. Curr Hypertens Rep. 2006 Oct;8(5):368-76.
Bandini LG, Must A, Naumova EN, et al. Change in leptin, body composition and other hormones around menarche--a visual representation. Acta Paediatr. 2008; 97:1454–9. [PubMed: 18657126]

Bandini LG, Must A, Naumova EN, et al. Change in leptin, body composition and other hormones around menarche--a visual representation. Acta Paediatr. 2008; 97:1454–9. [PubMed: 18657126]

Cabrera CP1, Ng FL, Warren HR, Barnes MR, Munroe PB, Caulfield MJ. Exploring hypertension genome-wide association studies findings and impact on pathophysiology, pathways, and pharmacogenetics. Wiley Interdiscip Rev Syst Biol Med. 2015 Mar-Apr;7(2):73-90. doi: 10.1002/wsbm.1290. Epub 2015 Feb 6.

Dunger DB, Ahmed ML, Ong KK. Early and late weight gain and the timing of puberty. Mol Cell Endocrinol. 2006; 254–255:140–5.

Daniels, S.R., et al., Longitudinal correlates of change in blood pressure in adolescent girls. Hypertension, 1998. 31(1): p. 97-103.

Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011; 478: 103–109. nature10405 [pii];10.1038/nature10405 [doi].

Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet. 2010;42:1077–85. [PMC free article] [PubMed]

Ewald DR, Haldeman LA. Risk Factors in Adolescent Hypertension.
Glob Pediatr Health. 2016 Feb 16;3:2333794X15625159. doi: 10.1177/2333794X15625159. eCollection 2016.

Fontana V, Luizon MR, Sandrim VC. J An update on the pharmacogenetics of treating hypertension. Hum Hypertens. 2015 May;29(5):283-91. doi: 10.1038/jhh.2014.76. Epub 2014 Aug 28. Review.

GWAS catalog. A Catalog of Published Genome-Wide Association Studies. Available at: http://www.ebi.ac.uk/gwas or http://www.genome.gov/gwasstudies (Accessed July 30, 2016).

Hall JE. The kidney, hypertension, and obesity. Hypertension. 2003; 4:625–633. [PubMed: 12623970]

Hall JE, da Silva AA, do Carmo JM, Dubinion J, Hamza S, Munusamy S, Smith G, Stec DE. Obesity-induced hypertension: role of sympathetic nervous system, leptin, and melanocortins. J Biol Chem. 2010; 285:17271–17276. [PubMed: 20348094]

Herman-Giddens ME. Recent data on pubertal milestones in United States children: the secular trend toward earlier development. Int J Androl. 2006 Feb;29(1):241-6; 286-90.

Johnson RJ. Why focus on uric acid? Curr Med Res Opin. 2015;31 Suppl 2:3-7. doi: 10.1185/03007995.2015.1087979.

Koziel, S., H. Kolodziej, and S. Ulijaszek, Body size, fat distribution, menarcheal age and blood pressure in 14-year-old girls. Eur J Epidemiol, 2001. 17(12): p. 1111-5.

Hu, F.B., et al., Age at natural menopause and risk of cardiovascular disease. Arch Intern Med, 1999. 159(10): p. 1061-6.

Izumi, Y., et al., Effect of age at menopause on blood pressure in postmenopausal women. Am J Hypertens, 2007. 20(10): p. 1045-50.

Lakshman, R., et al., Early age at menarche associated with cardiovascular disease and mortality. J Clin Endocrinol Metab, 2009. 94(12): p. 4953-60.

Lewington S, Clarke R, Qizilbash N, Peto R, Collins R; Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002;360:1903–1913.

Lindquist, O., Intraindividual changes of blood pressure, serum lipids, and body weight in relation to menstrual status: results from a prospective population study of women in Goteborg, Sweden. Prev Med, 1982. 11(2): p. 162-72.

Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet. 2003;33:177–182.

Manatunga AK, Jones JJ, Pratt JH. Longitudinal assessment of blood pressures in black and white children. Hypertension. 1993; 22:84–89. [PubMed: 8319996]

Munroe PB, Barnes MR, Caulfield MJ. Advances in blood pressure genomics. Circ Res 2013, 112:1365–1379. doi:10.1161/circresaha.112.300387.

Padmanabhan S, Caulfield M, Dominiczak AF. Genetic and molecular aspects of hypertension. Circ Res. 2015 Mar 13;116(6):937-59. doi: 10.1161/CIRCRESAHA.116.303647.

Pratt JH, Jones JJ, Miller JZ, Wagner MA, Fineberg NS. Racial differences in aldosterone excretion and plasma aldosterone concentrations in children. N Engl J Med. 1989; 321:1152–1157.[PubMed: 2677724]

Reckelhoff JF, Roman RJ. Androgens and hypertension: role in both males and females? Hypertension. 2011 Apr;57(4):681-2

Remington PL, et al. Chronic disease epidemiology and control (Third Edition). Publisher: American public health association. 2010. Page 335-362

Rogol AD. Growth at puberty: interaction of androgens and growth hormone. Med Sci Sports Exerc. 1994; 26:767–70. [PubMed: 7914344]

Shankar, R.R., et al., The change in blood pressure during pubertal growth. J Clin Endocrinol Metab, 2005. 90(1): p. 163-7.

Singh M, Singh AK, Pandey P, Chandra S, Singh KA, Gambhir IS. Molecular genetics of essential hypertension. Clin Exp Hypertens. 2016 Mar 30:1-10. [Epub ahead of print]

Spencer KL1, Malinowski J, Carty CL, Franceschini N, Fernández-Rhodes L, Young A, Cheng I, Ritchie MD, Haiman CA, Wilkens L, Chunyuanwu, Matise TC, Carlson CS, Brennan K, Park A, Rajkovic A, Hindorff LA, Buyske S, Crawford DC. Genetic variation and reproductive timing: African American women from the Population Architecture using Genomics and Epidemiology (PAGE) Study. PLoS One. 2013;8(2):e55258. doi: 10.1371/journal.pone.0055258. Epub 2013 Feb 12.

Staessen, J., et al., The influence of menopause on blood pressure. J Hum Hypertens, 1989. 3(6): p. 427-33.

Stolk L, Perry JR, et al Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. Nat Genet. 2012 Jan 22;44(3):260-8. doi: 10.1038/ng.1051.

Thiel B, Weder AB. Genes for Essential Hypertension: Hype, Help, or Hope? J Clin Hypertens (Greenwich). 2000 May;2(3):187-193.

Tu W, Eckert GJ, Saha C, Pratt JH. Synchronization of adolescent blood pressure and pubertal somatic growth.J Clin Endocrinol Metab. 2009;94:5019-5022.

Tu W, Eckert GJ, DiMeglio LA, Yu Z, Jung J, Pratt JH. Intensified effect of adiposity on blood pressure in overweight and obese children. Hypertension. 2011 Nov;58(5):818-24

Tu, W, Pratt JH. A Consideration of Genetic Mechanisms Behind the Development of Hypertension in Blacks. Curr Hypertens Rep. 2013 Apr; 15(2): 108–113.

Tu W, Eckert GJ, Hannon TS, Liu H, Pratt LM, Wagner MA, Dimeglio LA, Jung J, Pratt JH.Racial differences in sensitivity of blood pressure to aldosterone. Hypertension. 2014 Jun;63(6):1212-8.

Tu W, Wagner EK, Eckert GJ, Yu Z, Hannon T, Pratt JH, He C. Associations between menarche-related genetic variants and pubertal growth in male and female adolescents. J Adolesc Health. 2015 Jan; 56(1):66-72

Zhang L, Spencer KL, Voruganti VS, Jorgensen NW, Fornage M, Best LG, Brown-Gentry KD, Cole SA, Crawford DC, Deelman E, Franceschini N, Gaffo AL, Glenn KR, Heiss G, Jenny NS, Kottgen A, Li Q, Liu K, Matise TC, North KE, Umans JG, Kao WH. Association of functional polymorphism rs2231142 (Q141K) in the ABCG2 gene with serum uric acid and gout in 4 US populations: the PAGE Study. Am J Epidemiol. 2013 May 1;177(9):923-32. doi: 10.1093/aje/kws330. Epub 2013 Apr 3.

Study 2: Discovery Bivariate Meta-analysis of GWAS on Blood Pressure and Age at Menarche/ Natural Menopause

Introduction

It was well established that increased blood pressure (BP) increases risk of cardiovascular diseases such as myocardial infarction and stroke, chronic renal disease, premature death and disability. Hypertension is the one of the leading causes of death in the US. The total costs of health care services, medications to treat high blood pressure, and missed days of work due to hypertension are about $48.6 billion each year [CDC blood pressure fact sheet, 2016]. Multiple environment, lifestyle, and genetic factors contribute to hypertension. The environment and lifestyle factors include obesity, excessive salt intake, less potassium intake, excessive alcohol intake, and insufficient physical activity and stress [Remington 2010]. Genetically, the heritability of BP has been estimated to be 30–50% [Munroe, 2013]. The accumulated data suggest that blood pressure regulation in general is likely to be influenced by multiple genetic variants, and the most prominent gene candidate that has been identified so far is relevant to the polymorphisms in the renin–angiotensin–aldosterone system [Sigh, 2016]. However, the identified genetic variants from GWAS together only explain less than 3% of the total variance of BP and many of genetic variants that can explain inheritability have been missed in the GWAS studies [Munroe, 2013].

Reproductive timing, age at menarche (AAM) as well as age at natural menopause (AANM), are found associated with increased BP and risk of cardiovascular diseases [Lakshman, 2009; Hu 1999]. BP measures, both systolic and diastolic BP, increase at an accelerated rate with onset of sexual maturation [Shankar 2005]. Early menarche is associated with elevated BP in adolescent

and adulthood [Koziel 2001; Dianels1998]. BP is also known to increase more steeply around age at menopause [Lindquist 1982]. Earlier menopause is associated with higher BP [Staessen, 1989; Izumi, 2007], although it is still unclear whether menopause accelerates BP increase or increased BP leads to earlier menopause. The observed synchronization between reproductive aging and BP increase raises questions about the possibility of common regulating mechanisms shared by these processes.

Previous GWAS studied the associations between common genetic variants, and AAM, AANM and BP, separately (Elks 2010, Stolk 2012 and Ehret 2011). However, no study was conducted to explore if common genetic variants, with pleiotropic effects, are associated with both traits BP and reproductive timing. Therefore, we conducted a bivariate analysis to evaluation association between the traits (i.e., BP and reproductive timing) and the common genetic variants from previous GWAS.

Materials and Methods

GWAS data sources

Full details of the individual GWAS were given in the original publications [Perry 2014, Ehret 2011, Elks 2010, Stolk, 2012], but briefly, the BP GWAS included 29 studies, comprising 69,395 individuals the menarche GWAS included 32 studies, comprising 87 802 women; and the menopause GWAS included 22 studies, comprising 38 968 women. All subjects were of white European ancestry. Blood pressure was measured directly in the BP GWAS subjects. The ages at menarche (AAM), ranging from 9-17 years as recalled by the women in the menarche GWAS, were included in the analysis. Age at natural menopause (AANM) was defined as the age at the last menstrual period that occurred naturally. Those AANMs, ranging from 40 to 60 years as reported by the women in menopause GWAS, were included in the analysis. The woman subjects

were excluded if their menopause was due to hysterectomy and/or bilateral ovariectomy, or chemotherapy/irradiation, or if they received hormone replacement therapy before menopause as ascertained by medical records.

The study subjects were genotyped using a variety of Affymetrix (6.0, GeneChip 500K, 250K, MIP50K and 10K) and Illumina (HumanHap 550K, 318K, 370 K, HumanHap 300K, HumanHap 370K CNV, HumanHap610 quad, Human660W-Quad BeadChip, 6K and Human 1Mv1_C, HumanHap300 Duo "+") genotyping arrays. For the unmeasured SNPs, genotypes were imputed in these studies. Altogether, 2.5 million genotyped or imputed single nucleotide polymorphisms (SNPs) were included in the GWAS meta-analyses [Perry 2014, Ehret 2011, Elks 2010, Stolk, 2012].

Written informed consent was provided by all participants and the studies were approved by local Research Ethics Committees and/or Institutional Review Boards.

Statistical analysis

Overall, the associations between the SNPs and the trait pairs of BP and reproductive timing were explored by using a multivariate analysis approach, which is similar with other multivariate approaches for potential pleiotropic effects. We essentially tested the null hypothesis (H_0) that the parameter estimate for genetic effect was 0 for both traits (e.g., $\beta1 = 0$ for SBP and $\beta2 = 0$ for AAM). The goal of this bivariate approach was to identify more GWAS loci with potential pleiotropic effects when two correlated traits together were combined. However, the SNPs with significant bivariate GWAS p-values were not necessary to be pleiotropic SNPs because there were 3 potential alternative hypotheses (H_1) ($\beta1 \neq 0$ and $\beta2 \neq 0$; $\beta1 = 0$ and $\beta2 \neq 0$; $\beta1 \neq 0$ and $\beta2 = 0$). To accommodate these multiple alternative H_1, we compared the p-values from bivariate GWAS with the p-values from univariate GWAS. When a SNP with a potential pleiotropic effect, its

bivariate GWAS p-value should be smaller than its univariate GWAS from each of the phenotypes. A rule was set up to consider a SNP with potential pleiotropic effect if its bivariate GWAS p-value was one order of magnitude smaller than the p-values from both univariate GWAS. The simulation suggested that this screening process is able to capture the most but not all of SNPs with pleiotropic effects and reduce the false positive rates for those SNPs without pleiotropic effects [Personal correspondence with Dr. Yi-Hsiang Hsu]. In this bivariate analysis, empirical-weighted linear-combined test statistics (eLC), as proposed by Dr. Hsu [Perry 2014], directly combined correlated test statistics that were obtained from univariate GWAS meta-analyses, with a weighted sum of univariate test statistics to maximize the overall association signals and accounted for the correlation between phenotypes. An optimal weighting was estimated empirically, so that the smallest variance of the combined test statistics among all the possible combinations was used for the new test statistics. An empirical permutation test was then used to estimate p-values for the eLC new test statistics. Bivariate P-values $<5 \times 10^{-8}$ for the SNPs were considered genome-wide significant with potential pleiotropic effects. Bivariate P-values $<1 \times 10^{-5}$for the SNPs were also reported in the discovery bivariate analyses.

The data used in the discovery bivariate analyses were aggregated from univariate GWAS meta-analyses that were conducted for the 2.5 million SNPs in BP consortium and ReproGen consortium. Detailed statistics method on univariate GWAS meta-analyses was described in the published literature [Perry 2014, Ehret 2011, Elks 2010, Stolk, 2012], but briefly, linear regression under additive genetic modelling estimated statistics (regression effect betas, standard errors and P values) for the associations between SNPs and each of the individual traits such as BP, and reproductive timing trait (AAM or AANM), separately. These univariate analyses were conducted for each of 2.5 million genotype or imputed SNPs, and thus generated the aggregated data for the discovery bivariate analyses, in which 4 pairs of traits of interest were studied:

diastolic blood pressure (DBP) and AAM; DBP and AANM; systolic blood pressure (SBP) and AAM; and SBP and AANM.

Results

At genome-wide significance level ($P< 5X10^{-8}$), the bivariate analyses on the aggregated data showed that 49 SNPs were associated with the trait pair SBP_AAM, 25 SNPs were associated with the pair SBP_AAM; 65 SNPs with the pair DBP_AANM; and 28 SNPs with the pair SBP_AANM (Table 2-1). No matter it was DBP or SBP, 22 SNPs of the above significant SNPs ($P < 5X10^{-8}$) were consistently associated with both pairs DBP_AAM, and SDP_ AAM; and 17 SNPs with both pairs DBP_AANM, and SDP_AANM (Table 2-2).

At genome-wide significance level ($P< 1X10^{-5}$), the bivariate analyses suggested many more SNPs associated with the trait pairs: 525 SNPs were associated with the trait pair SBP_AAM, 508 SNPs were associated with the pair SBP_AAM; 591 SNPs with the pair DBP_AANM; and 437 SNPs with the pair SBP_AANM. No matter it was DBP or SBP, 192 SNPs of the above significant SNPs ($P < 5X10^{-5}$) were consistently associated with both pairs DBP_AAM, and SDP_ AAM; and 212 SNPs with both pairs DBP_AANM, and SDP_AANM.

Discussion

In this analysis, we identified more than one hundred SNPs were associated with both traits of reproductive timing and BP. It suggested that those genetic variants that were previously associated with reproductive timing, and associated with BP, separately, could have potential pleiotropic effects on both traits. Identifying the novel genetic variants that explain BP more is important because it may bring about the clues for developing better solutions for hypertension prevention and control. Our bivariate analysis approach leading to these findings may help identifying more novel variants associated with BP.

Epidemiological data on phenotypes suggests that [Lakshman, 2009; Hu 1999; Shankar, 2005; Koziel, 2001; Dianels,1998; Lindquist, 1982; Staessen, 1989; Izumi, 2007] both factors of age at menarche and age at natural menopause are associated with the risk of cardiovascular disease as well as its risk factors including blood pressure (BP). The observed synchronization between reproductive timing and hypertension development raises questions about the possibility of common pathophysiology process involving both reproductive timing and hypertension development. To date, no study has directly addressed the association between common genetic variation related to both reproductive timing and BP development [Zhang, 2013, Johnson, 2015, Ehret 2011, Spencer 2013]. However, understanding more about the association between these genetic variants and BP development is important for the following reasons. First, more genes that can contribute to blood pressure variation remain to be discovered. The heritability of BP has been estimated to be 30–50%, but the genetic variants identified through GWAS together explain less than 3% of the total variance of BP. Thus, many of the genetic variants that can explain heritability have been missed in the GWAS studies [Munroe, 2013]. Second, any seemly trivial novel gene discovery, even with a small magnitude of association, could have utility for disease prevention and clinical management. Observational data suggests that a slight increase in BP can

execute a great impact on health outcome: an increase in SBP by 2 mm Hg increases stroke risk by 10% and coronary risk by 7% (Lewington, 2002). Therefore, any novel genes associated with blood pressure that does not seem impressive at first discovery could have great impact on identifying high risk population for implementing prevention in early life before full-blown presentations of clinical BP and its complications or can be applied in anti-hypertensive medication development. In an analog example, widely used anti-diabetic medication class glitazones has been developed based on original finding on the gene product of PPARG, a gene with an odds ratio only about 1.2 for association with type 2 diabetes mellitus [Lohmueller, 2002].

In this study, we hypothesized that some common genetic variants have pleiotropic effects both on BP and reproductive timing. This hypothesis is supported by recent meta-analysis of GWAS in which genetic variants were associated with both age at menopause and serum urate [Zhang, 2013], a trait correlated with blood pressure [Johnson, 2015]. In addition, genetic variant intronic ARHGAP42 rs633185 was reported associated with a blood pressure in a GWAS study [Ehret 2011], and was also associated with age at menarche and age at natural menopause in another meta-analysis of GWAS [Spencer 2013]. By using the eLC approach, we speculate that we will be able to discover more common genetic variants that could be associated with both BP and reproductive timing because eLC approach has the following advantages: (1) eLC does not require individual level genotype and phenotype information, so it can be conveniently applied to re-analyze the aggregated data from univariate meta-analysis on existing GWAS; (2) eLC avoids the loss of statistical power due to missing data for one of the studied phenotypes [Perry 2014]. At genome-wide significance level ($P< 5X10^{-8}$), our bivariate analyses demonstrated that more than one hundred SNPs were associated with both traits of BP and reproductive timing. It turned out that some of the significant SNPs (e.g., rs1046089, rs1077393, rs11748651, rs2736176, rs2763979, rs2844477, rs3751813, rs707929, and rs805294) from this bivariate analysis had not

been found associated with BP in the previous GWAS meta-analyses [PhenoScanner 2017]. Therefore, our study suggested some of these SNPs could become novel genes that are contributable to blood pressure regulation.

Although, eLC, as a screening tool, was able to screen more than one hundred SNPS both associated with BP and reproductive timing, this bivariate GWAS meta-analysis should be considered preliminary at a discovery stage like other GWAS aiming to discover novel genes associated with a phenotype or phenotypes. Our findings are subject to validation in larger sample sized studies and different study populations with different racial backgrounds. For validation, we propose that we use two criteria to define SNPs with potential pleiotropic effects: (1) bivariate p values $< 5x10^{-8}$ from the eLC test and (2) p-values from bivariate analysis must be smaller than the p-values from each univariate analyses. We also propose to prioritize SNPs for replication that meet this criterion based on functional relevance and importance to trait biology (see discussion on biological mechanisms). The validation would require individual level data from additional studies that ideally do not contribute to our preliminary results. Again, Bonferroni correction will be used to account for multiple testing. Because of the limited statistical power inherently in the GWAS, lack of statistical significance for other genetic variants not found genome-wide significant ($P< 5X10^{-8}$) should not be interpreted as evidence for lack of association. At a liberal genome-wide significance level ($P< X10^{-5}$), more than one thousand SNPs were found associated with both traits of BP and reproductive timing. Therefore, more SNPs can be considered at the validation stages. In addition to the bivariate analyses, tri-variate analyses (e.g., association between an SNP and trivariates menarche, SBP and DBP) or even qua-variate analyses may be explored to enhance statistical power to discover more common genetic variant candidates for validation.

To date, the variance of BP explained by the genetic variants has remained small (~3%), and how the genetic variants play the role in the pathophysiology of developing hypertension has remained largely unknown [Munroe, 2013]. It is important to link any potential discoveries to existing or novel pathways that regulate hypertension in the process of discovering attributable novel genes. A brief functional review on the genes near the discovered genome-wide significant SNPs ($P< 5X10^{-8}$) revealed that the gene DDAH2 encodes a dimethylarginine dimethylaminohydrolase. This encoded enzyme functions in generation of nitric oxide (NO), which is known to be involved in BP regulation. NO affects the cardiac changes and endothelium vasodilation [NCBI gene website 2017]. However, it is hard to directly map most of the discovered genes to the well-established pathways for BP including renin-angiotension-aldeosterone system, kallikrein-kinn system, ion channels, transporters, natriuretic proteins, nitric oxide, and endolthelin systems [Cabrera 2015]. Even so, we noticed that numerous genes are associated with the common diseases/conditions coexisting with hypertension. For example, the gene ARNTL was found associated with problems with gluconeogenesis and lipogenesis, and altered sleep pattern; DLK1 with adult and adolescent obesity; and FTO with obesity and type 2 diabetes. Although it is challenging to distinguish between the causal mechanisms specifically related to hypertension and those linked to other diseases/conditions, it is biologically plausible that these genes could have direct or indirect effect on BP because BP is likely determined by many susceptibility genes, each of which has a small effect size. As mentioned above these discovered genes need validation in future studies. Meanwhile, other genes such as RXRG, DCAKD, BSX, RP34P23, LY6G6C, PRRC2A, BAG6, BCL2, VWA7, NRG1, and ULK4 appeared limited in evidence that supports an association with BP based on the current knowledge of hypertension etiology and pathophysiology. These findings could be either from false-positive results or implied that, if there were true effects, they could be involved in the novel pathways for BP. To understand more about the biological mechanisms for any novel genes., future studies can be implemented to focus on sequencing whole genomes, zooming in to deeply sequence target regions, studying the

variants expressions, and study microbial diversity in humans or in the environment [Cabrera, 2015; Padmanabhan,2015; Munroe, 2013; Fontana 2015; Arwood,2015]. We wish that these GWAS and biological mechanism studies help identify new drug targets, and give clues to help hypertension prevention and clinical management.

Conclusion

Our preliminary discovery bivariate analysis results suggested that more than a hundred of SNPs may have potential pleiotropic effects both on BP and reproductive timing. Our approach of bivariate GWAS meta-analysis may help identify more genetic variants attributable to BP that will be eventually used for searching more effective prevention and management of hypertension. However, the SNPS identified in our analysis will be subject to validation in different study samples with individual-level data that do not contribute to our observations, and in biological mechanism studies.

Table 2-1. Genome-wide significant SNPs ($P< 5 X 10^{-8}$) by trait pair in discovery bivariate analyses

Trait pair	SNP	Locus	Position	Near Gene	P			Note
DBP _AAM					DBP_AAM*	AAM only#	DBP only#	49 significant SNPs^
	rs900145	11p15.2	13250481	ARNTL	1.39E-10	7.66E-09	0.000873	33 other SNPs in high LD
	rs285482	1	163677347	RXRG	5.36E-10	9.81E-10	0.00703	
	rs16948048	17q21.32	44795465	ZNF652	1.44E-09	0.0001717	1.38E-06	2 other SNPs in high LD
	rs1190903	14q32.2	100166167	Intergenic between BEGAIN and DLK1	3.39E-09	9.56E-07	0.000489	3 other SNPs in high LD
	rs3751813	16q12.2	52376209	FTO	5.81E-09	7.75E-08	0.00388	
	rs4905976	14	100130358	Intergenic between BEGAIN and DLK1	1.55E-08	1.60E-08	0.0176	5 other SNPs in high LD
SBP _AAM					SBP_ AAM*	AAM only#	SBP only#	25 significant SNPs^
	rs285482	1	163677347	RXRG	7.64E-10	0.00886	9.81E-10	
	rs900145	11	13250481	ARNTL	1.10E-09	0.00408	7.66E-09	19 other SNPs in high LD
	rs2194899	1	163677409	RXRG	5.62E-09	0.0112	9.85E-09	
	rs4793172	17q21.31	40487006	DCAKD	2.39E-08	1.11E-06	0.002302	
	rs7111924	11	122371312	Intergenic between BSX and RPL34P23	4.34E-08	0.019	5.78E-08	

	rs1190903	14q32.2	100166167	Intergenic between BEGAIN and DLK1	4.51E-08	0.00422	9.56E-07	
DBP_ AANM					DBP_AANM*	AANM only#	DBP only#	65 significant SNPs ^
	rs1046089	6	31710946	PRRC2A	7.59E-14	0.00119	1.31E-13	5 other SNPs in high LD
	rs805294	6	31796196	LY6G6C	7.26E-11	4.31E-05	2.14E-07	4 other SNPs in high LD
	rs805305	6	31805366	DDAH2	5.20E-10	9.64E-05	7.55E-07	
	rs2763979	6	31902571	Intergenic between HSPA1A and HSPA1B	6.54E-10	6.95E-05	1.46E-06	
	rs2736155	6	31713178	PRRC2A	1.55E-09	0.000563	2.62E-07	
	rs1077393	6	31718508	BAG6	1.64E-09	0.000562	2.79E-07	
	rs707929	6	31850046	VWA7	4.22E-09	0.000469	1.13E-06	
	rs2471980	6	31908847	C6orf48	7.01E-09	0.00071	1.18E-06	
	rs776387	8	31857593	NRG1	2.41E-08	0.000838	4.14E-06	2 other SNPs in high LD
	rs9856088	3	41835486	ULK4	2.93E-08	1.90E-07	0.007213	45 other SNPs in high LD
SBP_ AANM					SBP_ AANM*	AANM only#	SBP only#	28 significant SNPs ^
	rs2242660	6	31705732	PRRC2A	6.70E-15	1.30E-06	3.94E-10	5 other SNPs in high LD
	rs2736155	6	31713178	PRRC2A	3.90E-12	2.17E-06	2.62E-07	
	rs1077393	6	31718508	BAG6	4.25E-12	2.22E-06	2.79E-07	
	rs805294	6	31796196	LY6G6C	5.75E-11	3.42E-05	2.14E-07	4 other SNPs in high LD
	rs805305	6	31805366	DDAH2	1.08E-10	2.15E-05	7.55E-07	

	rs2763979	6	31902571	Intergenic between HSPA1A and HSPA1B	5.07E-10	5.38E-05	1.46E-06	
	rs2471980	6	31908847	Intergenic between HSPA1B and C6orf48	7.76E-10	9.61E-05	1.18E-06	
	rs707929	6	31850046	VWA7	2.03E-09	0.000241	1.13E-06	
	rs2844477	6	31686751	Intergenic between NCR3 and UQCRHP1	2.66E-08	0.00939	1.00E-07	4 other SNPs in high LD
	rs11748651	5	171864544	Intergenic between SH3PXD2B and NEURL1B	2.88E-08	0.0111	8.15E-08	1 other SNP in high LD
	rs781427	4	84762727	Intergenic between AGPAT9 and NKX6-1	3.30E-08	0.0133	6.89E-08	
	rs7029	6	31737932	GPANK1	4.31E-08	0.000374	2.19E-05	1 other SNP in high LD
	rs2736176	6	31695540	Intergenic between AIF1 and PRRC2A	4.44E-08	0.0202	4.44E-08	

*P value from bivariate analysis
#P value from univariate GWAS meta-analysis
^ genome-wide significance level < 5 X 10^{-8}
AAM: age at menarche
AANM: age at natural menopause;
DBP: diastolic blood pressure
DBP_AAM: trait pair of diastolic blood pressure and age at menarche

DBP_AANM: trait pair of diastolic blood pressure and age at natural menopause
High LD: high linkage disequilibrium (R^2>0.8)
SBP: systolic blood pressure
SBP_AAM: trait pair of systolic blood pressure and age at menarche
SBP_AANM: trait pair of systolic blood pressure and age at natural menopause
SNP: single nucleotide polymorphisms

Table 2-2. Summary of SNPs ($P< 5 X 10^{-8}$) that were consistently genome-wide significant^ for trait blood pressure and reproductive aging regardless of it was SBP or DBP*

Trait pairs	SNP	Locus	Position	Near Gene	P value					Note
DBP_AAM and SBP_AAM					SBP_AAM*	DBP_AAM*	AAM #	SBP #	DBP #	22 significant SNPs ^
	rs285482	1	163677347	RXRG	7.64E-10	5.36E-10	9.81E-10	0.00886	0.00703	
	rs900145	11p15.2	13250481	ARNTL	1.10E-09	1.39E-10	7.66E-09	0.00408	0.000873	12 other SNPs in high LD
	rs6486120	11p15.2	13280718	ARNTL	5.68E-09	4.52E-10	2.37E-08	0.00726	0.0011	6 other SNPs in high LD
	rs1190903	14q32.2	100166167	Intergenic between BEGAIN and DLK1	4.51E-08	3.39E-09	9.56E-07	0.00422	0.000489	
DBP_AANM and SBP_AAM					SBP_AAM*	DBP_AAM*	AAM #	SBP#	DBP#	17 significant SNPs ^
	rs1046089	6	31710946	PRRC2A	6.99E-16	7.59E-14	1.31E-13	4.30E-05	0.00119	5 Other SNPs in high LD
	rs2736155	6	31713178	PRRC2A	3.90E-12	1.55E-09	2.62E-07	2.17E-06	0.000563	
	rs1077393	6	31718508	BAG6	4.25E-12	1.64E-09	2.79E-07	2.22E-06	0.000562	

	rs707916	6	31805537	DDAH2	9.69E-11	1.14E-10	2.98E-07	4.25E-05	5.03E-05	4 Other SNPs in high LD
	rs805305	6	31805366	DDAH2	1.08E-10	5.20E-10	7.55E-07	2.15E-05	9.64E-05	
	rs2763979	6	31902571	Intergenic between HSPA1A and HSPA1B	5.07E-10	6.54E-10	1.46E-06	5.38E-05	6.95E-05	
	rs2471980	6	31908847	Intergenic between HSPA1B and C6orf48	7.76E-10	7.01E-09	1.18E-06	9.61E-05	0.00071	
	rs707929	6	31850046	VWA7	2.03E-09	4.22E-09	1.13E-06	0.000241	0.000469	

* P values for SNPs that were consistently genome-wide significant ($P<5X10^{-8}$) for both trait pairs of DBP_AAM and SBP_AAM, or both trait pairs of DBP_AANM and SBP_AANM in bivariate analyses.
#P value from univariate GWAS meta-analysis
^ Genome-wide significance level < 5 X 10^{-8}. No matter it was DBP or SBP, 22 were consistently associated with both pairs DBP_AAM, and SDP_ AAM; and 17 SNPs with both pairs DBP_AANM, and SDP_AANM.
AAM: age at menarche
AANM: age at natural menopause;
DBP: diastolic blood pressure
DBP_AAM: trait pair of diastolic blood pressure and age at menarche
DBP_AANM: trait pair of diastolic blood pressure and age at natural menopause
High LD: high linkage disequilibrium ($R^2>0.8$)
SBP: systolic blood pressure
SBP_AAM: trait pair of systolic blood pressure and age at menarche
SBP_AANM: trait pair of systolic blood pressure and age at natural menopause
SNP: single nucleotide polymorphisms

References

Arwood MJ, Cavallari LH, Duarte JD. Pharmacogenomics of hypertension and heart disease. Curr Hypertens Rep. 2015 Sep;17(9):586. doi: 10.1007/s11906-015-0586-5.

CDC blood pressure fact sheet. http://www.cdc.gov/dhdsp/data_statistics/fact_sheets/docs/fs_bloodpressure.pdf. Accessed on July 10 2016.

Daniels, S.R., et al., Longitudinal correlates of change in blood pressure in adolescent girls. Hypertension, 1998. 31(1): p. 97-103

Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011; 478: 103–109. nature10405 [pii];10.1038/nature10405 [doi].

Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet. 2010;42:1077–85. [PMC free article] [PubMed]

Fontana V, Luizon MR, Sandrim VC. J An update on the pharmacogenetics of treating hypertension. Hum Hypertens. 2015 May;29(5):283-91. doi: 10.1038/jhh.2014.76. Epub 2014 Aug 28. Review.

Hu, F.B., et al., Age at natural menopause and risk of cardiovascular disease. Arch Intern Med, 1999. 159(10): p. 1061-6.

Izumi, Y., et al., Effect of age at menopause on blood pressure in postmenopausal women. Am J Hypertens, 2007. 20(10): p. 1045-50

Johnson RJ. Why focus on uric acid? Curr Med Res Opin. 2015;31 Suppl 2:3-7. doi: 10.1185/03007995.2015.1087979.

Koziel, S., H. Kolodziej, and S. Ulijaszek, Body size, fat distribution, menarcheal age and blood pressure in 14-year-old girls. Eur J Epidemiol, 2001. 17(12): p. 1111-5.

Lakshman, R., et al., Early age at menarche associated with cardiovascular disease and mortality. J Clin Endocrinol Metab, 2009. 94(12): p. 4953-60.

Lewington S, Clarke R, Qizilbash N, Peto R, Collins R; Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002;360:1903–1913.

Lindquist, O., Intraindividual changes of blood pressure, serum lipids, and body weight in relation to menstrual status: results from a prospective population study of women in Goteborg, Sweden. Prev Med, 1982. 11(2): p. 162-72.

Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet. 2003;33:177–182.

Munroe PB, Barnes MR, Caulfield MJ. Advances in blood pressure genomics. Circ Res 2013, 112:1365–1379. doi:10.1161/circresaha.112.300387.

NCBI gene website: https://www.ncbi.nlm.nih.gov/gene/. Accessed on 2017/02/26

Perry JRB, Hsu YH. et al. DNA mismatch repair gene MSH6 implicated in determining age at natural menopause. Human Molecular Genetics, 2014. 23 (9): p. 2490–2497.

PhenoScanner. Website: http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner assessed on April 23 2017

Remington PL, et al. Chronic disease epidemiology and control (Third Edition). Publisher: American public health association. 2010. Page 335-362

Shankar, R.R., et al., The change in blood pressure during pubertal growth. J Clin Endocrinol Metab, 2005. 90(1): p. 163-7.

Singh M, Singh AK, Pandey P, Chandra S, Singh KA, Gambhir IS. Molecular genetics of essential hypertension. Clin Exp Hypertens. 2016 Mar 30:1-10.

Spencer KL1, Malinowski J, Carty CL, Franceschini N, Fernández-Rhodes L, Young A, Cheng I, Ritchie MD, Haiman CA, Wilkens L, Chunyuanwu, Matise TC, Carlson CS, Brennan K, Park A, Rajkovic A, Hindorff LA, Buyske S, Crawford DC. Genetic variation and reproductive timing: African American women from the Population Architecture using Genomics and Epidemiology (PAGE) Study. PLoS One. 2013;8(2):e55258. doi: 10.1371/journal.pone.0055258. Epub 2013 Feb 12.

Staessen, J., et al., The influence of menopause on blood pressure. J Hum Hypertens, 1989. 3(6): p. 427-33.

Stolk L, Perry JR, et al Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. Nat Genet. 2012 Jan 22;44(3):260-8. doi: 10.1038/ng.1051.

Zhang L, Spencer KL, Voruganti VS, Jorgensen NW, Fornage M, Best LG, Brown-Gentry KD, Cole SA, Crawford DC, Deelman E, Franceschini N, Gaffo AL, Glenn KR, Heiss G, Jenny NS, Kottgen A, Li Q, Liu K, Matise TC, North KE, Umans JG, Kao WH. Association of functional polymorphism rs2231142 (Q141K) in the ABCG2 gene with serum uric acid and gout in 4 US populations: the PAGE Study. Am J Epidemiol. 2013 May 1;177(9):923-32. doi: 10.1093/aje/kws330. Epub 2013 Apr 3.

Study 3: Menarche Related Genetic Variants from Genome-Wide Association Studies Are Associated with Height Growth Catchup in Healthy Adolescents

Introduction

Parents may be concerned or anxious about the short stature of their short children (Woolford 2007). Adolescents undergo rapid changes in linear body growth as well as psychological and social functions (Nelson, 2016). Accelerated growth among adolescents usually starts from first visible sign of puberty when testicular enlargement in boys and appearance of breast buds (thelarche) in girls until an adult height is achieved (Nelson, 2016). However, the timing of growth acceleration varies widely (Nelson, 2016), and those children who appear short may be experiencing constitutional growth delay and will catch up later in the adolescence period (Kappy, 2010). Other children could be constantly short during adolescence, and be adversely affected in psychological and social function development. Short children could have maladaptive behaviors and emotional problems because of teasing and low self-esteem (Tanaka, 2009; Ross, 2004). Unproven or controversial treatments (Kappy, 2010, Ali 2011) could have been implemented on the children who appear short. Importantly, expensive treatments including growth hormone could impose financial burden on the treated children, their families, and our health care system (Kelnar, 1999). Identifying those adolescents who appear short from adolescence and will continue to be short in adulthood is important for efficient and efficacious clinical consultation and management.

Final adult height is determined by a complex combination of genetic and environment factors, but mainly by genetics which explains 60%-90% of height variation (Macgregor 2006; Roberts 1978; Silventoinen 2003). A meta-analysis published in 2014 (Wood 2014) included more than

250,000 individuals with European ancestry and revealed 697 common single nucleotide polymorphisms (SNPs) clustered in 423 different loci across the human genome that influence adult height. These loci are enriched for genes that are connected in biological pathways and trigger skeletal growth defects, and explained approximately 16% of the height variation. The genome-wide association studies (GWASs) suggested future genetics epidemiology studies could explain more variance of height. Other GWASs identified 31 genetic variants that were associated with age at menarche in women of European ancestry (Elks, 2010). Some of these SNPs were also associated with adult height and BMI (Elks, 2010). But the effects of these genetic variations on height growth catchup failure (HGCF) among adolescents have not been investigated. We hypothesize that common genetic variants have pleiotropic effects on age at menarche and HGCF. Therefore, we conducted a study to evaluate association between HGCF and SNPs previously identified from GWAS that were associated with age at menarche in healthy adolescents.

Patients and Methods

Study population

The study subjects were from a longstanding prospective cohort established in 1986 to study blood pressure development in children and adolescents. The detailed study design and data collection process have been described elsewhere [Pratt, 1989; Tu, 2011, Tu 2015]. Briefly, healthy children, aged 4-17 years were enrolled from 33 schools in Indianapolis, IN. Their participation was voluntary. Informed consent was obtained from each child as well as from his or her parents or a legal guardian. The study was approved by the institutional review board of Indiana University-Purdue University of Indianapolis. Self-reported race categories were recorded and validated [Tu 2009; T2014]. Children were excluded from the study if they had a history of renal or cardiac disease or diabetes mellitus [Pratt, 1989; Manatunga, 1993; Tu, 2011].

Body height and weight assessment

Semiannual assessment of body height (cm) was conducted. Standing height measurements were carried out by using a stadiometer at enrollment and semiannually at the subject's school, in some instances in the Indiana University Clinical Research Center, or in the subject's home. Semiannual assessment of growth in weight was also conducted in these subjects [Manatunga, 1993; Tu 2011]. BMI, defined as weight (kilograms)/height (meters)^2 was calculated for each follow-up visit.

We identified subjects who had body height at puberty onset (BHAPO) as well as body height at adult age (BHAAA). BHAPO was estimated at the age where first vital sign of puberty onset appears (i.e., when testicular enlargement appears in boys and breast buds (thelarche) appear in girls). Because no sexual maturity rating or Tanner stage data were collected in the original cohort, we cannot directly observe puberty onset age in order to obtain a body height directly relevant to the puberty onset age. We, instead, estimated the BHAPO by using the body height observed close to the mean puberty onset age (i.e., 11.5 years for white boys, 10.5 years for black boys, 10.5 for white girls and 9.5 years for black girls) (Kappy, 2010). We specifically used the periods one year before to one year after the mean puberty onset age by sex and race (10.5 – 12.5 years for white boys, 9.5 – 11.5 years for black boys, 9.5 – 11.5 years for white girls, and 8.5 – 10.5 years for black girls, respectively). Puberty onset weights within these periods were also measured. BHAAA were estimated by using the body height that was lastly observed after age 16 for girls and after age 18 for boys when an adult body height is likely to have achieved (CDC growth chart; Benabbad, 2016). Center of Disease Control Growth Charts 2000 were used to give percentiles for the body heights measured (CDC growth chart).

Blood samples, genotyping and SNP selection

DNA was extracted for participants with blood samples stored at -20° C. Genotypes of the candidate SNPs were determined using the Sequenom MassArray iPLEX Platform (Sequenom, San Diego, CA). The genotyping success rate for each SNP was over 95% [Tu 2015]. Samples with missing genotypes higher than 2% were removed from the study. In the retained samples, all SNPs were in Hardy–Weinberg equilibrium (P value > 0.05). The allele frequency for each SNP in our data was consistent with that reported for populations of European and African descent in the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). We selected 31 independent SNPs that have been reported to be associated with age at menarche in the meta-analyses of GWAS of women of European ancestry [Elks, 2010]. These SNPs were either strongly associated with age at menarche (with reported p-value $\leq 1\times10-8$), or associated with both age at menarche and another adult growth outcome (height or BMI). Based on their associations with age at menarche and other correlated growth traits, we categorized these SNPs into 3 groups: Group A included 16 SNPs associated with age at menarche only [Elks, 2010]; Group B included 8 SNPs associated with both age at menarche and adult BMI [Elks, 2010]; Group C included 7 SNPs associated with both age at menarche and adult height [Elks, 2010]. Detailed descriptions of the SNPs are listed in Table 3-1.

Data Analysis

Patient characteristics were summarized using sample mean and standard deviation (SD) for continuous variables; and sample proportions for discrete variables.

For the subjects whose BHAPO and BHAAA were both measured, logistic regression modelling was used to compare the subjects whose BHAPO and BHAAA were both below 25th percentile and the rest. Odds ratios for SNPs were estimated adjusting for covariates sex, race, BHAPO, BMI at puberty onset. Because we did not know the real underlying genetic model, we tested dominant, recessive, and additive genetic models. We then tested interactions of SNP*race and

SNP*sex while adjusting for the covariates. These interaction terms allowed us to determine the heterogeneity of the genotype effect on HGCF between males and females or between whites and blacks. Statistical significance level was at alpha 0.05. All statistical analyses were performed using SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of subjects

We identified 245 subjects (n=121 for boys; and 124 for girls) who had body height both at puberty onset (BHAPO) and body height at adult age (BHAAA). Of these subjects, 184 (75.1%) were whites, and 61 (24.9%) were blacks. 17 subjects (6.9%) had HGCF. At puberty onset, the HGCF group and the non-HGCF group were comparable for variable age (years) (mean 10.9 ± SD 0.7 versus 10.9 ± 0.8), sex (male 52.9% versus 49.1%), race (white 94.1% versus 73.7%), BMI (kg/m2) (18.2 ± 2.7 versus 18.7 ± 3.2); the HGCF group was statistically significantly lower in measures of height (cm) (133.1 ± 3.6 versus 147.2 ± 8.3, P<0.0001) and weight (kg) (32.3 ± 4.9 versus 41.0 ± 9.6, P=0.0003) (Table 3-2). At the time of BHAAA, the HGCF group and the non-HGCF group were also comparable for age (years) (mean 20.7 ± SD 2.9 versus 21.4 ± 3.7), and BMI (kg/m2) (24.5 ± 3.9 versus 25.6 ± 5.5); the HGCF group was statistically significantly lower than the non-HGCF group in measures of adult height (cm) (160.4 ± 7.6 versus 171.7 ± 9.8, P<0.0001) and weight (kg) (63.0 ± 9.5 versus 75.8 ± 18.1, P=0.0044) (Table 3-2).

Genetic associations with HGCF

Table 3-3 presents the results for SNPs that were statistically significantly associated with HGCF. Of the 31 SNPs, 8 SNPs were found significantly associated with HGCF (P<0.05) and presented either increased risk or decreased risk of HGCF: 4 SNPs (rs7642134 near gene VGLL, rs7647305 near ETV5, rs9939609 near FTO, and rs6440003 near ZBTB38) were associated with increased risk of HGCF (ORs = 4.89, 12.0, 14.0 and 7.02, respectively). 1 SNP, rs987237 near TFAP2B was associated with a decreased risk of HGCF (OR= 7.02). The rest 3 SNPs presented opposite directions of associations by sex: rs7759938 near LIN28B presented increased risk for boys (OR = 53.6) but decreased risk for girls (OR = 0.03); in contrast, rs1042725 near HMGA2 and

rs4794665 near NOG presented decreased risk for boys (OR = 0.02 and 0.002, respectively) but increased risk for girls (OR= 4.55 and 3.74, respectively). The magnitude of estimated ORs ranged widely from 0.002 to 117. Different genetic models (additive, recessive and dominant) were observed across these associations.

Table 3-1. Groups of SNPs associated with traits of age at menarche

SNP Group	SNP	Locus	Near Gene	Function	Major/Minor Allele	MAF	Risk Allele *
Group A (n=16): associated with age at menarche	rs466639	1q23.3	RXRG	Intronic	C/T	0.13	T
	rs17268785	2p16.1	CCDC85A	Intronic	A/G	0.17	A
	rs7642134	3p12.1	VGLL3	Intergenic	G/A	0.43	A
	rs6438424	3q13.32	LOC100421670	Intergenic	A/C	0.46	A
		5q31.1	PHF15	Intergenic	C/G	0.23	C
	rs1079866	7p14.1	INHBA	Intergenic	C/G	0.12	C
	rs7821178	8q21.11	PEX2	Intergenic	C/A	0.42	A
	rs2090409	9q31.2	TMEM38B	Intergenic	C/A	0.39	A
	rs10980926	9q31.3	ZNF483	Intronic	G/A	0.40	G
	rs10899489	11q14.1	GAB2	Intronic	C/A	0.12	C
	rs6589964	11q24.1	BSX	Intergenic	C/A	0.48	A
	rs9635759	17q21.33	CA10	Intergenic	G/A	0.30	G
	rs1398217	18q21.1	SKOR2	Intronic	C/G	0.40	G
	rs10423674	19p13.11	CRTC1	Intronic	C/A	0.39	C
Group B (n=8): associated with age at menarche and BMI	rs2815752	1p31.1	NEGR1	Intergenic	A/G	0.36	A, A
	rs10913469	1q25.2	SEC16B	Intronic	T/C	0.25	C, C
	rs6548238	2p25.3	TMEM18	Intergenic	C/T	0.15	C, C
	rs7647305	3q27.2	ETV5	Intergenic	C/T	0.20	C, C
	rs10938397	4p12	GNPDA2	Intergenic	A/G	0.45	G, G
	rs987237	6p12.3	TFAP2B	Intronic	A/G	0.16	G, G
	rs7138803	12q13.12	FAIM2	Intergenic	G/A	0.35	A, A
	rs9939609	16q12.2	FTO	Intronic	T/A	0.46	A, A
Group C (n=7): associated with	rs6440003	3q23	ZBTB38	Intronic	A/G	0.43	G,G
	rs10946808	6p22.2	HIST1H1D	Intergenic	A/G	0.30	G, G

age at menarche and height	rs7759938	6q16.3	LIN28B	Intergenic	T/C	0.36	T, C
	rs4549631	6q22.32	LOC728666	Intergenic	C/T	0.46	C, T
	rs1042725	12q14.3	HMGA2	Intergenic	T/C	0.49	T, T
	rs1659127	16p13.12	MKL2	Intergenic	G/A	0.30	G, G
	rs4794665	17q22	NOG	Intergenic	G/A	0.46	A, G
	rs757608	17q23.2	TBX2	Intergenic	G/A	0.31	A, G
	rs4800148	18q11.2	CABLES1	Intronic	A/G	0.24	A, G

MAF: Major allele frequency; SNP: single nucleotide polymorphism

* Risk allele refers to the allele that was associated with a younger age at menarche or at natural menopause in previous GWAS [Elks, 2010]

Table 3-2. Characteristics of study subjects

Characteristics	HGCF group (N= 17)	Non-HGCF group (N= 228)	P value
At puberty onset *			
Age (years) (Mean ± SD)	10.9 ± 0.7	10.9 ± 0.8	NS
Sex [n, (%)] Male	9 (52.9%)	112 (49.1%)	NS
Female	8 (47.1%)	116 (50.9%)	
Race (n, %) White	16 (94.1%)	168 (73.7%)	NS
Black	1 (5.9%)	60 (26.3%)	
Height (cm)	133.1 ± 3.6	147.2 ± 8.3	<0.0001
Weight (kg)	32.3 ± 4.9	41.0 ± 9.6	0.0003
BMI (kg/m2)	18.2 ± 2.7	18.7 ± 3.2	NS
At time of BHAAA *			
Age (years)	20.7 ± 2.9	21.4 ± 3.7	NS
Height (cm)	160.4 ± 7.6	171.7 ± 9.8	<0.0001
Weight (kg)	63.0 ± 9.5	75.8 ± 18.1	0.0044
BMI (kg/m2)	24.5 ± 3.9	25.6 ± 5.5	NS

BHAAA: body height at adult age (lastly observed body height for boys older than 18 years and girls older than 16 years)
BMI: body mass index
HGCF: height growth catchup failure
Puberty onset: estimated when onset of breast enlargement or onset of testicular enlargement occurs (10.5 to 12.5 years old for the white boys, and 9.5 to 11.5 years for black boys; and 9.5 to 11.5 years for white girls and 8.5 to 10.5 for black girls)
SD: standard deviation

Table 3-3. Significant SNP associations with HGCF

SNP	Genetic model	Effect allele	OR	P-value	P-Value for heterogeneity for sex difference	P-Value for heterogeneity for race difference
rs7642134	additive	A	4.89	0.04	NS	NS
rs7647305	recessive	C	117	0.03	NS	NS
rs987237	dominant	G	0.05	0.04	NS	NS
rs9939609	dominant	A	14.0	0.04	NS	NS
rs6440003	recessive	G	7.02	0.05	NS	NS
rs7759938	recessive	C	53.6 for male 0.03 for female	0.006	0.006	NS
rs1042725	dominant	T	0.02 for male 4.55 for female	0.02	0.02	NS
rs4794665	dominant	G	0.002 for male 3.74 for female	0.03	0.03	NS

HGCF: Height growth catchup failure;
OR: odds ratio estimated from logistic regression models

Discussion

Epidemiology studies have been conducted to explore the association between genetic variations and adult height (Lui 2015; de Bruin 2016). However, to our best knowledge, no studies were published on the association of genetic variations and catch-up in body height growth in adolescents who are relatively short in reference to their chorological age. Our study showed that 8 SNPs previously found associated with age at menarche were also associated with HGCF (Table 3-3). In addition to originality in our study, our research is clinically important because, by using genetic variants from previous GWAS studies, we might be able to timely identify those adolescents who would remain relatively short from puberty onset to adulthood, and make them accessible to effective and efficacious treatments that would be otherwise missed. Earlier data showed that by the time when the short adolescents received medical attention and started their growth enhancement, they were already on average older than 14 years old (Albanese 1995). These subjects could have passed the growth spurt period, and have missed their optimum timing for growth enhancement treatments (e.g., testosterone, and growth hormone) (Grimberg 2016; Kappy 2010). Starting the growth enhancement treatments late at this age partially explained the unsatisfactory treatment result for growth catch up in these adolescents (Albanese 1995). In recent years, younger ages at starting growth enhancement treatments (approximately 10 to 11 years old) were observed (Şıklar 2015) probably because more parents started to bring their short children to medical attention at a younger age, and then have more accessibility to growth hormone therapy. By using the HGCF-associated SNPs as a biomarker, we would be able to practically restrict the growth enhancement treatments to the subjects who are likely to develop HGCF. Therefore, we would avoid the high costs of growth hormone therapy unnecessarily prescribed to those who can catch up in growth, and would save health resources. With insight in the SNPs associated with HGCF, we may also be able to identify those who appear short but would catch up in height growth, thus help mitigate concern and anxiety arising from being short

that is observable at puberty onset. Therefore, studying these SNPs associated with HGCF is valuable for improving clinical consultation and management.

Of the 31 menarche-related SNPs, 8 SNPs were found significantly associated with HGCF ($P<0.05$). 1 of these 8 significant SNPs was from group A consisting of 16 SNPs associated with menarche only; 3 were from group B consisting of 8 SNPs associated with both menarche and BMI; and 4 from group C consisting of 7 SNPs associated with both menarche and height. The observed associations in various groups supported the hypothesis that the common genetic variants have pleiotropic effects on age at menarche, body growth in height, weight as well as HGCF.

It is interesting that these 8 SNPs presented either increased or decreased risk of HGCF. SNP rs7642134 from group A presented an increased risk of HGCF (Table 3-3). This is plausible because high-level exposure to estrogen exists among the girls with menarche at a younger age (Onland-Moret, 2005) and presumably in the boys with menarche-associated genetic variants. The high-level estrogen promotes growth plate senescence at epiphyses of long bones. Consequently, it causes chondrocyte proliferation to stop at a younger age and results in a shorter adult height (Lui 2015). In other words, a delay in menarche or lack of menarche-associated genetic variation allows more growth of the long bones before the epiphyses closure and results in a taller adult height. A large epidemiology study including 286,202 European women subjects, showed that younger age at menarche was associated with shorter adult height (Onland-Moret, 2005). Of note, we hypothesized the effects of menarche-associated SNPs are applicable to both girls and boys. Recently studies including one large-scale GWAS of puberty timing in men suggested overlapped gene loci between males and females and these shared loci were associated adverse health outcomes (higher BMI, polycystic ovary syndrome, type 2 diabetes, lipid profiles and cardiovascular disease) (Day 2015; Perry 2014; Ong 2009). However, the rest 7 SNPs in

group B and C was either positively or negatively associated with HGCF (Table 3-3). The precise mechanisms of how these SNPs play their roles in developing HGCF are unknown. Of note, the SNPs in the group B were previously associated both age at menarche and BMI, and the SNPs in group C were associated with both age of menarche and height. Therefore, it is speculated that, in addition to the role of estrogen, other common hormones (e.g., testosterone, growth hormone and insulin-like-growth factor-1 (IGF-1)) that regulate puberty development and body growth (linear height and BMI) are also involved in the process of reaching an adult height (Kappy 2010). These hormones could interact with estrogen in a sophisticated way that determines HGCF. In group C, we observed the opposite associations by sex. This suggested that the effects of these SNPs on HGCF, if any, could vary by sex. Again, the precise mechanism behind it is unknown. It is known that girls and boys differ in puberty onset timing and duration of growth spurt, all of which affects adult height (Kappy 2010 and Nelson 2016). Further studies on how boys and girls differ in the SNPs roles in estrogen and androgen generation, and subsequent interaction with related growth pathway (e.g. growth-hormone-IGF-1 axis) as well as local growth plate at long-bone epiphyseal will advance our understanding about sex differences.

Our study has the following limitations. First, the study was retrospective and should be considered exploratory for scientific assumption generation. We adjusted for a few covariates. However, important risk factors such as exercise, and nutrition were not available in this study. Thus, residual confounding could not be ruled out. Second, a small sample size is the major limitation. The findings from this study will be subject to replication and validation in large-scaled studies with different study populations and different racial backgrounds. Also because of the small sample size, we cannot perform more clinically meaningful analysis by selecting those subjects who had a body height that remained much lower than 25^{th} percentile from adolescence to adulthood and would draw much more medical attention. For example, we could not have a sample size large enough for those with a body height < 1^{st} percentile in reference to the CDC

growth chart for height from adolescence to adulthood, or <-2.25 standard deviation below the general population mean. The cutoff <-2.25 standard deviation sometimes is referred as clinical cutoff for idiopathic short stature that is reimbursable for growth hormone treatment by the US FDA (Foods and Drugs Administration) (Allen 2013). Also because of the small sample size, no effort was executed to account for multiplicity. Third, our study measures of BHAPO and BHAAA were limited by lack of direct measures of puberty onset and adult height because we did not have data on when testicular enlargement occurred in boys and breast buds (thelarche) occurred in girls, or bone age data indicating an adult height had been reached. Nevertheless, our method of selecting those subjects who were consistently relatively short from early adolescence to adulthood should be considered robust because we selected those subjects whose body height can be reasonably estimated around the time of puberty onset (Kappy, 2010), and at the time when an adult height was likely to have achieved (CDC growth chart; Benabbad, 2016). Fourth, no precise biological mechanisms are known how these genetic variants played the role in HGCF. As recent meta-analyses suggested that adult height could be related to >600 hundreds of SNPs clustered in >400 different loci across the human genome, and these loci are enriched for genes that are connected to multiple biological pathways to height growth (e.g., growth hormone-IGF-1, transforming growth factor beta, C-natriuretic peptide signaling, rapamycin, osteoglycin, and hyaluronic acid) (Wood 2014, Lui 2015, De Bruin 2016), our effectors of studying only 31 SNPs appeared small. Before studying the precise mechanisms for these, we probably need to expand our study to include more height-associated SNPs from previous GWAS studies and evaluate if more SNPs are associated with HGCF. However, our study can serve as a pilot study and give us clues for future researche.

Conclusion

Some but not all the genetic variants from previous GWAS that were associated with menarche age showed significant associations with HGCF in healthy adolescents. The results from this study suggest that the genetic variants associated with age at menarche, BMI and adult height may add value to clinical growth evaluation and management for the subjects at risk of HGCF. However, it is necessary to replicate and validate the results from this study by conducting further studies with larger sample sizes and different populations, and studies on mechanisms of how these genetic variants influence HGCF

References

Allen DB, Cuttler L. Clinical practice. Short stature in childhood--challenges and choices. N Engl J Med. 2013 Mar 28;368(13):1220-8. doi: 10.1056/NEJMcp1213178. Review

Albanese A1, Stanhope R. Predictive factors in the determination of final height in boys with constitutional delay of growth and puberty. J Pediatr. 1995 Apr;126(4):545-50.

Ali O, Wyatt D. Therapy of growth disorders. Curr Opin Endocrinol Diabetes Obes. 2011 Feb;18(1):3-8. Review.

Benabbad I, Rosilio M, Child CJ, Carel JC, Ross JL, Deal CL, Drop SL, Zimmermann AG, Jia N, Quigley CA, Blum WF. Safety Outcomes and Near-Adult Height Gain of Growth Hormone-Treated Children with SHOX Deficiency: Data from an Observational Study and a Clinical Trial. Horm Res Paediatr. 2016 Dec 22. doi: 10.1159/000452973. [Epub ahead of print]

CDC growth charts 2000. Centers for Disease Control and Prevention, National Center for Health Statistics: (website access Feb 28 2016), https://www.cdc.gov/growthcharts/cdc_charts.htm

Day FR, Bulik-Sullivan B, Hinds DA, Finucane HK, Murabito JM, Tung JY, Ong KK, Perry JR. Shared genetic aetiology of puberty timing between sexes and with health-related outcomes. Nat Commun. 2015 Nov 9; 6:8842.

de Bruin C1, Dauber A.Genomic insights into growth and its disorders: an update. Curr Opin Endocrinol Diabetes Obes. 2016 Feb;23(1):51-6.

Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet. 2010;42: 1077–85.

Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, Rossi WC, Feudtner C, Murad MH; Drug and Therapeutics Committee and Ethics Committee of the Pediatric Endocrine Society.
Guidelines for Growth Hormone and Insulin-Like Growth Factor-I Treatment in Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature, and Primary Insulin-Like Growth Factor-I Deficiency. Horm Res Paediatr. 2016;86(6):361-397.

Kelnar CJH, Albertsson-Wikland K, Hintz RL, Ranke MB & Rosenfeld RG. Should we treat children with idiopathic short stature? Hormone Research 1999 52 150±157.

Kappy M, Allen DB, Geffner Ml. Pediatric Practice – Endocrinology 2010. Page 257-297

Manatunga AK, Jones JJ, Pratt JH. Longitudinal assessment of blood pressures in black and white children. Hypertension. 1993; 22:84–89. [PubMed: 8319996]

Macgregor S, Cornes BK, Martin NG, Visscher PM. Bias, precision and heritability of self-reported and clinically measured height in Australian twins. Hum Genet. 2006 Nov;120(4):571-80. Epub 2006 Aug 25

Lui JC, Garrison P, Baron J. Regulation of body growth. Curr Opin Pediatr. 2015 Aug;27(4):502-10

Nelson Textbook of Pediatrics. 20th edition. Published by Elsevier in 2016. P926-936 Therapy of growth disorders.

Ong KK, Elks CE, Li S, Zhao JH, Luan J, Andersen LB, Bingham SA, Brage S, Smith GD, Ekelund U, Gillson CJ, Glaser B, Golding J, Hardy R, Khaw KT, Kuh D, Luben R, Marcus M, McGeehin MA, Ness AR, Northstone K, Ring SM, Rubin C, Sims MA, Song K, Strachan DP, Vollenweider P, Waeber G, Waterworth DM, Wong A, Deloukas P, Barroso I, Mooser V, Loos RJ, Wareham NJ. Genetic variation in LIN28B is associated with the timing of puberty. Nat Genet. 2009 Jun;41(6):729-33

Onland-Moret NC1, Peeters PH, van Gils CH, Clavel-Chapelon F, Key T, Tjønneland A, Trichopoulou A, Kaaks R, Manjer J, Panico S, Palli D, Tehard B, Stoikidou M, Bueno-De-Mesquita HB, Boeing H, Overvad K, Lenner P, Quirós JR, Chirlaque MD, Miller AB, Khaw KT, Riboli E. Age at menarche in relation to adult height: the EPIC study. Am J Epidemiol. 2005 Oct 1;162(7):623-32. Epub 2005 Aug 17.

Perry, J. R. Day F, et al. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. Nature. 2014 Oct 2;514(7520):92-7

Pratt JH, Jones JJ, Miller JZ, Wagner MA, Fineberg NS. Racial differences in aldosterone excretion and plasma aldosterone concentrations in children. N Engl J Med. 1989; 321:1152–1157.[PubMed: 2677724]

Roberts DF, Billewicz WZ, McGregor IA. Heritability of stature in a West African population. Ann Hum Genet. 1978 Jul;42(1):15-24

Ross JL, Sandberg DE, Rose SR, et al. Psychological adaptation in children with idiopathic short stature treated with growth hormone or placebo. J Clin Endocrinol Metab. 2004;89(10):4873-4878.

Şıklar Z, Kocaay P, Çamtosun E, İsakoca M, Hacıhamdioğlu B, Savaş Erdeve Ş, Berberoğlu M. The Effect of Recombinant Growth Hormone Treatment in Children with Idiopathic Short Stature and Low Insulin-Like Growth Factor-1 Levels. J Clin Res Pediatr Endocrinol. 2015 Dec;7(4):301-6. doi: 10.4274/jcrpe.2111.

Silventoinen K1, Sammalisto S, Perola M, Boomsma DI, Cornes BK, Davis C, Dunkel L, De Lange M, Harris JR, Hjelmborg JV, Luciano M, Martin NG, Mortensen J, Nisticò L, Pedersen NL, Skytthe A, Spector TD, Stazi MA, Willemsen G, Kaprio J. Heritability of adult body height: a comparative study of twin cohorts in eight countries. Twin Res. 2003 Oct;6(5):399-408.

Tanaka T, Tai S, Morisaki Y et al. Evaluation of quality of life in children with GH deficiency and idiopathic short stature using the child behavior checklist. Clin Pediatr Endocrinol. 2009;18(1):15-22

Tu W, Eckert GJ, Saha C, Pratt JH. Synchronization of adolescent blood pressure and pubertal somatic growth.J Clin Endocrinol Metab. 2009;94:5019-5022.

Tu W, Eckert GJ, DiMeglio LA, Yu Z, Jung J, Pratt JH. Intensified effect of adiposity on blood pressure in overweight and obese children. Hypertension. 2011 Nov;58(5):818-24

Tu W, Eckert GJ, DiMeglio LA, Yu Z, Jung J, Pratt JH. Intensified effect of adiposity on blood pressure in overweight and obese children. Hypertension. 2011 Nov;58(5):818-24

Tu W, Eckert GJ, Hannon TS, Liu H, Pratt LM, Wagner MA, Dimeglio LA, Jung J, Pratt JH.Racial differences in sensitivity of blood pressure to aldosterone. Hypertension. 2014 Jun;63(6):1212-8.

Tu W, Wagner EK, Eckert GJ, Yu Z, Hannon T, Pratt JH, He C. Associations between menarche-related genetic variants and pubertal growth in male and female adolescents. J Adolesc Health. 2015 Jan; 56(1):66-72

Wood AR, Esko T, Yang J, et al. Defining the role of common variation in the genomic and biological architecture of adult human height. Nat Genet 2014; 46:1173–1186.

Woolford SJ, Clark SJ, Lumeng JC, Williams DR, Davis MM. Maternal perspectives on growth and nutrition counseling provided at preschool well-child visits. J Natl Med Assoc.2007;99 (2):153– 158

Curriculum Vitae

Daojun Mo

Education

PhD, Indiana University-Purdue University Indianapolis, 2017

MSc, McGill University, Canada, 1999

MD, Tongji Medical University, China, 1991

Professional Experience

Research Scientist, Eli Lilly and Company, 2002-2017

Statistical consultant: MedFocus, 2001-2002

Epidemiologist: CancerCare Hospital, Manitoba, Canada, 1999-2001

Research assistant: McGill University, Canada, 1996-1999

Clinician: Internal Medicine Department, No. 2 Hospital of Hubei Medical University,

Wuhan, China, 1991-1996

Selected Published Papers

Fracture Risk in Adult Patients Treated with Growth Hormone Replacement Therapy for Growth Hormone Deficiency: An Analysis of the Hypopituitary Control and Complications Study (HypoCCS). Daojun Mo, Maria Fleseriu, Rong Qi, Roger Bouillon, Nan Jia, Christopher Jeremy Child, Dana Sue Hardin. The Lancet Diabetes & Endocrinology. 2015 May;3(5):331-8. doi: 10.1016/S2213-8587(15)00098-4. Epub 2015 Apr 12

Ten-Year Change in Quality of Life in Adults on Growth Hormone (GH) Replacement for GH Deficiency: An Analysis of the Hypopituitary Control and Complications Study (HypoCCS).
Daojun Mo, Werner F. Blum, Myriam Rosilio, Susan M. Webb, Rong Qi, Christian J. Strasburger. JCEM (Journal of Clinical Endocrinology & Metabolism), 2014 Dec;99(12):4581-8. doi: 10.1210/jc.2014-2892.;

Adult mortality or morbidity is not increased in childhood-onset growth hormone deficient patients who received pediatric GH treatment: an analysis of the Hypopituitary Control and Complications Study (HypoCCS). Daojun Mo, Dana Sue Hardin, Eva Marie Erfurth, Shlomo Melmed. Pituitary. Pituitary. 2014 Oct;17(5):477-85.