α-ADDUCIN METHYLATION AND BLOOD PRESSURE IN YOUNG ADULTS | Less is More

WAN FATEIN NABEILA WAN OMAR[1], NORLELAWATI A. TALIB[2], JAMALLUDIN AB. RAHMAN[3], AZARISMAN SHAH MOHD SHAH[4], ASZRIN ABDULLAH[1].

[1]Dept of Basic Medical Sciences | [2]Dept of Pathology and Laboratory Medicine | [3]Dept of Community Medicine | [4]Dept of Internal Medicine, Kulliyyah of Medicine, International Islamic University Malaysia

OUTLINE

- Introduction
- Literature review
- Methods
- Results and Discussion
- Strength and Limitation
- Conclusion

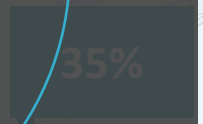
INTRODUCTION

Cardiovascular risk factors	Prevalence (%)	
	Malaysia[2]	Other countries[4]
Age, years (mean/median)	58.5	65
Hypertension	65.0	52
Smoking	38	62
Diabetes mellitus	45.8	21
Dyslipidaemia	37.4	38
Male	78.8	70
Family history	13.2	-
Body mass index (mean (SD))	26.1 (4.3)	-

[1]World Health Organisation (2008); [2]National Cardiovascular disease-Acute coronary syndrome Registry 2011-2013; [3]Hoo et al (2016); [4]Global Registry of Acute Coronary Events 1999-2009

HYPERTENSION

Malaysia >18 y



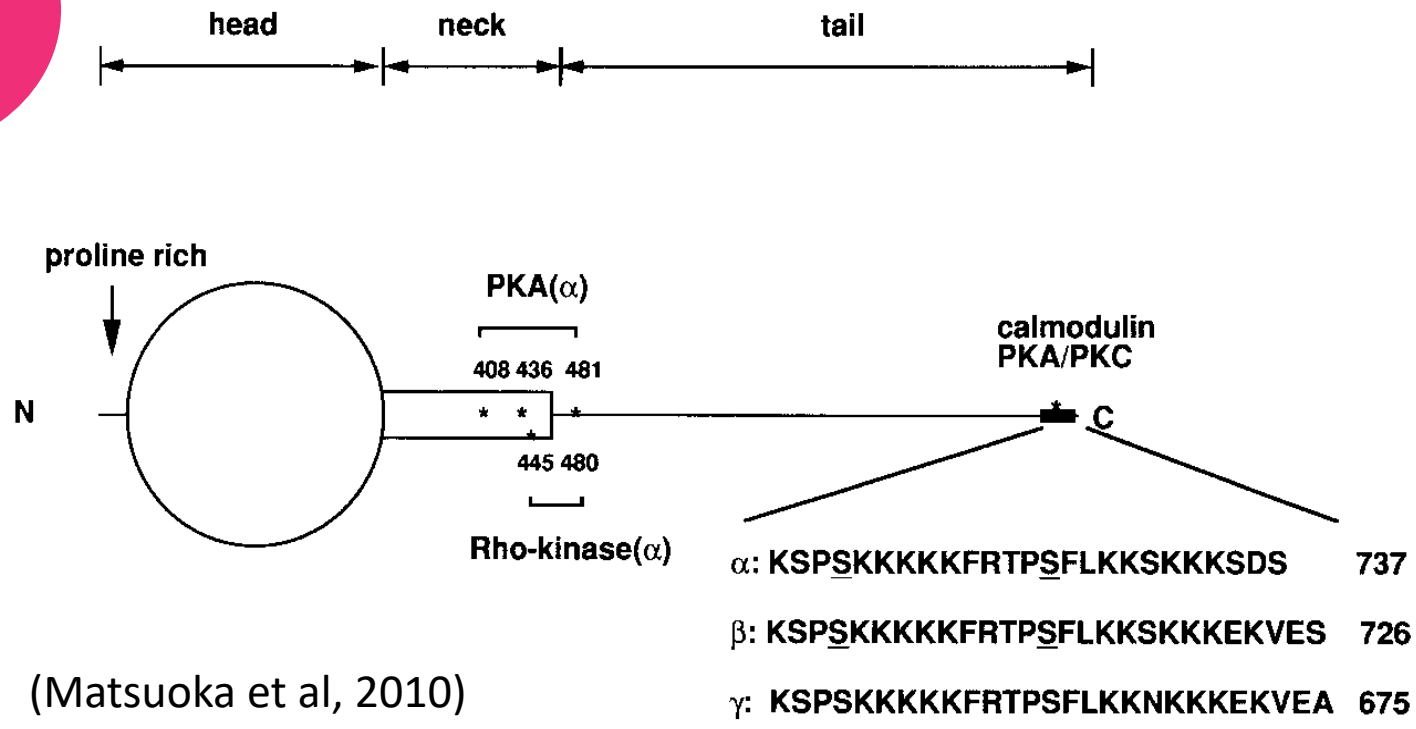
Normotension	23.9%
Prehypertension	45.8%
Hypertension	30.3%

Age group (years)	Prevalence of hypertension (%)[2]		Ratio unknown:known
	Known	Unknown	
18-19	0.7	6.0	8.6
20-24	1.9	7.5	3.9
25-29	2.8	10.4	3.7
30-34	3.9	12.0	3.1
35-39	5.8	18.1	3.1
40-44	11.9	20.3	1.7
45-49	15.0	23.8	1.6
50-54	23.1	26.2	1.1
55-59	29.3	26.2	0.9
60-64	37.1	27.9	0.8
65-69	39.1	28.7	0.7
70-74	50.4	25.0	0.5
≥ 75	46.1	27.3	0.6

[2]National Health and Morbidity Survey 2015

α-ADDUCIN (ADD1)

ADD



(Matsuoka et al, 2010)

- Adducin is a cytoskeletal protein
- Exists as α–γ and α–β heterodimers
- Tail – important interaction site with other proteins

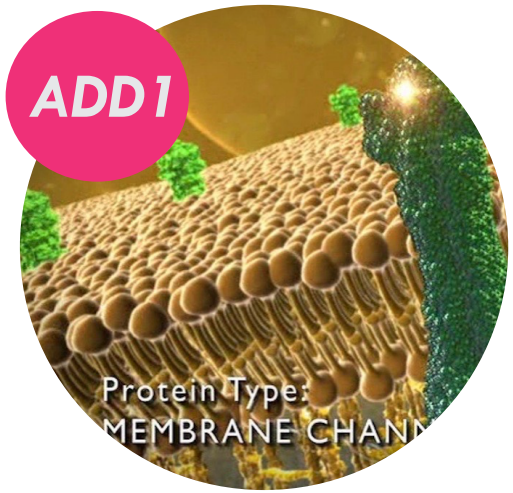
ADD1 & ESSENTIAL HYPERTENSION

ADD1 References into Functions		References
Polymorphism	Gly460Trp polymorphism is associated with Essential Hypertension in a Caucasian population from Madeira Island.	Sousa et al. (2017)
	rs4963 polymorphism showed an increased hypertension risk.	Qu et al. (2017)
	G460T polymorphism is associated with essential hypertension.	Soualmia et al.(2016)
	The T allele is associated with essential hypertension in Asians.	Liao et al. (2016)
	No difference in Gly460Trp polymorphism between control and pediatric hypertensive group.	Kaplan et al. (2015)
	G614T polymorphism is associated with essential hypertension in Chinese patients.	Wang et al. (2015)
	rs4961 has a protective role in development of EH; interactions between alcohol consumption	Han et al. (2016)
	rs4963 polymorphism is associated with essential hypertension in the Chinese population	Zhang et al. (2013)
	G460W gene polymorphism was linked to essential hypertension	Li (2012)
	G460W polymorphism was associated with hypertension in female Japanese subjects.	Shioji et al (2010)
	G460W polymorphism as predisposition gene to hypertension among Russians, but is influenced by environmental factors.	Polonikov et al. (2012)
	Null association of G460T with hypertension in Chinese.	Niu et al. (2011)

ADD1 & ESSENTIAL HYPERTENSION

ADD1 References into Functions		References
Polymorphism	Gly460Trp polymorphism might increase the risk of hypertension in Han Chinese populations.	Liu et al. (2011)
	Genetic variation in *ADD1* alter renal function and/or vasoreactivity	Alioglu et al. (2011)
	Gly460Trp polymorphism is associated with salt-sensitivity.	Wang et al. (2010)
	460Trp allele was associated with lower levels of central systolic pressure and pulse pressure in JingNing population.	Guo et al. (2010)
	Role for the *ADD1* variants in blood pressure salt sensitivity	Kelly et al. (2010)
	rs4961 polymorphism is associated with essential hypertension	Gong et al. (2010)
	TT genotypes might be genetic susceptibility factors to hypertension accompanying renal injury.	Lu, Chen & Yu (2010)
	Gly460Trp polymorphism is significantly associated with an increased risk of coronary artery disease as well as blood pressure in Koreans	Cha et al. (2010)
	G460W polymorphism influences blood pressure when BMI and sex are taken into account	Fava et al. (2010)
	Gly460Trp polymorphism is associated with low renin hypertension.	Sugimoto et al. (2010)
	The ACE I/D, alpha-adducin Gly460Trp and aldosterone synthase -344C/T polymorphisms interact to influence SBP in Chinese.	Wang et al. (2010)
Mutation	Increased CFTR surface expression and activity in HEK (G460W) and cultured rat distal convoluted tubule cells (F316Y).	Mondini et al. (2013)

ADD1 & ESSENTIAL HYPERTENSION



SODIUM HANDLING

Sodium retention

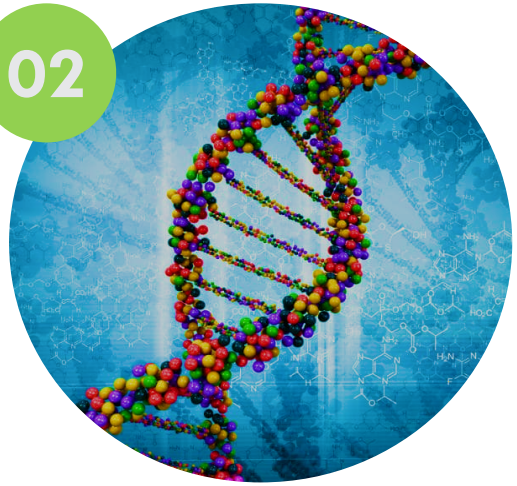
Mutated ADD variant induced constitutive reduction of the Na/K pump endocytic rate (Torielli et al., 2010).

Physiological interaction between the *ADD1* and WNK1-NEDD4L pathways influences the effects of variants in these genes on Na-related BP regulation. (Manunta, Lavery et al., 2010).

Patients with *ADD1* Trp alleles are sensitive to salt and tubular Na reabsorption remains elevated after volume expansion (Manunta, Milliard et al. 2010).

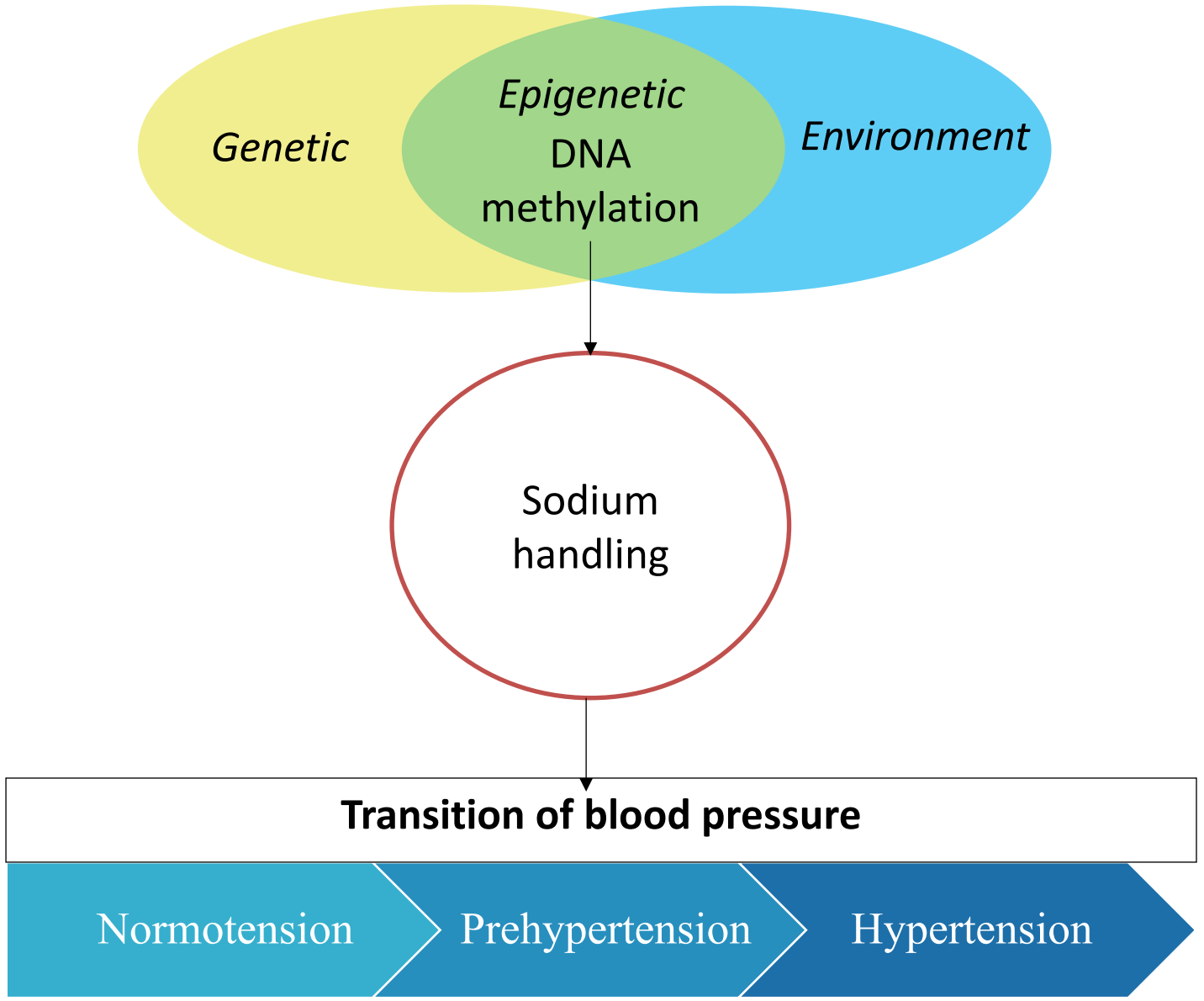
ADD1 Trp460Trp genotype is significantly associated with reduced renal plasma flow and glomerular filtration rate. (Beeks et al, 2010).

02



EPIGENETICS

Links genetic and environment



ADD1 METHYLATION IN ESSENTIAL HYPERTENSION

1

HYPOMETHYLATED

Chinese adults with essential hypertension (Zhang et al., 2013).

2

HYPOMETHYLATED

Middle-age Egyptian with essential hypertension (Bayoumy et al. 2017).

3

INTERACTION WITH ALCOHOL

Modifies EH susceptibility in Han Chinese adults (Han et al., 2015).

GAPS OF KNOWLEDGE

GAP 1

YOUNG ADULTS

Most studies: general adults

GAP 2

METHYLATION METHODS

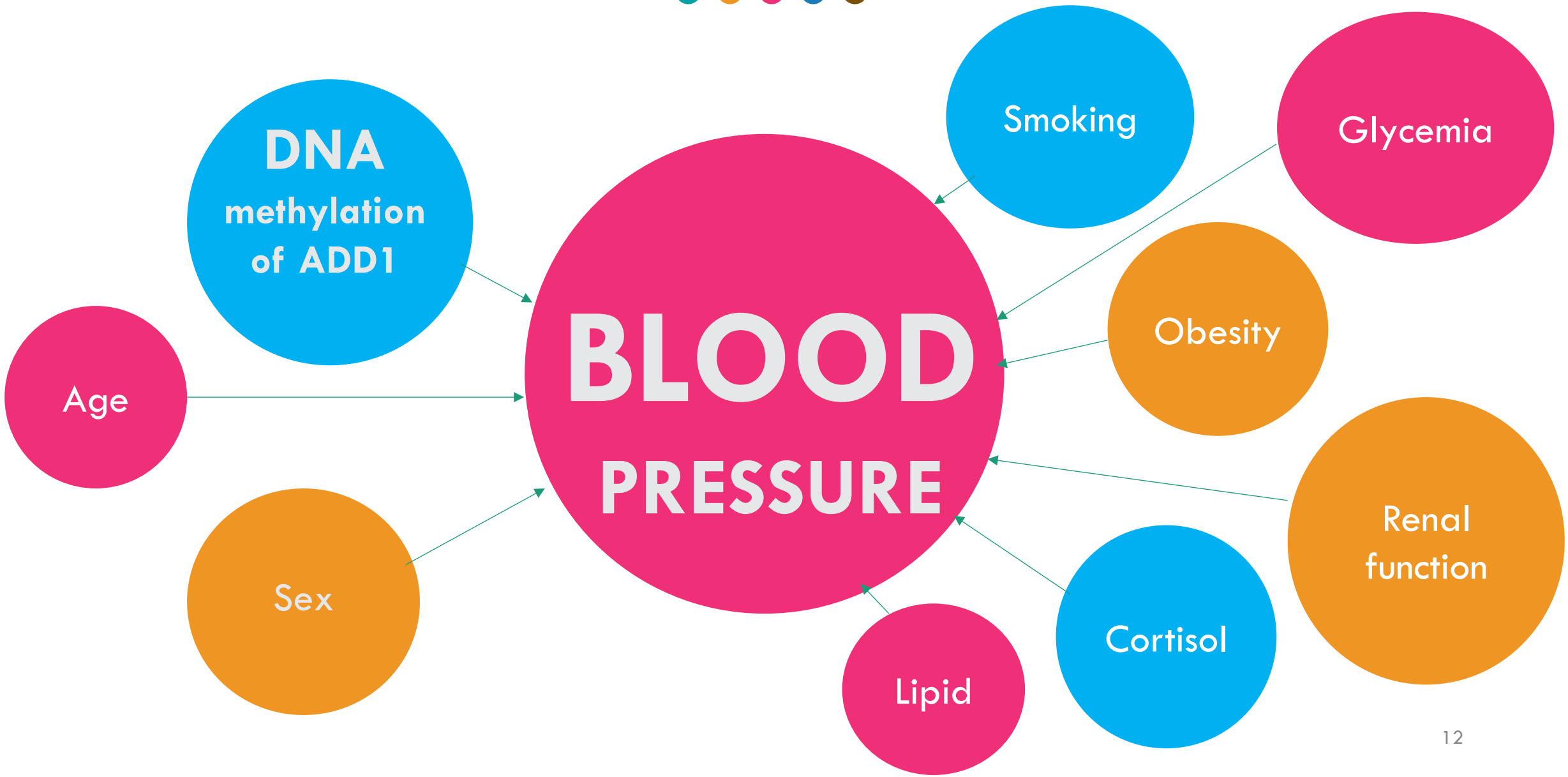
Incomparable results

GAP 3

EFFECT ON GENE EXPRESSION

Unknown

CONCEPTUAL FRAMEWORK



RESEARCH OBJECTIVES

1. To investigate the association between DNA methylation of the promoter of α-adducin (*ADD1*) with blood pressure in young adults

2. To investigate the association between the level of DNA methylation of the promoter of *ADD1*, and other covariates, with blood pressure in young adults

3. To investigate the association between level of *ADD1* methylation with the gene expression level.

METHODOLOGY

01 ETHICAL APPROVAL

Medical Research Ethical Committee (NMRR-16-2572-32869) Institutional Research Ethic Committee (IREC544)

02 RECRUITMENT

N = 80 for each Nt and Hpt group

Sample Size For Comparing Two Means

Input Data

Confidence Interval (2-sided)	95%
Power	80%
Ratio of sample size (Group 2/Group 1)	1

	Group 1	Group 2	Difference*
Mean	16	14	2
Standard deviation	4.5	4.5	
Variance	20.25	20.25	

Sample size of Group 1	80
Sample size of Group 2	80
Total sample size	160

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean

Values adopted from previous methylation study by Mao et al (2017)[10]

METHODOLOGY

Inclusion criteria	Exclusion criteria
1. Age **18–45** years as determined by the year of birth 2. Consented	1. Previous or current **diagnosis of hypertension** and/or current use of **anti–hypertensive medication** 2. History of **ischemic heart disease** as reported by subject 3. History of Type 1 or 2 **diabetes mellitus** as reported by subject 4. History of **chronic renal failure** as reported by subject or serum creatinine more than 112 mmol/L as reported by subject 5. Current use of **steroid** medication or history of conditions **hyper–or hypocortisolism** as reported by subject including but not limited to Cushing's syndrome 6. Female subjects who are **pregnant** as reported by subject 7. Female subjects who are on **hormonal contraception** as reported by subject

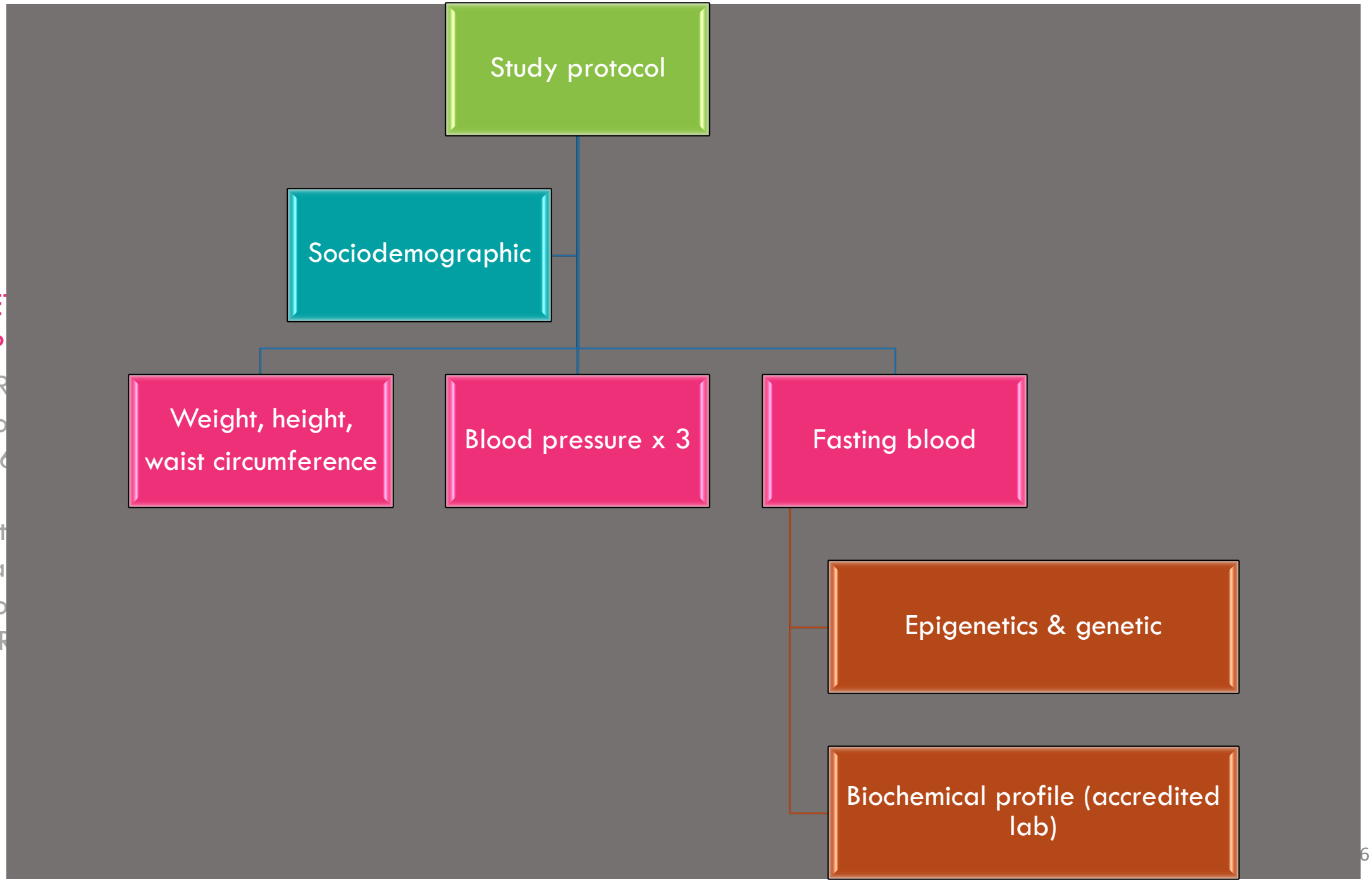
ETHI
APPRO
Medical Rese
Ethical Comm
(NMRR-16-2
32
Institut
Research
Committee
(IREC5

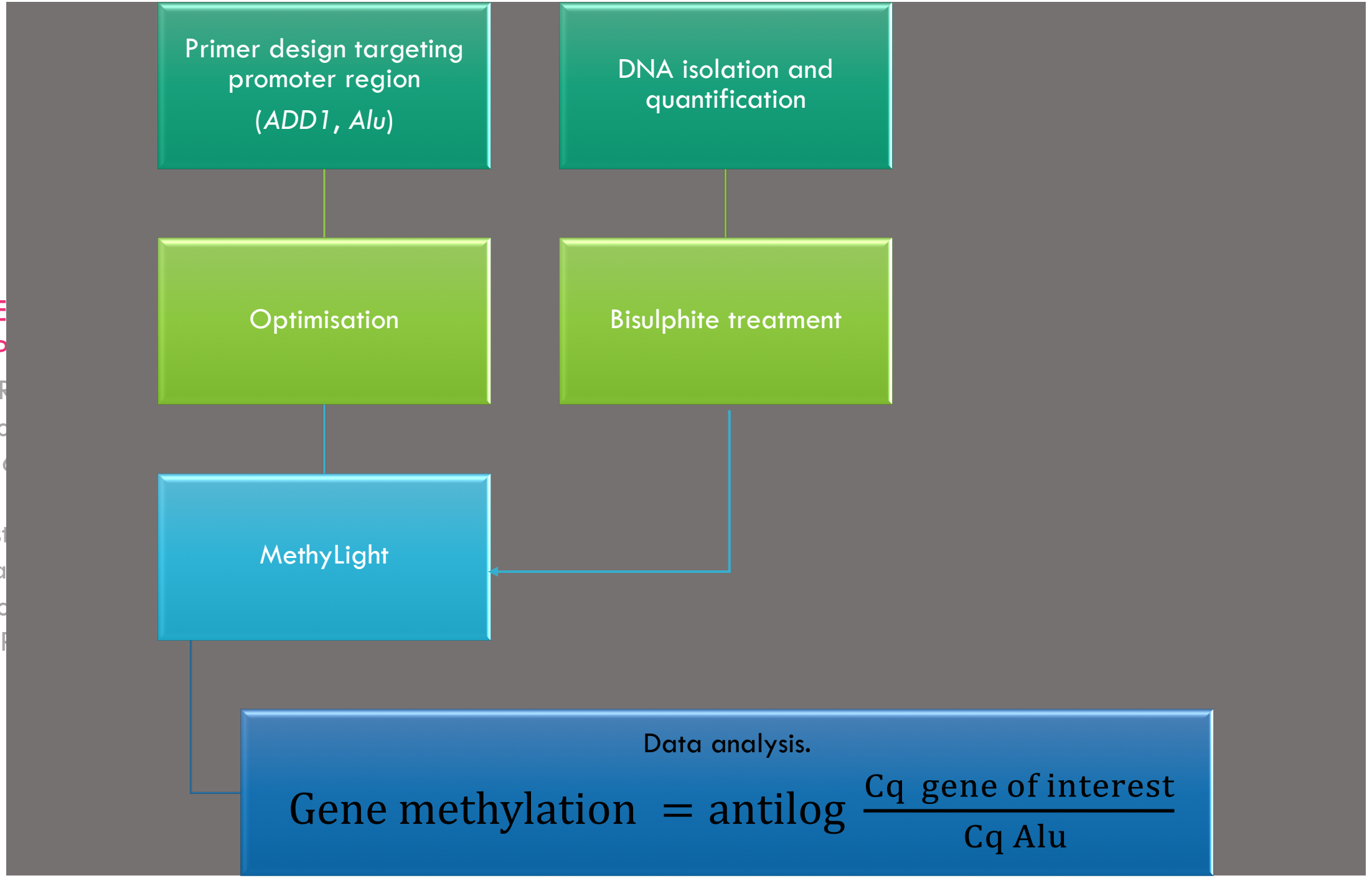
Blood pressure class according to CPG 4th ed[17] and JNC7[18]	Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)
Normotension (Nt)	< 120	and	< 80
Hypertension (Hpt)	≥ 140	and/or	≥ 90

each Nt and
Hpt group

E
APP
Medical R
Ethical Co
(NMRR-16

Inst
Resea
Co
(IR




Primer design targeting promoter region (*ADD1*, *Alu*)
DNA isolation and quantification
Optimisation
Bisulphite treatment
MethyLight
Data analysis.
$\text{Gene methylation} = \text{antilog} \frac{\text{Cq gene of interest}}{\text{Cq Alu}}$

01

ETHICAL APPROVAL

Medical Research Ethical Committee (NMRR-16-2572-32869)

Institutional Research Ethic Committee (IREC544)

RECRUI

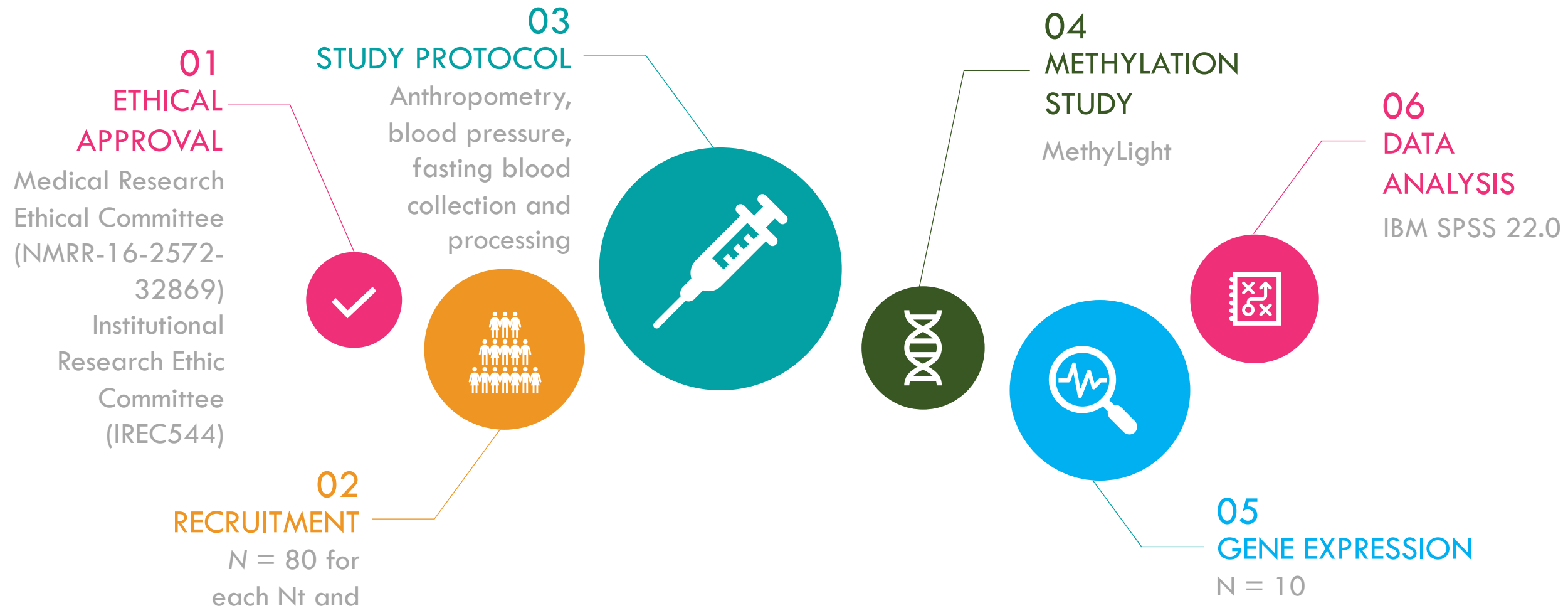
N =

each

Hp

ESSION

METHODOLOGY



01
ETHICAL APPROVAL
Medical Research Ethical Committee (NMRR-16-2572-32869) Institutional Research Ethic Committee (IREC544)

02
RECRUITMENT
N = 80 for each Nt and Hpt group

03
STUDY PROTOCOL
Anthropometry, blood pressure, fasting blood collection and processing

04
METHYLATION STUDY
MethyLight

05
GENE EXPRESSION
N = 10

06
DATA ANALYSIS
IBM SPSS 22.0

RESULTS | Sociodemographic

Sociodemographic aspect		Blood pressure status		
		Nt	Htn	p–value
Age (years)$^{\Phi}$		31 (7)	**35 (6)**	<0.001[c]
Male$^{\Psi}$		40 (39.6)	**61 (60.4)**	0.001[a]
Malay$^{\Psi}$		78 (53.1)	**69 (46.9)**	0.016[a]
Education level$^{\Psi}$	Primary	0 (0.0)	**1 (100.0)**	0.006[b]
	Secondary	12 (30.8)	**27 (69.2)**	
	Tertiary	68 (56.7)	52 (30.1)	
Smoking$^{\Psi}$		13 (39.4)	20 (60.6)	0.171[a]
Consume alcohol$^{\Psi}$		1 (12.5)	**7 (87.5)**	0.030[b]
Body mass index (kg/m^2)$^{\Phi}$		25.2 (5.6)	**29.4 (5.0)**	<0.001[c]
Body mass index category$^{\Psi}$	Underweight/Normal (<23 kg/m^2)	31 (93.9)	2 (6.1)	<0.001[a]
	Overweight (23–27.49 kg/m^2)	28 (47.5)	**31 (52.5)**	
	Obese (≥27.5kg/m^2)	21 (30.9)	**47 (69.1)**	
Waist circumference (cm)$^{\Phi}$		84.9 (10.5)	94.5(13.3)	<0.001[c]
Female		83.4 (11.6)	85.8(10.0)	0.446[c]
Male		86.4 (9.1)	**97.3(13.0)**	<0.001[c]

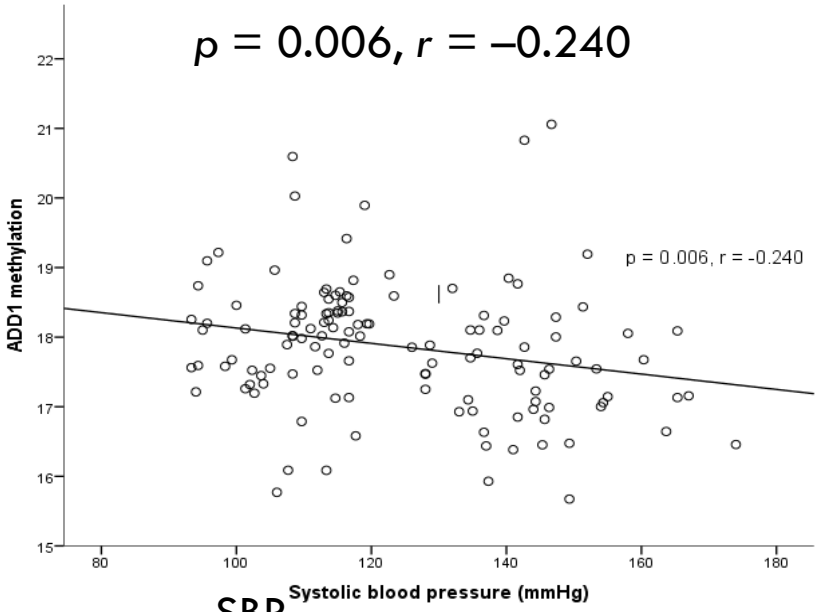
Note $^{\Phi}$Mean(standard deviation). $^{\Psi}$n(%). [a]Analysed using Chi–squared test, [b]Analysed using Fisher's exact test, [c] Analysed using student's t-test. Body mass index status is according to World Health Organisation recommendation for Asian population.

RESULTS | Biochemical

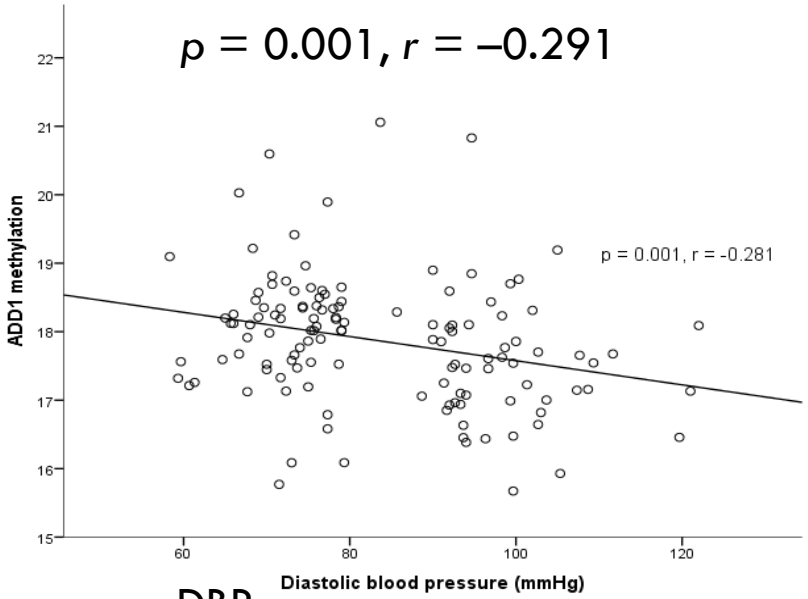
Biochemical profile	Blood pressure status		
	Nt	Htn	p–value
Creatinine (µmol/L)$^{\Omega}$	66.0 (27.0)	77.50(30.0)	0.032[a]
Fasting blood glucose (mmol/L)$^{\Phi}$	4.8 (0.5)	5.0(0.56)	0.043[b]
HbA1c (%)$^{\Phi}$	5.3 (0.3)	5.5(0.3)	<0.001[b]
Total cholesterol (mmol/L)$^{\Phi}$	5.51 (0.92)	6.01(1.08)	0.002[b]
HDL–cholesterol (mmol/L)$^{\Omega}$	1.41 (0.43)	1.21(0.34)	<0.001[a]
LDL–cholesterol (mmol/L)$^{\Omega}$	3.43 (1.28)	3.89(1.09)	0.002[a]
Triglycerides (mmol/L)$^{\Omega}$	0.95 (0.67)	1.68(1.04)	<0.001[a]
TC/HDL ratio$^{\Omega}$	3.95 (1.4)	4.85(1.7)	<0.001[a]
Cortisol (nmol/l)$^{\Omega}$	254.0 (177.0)	314.5(159.0)	0.014[a]
hsCRP (mg/l) $^{\Omega}$	0.9 (2.1)	2.6(4.8)	<0.001[a]

Note $^{\Phi}$mean (standard deviation). $^{\Omega}$ median (IQR). [a]Analysed using Wilcoxon-Mann-Whitney test, [b]Analysed using student's t-test. HbA1c glycated haemoglobin. HDL high density lipoprotein. LDL low density lipoprotein. TC/HDL ratio total cholesterol to HDL ratio, HsCRP high sensitivity C-reactive protein.

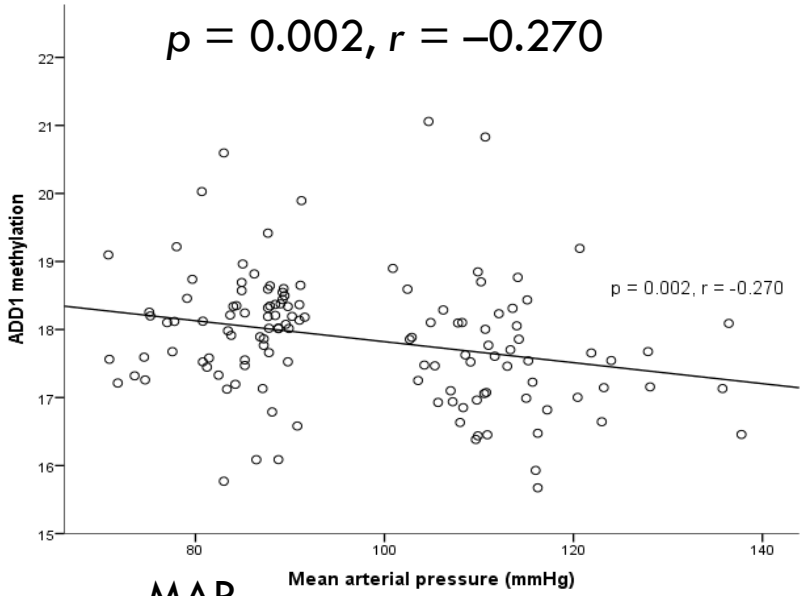
RESULTS | Association between *ADD1* methylation and blood pressure



SBP

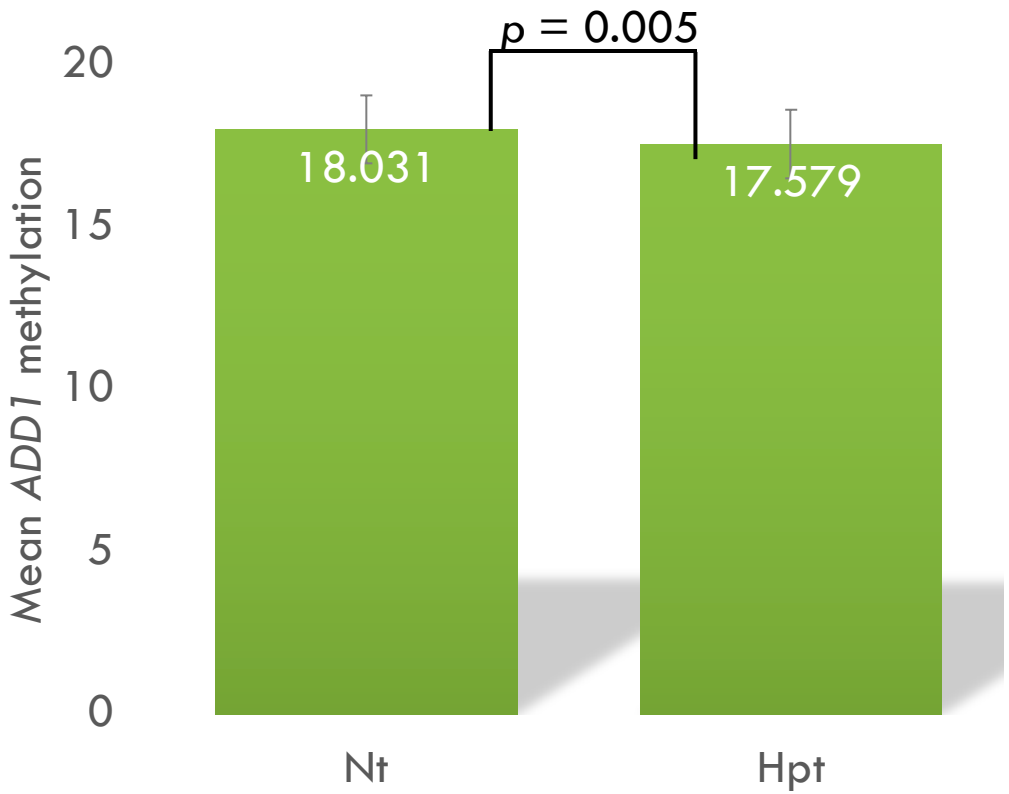


DBP



MAP

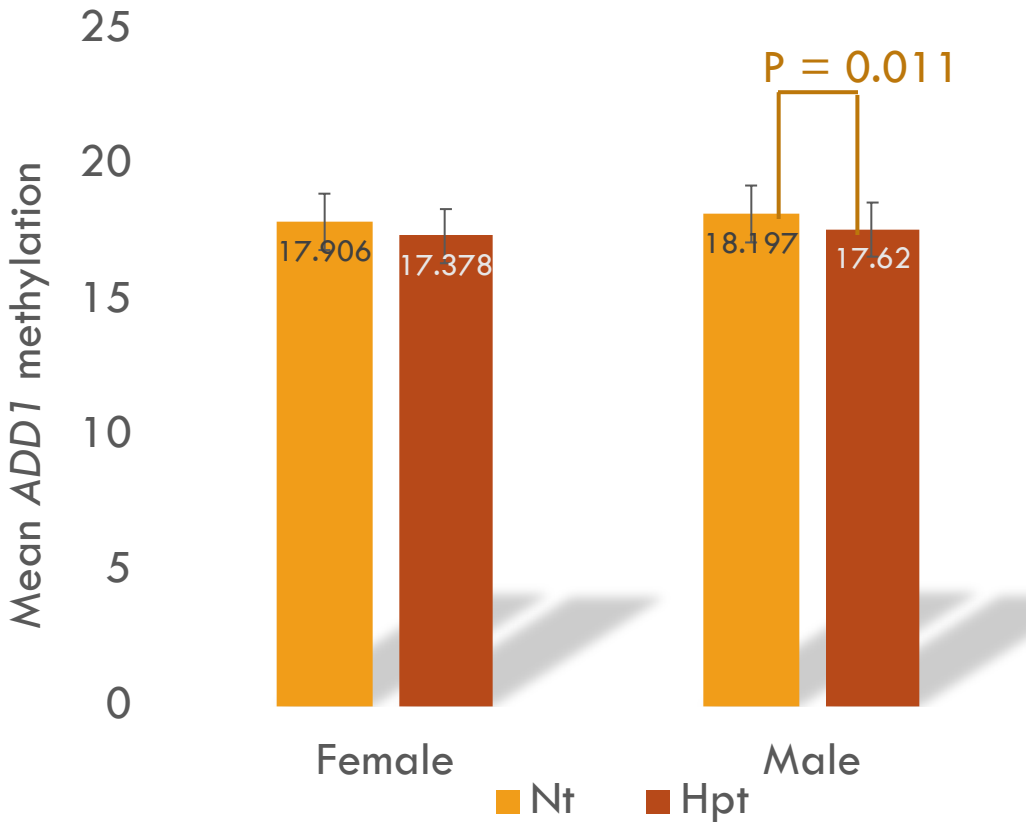
RESULTS | Association between *ADD1* methylation and blood pressure



Hpt < Nt

Error-bars indicate standard deviation. Difference in mean were analysed by student's t-test.

RESULTS | Association between *ADD1* methylation and blood pressure



Hpt < Nt

Error-bars indicate standard deviation. Difference in mean were analysed by student's t-test.

RESULTS | Association between *ADD1* methylation and blood pressure

Variable	Hypertension[a]			
	p	OR	95 % C.I. of OR	
			Lower	Upper
ADD1 methylation	0.008	0.516	0.316	0.844
Age	0.006	1.110	1.031	1.194
BMI	<0.001	1.207	1.094	1.331
Creatinine	0.730	1.006	0.970	1.044
FBG	0.799	1.174	0.383	3.596
LDLC	0.316	1.326	0.764	2.304
Female[b]	0.080	0.305	0.081	1.154
Non–smoker[c]	0.708	0.795	0.240	2.633

[a]Compared to normotension, [b]compared to male, [c]compared to current smoker.

B = coefficient, S.E. = standard error, df = degree of freedom, p = significance, OR = odd ratio, CI = confidence interval.

BMI = body mass index, FBG = fasting blood glucose, LDLC = low-density lipoprotein cholesterol.

Classification table: 80.3% correct. Nagelkerke R square, p = 0.461, Cox & Snell p = 0.344. Hosmer lemeshow 0.165

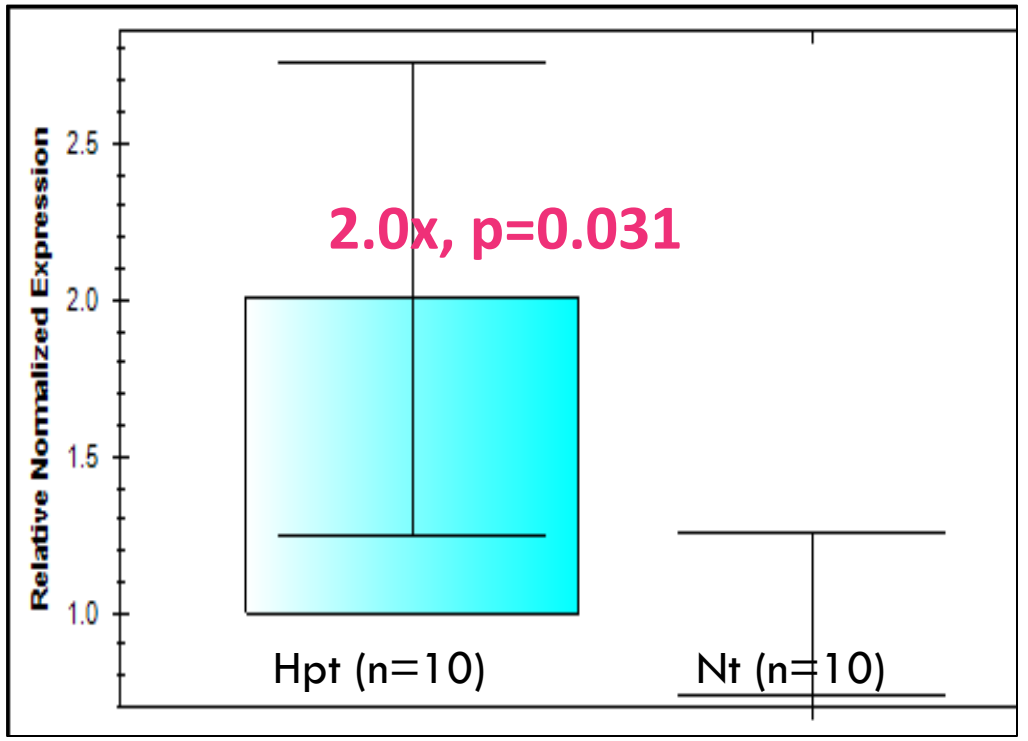
RESULTS | Association between *ADD1* methylation and blood pressure

Variable	Female, hypertension[a]				Male, hypertension[a]			
	p	OR	95 % C.I. of OR		p	OR	95 % C.I. of OR	
			Lower	Upper			Lower	Upper
ADD1 methylation	0.803	0.866	0.280	2.683	0.024	0.509	0.282	0.916
Age	0.065	1.205	0.989	1.468	0.007	1.138	1.036	1.250
BMI	0.296	1.103	0.918	1.326	<0.001	1.317	1.131	1.533
Creatinine	0.846	1.102	0.899	1.138	0.570	1.012	0.971	1.054
FBG	0.641	1.960	0.118	32.280	0.546	0.650	0.160	2.632
LDLC	0.058	3.888	0.953	15.858	0.689	0.867	0.430	1.746
Non-smoker[b]					0.608	0.714	0.197	2.587

[a]Compared to normotension, [b]compared to current smoker.
B = coefficient, S.E. = standard error, df = degree of freedom, p = significance, OR = odd ratio, CI = confidence interval.
BMI = body mass index, FBG = fasting blood glucose, LDLC = low-density lipoprotein cholesterol.

Gene expression of *ADD1*

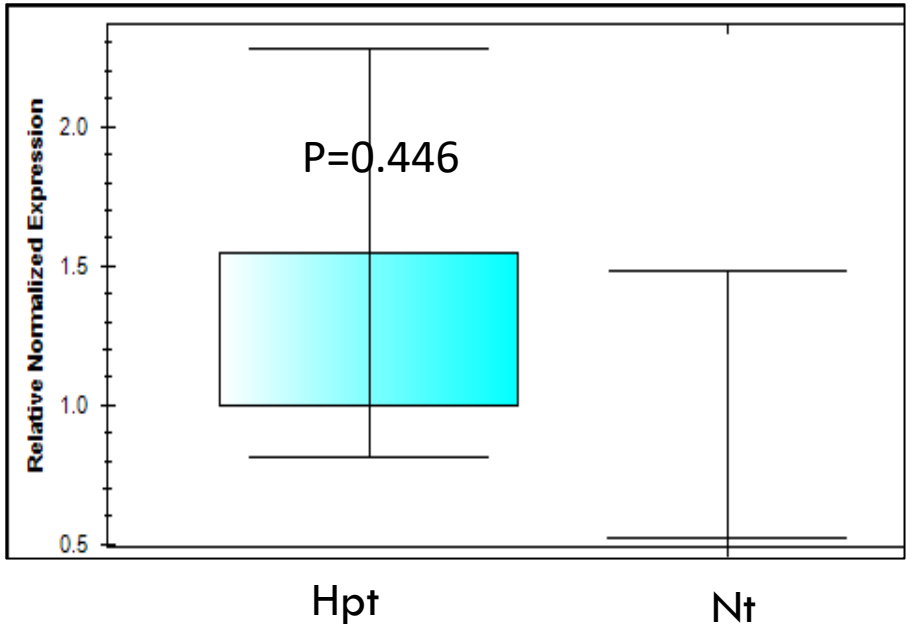
normalised to *ACTB* and *GAPDH*



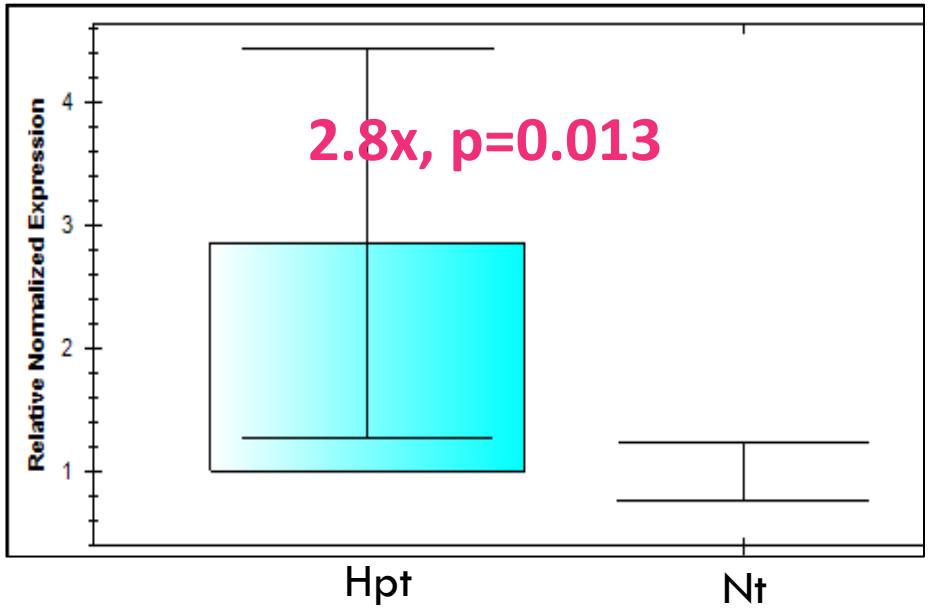
Graph was generated by CFX Manager ver 3.0 (BioRad, USA). Error bar indicates standard deviation.

RESULTS | Association between *ADD1* methylation and gene expression

Gene expression of *ADD1* normalised to *ACTB* and *GAPDH* in low–methylated samples (n = 5)

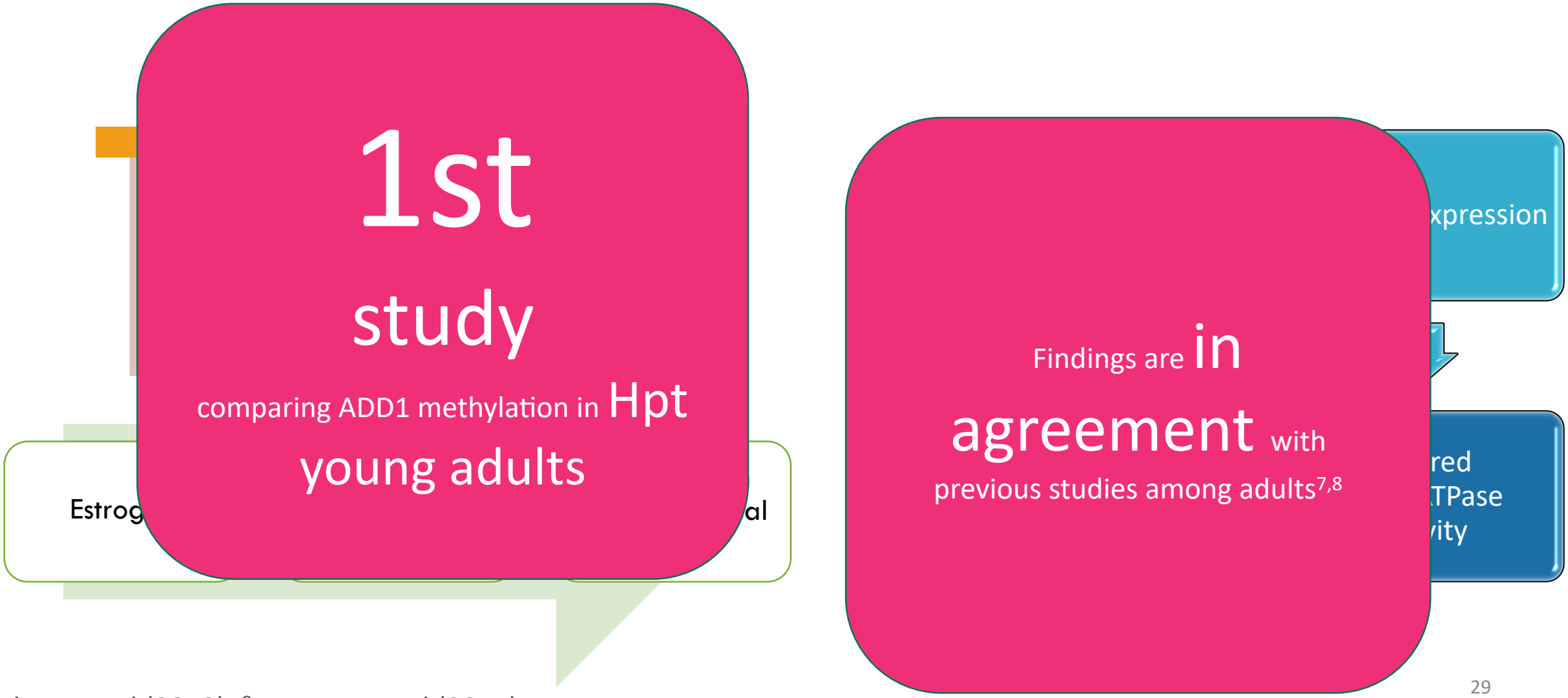


Gene expression of *ADD1* normalised to *ACTB* and *GAPDH* in high–methylated samples (n = 5)



Expression normalised to normotension group (Nt). Graph was generated by CFX Manager ver 3.0 (BioRad, USA). Error bar indicates standard deviation.

DISCUSSION



[7]Zhang et al (2013); [8]Bayoumy et al (2017)

CONCLUSION

01

LOWER *ADD1* methylation

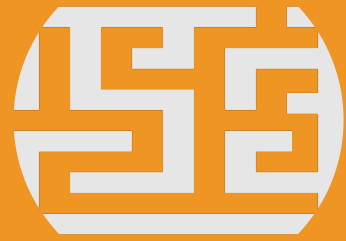
in Hpt male young adults

02

ADD1 HYPOMETHYLATION

Predicts Hpt in male young adults

03



COMPLEX RELATIONSHIP

between *ADD1* methylation and gene expression

STRENGTH

01 First in Young Adults

Previous methylation study in general adult

02 Anti-HPT naïve subjects

Eliminate anti-HPT confounding effects

03 MethyLight

Real-time, high sensitivity, quantitative

04 Gene expression study

Following methylation study

LIMITATION

01 **Cross-sectional design**
Cause & effect is not certain

Longitudinal study
or experimental animal study

02 **Non-generalizable**
Single centre study

Multi-centre study
For national data

03 **? Environment effects on *ADD1* methylation**
Sociodemographic, hormonal

⏭ **Role of *ADD1* methylation as mediator**
on blood pressure

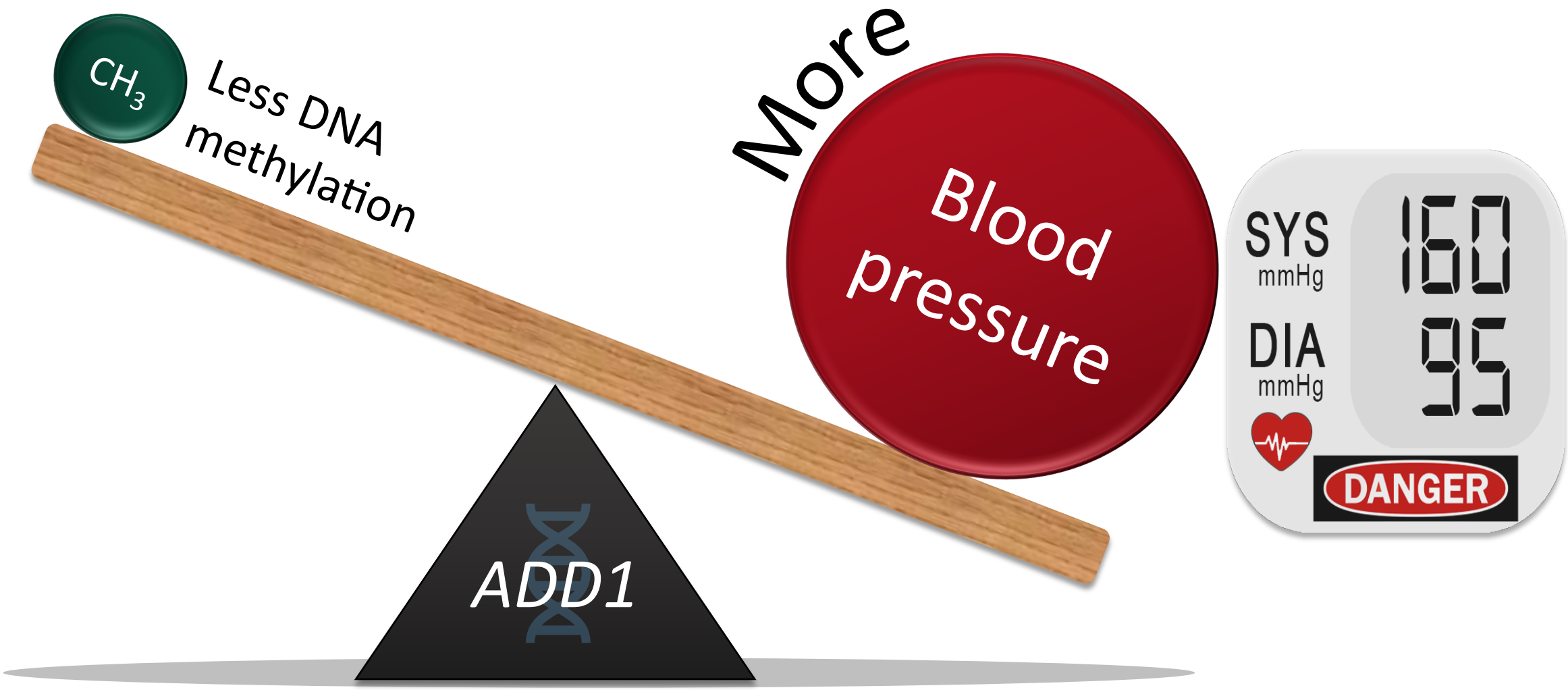
THANK YOU

fateinwanomar@iium.edu.my

Wan Fatein Nabeila Wan Omar

REFERENCES

1. World Health Organization. (2013). *A Global Brief on Hypertension. WHO Press.*
2. Wan Ahmad, W. A., & Sim, K. H. (Eds). (2015). *Annual Report of The NCVD-ACS Registry, 2011-2013*. Kuala Lumpur, Malaysia.
3. Hoo, F. K., Foo, Y. L., Lim, S. M. S., Ching, S. M., & Boo, Y. L. (2016). Acute coronary syndrome in young adults from a Malaysian tertiary care centre. *Pakistan Journal of Medical Sciences*, *32*(4), 841–845
4. Fox, K. A. A., Eagle, K. A., Gore, J. M., Steg, P. G., & Anderson, F. A. (2010). The global registry of acute coronary events, 1999 to 2009-GRACE. *Heart*, *96*(14), 1095–1101.
5. Institute for Public Health (IPH). (2015). *National Health and Morbidity Survey 2015 (NHMS 2015). Vol. II: Non-Communicable Diseases, Risk Factors & Other Health Problems. Ministry of Health* (Vol. II).
6. Mao, S., Sun, J., Gu, T., Zhu, F., Yin, F., & Zhang, L. (2017). Hypomethylation of interleukin-6 (IL-6) gene increases the risk of essential hypertension: a matched case-control study. *Journal of Human Hypertension*, 1–7.
7. Zhang, L.-N., Liu, P.-P., Wang, L., Yuan, F., Xu, L., Xin, Y., … Duan, S. (2013). Lower ADD1 gene promoter DNA methylation increases the risk of essential hypertension. *PloS One*, *8*(5), e63455.
8. Bayoumy, N. M. K., El-Shabrawi, M. M., Leheta, O. F., & Omar, H. H. (2017). α-Adducin gene promoter DNA methylation and the risk of essential hypertension. *Clinical and Experimental Hypertension*, *39*(8), 764–768.
9. Malaysian Society of Hypertension, Ministry of Health Malaysia, & Academy of Medicine Of Malaysia. (2013). *CPG Management of Hypertension (4th Edition). Malaysia Ministry of Health.*
10. Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., … Roccella, E. J. (2003). The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*, *42*, 1206–1252.


CH_3
Less DNA methylation
More
Blood pressure
ADD1
SYS
mmHg
160
DIA
mmHg
95
DANGER