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DNA Methylation of the AGTR1 Gene in a Hypertensive Population of Kenyans

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Introduction

Cardiovascular disease (CVD), the leading cause of death worldwide, is rapidly increasing in low-and middle-income countries (LMICs), particularly those of Sub-Saharan Africa (SSA)(World Health Organization). Hypertension is the leading risk factor for CVD and is a multifactorial disease with no single genetic cause. Increasingly, evidence indicates that hypertension is predisposed by environmental regulation of genes through heritable, yet modifiable, epigenetic changes to DNA leading to changes in gene expression, e.g. methylation. While understanding the etiology of hypertension in LMICs is a global priority, few epigenetic studies exist from populations living in SSA (Fan). The renin-angiotensin system (RAS) is the primary hormonal pathway that regulates blood pressure through changes in salt and water retention. Previously, we have found a high prevalence of hypertension (55 % had systolic blood pressure (SBP) >130 mmHg) in a rural population of Kenyans that was not correlated with lifestyle or behavioral factors (Williams).

Hypothesis

The Renin-Angiotensin System (RAS) Gene AGTR1, as a result of exposure to household air pollution (HAP), is hypomethylated in individuals with hypertension.

Study Population

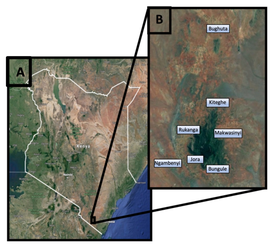

Figure 1. (A) Map of Kenya. (B) Mount Kasigau and the seven surrounding villages of the study population.

Sample Collection

- 64 samples
- 50% male / 50% female
- Age 45-75 years old

Methods

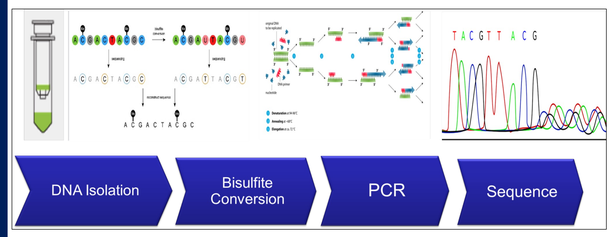

Figure 2. Basic Flow of Procedure

Saliva Sample Collection
In 2018, 2 mL saliva samples were collected using the DNA Genotek's Oragene ORG-500 collection kit.

DNA Isolation
DNA Genotek prepIT•L2P isolation kit used to isolate Genomic DNA from samples. The purity verified using DNA NanoDrop One by ThermoFisher Scientific.

Bisulfite Conversion
Unmethylated cytosines converted into uracil in bisulfite-converted products.

PCR Amplification and Gel Electrophoresis
J1 primer set amplified 4 CpG sites in the promoter region of Exon 1. Amplicon length verified using gel electrophoresis.

Pyrosequencing
Quantitative methylation analysis for 4 CpG sites 34 samples (n=34).

Analyze Spending Habits as Proxy for HAP Exposure
Data obtained from questionnaire about daily life practices administered same time as sample collection.

J1 Primer Set Target Region

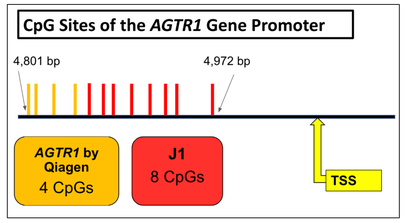

Figure 3. Qiagen AGTR1 and J1 primer set target regions

Results

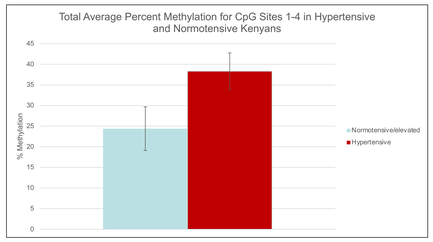

Figure 4. Average Percent Methylation for CpG 1-4

Increased methylation in hypertensive versus normotensive Kenyans (18.4 ± 2 and 12.92 ± 1.78, respectively) when four cytosine-phosphate guanine (CpG) sites of the AGTR1 promoter were analyzed (n=34).

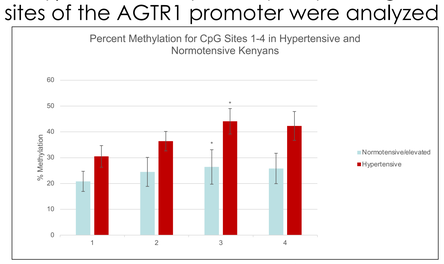

Figure 5. Average Percent Methylation at each CpG 1-4

Increased methylation in hypertensive versus normotensive Kenyans for CpG 3 was observed (44.1 ± 4.95 and 26.42 ± 6.68, respectively) (p=0.047)

Pearson's Correlation Between CpG Q1-4 (* = statistical significance)							
	Q1	Q2	Q3	Q4	1	2	3
Q1							
Q2	0.0187						
Q3	-0.104	.438*					
Q4	0.219	0.536*	0.462*				
1	-0.056	0.138	0.183	0.271			
2	-0.047	-0.154	0.305	0.149	0.743*		
3	0.0279	-0.0624	0.318	0.182	0.841*	0.969*	
4	0.0395	-0.203	0.211	0.203	0.838*	0.944*	0.961*

Figure 6. Pearson's Correlation between CpG sites 1-4

9 positive, statistically significant correlations were found between CpG sites indicating linear relationships.

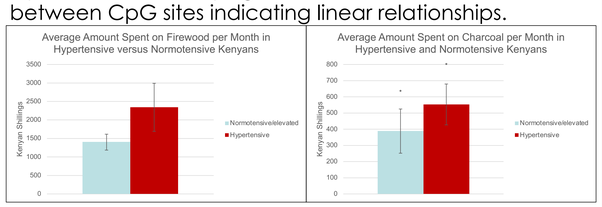

Figure 7 and 8. Average Amount Spent on Firewood and Charcoal

When monthly spending on firewood and charcoal were analyzed as proxies for HAP exposure, spending on charcoal was increased for hypertensive versus normotensive Kenyans (552.94 ± 126.35 and 388.24 ± 136.62, respectively).

Discussion

This study's work contributed data on four additional CpG sites. Using this data in combination with preliminary data from the methylation patterns in 4 other CpG sites there is broader coverage along the promoter region of the *AGTR1* gene. Thus, a deeper understanding of the relationship between methylation levels and the prevalence of hypertension. Overall, DNA methylation was not able to be concluded to correlate to the prevalence of hypertension. During pyrosequencing, the first four CpG sites were able to be amplified. The methylation data obtained needs to be re-run with a redesigned, shorter primer set.

Conclusions

- Hypomethylation of the *AGTR1* promoter was not observed.
- Overall, DNA methylation of the *AGTR1* promoter is possibly elevated in hypertensive vs. normotensive/elevated individuals.
- CpG 3 methylation was statistically elevated in hypertensive versus normotensive/elevated individuals.
- No predictive relationship appears to exist between methylation at any CpG site and an increase in blood pressure.
- Increased expenditure of biomass fuels in hypertensive individuals may support the role that HAP exposure in the development of high blood pressure.

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