ARE CYTOKINE GENE POLYMORPHISMS ASSOCIATED WITH HYPERTENSION? AN AFRICAN AMERICAN COHORT STUDY

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ABSTRACT

Objective

Inflammation plays a significant role in the development and the progression of cardiovascular diseases including hypertension. Thus we sought to investigate the relationship between cytokine gene polymorphisms and hypertension.

Study Design and Methods

This study was designed to evaluate cytokine gene polymorphism association with hypertension in an African American population in the State of Mississippi. Cytokine genotypes were detected by primers specific for single nucleotide polymorphisms (SNPs) and multiplex-PCR analysis assays. Allele frequencies were compared using contingency 2 x 2 table and Fisher's exact test.

Results

Globally, we have shown that the IL-18 high producer and IFN-γ low producer genotypes were significantly associated with hypertension. There was a borderline association for the TGF-β1 TC/GG high producer haplotype in female patients with hypertension ($p<0.053$). In male patients, IFN-γ intermediate/low and IL-18 high producer genotypes were significantly associated with hypertension. The TGF-β1 TT/GG high producer and IL-10 ACC/ATA low producer haplotypes were also significantly increased in female patients as compared with male patients with hypertension.

Conclusion

Our data indicate that inflammation might induce hypertension which is genetically determined, and African Americans genetically are predisposed to higher susceptibility risk markers that cause hypertension in the population.

INTRODUCTION

Hypertension is the number one public health disparity in the United States, particularly among African-Americans (Kong, 1997, Hertz, 2005). It is a major contributing cause for stroke, cardiovascular disease, and kidney failure. Hypertension is a clinical condition defined by an elevated blood pressure reading of greater than 140/90 mmHg systolic/diastolic for adults. Occurrence of hypertension is observed in both children and adults (Lurbe, 2007; Morenoff, 2007), but it is particularly prevalent in the middle-aged and elderly, obese individuals, heavy drinkers, and women who are taking birth control pills and women who are preqnant. In addition, in African Americans the age of onset is younger, disease is more aggressive and the treatment is often difficult leading to organ damage and organ failure (Jamerson, 2004).

The National High Blood Pressure Education Program was started in 1972, in an effort to significantly decrease the mortality and morbidity of this disease, but the percentage of African-Americans suffering from this disease and whose conditions are detected, treated and controlled continues to lag behind that of the Caucasian population with hypertension (Kong, 1997; Hajjar and Kotchen, 2003). Blood pressure tends to rise more rapidly with advancing age in African-Americans than in Caucasians, with an excess mortality six to thirteen times greater in African-Americans than Caucasians (Anderson, 1997).

Hypertension may have genetic linkage, however, several research studies have reported that neither blacks nor whites are genetically prone to developing the disease due to overt gene expression (August, 1999). Nevertheless, a number of pathophysiologic features that are genetically determined have the characteristic of hypertension in blacks. Some of the characteristics that suggest a common

pathway of genetic disposition of hypertension in blacks may be salt sensitivity, obesity and hyperlipidemia (August, 1999; Thompson, 2004; Franco, 2006; Bindon, 2007). Other factors that account for hypertension include: 1) sympathetic nervous system compensation which might be due to increased exposure to and response to psychosocial stress (Pickering, 1999; Plante, 2002), 2) adrenal compensation, such as overproduction of sodium retaining hormones and vasoconstrictors such as endothelin and thromboxanes (Dao, 2001), 3) renal compensation, causing either renovascular hypertension, usually seen in pregnancy or renal parenchymal hypertension causing decreased renal perfusion leading to activation of rennin-angiotensin-aldosteron pathways (Halushka, 1999; Timberlake, 2001; Ho, 2005; Timofeeva, 2006). Furthermore, other clinical conditions such as congenital abnormalities of the resistance vessels, diabetes mellitus, insulin resistance, increased activity of vascular growth factors, and altered cellular transport have contributed to the cause of extended elevation of blood pressure (Oparil, 2003; Chobanian, 2003; Oparil, 2005).

A recent study utilizing genome-scan microsatellite markers suggested that the genes influencing risk of hypertension might be located in chromosome 6q24 and chromosome 21q21 (Zhu, 2005). It is clear that the development of hypertension is most likely influenced by an interaction of multiple genes and environmental factors that are influential in health disparities in the population.

Cytokines, growth factors and hormones also contribute to elevated blood pressure. The effective role of cytokines and growth factors in the induction of hypertension is unknown. However, one could relate role of cytokines to a comparable physiologic process that is controlled through the neural output of the CNS that maintains homeostasis for body temperature, heart rates, blood pressure etc. A similar mechanism could control

cytokine release in the blood vessels leading to activation of leukocytes and inflammatory responses (Tracy, 2002), release of mediators of oxidative stress and endothelial cell damage (Kristal, 1998).

Impact of cytokines and growth factors on hypertension is an area of great interest, particularly, in women with preeclampsia (Conrad, 1998; Granger 2001). In the United States, approximately, 5-10% of all pregnancies are expected to be affected by preeclampsia. It is characterized by hypertension, proteinurea, and edema, and is the leading cause of maternal and prenatal morbidity, particularly, in African American young females (Shen, 2005; Bodnar, 2007; Tucker, 2007). Thus, cytokine profiling might provide an alternative view for the effects of cytokine genes on hypertension and might allow the development of anti-hypertension therapeutic options, contributing to the primary prevention of this condition.

There is evidence that cytokine production is under genetic control and a gene polymorphism within the regulatory sequences affects the levels of cytokine production (Wilson, 1997). For example, a transversion of G→C at position -174 of the IL-6 gene and a transition of G→A at position –308 of the TNF-α gene promoter regions are associated with different transcription rates, producing high or low responder genotypes (Wilson, 1997; Perry, 1998). Polymorphisms with transcriptional relevance have also been reported for IFN-γ, IL-10 and IL-18 in association with a number of inflammatory immune responses including the development of fibrosis after lung transplantation (Awad, 1998), coronary vasculopathy (Densem 2000; Asderakis, 2001; Jackson, 2007), organ failure (Sankaran, 1997; McDaniel, 2003), and trauma induced clinical complications (McDaniel, 2007). However, due to the role that these cytokines play in pro and anti-inflammatory responses, in human diseases their relationships in hypertension require further investigation.

In this study, we hypothesized that cytokine production could influence the induction of hypertension in African Americans due to the fact that African Americans would be more likely than Caucasians to carry cytokine gene variants that are known to effect level of cytokine production. We carried out cytokine gene polymorphisms using an existing set of gene mutations known as single nucleotide polymorphisms (SNPs) to evaluate the influence of these SNPs on hypertension in the African American population.

MATERIALS AND METHODS

Study Population

Patients attending the Emergency Room at the University of Mississippi Medical Center (UMMC) were studied. One hundred and sixty two individuals diagnosed with hypertension and 77 individuals with no hypertension were included in this study. Patients were all African Americans and from the same geographic region in the state of Mississippi. Demographic and clinical characteristics of study population are given in Table 1.

The blood sample collection process was performed according to the guidelines approved by the University of Mississippi Medical Center Institutional Review Board (IRB).

Table 1
Demographic and clinical characterization of study population

Variants	Hypertension N=162	No hypertension N=77
Age range (year)	22-79	22-81
Gender (F%)	47.5%	54.45%
Race (100%)	AA*	AA*
HTN**	100%	0%
DML***	48%	0%

* AA African American; ** HTN Hypertension; *** DML diabetes mellitus

DNA extraction and Genotyping

Genomic DNA was isolated from whole blood using a modification previously reported (Blin and Stafford, 1976), followed by phenol extraction and precipitation with 3M sodium acetate and 100% ethanol. DNA was stored in TE buffer at 4^{o}C until analysis of genotypes by PCR technique. A total of 30 genotypes given in Table 2 were tested.

Cytokine genotypes were detected using primers specific for single nucleotide polymorphisms (SNPs) in multiplex-PCR analysis trays (Cytogen, One Lambda, Inc., Canoga Park, CA).

Table 2
Cytokine gene variants and genotype/phenotype association

Cytokines	Codon	Polymorphism	Haplotypes	Phynotypes
TNF-α	-308	G→A	G/G	(L) low producer
			G/A	(H) high producer
			A/A	(H) high producer
TGF-β1[a]	(codon 10)	T→C	TT/GG	(H) high producer
	(codon 25)	G→C	TC/GG	(H) high producer
			TC/GC	(I) intermediate
			CC/GG	(I) intermediate
			TT/GC	(I) intermediate
			CC/GC	(L) low producer
			CC/CC	(L) low producer
			TT/CC	(L) low producer
			TC/CC	(L) low producer
IL-6	-174	G→C	G/G	(H) high producer
			G/C	(H) high producer
			C/C	(L) low producer
IL-10[b]	-1082	G→A	GCC/GCC	(H) high producer
	-819	C→T	GCC/ATA	(I) intermediate
	-592	C→A	GCC/ACC	(I) intermediate
			ACC/ACC	(L) low producer
			ACC/ATA	(L) low producer
			ATA/ATA	(L) low producer
IFN-γ	+874	T→A	T/T	(H) high producer
			T/A	(I) intermediate
			A/A	(L) low producer
IL-18[c]	-607	C→A	CC/GG	(H) high producer
	-137	G→C	CC/GC	(H) high producer
			CA/GG	(I) intermediate
			CA/GC	(I) intermediate
			AA/GG	(L) low producer
			AA/CC	(L) low producer

a: Two polymorphisms at codon 10T and 25G of TGF-β1 have been identified which generated nine putative haplotypes. b: Three polymorphisms at the promoter region of IL-10 have been identified at position -1082G, -819C and -592C which generated three putative haplotypes: GCC, ACC and ATA. c: Two polymorphisms at the promoter region of IL-18 have been identified which generated three putative haplotypes: CG, AG and AC.

Amplification primers were selected to detect polymorphisms in the genes coding for transforming growth factor-β1 (TGF-β1) at codon 10 and 25 (Awad, 1998), Tumor necrosis factor-α (TNF-α) at -308 (Abdallah, 1999), Interferon-γ, (IFN-γ) in intron 1 at +874 (Pravica, 2000), Interleukin-10 (IL-10) at -1082, -819, -592 (Turner, 1997), IL-6 at -174 (Fernández-Real, 2000) and IL-18 at -607 and -137 (Kretowski, 2002). PCR was performed according to the manufacturer's instructions. The amplified DNA fragments were visualized by 2.5% agarose gel based on the presence or absence of the target DNA fragment. The IL-18 genotypes were tested separately using a similar detection mechanism same as described previously (McDaniel, et al. 2003).

Statistical Analysis

Cytokine alleles were analyzed using an InSTAT version 5 statistical program to determine the effect on clinical outcomes. Allele frequency or genotypes were compared using contingency 2 x 2 table and Fisher's exact test. A $p<0.05$ was considered statistically significant.

RESULTS

One hundred and sixty two patients with established hypertension (HTN) and 77 with no hypertension (no-HTN) were included in this study. The demographic data presented in Table 1 demonstrates an age-matched group of patients with hypertension and with no hypertension. The study was conducted with the notion that the individual's inflammatory response genes beyond the mechanical/physiological mechanisms might have additional effect on the development of hypertension.

Analysis Of Cytokine Genotypes

Six cytokines and growth factors including TNF-α, TGF-β1, IL-10, IL-6, IL-18 and IFN-γ were tested. As shown in Table 2 of the 30 different genotypes three were associated with TNF-α; nine with TGF-β1; six with IL-10; three with IL-6; six with IL-18, and three with IFN-γ. From a total of six pro and anti-inflammatory cytokine genotypes only four demonstrated clinical correlation with HTN. As shown in Table 3, there are indications that the genotypes of IFN-γ and IL-18 could have potentials as disease risk factors in the development of HTN. The IFN-γ, genotype A/A and IL-18 genotype G/C were found significantly elevated in patients with HTN than those with no HTN (Table 3.)

IFN-γ A/A, which is a low producer genotype, was 1.4 fold greater in patients with HTN as compared with patients with no HTN ($p<0.001$, Odds Ratio:2.82). IL-18 -137 G/C, a high producer genotype was 1.9 fold higher in patients with HTN as compared with patients with no HTN. Although there were clinical correlations between the genotypes of TGF-β1 TC/GG, a high producer genotype (HTN: 50.6% vs. no HTN: 37.7%), and IL-10 GCC/ATA, an intermediate producer genotype (HTN: 29.8% vs. no HTN: 20.8%), statistically, the data were not significant.

Table 3
Cytokine genotype frequency distribution in all patients

Cytokine Genotypes	Hypertension N=162	No-hypertension N=77	
TGF-β1 (codon 10 & 25)	+ (%)	+ (%)	P value
T/T G/G	53 (32.8)	26 (33.8)	NS
T/C G/G	82 (50.6)	29 (37.7)	<0.06*
IFN-γ (intron +874)			
T/A	26 (16)	30 (39)	NS
A/A	128 (79)	44 (57)	<0.001**
IL-10 (-1082, -819, -592)			
GCC/ATA	48 (29.8)	16 (20.8)	<0.1***
IL-18 (-137)			
G/G	110 (68)	61 (79)	NS
G/C	46(29.9)	12 (15.6)	<0.036****

* Odds Ratio (OR): 1.74, 95% Conf. Intervals: (1-3.0)
** OR: 2.82, 95% Conf. Intervals: (1.57-5.1)
*** OR: 1.6, 95% Conf. Intervals: (0.8-3.0)
**** OR: 2.15 95% Conf. Intervals: (1-4.3)

Cytokine Genotype Frequency In Female Patients

The data for cytokine genotypes were further analyzed by stratifying into male and female groups. The sample size was perhaps too restrictive for interpretation of the data. Some of the odds ratios were high enough to indicate that there may be a relationship but it was not definitive due to small the sample size. The data were summarized in Table 4 and Table 5. For females, there was a differential expression of TGF-β1 TC/GG based on the odds ratio but with a borderline significance ($p<0.053$, odds

ration= 2.29) possibly due to small sample size. The INF-γ A/A genotype was significantly increased in female patients with hypertension as compared with patients with no hypertension ($p<0.025$, odds ratio: 2.7).

Table 4
Cytokine genotype frequency distribution in female patients with HTN and with no HTN

Cytokine Genotypes	Hypertension N=77	No- Hypertension N=42	
TGF-β1 (codon 10 & 25	+ (%)	+ (%)	P value
T/T G/G	33 (42.8)	19 (45.2)	
T/C G/G	39 (50.6)	13 (30.95)	<0.053*
IFN-γ (intron +874)			
T/A	13 (16.9)	13 (31)	
A/A	64 (83.1)	27 (64)	<0.025**
IL-10 (-1082, -819, -592)			
GCC/ATA	22 (28.5)	7 (16.6)	<0.1***
ACC/ATA	22 (28.5)	6 (14.3)	
IL-18 (-137)			
G/G	51 (66.2)	33 (78.6)	
G/C	23 (29.9)	8 (19.05)	<0.2****

* Odds Ratio (OR): 2.29, 95% Conf. Intervals: 1-5.0
** OR: 2.7, 95% Conf. Intervals: (1.16-6.45)
*** OR: 2.4, 95% Conf. Intervals: (0.9-6.31)
**** OR: 1.8, 95% Conf. Intervals: (0.7-4.4)

Cytokine Genotype Frequency Distribution In Male Patients

For male patients there was a statistically significant difference in the genotypes of IFN-γ between patients with

HTN and those with no HTN. The A/A, low producer and the T/A intermediate producer genotypes were inversely associated with hypertension. A stepwise logistic regression analysis demonstrated that for males, the IFN-γ could play a high risk factor as a predictor of hypertension. There was also an evidence that IL-18 -137 G/C, a high producer genotype that occurred more frequently in patients with hypertension ($p<0.048$, see Table 5.) was a predictive risk factor, and patients who had this genotype were three times at higher risk of developing hypertension as compared with patients without having the genotype.

Table 5
Cytokine genotype frequency distribution in male patients with HTN and with no HTN

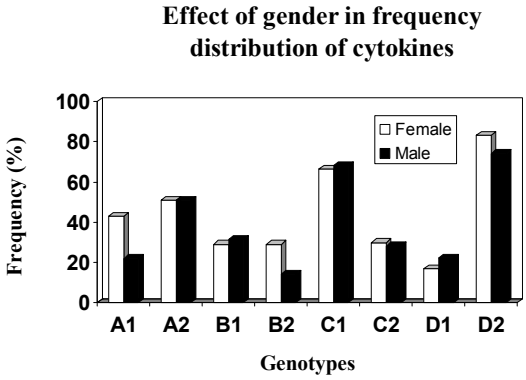
Cytokine Genotypes	Hypertension N=85	No Hypertension N=35	
TGF-β1 (codon 10 & 25	+ (%)	+ (%)	P vale
T/T G/G	18 (22)	9 (25.7)	
T/C G/G	43 (50.6)	16 (45.7)	<0.5*
IFN-γ (intron +874)			
T/A	19 (22.3)	17 (48.5)	<0.008
A/A	63 (74)	17 (48.5)	<0.01**
IL-10 (-1082, -819, -592)			
GCC/ATA	27 (31.5)	9 (25.7)	<0.5***
ACC/ATA	12 (14.3)	9 (25.7)	
IL-18 (-137)			
G/G	58 (68.2)	28 (80)	
G/C	24 (28.2)	4 (11.4)	<0.048****

* Odds Ratio (OR): 1.28, 95% Conf. Intervals: (0.6-2.8)
** OR: 3.0, 95% Conf. Intervals: (1.3-6.8)
*** OR: 1.3, 95% Conf. Intervals: (0.6-3.2)
**** OR: 3.05, 95% Conf. Intervals: (1-9.1)

Gender Effect In Frequency Distribution Of Cytokines

It was important to evaluate whether there was a gender effect influencing the frequency distribution of cytokine genotypes in the study cohort. Therefore, a stepwise logistic regression analysis for female and male was performed. In this model, a high producer T/T G/G haplotype of the TGF-β1 gene and a low producer ACC/ATA haplotpe of the IL-10 were significantly increased in female patients with hypertension (Figure 1). Although GCC/ATA high to intermediate producer haplotypes of the IL-10 were clinically correlated with hypertension in male patients, it was not statistically significant. It should be noted that the IFN-γ low producer genotype was significantly increased in both male and female with hypertension as compared with patients with no hypertension.

Figure 1



A1: p<0.004; Odds ratio= 2.79; 95% Conf. Interval (1.4-5.53)
B2: p< 0.033; Odds ratio= 2.4; 95% Conf. Interval (1.1-5.27)
A1 and A1: TGF-β1 TT/GG and TC/GG haplotypes B1 and B2: IL-10 GCC/ATA and ACC/ATA haplotypes C1 and C2: IL-18 (-137) G/G and G/C haploypes
D1 and D2 : IFN-γ T/A and A/A haplotypes respectively.

DISCUSSION

This study provides a unique look at risk factors for hypertension in a small study cohort of African Americans in Mississippi. Our data is suggestive of the fact that particular cytokines and growth factors may play a role in the etiology of HTN. Frequency distribution of the TGF-β1, IFN-γ and IL-18 genotypes were significantly increased in patients with HTN as compared with patients with no HTN. Thus, suggesting that the effects of these genotypes on hypertension might have an inflammatory framework association that could possibly be triggered by health disparities. It has been well established that IFN-γ is involved in up-regulation and induction of environmental antigens in the context of the major histocompatibility complex (MHC) (Kirk, 2001). In addition, antigen presenting markers associated with MHC such as CD80 and CD86 are expressed more in African Americans than in Caucasians (Hutchings, 1999). Thus activation of cellular elements resident in the blood vessels upon stimulation might release the cytokines and growth factors, leading to pathophysiologic mechanisms that cause inflammation in the vessel wall resulting in an elevated blood pressure.

Indeed, it has been shown presence of elevated levels of circulating C-reactive protein (CRP) (Sesso, 2003), TNF-α, IL-6 (Pfab, 2005), and TGF-β1 (Cambien, 1996; Suthanthiran, 2000) in plasma samples from patients with hypertension. In this study we have shown an increased frequency distribution of the TGF-β1 high producer genotypes in female patients. The TGF-β1 is known to activate endothelial cells, phagocytic cells and lymphocytes (Kristal, 1998). Subsequently this could induce expression of many pro-inflammatory molecules whose excess activity may cause tissue injury leading to endothelial dysfunction and thrombotic complications.

The question as to whether cytokines and growth factor polymorphism are associated with hypertension in African-Americans might further be answered by referring to the studies demonstrating variations in cytokine gene polymorphisms in African American vs. Caucasian population who had end-stage renal or heart failure (Densem, 2000; Cox, 2001; McDaniel, 2003). In these studies it was indicated that the majority of patients also suffered from a primary hypertension condition.

Elevated levels of pro-inflammatory cytokines including TNF-α and IL-6 have been reported to cause altered vascular function and hypertension in women with preeclampsia (Granger, 2004). This supports the cytokine hypothesis in hypertension. Studies have shown that genes associated with immune tolerance and inflammatory responses are involved in the regulation of blood pressure (Pauletto and Rattazzi, 2006). In addition, a higher prevalence of the preeclampsia in young African American women and the prevalence of high producer variants of the cytokines in females shown in this study, further support the role of the cytokines in the development of hypertension. In this study we did not observe any association with genotypes of IL-6 or TNF-α and hypertension. This could be a result from the fact that a great majority of African Americans carry the high producer/responder genotypes of IL-6 and TNF-α. Thus, such individuals are genetically at a higher risk of developing inflammatory responses which supports the hypothesis that genetically determined factors of the immune response genes are involved in the pathogenesis of the development of hypertension. In such individuals strong antiinflammatory response genes are required to maintain a balanced immunologic response and to overcome the consequences of inflammatory complications such as hypertension.

The inflammatory response genes all have a potential role in vascular pathology including coagulation, lipid metabolisms, and hypertension. Except for TGF-β1 genotypes (Cambien et al, 1996, Suthanthiran et al, 2000), associations of IFN-γ, IL-10, and IL-18 cytokine gene polymorphisms with hypertension have not been reported. We have not measured protein concentrations of these cytokines in plasma samples, which needs further investigation in a larger cohort study. Other investigators including us have demonstrated association of these cytokine genotypes with stronger inflammatory responses in African American population than in Caucasians (Cox, 2001; Simhan, 2003; McDaniel, 2003). Furthermore, cytokine gene polymorphisms have been reported in multiple studies that described the association with a number of health disparities such as atherosclerosis (Bidwell, 2001; Losito, 2003), preterm birth (Roberts, 1999; Simhan, 2003), diabetes (Fernández-Real, 2000; Kretowski, 2002), allograft rejection (Hutchinson, 1998; Turner, 1999; McDaniel, 2003) and sepsis (McDaniel, 2007) which commonly occur in African American population. Genetics, health disparities, cultural variances and environmental factors have been described to be related with a high prevalence of hypertension in the African American population (Oparil, 2005). Our observation supports the impact of TGF-β1, IL-18, IL-10, and IFN-γ in hypertension and suggests that an inflammatory pathway might be a leading contributor to the development of hypertension. Considering the commonness of high producer cytokine alleles in African Americans that tend to cause increased inflammatory responses suggests that inflammation might induce hypertension and that is genetically determined. Thus, African Americans are genetically predisposed to higher susceptibility risk markers that cause hypertension.

The technology is now available for assessing gene variants that encode the risk of common diseases including hypertension in the population.

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