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# Unique variation in genetic selection among Black North American women and its potential influence on pregnancy outcome

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ABSTRACT

We hypothesize that variations in the frequency of genetic polymorphisms, reflecting ancestral differences in living conditions and exposure to microorganisms, increase susceptibility to adverse pregnancy outcome among present day Black North American women. Striking differences were observed in the frequency of genetic variants between Black and White or Hispanic women in 5 genes (*IL1RN*, *MBL2*, *PPARA*, *ATG16L1*, *CIAS1*) associated with inflammation and anti-microbial immunity. The *CIAS1* and *IL1RN* polymorphisms were associated with altered interleukin-1β serum levels; the *MBL2* polymorphism resulted in a decreased serum mannose-binding lectin concentration. Gene polymorphisms associated with an alteration in innate immunity were most frequent in Black women. This may reflect an evolutionary selection in response to an ancient environment containing a high multitude of microorganisms, and may increase susceptibility of Black women to infection-associated preterm birth in the current North American environment.



## Hypothesis

Variations in the frequency of polymorphisms in individual genes between racial/ethnic groups may be a consequence of differences in evolutionary pressure. For example, variations in microbial density and rate of microbial exposure in different ancestral environments may have resulted in a selective pressure to favor the maintenance of genetic variants that optimize survival in response to the specific infectious insults. When individuals relocate to a new environment, specifically the migration of Blacks from Africa to the relatively low infection environment of the twenty first century United States, polymorphisms that were advantageous in the past may now be detrimental. It is well known that a too vigorous pro-inflammatory immune response to infection during pregnancy triggers a sequence of events that culminates in premature myometrial contractions and preterm delivery [1]. Thus, in a high infection-prone environment a genetic capacity to down-modulate pro-inflammatory immunity has survival benefit for the fetus. However, in an infection scarce environment the down-regulation of pro-inflammatory immunity in response to infection may result in a decreased anti-microbial immune defense during gestation and increase the risk for infection-related pregnancy complications.

## Racial disparity in pregnancy outcome in the United States

In the United States in 2003, the preterm birth rate was 17.9% for Blacks, 10.8% for Asians and Pacific Islanders and 11.8% for Whites [2]. Another study demonstrated that the proportion of deliveries that occurred at <28 weeks (very preterm birth) was 0.35% for Whites, 0.45% for Hispanics and 1.39% for Blacks and the overall neonatal mortality rate per 1000 live births was 3.24 for Whites, 3.45 for Hispanics and 8.16 for Blacks [3]. These disparities remain even after controlling for socio-economic and lifestyle variables. The disparity in birth outcome between Blacks and Whites is also evident among college educated women [4]. Black women seem to be especially susceptible to preterm birth as a result of infection [5–7]. Utilizing sophisticated pathway analyses, it was concluded that a maternal infection is the predominant cause of preterm birth in Black women, but not in Whites [7,8]. Several previous studies have identified variations in the rate of functional gene polymorphisms between Blacks and Whites that could at least partially account for this difference [9–12].

## Study protocol

In the present study we evaluated polymorphisms in 7 genes in White, Black and Hispanic women that influence pro-inflammatory immune responses or anti-microbial innate immunity. Our aim was to seek evidence of differences in the frequency of specific gene polymorphisms that may increase susceptibility to infection-mediated adverse pregnancy outcomes in Black women.

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Two hundred seventy nine women seen at the Weill Cornell Medical Center outpatient obstetrics clinic who self-reported as being either White (64), Black (77) or non-Black Hispanic (138) were available for genetic and protein testing. The study was approved by the Institutional Review Board of Weill Cornell Medical Center and written informed consent was obtained from all participants.

For gene polymorphism analyses, buccal swabs were obtained after rinsing the mouth with water. The samples were air dried for 30 min, kept at 4 °C and processed within 24 h. The procedure to liberate DNA from the buccal cells and prepare samples for gene polymorphism analysis was as described previously [13]. The specific polymorphisms selected for analysis are shown in Table 1. Published procedures based on gene amplification, endonuclease digestion and agarose gel electrophoresis were utilized for detection of the *MBL2* [14], *PPARA* [15], *ATG16L1* [16], *FABP2* [17] and *TLR4* [18] single nucleotide polymorphisms. For the *IL1RN* [13] and *CIAS1* [19] length polymorphisms the endonuclease step was omitted. Control DNA with known genotypes was always analyzed in parallel to the test samples and selected samples were assayed on 2–3 occasions to assure accuracy and reproducibility.

Peripheral blood was obtained by venipuncture and serum concentrations of mannose-binding lectin (MBL) and interleukin-1β (IL-1β) in each subject were determined by commercial ELISA kits (R&D Systems, Minneapolis, MN). The lower limit of sensitivity was 15.8 pg/ml for MBL and 3.9 pg/ml for IL-1β. Samples were assayed in duplicate and the average value determined. Optical density units were converted to pg/ml by reference to a standard curve that was analyzed in parallel to each assay.

Genotype and allele frequencies were determined by direct counting and then divided by the number of chromosomes to obtain allele frequency and by the number of women to obtain genotype frequency. Goodness of fit to Hardy–Weinberg equilibrium was determined by comparing the expected genotype frequencies with the observed values, using the chi-square test. Association between genotypes or alleles and race/ethnicity were analyzed by the Fisher's exact test. Serum IL-1β or MBL levels and genotypes were analyzed by the non-parametric Kruskal–Wallis test since the values were not normally distributed. A $p$ value <0.05 was considered significant.

## Results

Polymorphisms in 7 genes were evaluated. All genotypes were in Hardy–Weinberg equilibrium. In 5 of the genes the frequency of the variant allele differed between the White and Black subjects. Similarly, the variant allele frequency in 4 of the genes differed between Blacks and Hispanics. In contrast, the allele frequency between Whites and Hispanics differed for only one gene, *ATG16L1*. Results are shown in Table 2. The variant allele frequency in Blacks was elevated for *MBL2* and *CIAS1* and reduced for *IL1RN*, *PPARA* and *ATG16L1*; allele frequencies for *FABP2* and *TLR4* were similar in each of the three groups.

**Table 1**
Individual gene polymorphisms analyzed.

| Gene | Function | Polymorphism |
|---|---|---|
| IL1RN | Competitive inhibitor of IL-1 and down-regulator of inflammation | Variable number of an 86 base pair tandem repeat in intron 2 |
| MBL2 | Innate antimicrobial immunity and complement activation | Exon 1 codon 57, rs1800451 |
| PPARA | Regulation of lipid metabolism, insulin resistance, inflammation, obesity | Leu162Val, rs1800206 |
| ATG16L1 | Induction of autophagy – killing of intracellular microbes, cell survival, control of inflammation | T300A, rs2241880 |
| CIAS1 | Inflammasome formation, IL-1β production and release | A variable number of a 42 base pair tandem repeat in intron 4 |
| FABP2 | Glucose regulation, lipid oxidation, metabolism of dietary fat | Ala54Thr, rs1799883 |
| TLR4 | Lipopolysaccharide receptor, activation of pro-inflammatory immunity | Asp299Gly, rs4986790 |

Women homozygous for the 12 unit repeat (12,12) of the *CIAS1* gene had the highest median serum IL-1β concentration (29.0 pg/ml), followed by women positive for one (9.6 pg/ml) or none (4.0 pg/ml) 12 unit repeats ($p = 0.0122$). Similarly, the median serum IL-1β concentration was 47.0 pg/ml in women who were *IL1RN* 2,2, 13.0 pg/ml in women who were 1,2 and 8.0 pg/ml in those who were 1,1 ($p = 0.0331$). There were no associations between serum IL-1β levels and *ATG16L1*, *PPARA* or *MBL2* genotypes.

MBL serum levels in the study population differed significantly according to the *MBL2* genotype. Median concentrations were 1.1 ng/ml in women who had the wild type A,A genotype, 0.7 ng/ml in heterozygote women and 0.3 ng/ml in women who were homozygous for the variant C allele ($p < 0.0001$).

## Discussion

The frequency of allelic variations in three genes, *IL1RN*, *PPARA* and *ATG16L1*, were reduced in Black women. The *IL1RN* gene codes for interleukin-1 receptor antagonist (IL-1ra), a competitive inhibitor of IL-1β. Both IL-1ra and IL-1β compete for the same receptor and the ratio of IL-1ra to IL-1β determines the extent and duration of inflammation. The genes coding for IL-1β and IL-1ra are linked and the *IL1RN* 2,2 genotype is associated with increased IL-1β production and an enhanced pro-inflammatory immune response [20]. In our study carriage of the IL1RN 2,2 genotype was associated with an elevated serum IL-1β level. *PPARA* codes for peroxisome proliferator-activated receptor-α (PPAR-α), a nuclear receptor that plays a central role in the regulation of multiple metabolic processes [21]. PPAR-α activation induces expression of genes involved in fatty acid uptake, reduction in triglyceride levels and an increase in high density lipoprotein expression. It is also a down-regulator of inflammation by virtue of its inhibition of NF-κB activation as well as blocking expression of vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and pro-inflammatory mediators such as tumor necrosis factor-α, IL-1β and interleukin-6. The *PPARA L162V* polymorphism is associated with reduced gene activity [15]. Autophagy is a process whereby long-lived cytoplasmic components and intracellular microorganisms become sequestered in autophagosomes and are degraded after fusion with lysosomes [22]. The *ATG16L1* gene codes for the autophagy-related 16-like 1 protein, necessary for autophagosome assembly [23]. The *ATG16L1* T300A polymorphism is associated with defective autophagosome formation. Recent studies in mice have established that this polymorphism also results in production of high levels of pro-inflammatory IL-1β and IL-18 by macrophages following exposure to bacterial lipopolysaccharide [23].

The frequency of a length polymorphism in the *CIAS1* gene was elevated in Black women. This polymorphism was associated with reduced circulating IL-1β levels in our population. The *CIAS1* gene codes for the protein, NACHT-LRR-PYD-containing protein (NALP3), the rate limiting step in formation of the inflammasome [24]. Inflammasome formation is essential for the activation and release of biologically active IL-1β [25]. Black women with this polymorphism would be defective in inflammasome-mediated IL-1β production.

Although we were able to correlate polymorphisms in the *IL1RN* and *CIAS1* genes with alteration in serum IL-1β concentrations, this was not observed in women positive for the *PPARA* or *ATG16L1*