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Genetic polymorphisms in the serotonin receptor 7 (HTR7) gene are associated with cortisol levels in African American young adults [version 1; referees: awaiting peer review]

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Abstract

Introduction: Serotonin is a neurohormone involved in biological processes, such as behavior and immune function. Chronic psychosocial stressors may cause serotonin release resulting in immune system dysregulation, as evidenced by increased or far decreased levels of cortisol, a blood biomarker of stress and immune function. We hypothesize that genetic polymorphisms in the HTR7 gene are associated with both hypo- and hyper-cortisolism. Methods: The study population included 602 African American subjects between 18-34 years of age, living in Washington, D.C. Five single nucleotide polymorphisms (SNPs) in HTR7, rs2420367, rs12412496, rs2185706, rs7089533, and rs7093602 were genotyped by restriction fragment length polymorphism or the TaqMan assay. Statistical analysis, using the program SNPstat, was performed to determine their associations with cortisol measured in the study population. Results: While an increased risk of hypocortisolism was found to be associated with rs2420367, rs2185706, and rs7093602 in a gender specific manner, no genotypes could be associated with hypercortisolism. Inversely, a decreased risk of hypocortisolism was found with the haplotype CGGCC (p=0.033), which remained significant in males. When adjusting for gender, females associated with the haplotype AGACC. Hypercortisolism was also associated with a decreased risk for the haplotypes AAACC (p=0.042) and AAGTT (p=0.001). Discussion: Based on these results, genetic variation in the HTR7 gene may contribute to both stress and inflammation, and will provide a new glimpse into stress-related inflammation psychophysiology.
Genetic polymorphisms in the serotonin receptor 7 (HTR7) gene are associated with cortisol levels in African American young adults [version 1; referees: awaiting peer review] F1000Research 2017, 6:19 (doi: 10.12688/f1000research.10442.1)

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Introduction
Psychosocial stressors, such as exposure to interpersonal and community violence, may impact immune function (O’Connor et al., 2000), leading to the development of a number of health disparities (Black, 2003; Murali et al., 2007). During exposure to a stressor, many hormones are released in the brain, including serotonin (5-HT). 5-HT is a neurotransmitter that functions in the regulation of a variety of psychological and physiological processes, including behavior and inflammation (Idzko et al., 2004; Mikulski et al., 2010). Serotonin receptor 7 (HTR7) is the most recently described serotonin receptor, and it is found expressed on a number of immune cells, including macrophages and T lymphocytes (Ahern, 2011). Studies indicate that HTR7 activation results in the production of pro-inflammatory cytokines from immune cells, such as microglial cells, dendritic cells, and monocytes (Dürk et al., 2005; Mahé et al., 2005; Müller et al., 2009). Cytokines, such as interleukin-6 (IL-6), are then involved in the production of a variety of blood biomarkers, including cortisol, the major stress hormone (de Kloet et al., 2005; Howren et al., 2009; Maeda et al., 2010).

Cortisol is involved in the regulation of a number of biological processes, such as cellular metabolism and immune function (Anagnostis et al., 2009; Lundberg, 2005; Webster Marketon & Glaser, 2008). Both hypercortisolism and hypocortisolism have recently been associated with a number of health disparities, including cardiovascular disease and asthma (Buske-Kirschbaum et al., 2003; Manenschijn et al., 2013). During a stress response, cortisol concentration is increased resulting in a shift towards an inflammatory and humoral response (Murali et al., 2007; Straub et al., 2002). Under normal circumstances, this response will be limited and shut off when cortisol levels decrease back to normal levels (Ehlert et al., 2001). If a stressor persists for an extended period of time, the cortisol concentration will continue to increase until the system exhausts its supply (McEwen, 2004). Both cases, whether amplified or depressed, can lead to serious conditions, such as metabolic syndrome and irritable bowel syndrome (Anagnostis et al., 2009; Fries et al., 2005).

An increasing number of single nucleotide polymorphisms (SNPs) in genes for neurohormone receptors have also been linked to a number of health disparities. These diseases include breast cancer, depression, and asthma (Deming et al., 2012; Kim et al., 2011; Kring et al., 2009; Lucae et al., 2010). While SNPs in these genes have been identified, there is limited data on the molecular pathways involved in these relationships. With regard to SNPs found in serotonin receptors, the mechanisms used by serotonin receptor 2A (HTR2A) in relation to disease state have begun to be identified (Bertolla et al., 2008; Snir et al., 2013). It is possible that SNPs within HTR7 modulate the induction of pro-inflammatory cytokines from immune cells during times of stress, leading to a predisposition towards the development of health disparities. Previous findings prompted us to investigate whether genetic variation in HTR7 associates with the changes in function of the stress and immune system measured by cortisol levels in African American (AA) young adults.

Methods and materials

Study population
All DNA and sample data were collected during a previous study done between 2010 and 2012 entitled “Gender Differences in the Experience of Violence, Discrimination, and Stress Hormone in African Americans: Implications for Public Health”, which examined the genetic markers for alcohol and depression, violence exposure, and drug use in AA young adults (unpublished study; Jackson L, Shestov M, Abbas M and Saadatmand F). In total, DNA samples from 602 AA individuals living in the Washington, D. C. area were available for genotyping. All individuals, both male and female, ranged between the ages of 18 and 34 years old. In an Audio Computer-Assisted Self-Interviewing (ACASI) survey, only 590 participants provided information concerning behavioral data, including information concerning housing, income, and violence exposure (Table 1). Blood biomarkers, including cortisol (472 out of 602), were determined after blood collection in the morning (Dataset 1; Swanson et al., 2016a). All analyses were performed in a case-control manner for both hypercortisolism (case1, participant cortisol concentration >14 µg/dl; control1, participant cortisol concentration <14 µg/dl) and hypocortisolism (case2, participant cortisol concentration <5 µg/dl; control2, participant cortisol concentration >5 µg/dl). Consent was obtained from each participant in the study, and approval from Howard University’s Institutional Review Board was obtained (approval number, IRB-16-MED-03).

Dataset 1. Raw data for genetic polymorphisms in the serotonin receptor 7 (HTR7) gene are associated with cortisol levels in African Americans young adults

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Cortisol measurements were determined in Mic-Gr/dL and recorded as a numerical value or the three values as follows; -9, indicate participants in which no cortisol was measurable in the participant; NA, indicate participants in which blood samples were not taken; \( \sqrt{ } \), were taken as case values for analysis. NA for sex and age of participant indicate the participant did not wish to specify. Within SNP genotypes, NA indicates that no genotype was determined.

SNP selection
SNPs in HTR7 were downloaded from NCBI using the SNP Gene View (https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?chooseRs=all&locusId=3363&mrna=NM_019859.3&ctg=NT_030059.14&prot=NP_062873.1&ori=reverse&refresh=refresh) for the comparison of allele frequencies between the CEU, Caucasian descent, and YRI, Yoruban descent, populations. Differences between CEU and YRI populations was of interest as African American’s are likely to possess frequencies that fall between those of Caucasian and Yoruban descent. Four SNPs (rs12412496, rs2185706, rs7089533, and rs7093602) were selected for inclusion in the study based on a large difference in allele frequency between the CEU and YRI populations. The SNP rs2420367 was selected based on no frequency data being recorded with any population besides CEU (Table 2). All of the chosen SNPs were located in the first intron region of the gene, as indicated on NCBI’s SNP: Gene View.
### Table 1. Demographic data for the study population of 602 participants.
Demographics include gender, age, housing and income status, and violence exposure.

| Characteristic          | N   | %    | Mean (SD) | Range |
|-------------------------|-----|------|-----------|-------|
| Gender                  |     |      |           |       |
| Female                  | 197 | 32.72|           |       |
| Male                    | 317 | 52.66|           |       |
| Unspecified             | 88  | 14.62|           |       |
| Age, years              |     |      | 21.6      | 18-34 |
| Housing                 |     |      |           |       |
| Rent, own, or payed by the government | 366 | 62.03|           |       |
| Public housing          | 73  | 12.37|           |       |
| Unspecified             | 151 | 25.59|           |       |
| Income source           |     |      |           |       |
| Work                    | 380 | 64.41|           |       |
| Welfare or public assistance | 69  | 11.69|           |       |
| Part work part welfare  | 86  | 14.58|           |       |
| Unspecified             | 55  | 9.32 |           |       |
| Violence                |     |      |           |       |
| Partner violence        |     |      | 0.4 (0.8) | 0-6   |
| Community violence      |     |      | 0.7 (0.6) | 0-3   |
| Family violence         |     |      | 0.2 (0.3) | 0-1   |
| Police action           |     |      | 0.9 (0.5) | 0.08-2.91|
| Police attitude         |     |      | 2.4 (1.0) | 0-4   |
| Arrest history          |     |      | 1.9 (1.7) | 0-10  |

### Table 2. Summary of SNP data. Listed populations are as follows: YRI- Yoruban descent population listed in HapMap; CEU- Caucasian descent population listed in HapMap; AA- African American population from this study.

| SNP        | Allele | YRI Allele Frequency | CEU Allele Frequency | AA Allele Frequency | Genotype | YRI Genotype Frequency | CEU Genotype Frequency | AA Genotype Frequency | Number genotyped |
|------------|--------|----------------------|----------------------|--------------------|----------|------------------------|------------------------|----------------------|-----------------|
| rs24203767 | A      | 54.16                | 85.00                | 78.33              | A/A      | 28.33                  | 76.00                  | 21.66                | 516             |
|            | C      | 45.83                | 15.00                | 21.66              | C/C      | 20.00                  | 6.00                   | 31.66                |                 |
| rs12412496 | A      | 43.33                | 21.66                | 39.00              | A/A      | 18.33                  | 6.66                   | 21.00                | 600             |
|            | G      | 56.66                | 78.33                | 61.00              | A/G      | 50.00                  | 30.00                  | 37.00                |                 |
| rs2185706  | G      | 48.66                | 88.39                | 52.00              | G/G      | 31.66                  | 63.33                  | 42.00                | 600             |
|            | A      | 51.33                | 11.61                | 48.00              | G/A      | 56.42                  | 19.64                  | 66.00                |                 |
| rs7089533  | C      | 48.23                | 88.05                | 79.00              | A/A      | 24.28                  | 1.78                   | 15.00                | 593             |
|            | T      | 51.76                | 11.94                | 21.00              | C/T      | 55.75                  | 20.35                  | 42.00                |                 |
| rs7093602  | C      | 49.15                | 11.66                | 70.00              | C/C      | 20.33                  | 33.33                  | 48.00                | 602             |
|            | T      | 50.85                | 88.33                | 30.00              | C/T      | 57.62                  | 16.66                  | 43.00                |                 |
|            |        |                      |                      |                    | T/T      | 22.03                  | 80.00                  | 9.00                 |                 |
Genotyping
Genotypes were determined using one of two methods. Restriction fragment length polymorphisms (RFLPs) were used for the genotyping of rs2420367, rs7089533, and rs7093602. Samples were amplified by polymerase chain reaction (PCR) in 96 well plates. The Taq polymerase and dNTP mix utilized for PCR amplification was obtained from Thermo Fisher Scientific (Waltham, MA, USA). SNP primers were supplied by Integrated DNA Technologies (Coraville, IA, USA). The concentration of magnesium chloride was 1.8 mM (rs2420367; rs7089533) and 1.5 mM (rs7093602). A total of 40 cycles were used, in which the annealing temperature was decreased by 2°C every 5 cycles until reaching the optimum annealing temperature of 57°C for each primer set. After confirming proper DNA amplification, the PCR products were digested with the appropriate restriction enzyme. The restriction enzymes, SmII, AflIII, and AvaII, were used at a concentration of 0.2 µL for the three SNPs rs2420367, rs7089533, rs7093602, respectively, (New England BioLabs, Ipswich, MA, USA). Incubation time for SmII was increased to two hours at 55°C, while incubation for AflIII and AvaII remained at the recommended 15 minutes. Visualization of the digestion was done using a 3% agarose gel. The TaqMan SNP genotyping assay was used to genotype rs12412496 and rs2185706. A volume 1.5 µL of DNA was used, as well as the 10 µL of the prepared master mix as per manufacturer’s instructions. The plates were then run using the TaqMan SNP genotyping assay protocol by Applied Biosystems.

Statistical analysis
The genotype and allele frequencies for each SNP were determined using the data generated from all 602 DNA samples. Genotype and haplotype associations were made using the software program SNPstat, which tested the data for Hardy-Weinberg equilibrium, linear and logistic regression, and linkage disequilibrium statistics (http://bioinfo.iconcologia.net/SNPstats).

Results
All 602 samples were used for the determination of allele and genotype frequencies within the AA population (Table 2). In determining the haplotype frequencies within the population, the majority of the population possessed one of three haplotypes; AGGCC (18.19%), AGACC (16.72%), and AAGCC (13.22%) ordered by SNPs, rs2420367, rs12412496, rs2185706, rs7089533, rs7093602 (Dataset 2; Swanson et al., 2016a).

Hypocortisolism was found to be associated with rs2420367, rs2185706, and rs7093602 when adjusting for gender (Table 3 and Table 4). Females showed an association with the genotype A/C (rs2420367) by an 11-fold increase in the risk of hypocortisolism (OR=11.64[1.52-89.24]), when categorizing the interaction first by SNP then by gender (SNP within gender; Table 4). Similarly, males showed a 2-fold increased risk of hypocortisolism with the genotype A/A (rs2420367) when the interaction was categorized first by gender then by SNP (gender within SNP; Table 3). Only males showed an association to hypocortisolism when categorizing in a gender within SNP manner for both rs2185706

| Table 3. Associations between hypocortisolism and SNPs within HTR7. Data was analyzed as gender within the SNPs. |
|----------------------------------------------------------|
| **rs2420367** | **Case** | **Control** | **OR** |
| A/A | Female | 33 | 85 | 1 |
| | Male | 32 | 184 | 2.26(1.29-3.95) |
| A/C | Female | 1 | 31 | 1 |
| | Male | 10 | 38 | 0.12(0.01-1.02) |
| C/C | Female | 2 | 11 | 1 |
| | Male | 3 | 12 | 0.96(0.13-7.08) |
| Interaction in trend: 0.016 |

| **rs2185706** | **Case** | **Control** | **OR** |
|------------------|-----------|-------------|--------|
| G/G | Female | 5 | 25 | 1 |
| | Male | 12 | 49 | 0.90(0.28-2.89) |
| A/G | Female | 20 | 94 | 1 |
| | Male | 28 | 151 | 1.18(0.62-2.25) |
| A/A | Female | 10 | 14 | 1 |
| | Male | 5 | 38 | 5.23(1.50-18.21) |
| Interaction in trend: 0.029 |

| **rs7093602** | **Case** | **Control** | **OR** |
|------------------|-----------|-------------|--------|
| C/C | Female | 24 | 71 | 1 |
| | Male | 17 | 109 | 2.13(1.06-4.28) |
| C/T | Female | 9 | 52 | 1 |
| | Male | 23 | 109 | 0.87(0.37-2.03) |
| T/T | Female | 3 | 10 | 1 |
| | Male | 5 | 23 | 1.59(0.31-8.11) |
| Interaction in trend: 0.24 |

Cortisol, the major glucocorticoid in the stress response, is also an important biomarker of an immune response (Cavigelli & Chaudhry, 2012; Straub et al., 2002). Cortisol is shown to first be involved in the development of an inflammatory response, before eventually acting to depress this response (Elenkov, 2008; Kunz-Ebrecht et al., 2003). It has also been shown to shift the adaptive immune response towards the humoral response (Cavigelli & Chaudhry, 2012; Murali et al., 2007). As such, it was desired to identify potential associations between both hyper- and hypocortisolism and the five intronic SNPs in HTR7. None of the five SNPs were found to be associated with hypocortisolism.

Dataset 2. Haplotype frequencies for five SNPs in HTR7 in African Americans

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SNP order is as follows: rs2420367, rs12412496, rs2185706, rs7089533, rs7093602. 
Males with the genotype A/A (rs2185706) were found to be at a 5-times greater risk (OR=5.23 [1.50-18.21]), while those with the genotype C/C (rs7093602) were associated with a 2-times greater risk (OR=2.13 [1.06-4.28]; Table 3). This indicates that some SNPs within HTR7 are associated with hypocortisolism in a gender specific manner in the AA population.

When analyzing for haplotype associations to hypercortisolism, two haplotypes provided a decreased risk in the population. The haplotype AAACC (p=0.042) was found associated with a 77% decreased risk (OR=0.23 [0.06-0.95]; Table 5), while the AAGTT haplotype (p=0.001) was associated with a 98% decreased risk in the AA population (OR=0.02 [0.00-0.21]; Table 5). When analyzing

Table 4. Association between hypocortisolism and SNPs in HTR7. Data was analyzed as SNPs within gender.

| rs2420367 | Case  | Control | OR        |
|-----------|-------|---------|-----------|
| A/A       | 33    | 85      | 1         |
| A/C       | 1     | 31      | 11.64 (1.52-89.24) |
| C/C       | 2     | 11      | 2.03 (0.42-9.76)   |

| rs2420367 | Case  | Control | OR        |
|-----------|-------|---------|-----------|
| A/A       | 32    | 184     | 1         |
| A/C       | 10    | 38      | 0.63 (0.28-1.41) |
| C/C       | 3     | 12      | 0.87 (0.22-3.34) |

Interaction in trend: 0.0035

Table 5. HTR7 haplotype associations to cortisol level. SNP ordering for haplotype analysis as follows; rs2420367, rs12412496, rs2185706, rs7089533, rs7093602.

| Haplotype | Frequency | Case | Control | OR        | p-value |
|-----------|-----------|------|---------|-----------|---------|
| AGGCC     | 0.2001    | 0.0815 | 0.207 | 1         | ... ... ...
| AGACC     | 0.1518    | 0.1865 | 0.15  | 0.46 (0.10-2.13) | 0.32 |
| AAGCC     | 0.1193    | 0.0776 | 0.1206 | 0.95 (0.17-5.45) | 0.96 |
| AACC      | 0.1075    | 0.2505 | 0.1004 | 0.23 (0.06-0.95) | **0.042** |
| AGTT      | 0.0412    | 0.442  | 0.0399 | 0.24 (0.03-1.77) | 0.16 |
| AGCT      | 0.0408    | 0.0484 | 0.0386 | 0.53 (0.05-6.11) | 0.61 |
| CGACC     | 0.0397    | 0.0373 | 0.0375 | 0.22 (0.03-1.57) | 0.13 |
| CGGCC     | 0.026     | 0.0227 | 0.0256 | 0.80 (0.07-9.29) | 0.86 |
| AAGTT     | 0.0158    | 0.1492 | 0.0087 | 0.02 (0.00-0.21) | **0.001** |

| Haplotype | Frequency | Case | Control | OR        | p-value |
|-----------|-----------|------|---------|-----------|---------|
| AGGCC     | 0.2069    | 0.1768 | 0.2103 | 1         | ... ... ...
| AGACC     | 0.1466    | 0.1948 | 0.1378 | 0.45 (0.19-1.08) | 0.076 |
| AAGCC     | 0.1174    | 0.1158 | 0.1071 | 0.58 (0.23-1.47) | 0.25 |
| AAACC     | 0.1095    | 0.1068 | 0.1226 | 0.98 (0.41-2.32) | 0.95 |
| AGATT     | 0.0673    | 0.0698 | 0.0629 | 0.75 (0.23-2.43) | 0.63 |
| CGACC     | 0.0377    | 0.0398 | 0.0366 | 0.84 (0.21-3.26) | 0.8 |
| AGCT      | 0.0373    | 0.02   | 0.0475 | 0.86 (0.14-5.32) | 0.87 |
| AAATT     | 0.0304    | 0.034  | 0.0293 | 0.49 (0.11-2.25) | 0.36 |
| AAGCT     | 0.0272    | 0.0367 | 0.0285 | 0.39 (0.10-1.45) | 0.16 |
| CGGCC     | 0.0248    | 0.045  | 0.0203 | 0.19 (0.04-0.87) | **0.033** |
| AGACT     | 0.0193    | 0.0209 | 0.0193 | 0.62 (0.10-4.06) | 0.62 |
| AAGTT     | 0.017     | 0.0524 | 0.0135 | 0.27 (0.04-1.77) | 0.17 |
| CAGTT     | 0.0162    | 0.0066 | 0.0135 | 1.76 (0.18-17.50) | 0.63 |
| CAGCT     | 0.0137    | 0.0061 | 0.0166 | 0.72 (0.06-9.04) | 0.8 |
| rare      | 0.0487    | ...    | ...    | 0.47 (0.15-1.42) | 0.18 |
the data for hypocortisolism, only the haplotype CGGCC (p=0.033) was associated with 79% decreased risk (OR=0.19[0.04-0.87]; Table 5).

When adjusting for gender, males remained associated with hypocortisolism by the CGGCC haplotype (OR=0.01[0.00-0.19]) providing a 99% decreased risk (Table 6). This relationship held true regardless of the categorization of the interaction. While females did not remain associated with the CGGCC haplotype, a 91% decreased risk was found associated with hypocortisolism by the haplotype AGACC (OR=0.11[0.02-0.69]) and by 83% for the grouping of extremely rare haplotypes (OR=0.17[0.03-0.88]) (Table 6). These associations held when categorized in a haplotype within gender manner. Due to the low number of individuals denoted as case for both analyses (case1=22; case2=85), the majority of haplotypes were unable to be used for analysis purposes. The results obtained indicate that certain haplotypes are associated with cortisol level in the AA population by decreasing the risk of having either hyper- or hypocortisolism.

### Discussion

Despite the existing data on the impact of both genetic and environmental factors on immune function, few studies have examined relationships between these variables, especially in African Americans. We know relatively little about potentially sequential relationships between genetic risk factors, environmental stress, and immune function. In the brain, 5-HT is produced primarily by neurons in the midbrain, especially the dorsal raphe nucleus, which acts to innervate the majority of the brain, allowing 5-HT to modulate the response to stress (Holmes, 2008; Hornung, 2003). **HTR7** is located throughout the brain and periphery, functioning in the regulation of circadian rhythmicity and smooth muscle tone, both in the cardiovascular and gastrointestinal tract. (Abdouh et al., 2004; Hornung, 2003; Mahé et al., 2005)

During times of stress, **HTR7** expression is shown to increase and has been linked to depression (Guscott et al., 2005; Holmes, 2008). SNP genotypes and haplotypes in various genes, including serotonin receptors, have also been associated with changes in immune function or disease state (Ikeda et al., 2006; Snir et al., 2013; Zlojutro et al., 2011). As such, it should follow that SNP genotypes and haplotypes in the **HTR7** gene should also be associated with changes in the stress response, resulting in an effect on immune function. In this paper, we report the allele, genotype, and haplotype frequencies for five intronic SNPs of the **HTR7** gene in an African American population.

During times of stress, cortisol secretion will initially increase, and over time may lead to hypercortisolism if the stressor remains persistent (Fries et al., 2005). In the case of chronic stress, it is possible to reach a state of adrenal exhaustion or hypocortisolism (McEwen, 2004). Both states, hyper- and hypocortisolism, have been associated with a number of stress-related diseases. While hypercortisolism has been shown to contribute to diseases such as depression, heart disease, and type 2 diabetes (Lundberg, 2005; Tse & Bond, 2004), hypocortisolism contributes to post-traumatic stress disorder, asthma, and irritable bowel syndrome (Buske-Kirschbaum et al., 2003; Ehler et al., 2001; Fries et al., 2005; Heim et al., 1999). To test the hypothesis that there would be an association between SNPs in the **HTR7** gene and both hyper- and hypocortisolism, the program SNPStat was used. While hypercortisolism was not associated to any specific SNP genotypes, associations were found to hyper- and hypocortisolism with the haplotypes AAACC and AAGTT, respectively. Both haplotypes provided a decreased risk of 77% and 98%, respectively, and may identify individuals who are less likely to develop hypercortisolism, and the potential resulting health disparities.

An increased risk of hypocortisolism was found to associated with three SNP genotypes in a gender specific manner. For rs2420367, females with the A/C genotype and males with the A/A genotype showed an 11-fold and 2-fold increased risk, respectively. Males, but not females also showed a 5-fold and 2-fold increased risk of hypocortisolism with the genotypes A/A (rs2185706) and C/C (rs7093602), respectively. As such, it is possible to conclude that individuals with these genotypes will be at an increased risk of developing hypocortisolism, and this may also increase the likelihood of developing health disparity diseases.

The haplotype CGGCC was determined to provide a 79% decreased risk of hypocortisolism in the population, and this association remained in males when adjusting for gender. Females however, showed a decreased risk of 78% with the haplotype AGACC. These haplotypes may identify the individuals in the AA population who are at a decreased risk of developing hypocortisolism and associated diseases.

In this work, we demonstrate that genetic variation in **HTR7** influences the production of cortisol in response to chronic stress. While individual SNP genotypes indicate a risk towards the development of hypocortisolism, haplotypes within **HTR7** seem to be protective against both hyper- and hypocortisolism. This protective effect may be a biological indicator of resilience towards maladaptive effects of chronic stress. This supports previous findings that individual genetics are involved in the degree of adaptability seen in response to stress (Feder et al., 2009; Gillespie et al., 2009). To our knowledge, this is the first evidence for a functional link between

### Table 6. **HTR7** haplotype associations to hypocortisolism when adjusting for gender. Analysis was performed as a haplotype and gender cross-classification interaction.

| Haplotype | Frequency | Female OR | Male OR | Interaction p-value |
|-----------|-----------|-----------|---------|-------------------|
| AGGCC     | 0.205     | 0.11(0.02-0.69) | 0.32(0.02-4.21) | 0.088 |
| AGACC     | 0.1495    | 0.29(0.03-3.05) | 0.60(0.02-15.00) | 0.11 |
| AGACC     | 0.1197    | 0.16(0.01-1.17) | 0.59(0.04-7.99) | 0.32 |
| AAACC     | 0.1064    | 0.10(0.01-1.11) | 0.20(0.01-2.78) | 0.01 |
| AGCT      | 0.0425    | 0.01(0.00-0.19) | 0.01(0.00-0.19) | 0.01 |
| AATT      | 0.0349    | 0.14(0.01-2.27) | 0.10(0.01-2.78) | 0.01 |
| CGGCC     | 0.0253    | 0.01(0.00-0.19) | 0.01(0.00-0.19) | 0.01 |
| rare      | 0.0469    | 1.32(0.04-46.02) | 1.32(0.04-46.02) | 0.01 |
a genetic polymorphism in the HTR7 receptor gene and immunologically important subphenotypes related to differential levels of a blood biomarker for both stress and the immune response.

Further study is required to investigate this relationship between genetic polymorphisms in HTR7, the stress response, and the immune response. Due to the low number of individuals in the case grouping, haplotype analysis was incomplete. By increasing the sample set data with regards to cortisol measurements, a clearer picture of the relationship between these five SNPs and cortisol may be formed. This will enable a more detailed picture of the relationship between HTR7 polymorphisms and cortisol production. Furthermore, the cytokine shift induced by cortisol during a response to stress suggests that a relationship may exist between these five SNPs and both the inflammatory and humoral response (Maeda et al., 2010; Murali et al., 2007; Pepys & Hirschfield, 2003), and it would be beneficial to analyze whether any association between the five intronic SNPs and biomarkers of the inflammatory and humoral response. This would aid in the understanding of how stress exposure contributes to the development of disease, and the role in which genetics plays in this pathway.

Data availability
Dataset 1. Raw data for genetic polymorphisms in the serotonin receptor 7 (HTR7) gene are associated with cortisol levels in African Americans young adults. Cortisol measurements were determined in Mic-Gri/DL and recorded as a numerical value or the three values as follows: -9, indicate participants in which no cortisol was measurable in the participant; NA, indicate participants in which blood samples were not taken; √, were taken as case values for analysis. NA for sex and age of participant indicate the participant did not wish to specify. Within SNP genotypes, NA indicates that no genotype was determined. (doi, 10.5256/f1000research.10442.d146883; Swanson et al., 2016a).

Dataset 2. Haplotype frequencies for five SNPs in HTR7 in African Americans. SNP order is as follows: rs2420367, rs12412496, rs2187506, rs7089533, rs7093602. (doi, 10.5256/f1000research.10442.d146884; Swanson et al., 2016b).

Author contributions
MA conceived the project and designed the experiments. GS, SM, and AA carried out the experiments. GS performed the statistical analysis and prepared the manuscript. MA, CL, GD, FS, and BW were involved in the revision of the manuscript. All authors agreed to the final content.

Competing interests
No competing interests were disclosed.

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References

Abdoh M, Albert PR, Drobskay E, et al.: 5-HT<sub>2A</sub>-mediated promotion of mitogen-activated T and B cell survival and proliferation is associated with increased translocation of NF-kappaB to the nucleus. Brain Behav Immun. 2004; 18(1): 24–34. Published Abstract | Publisher Full Text

Ahem GP: 5-HT and the immune system. Curr Opin Pharmacol. 2011; 11(1): 29–33. Published Abstract | Publisher Full Text | Free Full Text

Anagnostis P, Athyros VG, Tsialos K, et al.: Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. J Clin Endocrinol Metab. 2009; 94(8): 2692–2701. Published Abstract | Publisher Full Text

Berrita L, Cossu M, Marchini M, et al.: A polymorphism in the human serotonin 5-HT<sub>2A</sub> receptor gene may protect against systemic sclerosis by reducing platelet aggregation. Arthritis Res Ther. 2008; 10(5): R103. Published Abstract | Publisher Full Text | Free Full Text

Black PH: The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. Brain Behav Immun. 2003; 17(5): 350–364. Published Abstract | Publisher Full Text

Buske-Kirschbaum A, von Auer K, Krieger S, et al.: Blunted cortisol responses to psychosocial stress in asthmatic children: a general feature of atopic disease? Psychosom Med. 2003; 65(5): 806–810. Published Abstract | Publisher Full Text

Cavigelli SA, Chaudhry HS: Social status, glucocorticoids, immune function, and health: can animal studies help us understand human socioecononimic-status-related health disparities? Horm Behav. 2012; 62(3): 295–313. Published Abstract | Publisher Full Text

de Klerx ER, Maël M, Holboer F: Stress and the brain: from adaptation to disease. Nat Rev Neurosci. 2005; 6(9): 463–475. Published Abstract | Publisher Full Text

Deming SL, Lu W, Beeghly-Fadiel A, et al.: Melatonin pathway genes and breast cancer risk among Chinese women. Breast Cancer Res Treat. 2012; 132(2): 693–699. Published Abstract | Publisher Full Text | Free Full Text

Dürk T, Panther E, Müller T, et al.: 5-Hydroxytryptamine modulates cytokine and chemokine production in LPS-primed human monocytes via stimulation of different 5-HT<sub>4</sub> subtypes. Int Immunol. 2005; 17(5): 599–606. Published Abstract | Publisher Full Text

Ehret U, Gaa B, Heinrichs M: Psychoneuroendocrinological contributions to the etiopathology of depression, posttraumatic stress disorder, and stress-related bodily disorders: the role of the hypothalamus-pituitary-adrenal axis. Biol Psychol. 2001; 57(1–3): 141–152. Published Abstract | Publisher Full Text

Elenkov IJ: Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. Neurochem Int. 2008; 52(1–2): 40–51. Published Abstract | Publisher Full Text

Maeda et al., 2010; Murali et al., 2007; Pepys & Hirschfield, 2003)
Feder A, Nestler EJ, Charney DS: Psychobiology and molecular genetics of resilience. Nat Rev Neurosci. 2009; 10(6): 446–457.
PubMed Abstract | Publisher Full Text | Free Full Text

Fries E, Hesse J, Heilhammer J, et al.: A new view on hypocortisolism. Psychoneuroendocrinology. 2005; 30(10): 1010–1016.
PubMed Abstract | Publisher Full Text

Gillespie CF, Phifer J, Bradley B, et al.: Risk and resilience: genetic and environmental influences on development of the stress response. Depress Anxiety. 2009; 26(11): 984–992.
PubMed Abstract | Publisher Full Text | Free Full Text

Guscott M, Bristow L, Hadingham K, et al.: Genetic knockout and pharmacological blockade studies of the 5-HT2C receptors suggest therapeutic potential in depression. Neuropharmacology. 2005; 48(4): 492–502.
PubMed Abstract | Publisher Full Text

Heim C, Ehlert U, Hanker JP, et al.: Psychological and endocrine correlates of chronic pelvic pain associated with adhesions. J Psychosom Obstet Gynaecol. 1999; 20(1): 11–20.
PubMed Abstract | Publisher Full Text

Holmes A: Genetic variation in cortico-amygdala serotonin function and risk for stress-related disease. Neurosci Biobehav Rev. 2008; 32(7): 1293–1314.
PubMed Abstract | Publisher Full Text | Free Full Text

Hornung JP: The human raphe nuclei and the serotonergic system. J Chem Neuroanat. 2003; 26(4): 331–343.
PubMed Abstract | Publisher Full Text

Howren MB, Lamkin DM, Suls J: Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med. 2009; 71(2): 171–186.
PubMed Abstract | Publisher Full Text

Idzko M, Panther E, Stratz C, et al.: The serotoninergic receptors of human dendritic cells: identification and coupling to cytokine release. J Immunol. 2004; 172(10): 6011–6019.
PubMed Abstract | Publisher Full Text

Ikeda M, Iwata N, Kitajima T, et al.: Positive association of the serotonin 5-HT2 receptor gene with schizophrenia in a Japanese population. Neuropsychopharmacology. 2006; 31(4): 866–871.
PubMed Abstract | Publisher Full Text

Kim TH, An SH, Cha JY, et al.: Association of 5-hydroxytryptamine (serotonin) receptor 4 (5-HTR4) gene polymorphisms with asthma. Respir Res. 2011; 12(4): 630–638.
PubMed Abstract | Publisher Full Text

Kring SI, Werge T, Holst C, et al.: Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. PLoS One. 2009; 4(6): e5696.
PubMed Abstract | Publisher Full Text | Free Full Text

Kunz-Ebrecht SR, Mohamed-Alli V, Feldman PJ, et al.: Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. Brain Behav Immun. 2003; 17(5): 373–383.
PubMed Abstract | Publisher Full Text

Lucas S, Ising M, Horstmann S, et al.: HTR2A gene variation is involved in antidepressant treatment response. Eur Neuropsychopharmacol. 2010; 20(1): 65–68.
PubMed Abstract | Publisher Full Text

Lundberg U: Stress hormones in health and illness: the roles of work and gender. Psychoneuroendocrinology. 2005; 30(10): 1017–1021.
PubMed Abstract | Publisher Full Text

Mahé C, Loetscher E, Dev KK, et al.: Serotonin 5-HT2 receptors coupled to induction of interleukin-6 in human microglial MC-3 cells. Neuropsychopharmacology. 2005; 49(1): 40–47.
PubMed Abstract | Publisher Full Text

Manservisi L, Schapa L, van Schoor NM, et al.: High long-term cortisol levels, measured in scalp hair, are associated with a history of cardiovascular disease. J Clin Endocrinol Metab. 2013; 98(5): 2078–2083.
PubMed Abstract | Publisher Full Text

McEwen BS: Protection and damage from acute and chronic stress: allostatic and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Ann N Y Acad Sci. 2004; 1032: 1–7.
PubMed Abstract | Publisher Full Text

Muller T, Durt K, Blumenthal B, et al.: 5-hydroxytryptamine modulates migration, cytokine and chemokine release and T-cell priming capacity of dendritic cells in vitro and in vivo. PLoS One. 2009; 4(7): e6453.
PubMed Abstract | Publisher Full Text | Free Full Text

Murrai R, Hanson MD, Chen E: Psychological stress and its relationship to cytokines and inflammatory diseases. Cytokines, Stress and Immunity. 2007; 29–49.
Reference Source

O’Connor TM, O’Halloran DJ, Shanahan F: The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melanocholia. QJM. 2000; 93(6): 323–333.
PubMed Abstract | Publisher Full Text

Pepys MB, Hirschfield GM: C-reactive protein: a critical update. J Clin Invest. 2003; 111(12): 1805–1812.
PubMed Abstract | Publisher Full Text | Free Full Text

Srir O, Hesselberg E, Amundrud P, et al.: Genetic variation in the serotonin receptor gene affects immune responses in rheumatoid arthritis. Genes Immun. 2015; 16(2): 83–89.
PubMed Abstract | Publisher Full Text | Free Full Text

Staub RH, Schulz A, Mullington J, et al.: The endotoxin-induced increase of cytokines is followed by an increase of cortisol relative to dehydroepiandrosterone (DHEA) in healthy male subjects. J Endocrinol. 2002; 175(2): 467–474.
PubMed Abstract | Publisher Full Text

Swanson G, Miller S, Aiyahyawi A, et al.: Dataset 1 in: Genetic polymorphisms in the serotonin receptor 7 (HTR7) gene are associated with cortisol levels in African Americans young adults. F1000Research. 2016a.
Data Source

Swanson G, Miller S, Aiyahyawi A, et al.: Dataset 2 in: Genetic polymorphisms in the serotonin receptor 7 (HTR7) gene are associated with cortisol levels in African Americans young adults. F1000Research. 2016b.
Data Source

Tse WS, Bond AJ: The impact of depression on social skills. J Nerv Ment Dis. 2004; 192(4): 260–268.
PubMed Abstract | Publisher Full Text

Webster Marketon JI, Glaser R: Stress hormones and immune function. Cell Immunol. 2008; 252(1-2): 16–26.
PubMed Abstract | Publisher Full Text

Publisher Full Text

Ziozuro M, Manz N, Rangaswamy M, et al.: Genome-wide association study of theta band event-related oscillations identifies serotonin receptor gene HTR7 influencing risk of alcohol dependence. Am J Med Genet B Neuropsychiatr Genet. 2011; 156B(1): 44–58.
PubMed Abstract | Publisher Full Text | Free Full Text