Inter-ethnic differences in lymphocyte sensitivity to glucocorticoids reflect variation in transcriptional response

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Glucocorticoids (GCs) are steroid hormones widely used as pharmaceutical interventions, which act mainly by regulating gene expression levels. A large fraction of patients (~ 30%), especially those of African descent, show a weak response to treatment. To interrogate the contribution of variable transcriptional response to inter-ethnic differences, we measured in vitro lymphocyte GC sensitivity (LGS) and transcriptome-wide response to GCs in peripheral blood mononuclear cells from African–American (AA) and European–American (EA) healthy donors. We found that transcriptional response after 8 h treatment was significantly correlated with variation in LGS within and between populations. We found that \( \text{NFKB1} \), a gene previously found to predict LGS within populations, was more strongly downregulated in EAs on average. \( \text{NFKB1} \) could not completely explain population differences, however, and we found an additional 177 genes with population differences in the average log2 fold change (false discovery rate < 0.05), most of which also showed a weaker transcriptional response in AAs. These results suggest that inter-ethnic differences in GC sensitivity reflect variation in transcriptional response at many genes, including regulators with large effects (for example, \( \text{NFKB1} \)) and numerous other genes with smaller effects.

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Introduction

Glucocorticoids (GCs) are steroid hormones that mediate physiological responses to the environment. Because of their potent anti-inflammatory properties, GCs are widely used as therapeutic agents. For example, GCs are the most commonly prescribed asthma controller medication1–3 and are commonly used in the treatment of inflammatory bowel syndrome, rheumatoid arthritis and other autoimmune diseases. GCs are also effective in the treatment of lymphoid malignancies, such as acute lymphoblastic leukemia.4 Although GCs are among the most successful drugs in history,5 there is large inter-individual variability in response to GC therapy,6,7 with ~ 30% of patients showing no response to treatment.8–10 The proportion of non-responders is similar across diseases,11 suggesting that GC resistance is an intrinsic property of the general population.

GC insensitivity is more common among individuals of African descent. For example, unresponsiveness to GC treatment is more common among African–American (AA) asthma patients compared with European–American (EA)
patients. Additionally, incidence of GC-induced side effects is significantly lower in acute lymphoblastic leukemia patients of African vs European descent. Characterizing the causes of variable GC sensitivity could aid in the development of treatment protocols that maximize efficacy while minimizing side effects across individuals and ethnic groups. Many potential explanations for higher rates of while minimizing side effects across individuals and ethnic groups. One explanation is that genetic differences contribute to inter-ethnic differences in GC sensitivity. To investigate this, we measured in vitro LGS and transcriptome-wide response to GCs in PBMCs from AA and EA healthy donors. We found that variation in transcriptional response to GCs is strongly correlated with LGS and tends to be weaker in AA donors, consistent with clinical and in vitro observations. Furthermore, we found that the transcriptional contribution to ethnic differences in GC sensitivity involves the response at few genes with major effects as well as many genes with smaller effects.

Materials and methods

Samples
Peripheral blood (100 ml) from each subject was obtained from Research Blood Components (http://researchblood-components.com/). All subjects were healthy donors and were not on any medication. Most samples were collected in the morning (0800 hours to 1200 hours) and time was recorded for use in subsequent analyses. We also recorded self-reported ethnicity, age, gender, ABO and Rh blood types and date and time of blood drawing. After quality control at Research Blood Components, whole blood was shipped overnight at 4 °C to the Human Immunological Monitoring Facility at the University of Chicago within 1 day of being drawn.

Practical considerations made it infeasible to process large numbers of samples in parallel. Therefore, we processed the samples in multiple successive batches. Batch number was recorded and used as a covariate in subsequent analyses. All conditions were kept as constant as possible across batches to minimize technical effects.

Cortisol levels were measured in the plasma of 12 of the samples (those used for transcriptional profiling) at the Clinical Chemistry Laboratory of the University of Chicago Medical Center using a standard immunoassay (Cat no. 11875116160, Roche Diagnostics Corporation, Indianapolis, IN, USA). Plasma samples were taken from the same blood draw used to isolate PBMCs. Measurements were taken in μg dl⁻¹.

Cell culturing and treatment
PBMCs were isolated from heparin-treated whole blood through a standard Ficoll protocol at the Human Immunological Monitoring Facility at the University of Chicago. PBMCs were washed in PBS and then transferred to RPMI supplemented with 10% charcoal-stripped fetal bovine serum. Each sample was then divided into one aliquot of 9 × 10⁶ cells for genome-wide transcriptional profiling and one aliquot of 1.8 × 10⁶ cells for measuring LGS. PBMCs were seeded at 1 × 10⁶ cells ml⁻¹ for all experiments.

Measuring LGS
LGS measurement was performed at the Human Immunological Monitoring Facility at the University of Chicago.
PBMCs from each donor were grown in 96-well plates with \(2 \times 10^6\) cells per well. For each donor, three replicates of each of the following treatments were performed: 0.5 \(\mu\)M Dex + 2.5 \(\mu\)g ml\(^{-1}\) PHA, EtOH + 2.5 \(\mu\)g ml\(^{-1}\) PHA and no treatment (blank). After 48 h of treatment, cell proliferation was measured by \(H^3\)-thymidine incorporation using standard protocols. Briefly, \(H^3\)-thymidine was added for the last 6 h of the 48 h treatment period. Afterward, PBMCs were harvested onto glass-fibre filter paper and radiolabel was counted in a \(\beta\)-spectrometer in units of counts per minute (cpm). The median value was taken from across the three replicates. On the 96-well plates used for these measurements, each column corresponded to a single treatment and each row to a single donor, with each row including all treatment conditions in triplicate for one donor. Position on the plate could introduce technical effects on cpm readings. To avoid these effects, we took the median of three different columns for each treatment (replicates). In addition, we used principal component analysis (PCA) to estimate and correct for the effect of row on cpm readings. Specifically, we used the loading on the first PC of the cpm values, which were rank transformed within columns to eliminate differences between treatments; then, we estimated the effect of being in a given row on overall cpm readings across treatments and corrected for this estimated row effect. \(\%d\) was calculated as \(1 - [\text{(proliferation in Dex + PHA)/(proliferation in EtOH + PHA)}]\) and fit to a normal distribution to avoid spurious results owing to outliers (mean and variance set equal to that observed in raw data). Simple linear regression was used to test for association between covariates and \(\%d\). Population differences in \(\%d\) were assessed using a one-tailed t-test.

**Transcriptional response profiling**

PBMCs from each donor were grown in 24-well plates with \(10^6\) cells per well. As for LGS, the following treatments were performed in three replicates per donor: 0.5 \(\mu\)M Dex + 2.5 \(\mu\)g ml\(^{-1}\) PHA and EtOH + 2.5 \(\mu\)g ml\(^{-1}\) PHA. Replicates were pooled and total RNA was extracted from each pool using the QiAgen RNeasy Plus mini kit. RNA was extracted from all 48 samples in 1 day. Total RNA was then reverse transcribed into cDNA, labeled, hybridized to Illumina (San Diego, CA, USA) HumanHT-12 v3 Expression BeadChips and scanned at the Southern California Genotyping Consortium (SCGC: http://scgc.genetics.ucla.edu/) at the University of California in Los Angeles. To avoid batch effects on RNA measurements, all microarrays were hybridized on the same day. Summary data (for example, mean intensity of each probe across within-array replicates) were obtained using the BeadStudio software (Illumina) at the SCGC. The microarray data have been deposited in the Gene Expression Omnibus (GEO), www.ncbi.nlm.nih.gov/geo, under accession number GSE33649.

Low-level microarray analysis was performed using the Bioconductor software package LUMI\(^\text{35}\) in R (http://www.r-project.org). Probes were annotated by mapping to the RNA sequences from RefSeq using BLAT. To avoid ambiguity in the source of a signal due to cross-hybridization of similar RNA species, probes that mapped to multiple genes were discarded (3879 of the 47,321 probes on the array). Probes that contained one or more HapMap SNPs were also discarded to avoid spurious associations between expression measurements and ethnicity owing to inter-ethnic differences in allele frequencies (1714 probes). We applied variance stabilizing transformation\(^\text{33}\) to all arrays, discarded probes with intensities indistinguishable from background fluorescence levels in all samples and performed quantile normalization across all arrays. After applying all these filters, 12,744 probes were used for downstream analysis.

**Measuring PBMC composition with flow cytometry**

We thawed aliquots from all 18 donors and cultured them in a single 96-well plate with PHA and EtOH for 8 h. We stained PBMCs with anti-CD3-PE-Cy7 (to mark T cells, BD 560910), anti-CD14-FITC (to mark monocytes, NC0088365), anti-CD20-PE (to mark B cells, BDB555623), anti-CD4-PerCP-Cy5.5 (to mark T helper cells, BD 560650) and anti-CD8-APC (to mark cytotoxic T cells, CO IM2469). All antibodies were obtained from Fisher Scientific, Pittsburgh, PA, USA. We then used fluorescence-activated cell sorting to measure the proportion of each cell type in PBMCs from each donor, using a BD LSRFortessa instrument maintained by the Flow Cytometry Core at the University of Chicago.

**Identification of differentially expressed genes**

In order to identify genes that, on average across individuals, changed expression levels upon treatment with GCs, we used the Bioconductor package LIMMA\(^\text{34}\) in R to perform multiple linear regression at each gene with treatment as the variable of interest and with batch, population, age and gender as covariates. False discovery rates (FDR) were estimated using the \(Q\)-value function\(^\text{35}\) in R.

**PCA to summarize overall transcriptional responsiveness**

We separated our data into two matrices of log_2 fold changes, with each row representing a gene and each column an individual, representing each of the treatment durations we assayed (that is, 8 h and 24 h). We then used the prcomp function in R to perform PCA, separately for each time point, on the correlation matrix corresponding to each of these log_2 fold change matrices. We took the loadings on the first principal component as a summary of overall responsiveness.

**Comparing transcriptional response between populations**

To identify genes with population differences in log_2 fold change, we used the Bioconductor package LIMMA\(^\text{34}\) in R to fit a linear model at each gene with log_2 fold change regressed on population and log_2 fold change at NFKB1 as a covariate. FDRs were estimated using the \(Q\)-value function\(^\text{35}\) in R.

**Gene ontology analyses**

We used the online tool DAVID\(^\text{36,37}\) to identify biological categories enriched among differentially expressed genes, using all genes expressed in PBMCs (based on microarray data) as a background.
Results

AAs show less GC-mediated inhibition of lymphocyte proliferation

To characterize patterns of variation in GC sensitivity within and between populations, we measured in vitro LGS in 18 healthy donors, including 9 AAs and 9 EAs. Specifically, we measured cellular proliferation in PBMCs following a 48-h exposure to PHA in the presence of either dexamethasone (dex) or its vehicle (EtOH) as a control. Consistent with previous work\textsuperscript{11,23} we found that dex treatment markedly inhibited PHA-mediated proliferation (mean log\textsubscript{2} fold change $= -3.9$, $P = 4.8 \times 10^{-15}$), variation in percent inhibition ($\%I$) was significantly greater between individuals versus within individuals (66\% of total variance was between individuals, $P = 1.2 \times 10^{-14}$), ethnicity was a significant predictor of $\%I$ (explaining 24\% of the interindividual variance) and PBMCs from AA donors tended to be less sensitive (Figure 1, mean $\%I$ in EA $= 98.1$ and in AA $= 94.9$, $P = 0.018$). None of the covariates we tested (that is, age, gender, circulating cortisol levels, baseline GR transcript levels, basal PBMC proliferation levels and collection batch) were significantly correlated with $\%I$ ($P > 0.36$); this is consistent with previous results, except for age.\textsuperscript{11} As previously reported,\textsuperscript{23} we found no evidence of population differences in fold increase in proliferation in response to PHA ($P = 0.62$), suggesting that differences in LGS reflect differing cellular function in the presence of GCs, not severity of the initial response counteracted by GCs (here modeled by PHA treatment).

Dex alters transcription in PHA-activated PBMCs

To characterize the contribution of transcriptional response to variation in GC sensitivity, we profiled gene expression in the presence of either dex or vehicle, following 8 h and 24 h of treatment, in PHA-treated PBMCs from 12 of the same 18 healthy donors above, assayed in parallel for LGS. Similar to Hakonarson et al.\textsuperscript{23} we found a large number of DE genes (2245 and 3373 at 8 h and 24 h, respectively; FDR $< 0.01$). We did not observe systematic differences in RNA quantity between treatment conditions (Supplementary Figure 1), likely because the dex effects on proliferation are not detectable after only 8 h of treatment. Differentially expressed genes included those with large log\textsubscript{2} fold changes as well as genes with small, but consistent changes in expression (Figure 2). Many well-established targets of GC-mediated transcriptional regulation are differentially expressed, including glucocorticoid-induced leucine zipper (GILZ)\textsuperscript{38} and serum/glucocorticoid regulated kinase 1 (SGK1).\textsuperscript{39} We also found differentially expressed genes with clear roles in lymphocyte proliferation, such as TNFSF9, a ligand that promotes T-cell proliferation.\textsuperscript{40} Consistent with the suppressive effects of GCs on lymphocyte proliferation, we found that downregulated genes are enriched for various biological processes related to lymphocyte-mediated immune response (listed in Supplementary Table 1), such as ‘immune response’ ($P = 2 \times 10^{-22}$; FDR $= 7.8 \times 10^{-19}$) or ‘regulation of T-cell activation’ ($P = 5.9 \times 10^{-5}$; FDR $= 4.7 \times 10^{-5}$). In contrast, we found that upregulated genes only show significant enrichments for the biological processes ‘endocytosis’ ($P = 3.2 \times 10^{-6}$; FDR $= 0.012$) and ‘membrane invagination’ ($P = 5.9 \times 10^{-8}$; FDR $= 0.012$).

To explore the tissue specificity of transcriptional response to GCs, we compared these data to the results from similar studies in EBV-transformed B cells (LCLs)\textsuperscript{41} and in osteoblasts.\textsuperscript{42} We found a significant overlap between the genes differentially expressed in PBMCs and in LCLs (69\% of genes; $P = 3.4 \times 10^{-55}$). The incomplete overlap likely reflects: (1) the presence of diverse cell types in PBMCs, (2) the effects of EBV transformation on GC response in LCLs and (3) incomplete power to detect differentially expressed genes.\textsuperscript{43} We found a much lower, although still significant (31\% of genes; $P = 8.7 \times 10^{-10}$), overlap with genes differentially expressed in osteoblasts. Interestingly, this overlap is very similar to that observed between LCLs and osteoblasts (28\%), suggesting that a large number of GC targets are shared across different types of lymphocytes, but not shared with osteoblasts.

PBMCs are comprised of multiple cell types, which may have different transcriptional responses to GCs. Variation in measurements of transcriptional response in PBMCs may, therefore, reflect variation in cell proportions, potentially causing spurious correlations between transcriptional response and ethnicity or $\%I$. To guard against this possibility, we measured the proportions of three major cell types in PBMCs (monocytes, B cells and T cells), as well as two subtypes of T cells (T helper cells and cytotoxic T cells), in each donor. For monocyte, B cell and T-cell proportions, we found no significant differences between populations (Supplementary Figure 2) or significant associations with $\%I$ ($P > 0.2$). We did observe significant differences between ethnic groups for T helper cell ($P = 0.05$) and cytotoxic T-cell proportions ($P = 0.03$). These differences are unlikely to confound our downstream analysis, however, as proportions of these cell types are not significantly correlated with $\%I$ (Supplementary Figure 2, $P > 0.7$) nor with log\textsubscript{2} fold change.
at any genes (FDR > 0.8). These results suggest that cell type heterogeneity is not likely to affect measurements of GC response in PBMCs.

Variation in early transcriptional response to dex is associated with LGS

We then sought to interrogate the relationship between transcriptional response to GCs and LGS. To obtain a summary of overall transcriptional response for each donor, we applied PCA to log2 fold changes in expression across all expressed genes at each time point. PCA has been shown to be an effective approach for identifying key explanatory variables from multi-dimensional datasets such as measurements of expression at many genes.44 We found that the loading on the first principal component at 8 h (that is, the value for each donor of the summary variable obtained through PCA, termed PC1₈₈, which explains 37% of the variance in log2 fold change across genes, was significantly correlated with %I across individuals (Figure 3a, $r^2 = 0.68$, $P = 1.7 \times 10^{-3}$). We also found that PC1₈₈ differs significantly between populations (Figure 3b, $P = 4.6 \times 10^{-6}$), raising the possibility that the correlation between PC1₈₈ and %I simply reflected population differences in both variables. However, when we corrected PC1₈₈ and %I for population, we found that the correlation was still significant ($r^2 = 0.36$, $P = 0.039$), despite a relatively small sample size. This implies that transcriptional response is correlated with inter-individual variability in LGS both within and between populations. Interestingly, the loading on the first principal component at 24 h (PC1₂₄₈) was not significantly correlated with %I ($r^2 = 0.23$, $P = 0.12$), suggesting that variation in GC sensitivity depends mostly on early events in the transcriptional response cascade.

Stronger suppression of NFKB1 is associated with greater sensitivity to GCs

Hakonarson et al.31 previously identified a set of 15 genes whose transcriptional response predicted LGS with high confidence in an Icelandic population sample. We tested these genes in our data, initially restricting our analysis to log2 fold change at 8 h, because overall transcriptional

Figure 2 (a) Volcano plots show the mean log₂ fold change (dex/vehicle) and corresponding evidence of differential expression (-log₁₀ P-value) for each gene (represented by a single point). Plots for response after 8 h and 24 h are shown separately. Transcriptional response is widespread, includes both up and downregulated genes and increases in intensity with duration of treatment. (b) Boxplots show examples of genes with large responses to dex treatment, the well characterized GC receptor (GR) target glucocorticoid-induced leucine zipper (GILZ, log₂ fold change = 2.8, $P = 1.4 \times 10^{-15}$) and the anti-apoptotic TNF-receptor TNFRSF4 (log₂ fold change = -1.6, $P = 2.6 \times 10^{-12}$). Dots in panel a corresponding to these genes are colored in blue. (c) Boxplots show examples of genes with small, but consistent responses to dex treatment, the mitogen-induced gene ERRF1 (log₂ fold change = -0.10, $P = 4.4 \times 10^{-3}$) and the apoptosis-associated RAD51 family member RAD51B (log₂ fold change = 0.096, $P = 8.4 \times 10^{-3}$). Dots in panel a corresponding to these genes are colored in red. (d) A Venn diagram shows the overlap between differentially expressed genes in peripheral blood mononuclear cells (PBMCs), EBV-transformed B cells (LCLs) and osteoblasts at an false discovery rates (FDR) < 0.01. Counts only reflect genes expressed in all three tissues.

Figure 3 (a) %I is plotted against loadings on PC1 at 8 h. African-American (AAs) are shown as open circles and European-American (EAs) are shown as closed circles. Transcriptional response (PC1) is correlated with lymphocyte GC sensitivity (LGS) (%I) at 8 h ($r^2 = 0.68$, $P = 1.7 \times 10^{-3}$), even after correcting for population ($P = 0.039$). (b) Boxplot shows population differences in PC1₈₈ ($P = 4.6 \times 10^{-6}$).
responsiveness at this time point was more strongly correlated with LGS. We found that log2 fold change at only one of these genes, NFKB1, was significantly correlated with %I ($\rho^2 = 0.45$, $P = 0.02$). Interestingly, Hakonarson et al. also found this gene to be the most accurate predictor of LGS in their data (81.25% accuracy). We found that NFKB1 is significantly downregulated by dex (mean log2 fold change = −0.5, $P = 5.8 \times 10^{-7}$, FDR < 0.01), and that individuals with more dramatic downregulation show greater LGS (see Figure 4a, $\rho = −0.67$). We also found that NFKB1 tends to be more markedly negative in EAs (Figure 4b, $P = 0.09$) and is significantly inversely correlated with %I within populations ($\rho^2 = 0.39$, $P = 0.029$). Log2 fold change at NFKB1 showed a slightly stronger negative correlation with %I in AAs, potentially reflecting a stronger effect of this gene on GC-mediated inhibition of proliferation in AAs ($p$ in AAs = −0.77 vs $\rho$ in AAs = −0.49); however, this difference is not statistically significant (95% CI for $\rho$ in AAs = (−0.97, 0.1) and CI for $\rho$ in AAs = (−0.93, 0.54)). These results are consistent with the molecular biology of lymphocyte response to GCs as NFKB1 codes for a subunit of NFkB, which is a transcriptional activator and a key positive regulator of inflammatory responses. In support of the hypothesis that transcriptional response at NFKB1 in turn affects GC response at other genes, we found that NFKB1 log2 fold change was significantly associated with log2 fold change at 110 genes at 8h (FDR < 0.05) and 133 genes at 24h (FDR < 0.05). (This is discussed in greater depth in Supplementary Text 1).

Many genes differ in transcriptional response between populations
Population differences at NFKB1 are unlikely to completely explain the tendency for lower LGS in AAs. Using the estimated effect of NFKB1 log2 fold change in expression on %I within populations (increase in %I of 9.1 per twofold decrease in NFKB1 transcript levels), we find that the average difference in log2 fold change between populations at this gene explains only 37.5% of the difference in LGS between AAs and EAs (expected difference based on NFKB1 = −1.2%, observed difference = −3.2%). We, therefore, sought to identify additional genes that differed in transcriptional response between populations. Correcting for variation in NFKB1 levels, we found population differences (FDR < 0.05) in the average log2 fold change in expression at an additional 177 genes after 8h treatment. These genes were significantly enriched only for the ‘immune response’ gene ontology category ($P = 7.9 \times 10^{-6}$, FDR = 9.3 \times 10^{-3}$). A number of these genes have clear relevance for lymphocyte-mediated immune response, including genes that encode inflammatory molecules (for example, CCL22) genes that encode regulators of the inflammatory response in lymphocytes (for example, TNFAIP3), and genes known to directly regulate cell growth (for example, CDKN1B).

A trend toward weaker transcriptional response in AAs
As an independent validation of the transcriptional contribution to inter-ethnic differences in GC sensitivity, we then asked if the direction of population differences in transcriptional response was consistent with the direction observed in clinical and in vitro studies. We found that transcriptional response was generally weaker in PBMCs from AAs. Specifically, we found that population differences at 8h were significantly more likely to reflect a stronger response in EAs: of 177 genes with significant inter-ethnic differences, 112 had higher absolute log2 fold change in EA ($P = 5.1 \times 10^{-4}$). We also found that population differences that followed this pattern tended to be of significantly larger magnitude (Figure 5a, median differences in absolute log2 fold change for genes with weaker response in AA = −0.19 vs median for genes with stronger response in AA = 0.11, $P = 3.3 \times 10^{-5}$). For example, GIMAP5, which encodes a positive regulator of lymphocyte proliferation, is downregulated in EAs but not, on average, differentially expressed in AAs (see Figure 5b). The tendency...
for weaker response in AAs across these genes could reflect a TF with differing regulatory activity across populations (for example, NFkB). To identify such a factor, we used the Molecular Signatures Database to test for an enrichment of TF motifs among the 112 genes with weaker response in AAs and found no significant enrichments.

Discussion

There are many potential mechanisms for the observed inter-ethnic differences in LGS, including genomic and non-genomic effects. Here, we provide the first evidence that differences in GC-mediated changes in gene expression contribute to lower average sensitivity to GCs in AAs in an assay known to predict clinical response.\(^{11,13-22}\) We found that both LGS and overall transcriptional response differed significantly between donors from different ethnic groups, and that LGS was significantly correlated with overall transcriptional responsiveness within populations. Our results indicate that variation in GC sensitivity between populations depends on variable transcriptional response to GCs. Providing greater insights into the molecular mechanisms of variable GC sensitivity, we found that LGS within populations was correlated with the magnitude of transcriptional repression at NFKB1, a gene that encodes a subunit of the transcriptional activator NFkB and that was previously reported to be predictive of GC sensitivity.\(^{30}\) Furthermore, we found that AA individuals tended to show less NFKB1 repression, consistent with a general tendency for less GC sensitivity. The tendency for weaker repression of NFKB1 in AAs could reflect a regulatory variant with differences in allele frequency between these populations. We previously found an eQTL for NFKB1 in LCLs (rs17032603, posterior probability = 0.81), that showed some allelic differentiation between the Yoruba (a West African population, C allele frequency = 0.73) and the CEPH (an American population of Northern European ancestry, C allele frequency = 0.92). Although intriguing, we do not know if this eQTL affects the transcriptional response to GCs in PHA-stimulated PBMCs. We estimated that differences between populations at NFKB1 were of limited magnitude, however, and could not explain all of the observed differences in LGS. When we corrected for log\(_2\) fold change at NFKB1, we found that a large number of additional genes showed population differences in transcriptional response. The tendency for weaker response in AAs across these genes could reflect a TF with differing regulatory activity across populations (for example, NFkB). We found no significant enrichment for TF motifs (P > 0.05), suggesting independent differences between populations in the regulation of these 112 genes (for example, cis-regulatory polymorphisms with different allele frequencies across populations). Overall, our results suggest that lower GC sensitivity in individuals of African ancestry reflects weaker transcriptional response at a large number of genes. This likely includes a combination of regulatory proteins with large effects on GC sensitivity, such as NFKB1, as well as numerous other genes with smaller effects.

Although a variety of environmental or disease-related factors may contribute to variation in GC sensitivity, there is a strong evidence for a genetic contribution.\(^{7,49}\) Direct estimates of the heritability of patient response to GC treatment (for glaucoma) are between 0.17 and 0.37.\(^{50}\) Indeed, several genetic polymorphisms have been implicated in variable response to GCs, including those in the genes coding for the GR\(^{51-53}\) (NR3C1), for adaptor and chaperon proteins that regulate GR-mediated signaling,\(^{54}\) and for the corticotropin-releasing hormone receptor,\(^{55}\) which is a regulator of endogenous GC synthesis. However, these variants tend to have extreme effects and explain only a small fraction of the inter-individual variance in response to GC therapy.\(^{7}\) Instead, cis-regulatory polymorphisms at individual GC target genes, that is, downstream of GR activation, could make a major contribution to variation in GC response, especially given the large number of direct and indirect GR targets. We previously showed that cis-regulatory polymorphisms contribute to variation within and between populations in GC transcriptional response in a cell line system.\(^{41}\) Therefore, the population differences in transcriptional response we observed here could, in part, reflect differences in allele frequency at cis-regulatory polymorphisms. Under this model, lower GC sensitivity would reflect higher frequency of the allele associated with lower responsiveness in AAs across many cis-regulatory polymorphisms. However, inter-ethnic differences in transcriptional response, and LGS, could also reflect a number of non-genetic factors. Importantly, Gould et al.\(^{14}\) found no significant correlation between proportion of African ancestry and clinical response to inhaled corticosteroids in asthma patients, whereas baseline bronchodilator responsiveness explained much of the variation in GC response in their sample. Although population differences in LGS among healthy volunteers suggest that variation in disease status does not completely account for inter-ethnic differences in patients, they do not necessarily imply a genetic origin. For example, previous studies have found that environmental factors, such as social isolation\(^{56}\) and fatigue,\(^{57}\) are correlated with changes in the expression of genes involved in inflammatory responses and of genes with nearby GR-binding elements; and that this relationship is not because of differing levels of circulating cortisol. Further work is needed to directly interrogate the role of genetics, such as testing for an association between GC response (LGS and/or transcriptional response) and the proportion of African ancestry in AAs (using a similar approach to\(^{58}\)).

Consistent with clinical and in vitro observations, we found an excess of genes with weaker response in AA among those that differed across populations. Interestingly, we found the opposite pattern (a tendency for stronger response to GC treatment in individuals of West African ancestry) in a previous study that compared transcriptional response in LCLs between populations.\(^{41}\) Furthermore, we found very little overlap between studies, with only six genes showing significant inter-ethnic differences in both cell types. Although a different set of populations were analyzed in LCLs, namely the Yoruba (Nigeria) and the
Toscani (Italy), these populations are closely related to the ancestral populations of those used in the current study (EAs and AAs). Although this discrepancy could reflect the differences in the genetic make up of the populations analyzed in the two studies (for example, different average patterns of response in AAs compared with Yoruba following admixture due to epistasis), we find this explanation unlikely. The discrepancy between these studies could also reflect artifacts of EBV transformation. To test this hypothesis, we compared the genes with population differences in response only in LCLs to a list of genes previously shown to change in expression following EBV transformation of B cells. We found no significant overlap between these gene sets (the proportion of genes with changes in expression after EBV transformation was 65.3% for genes with population differences only in LCLs vs 60.7% for all expressed genes, \( P = 0.19 \)), suggesting that EBV transformation does not explain the discrepancy between studies. Alternatively, differences between the results of these studies could simply reflect the differing experimental systems. For example, we used PHA-stimulated lymphocytes in the present study and virus-infected lymphocytes in the previous study. West African ancestry could be correlated with stronger GC modulation of the intra-cellular response to viral infection and also be correlated with weaker GC suppression of a lymphocyte-mediated immune response (modeled by PHA). In support of this explanation, we found that genes with population differences in LCLs were enriched for genes in the GO category ‘regulation of viral reproduction’ (\( P = 2.1 \times 10^{-4} \), FDR<0.083) whereas genes with population differences in PBMCs were not. Cell-type specificity could also have a role. B cells were used in the previous study while PBMCs, which are largely comprised of T lymphocytes (data not shown), were interrogated in the present study.

We found significant inter-ethnic differences in the in vitro cellular response to GCs, with no evidence of ethnic differences in PHA response (that is, mitogen-activated T-cell proliferation). Although other factors are likely to also contribute (for example, socioeconomic status), together with similar results in a previous study, our findings suggest that inter-ethnic differences in patient response to GC treatment reflect variation in their intrinsic cellular sensitivity to GCs. We provide strong evidence that these inter-ethnic differences in cellular sensitivity reflect differing patterns of GC-mediated transcriptional response between populations. Specifically, we found that lower average GC sensitivity in AAs seems to reflect a general tendency for weaker transcriptional response in AAs at a small number of regulators with large effects (for example, NFKB1) and at many additional genes with smaller effects. This work provides important new insights into the molecular mechanisms that underlie inter-ethnic differences in sensitivity, and could aid future efforts to improve outcomes in AA patients treated with GCs. For example, following further testing, transcriptional response at these genes could be used as a diagnostic tool to identify GC insensitive patients before treatment. Furthermore, genes with population differences in transcriptional response are excellent candidates for future efforts to identify targets for pharmaceutical interventions tailored to GC-insensitive patients, especially those of African descent.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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