Published in final edited form as:

Pharmacogenomics J. 2013 April ; 13(2): 121–129. doi:10.1038/tpj.2011.55.

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

Joseph C. Maranville, BS[1], **Shaneen S. Baxter, MS**[1], **Jason M. Torres, BA**[1], and **Anna Di Rienzo, PhD**[1]

[1]Departments of Human Genetics. The University of Chicago, Chicago, IL, USA

Abstract

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 (PBMCs) from African-American and European-American healthy donors. We found that transcriptional response after 8hrs treatment was significantly correlated with variation in LGS within and between populations. We found that *NFKB1*, a gene previously found to predict LGS within populations, was more strongly down-regulated in European-Americans on average. *NFKB1* could not completely explain population differences, however, and we found an additional 177 genes with population differences in the average $\log_2$ fold change (FDR<0.05), most of which also showed a weaker transcriptional response in African-Americans. These results suggest that inter-ethnic differences in GC sensitivity reflect variation in transcriptional response at many genes, including regulators with large effects (e.g. *NFKB1*) and numerous other genes with smaller effects.

Keywords

regulatory variation; gene expression; glucocorticoids; ethnic disparities; asthma; NFκB

Introduction

Glucocorticoids (GCs) are steroid hormones that mediate physiological responses to the environment. Due to their potent anti-inflammatory properties, GCs are widely used as therapeutic agents. For example, GCs are the most commonly prescribed asthma controller medication (1–3) and are commonly used in the treatment of inflammatory bowel syndrome, rheumatoid arthritis and other autoimmune diseases. GCs are also effective in the treatment

Correspondence: Anna Di Rienzo, Department of Human Genetics, University of Chicago, 920 E. 58th Street, Rm.424, Chicago, IL, 60637. Phone: 773-834-1037, Fax: 773-834-0505, dirienzo@uchicago.edu.

Conflict of interest

The authors declare no conflict of interest.

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 approximately 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 to European-American (EA) patients (9). Additionally, incidence of GC-induced side effects is significantly lower in acute lymphoblastic leukemia patients of African versus European descent (12). 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 (13, 14). Many potential explanations for higher rates of GC insensitivity in AAs have been proposed, including differing disease severity, access to and quality of healthcare, socioeconomic status and genetic factors. However, the degree to which inter-ethnic differences in transcriptional response contribute to disparities in clinical response to GC is unknown.

Clinical responsiveness to GC therapy is poorly correlated with disease severity, but is significantly correlated with *in vitro* lymphocyte GC sensitivity (LGS). This correlation has been observed in patients with a wide range of diseases, including asthma (15–19), rheumatoid arthritis (20), systemic lupus erythematosous (21), ulcerative colitis (11), and renal transplant rejection (22). LGS is most commonly assessed by measuring GC inhibition of phytohemagglutinin (PHA)-induced proliferation of peripheral blood mononuclear cells (PBMCs). Percent inhibition at a high dose (e.g. I_{max} - a measure of efficacy) is a particularly accurate predictor of clinical response, although other metrics (e.g. IC_{50} - a measure of potency) are also predictive (11).

Consistent with clinical observations of inter-ethnic differences, Federico *et al* found that LGS was, on average, significantly lower in AA compared to EA asthma patients (23). Interestingly, no inter-ethnic differences were observed in basal activity (i.e. T cell proliferation in the absence of PHA) or in the proliferative response to PHA, implying that inter-ethnic differences in LGS are due to variation in the cellular response to GCs. Because the same was observed in healthy controls, it was concluded that variation in GC response is not correlated with disease status. These results are of particular interest with regard to the known 4-fold difference in asthma mortality and hospitalization rate for AA vs. EA children with asthma (24), a disparity that persists after controlling for known socioeconomic factors (25, 26).

Although GCs can affect target cells through a variety of mechanisms, including ‘non-genomic’ effects (e.g. direct disruption of cell membranes or interaction or modulation of T cell receptor activity (27)), they act primarily through the regulation of gene expression (28). GCs enter target cells through passive diffusion and bind the GC receptor (GR) in the cytoplasm, allowing it to be translocated into the nucleus where it acts as a transcriptional regulator. The activated GR, together with cooperating transcription factors (TFs), modulates transcription at target genes through direct DNA binding. The activated GR can

also modulate and counteract the activity of other TFs, such as the NFκB complex (29) or the STAT proteins (30), usually leading to repression of transcription. Variation in transcriptional regulation is, therefore, likely to contribute to the variable patient sensitivity. In support of a transcriptional basis for inter-individual variation in GC sensitivity, Hakonarson *et al* (2005) (31) found that changes in gene expression in response to GCs in activated PBMCs were predictive of GC sensitivity. This raises the possibility that variation in transcriptional response also contributes to inter-ethnic differences in GC sensitivity. To characterize the transcriptional basis of inter-ethnic variability in GC response, we measured *in vitro* lymphocyte GC sensitivity (LGS) and transcriptome-wide response to GCs in PBMCs from AA and EA healthy donors. We find 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.

Methods

Samples

Peripheral blood (100ml) from each subject was obtained from Research Blood Components (http://researchbloodcomponents.com/). All subjects were healthy donors and were not on any medication. Most samples were collected in the morning (8am–12noon) 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 a quality control at Research Blood Components, whole blood was shipped overnight at 4°C to the Human Immunologic 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# 11875116160, Roche Diagnostics Corporation, Indianapolis, IN). Plasma samples were taken from the same blood draw used to isolate PBMCs. Measurements were taken in μg/dL.

Cell culturing and treatment

PBMCs were isolated from heparin-treated whole blood through a standard Ficoll protocol at the Human Immunologic 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 9×10^6 cells for genome-wide transcriptional profiling and one aliquot of 1.8×10^6 cells for measuring LGS. PBMCs were seeded at 1×10^6 cells/mL for all experiments.

Measuring LGS

LGS measurement was performed at the Human Immunologic Monitoring Facility at the University of Chicago. PBMCs from each donor were grown in 96 well plates with 2×10^5 cells per well. For each donor, three replicates of each of the following treatments were performed: 0.5μM Dex+2.5μg/ml PHA, EtOH+2.5μg/ml PHA, and no treatment (blank). After 48h of treatment, cell proliferation was measured by H^3-thymidine incorporation using standard protocols. Briefly, H^3-thymidine was added for the last 6h of the 48h treatment period. Afterward, PBMCs were harvested onto glass-fibre filter paper and radiolabel was counted in a β-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. %I was calculated as 1-[(proliferation in Dex+PHA)/(proliferation in EtOH+PHA)] and fit to a normal distribution to avoid spurious results due 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 %I. Population differences in %I 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: 0.5μM Dex+2.5μg/ml PHA and EtOH+2.5μg/ml 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 one day. Total RNA was then reverse transcribed into cDNA, labeled, hybridized to Illumina HumanHT-12 v3 Expression BeadChips and scanned at the Southern California Genotyping Consortium (SCGC: http://scgc.genetics.ucla.edu/) at the University of California at Los Angeles. To avoid batch effects on RNA measurements, all microarrays were hybridized on the same day. Summary data (e.g. mean intensity of each probe across within-array replicates) were obtained using the BeadStudio software (Illumina) at the SCGC. The microarray data has 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 (32) 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 (3,879 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 due to inter-ethnic differences in allele frequencies (1,714

probes). We applied variance stabilizing transformation(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 8h. 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. We then used fluorescence-activated cell sorting (FACS) 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 (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 qvalue function (35) in R.

Principal component analysis 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 (i.e. 8h and 24h). We then used the prcomp function in R to perform principal component analysis, 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 (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 qvalue function (35) in R.

Gene ontology analyses

We used the online tool DAVID (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

African-Americans 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 48h exposure to PHA in the presence of either dexamethasone (dex) or its vehicle (EtOH) as a control. Consistent with previous work (11) (23), we found that: dex treatment markedly inhibited PHA-mediated proliferation (mean $\log_2$ fold change=−3.9, $p=4.8\times10^{-15}$), variation in percent inhibition (%I) was significantly greater between compared to within individuals (66% of total variance was between individuals, $p=1.2\times10^{-14}$), ethnicity was a significant predictor of %I (explaining 24% of the inter-individual 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 (i.e. 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 (11). As previously reported (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 8hrs and 24hrs 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 (2005) (31), we found a large number of DE genes (2,245 and 3,373 at 8hrs and 24hrs, 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 8h of treatment. Differentially expressed genes included those with large $\log_2$ 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*) (38) and serum/glucocorticoid regulated kinase 1 (*SGK1*) (39). We also found differentially expressed genes with clear roles in lymphocyte proliferation, such as *TNFSF9*, a ligand that promotes T cell proliferation (40). Consistent with the suppressive effects of GCs on lymphocyte proliferation, we found that down-regulated genes are enriched for various biological processes related to lymphocyte-mediated immune response (listed in Supplementary Table 1), such as “immune response” ($p=2\times10^{-22}$, $FDR=7.8\times10^{-19}$) or “regulation of T cell activation” ($p=5.9\times10^{-8}$, $FDR=4.7\times10^{-5}$). (In contrast, we found that up-regulated genes only show significant enrichments for the biological processes “endocytosis” ($p=3.2\times10^{-6}$, FDR=0.012) and “membrane invagination” ($p=5.9\times10^{-8}$, 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) (41) and in

osteoblasts (42). We found a significant overlap between the genes differentially expressed in PBMCs and in LCLs (69% of genes; $p=3.4\times10^{-55}$). 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 (43). We found a much lower, although still significant (31% of genes; $p=8.7\times10^{-10}$), 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 $\log_2$ fold change at any genes (FDR>0.8). These results suggest that cell type heterogeneity is not likely to affect to 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 principal component analysis to $\log_2$ fold changes in expression across all expressed genes at each time point. Principal component analysis (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 8h (i.e. the value for each donor of the summary variable obtained through PCA, termed $PC1_{8h}$), which explains 37% of the variance in $\log_2$ fold change across genes, was significantly correlated with %I across individuals (Figure 3a, $\rho^2=0.68$, $p=1.7\times10^{-3}$). We also found that $PC1_{8h}$ differs significantly between populations (Figure 3b, $p=4.6\times10^{-6}$), raising the possibility that the correlation between $PC1_{8h}$ and %I simply reflected population differences in both variables. However, when we corrected $PC1_{8h}$ and %I for population, we found that the correlation was still significant ($\rho^2=0.36$, $p=0.039$), 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 24h ($PC1_{24h}$) was not significantly correlated with %I ($\rho^2=0.23$, $p=0.12$), 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 $\log_2$ fold change at 8h, because overall transcriptional responsiveness at this time point was more strongly correlated with LGS. We found that $\log_2$ fold change at only one of these genes, *NFKB1*, was significantly correlated with %I (ρ^2=0.45, p=0.02). Interestingly, Hakonarson and colleagues also found this gene to be the most accurate predictor of LGS in their data (81.25% accuracy). We found that *NFKB1* is significantly down-regulated by dex (mean $\log_2$ fold change = −0.5, $p=5.8\times10^{-7}$, FDR<0.01), and that individuals with more dramatic down-regulation show greater LGS (see Figure 4a, ρ = −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 (ρ^2=0.39, p=0.029). Log_2 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 (ρ in AAs = −0.77 vs. ρ in AAs = −0.49); however, this difference is not statistically significant (95% CI for ρ in AAs = [−0.97,0.1] and CI for ρ 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 NFκB, 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* $\log_2$ fold change was significantly associated with $\log_2$ 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* $\log_2$ fold change in expression on %I within populations (increase in %I of 9.1 per 2-fold decrease in *NFKB1* transcript levels), we find that the average difference in $\log_2$ 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 $\log_2$ 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 ($p=7.9\times10^{-6}$, $\text{FDR}=9.3\times10^{-3}$). A number of these genes have clear relevance for lymphocyte-mediated immune response, including genes that encode inflammatory molecules (e.g. *CCL22* (45)) genes that encode regulators of the inflammatory response in lymphocytes (e.g. *TNFAIP3* (46)), and genes known to directly regulate cell growth, (*CDKN1B* (47)).

A trend toward weaker transcriptional response in African-Americans

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 population differences in response, 112 had higher absolute $\log_2$ fold change in EA ($p=5.1\times10^{-4}$). We also found that population differences that followed this pattern tended to be of significantly larger magnitude (Figure 5a, median differences in absolute log fold change for genes with weaker response in AA = −0.19 versus median for genes with stronger response in AA = 0.11, $p=3.3\times10^{-5}$). For example, *GIMAP5*, which encodes a positive regulator of lymphocyte proliferation (48), is down-regulated 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 transcription factor with differing regulatory activity across populations (e.g. NFκB). To identify such a factor, we used the Molecular Signatures Database to test for an enrichment of transcription factor 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 lymphocyte GC sensitivity, 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, 15–22). 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 NFκB 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 transcription factor with differing regulatory activity across populations (e.g. NFκB). We found no significant enrichment for transcription factor motifs ($p > 0.05$), suggesting independent differences between populations in the regulation of these 112 genes (e.g. 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.

While a variety of environmental or disease-related factors may contribute to variation in GC sensitivity, there is 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 (*CRHR1*) (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 variants at individual GC target genes, i.e. 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* 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 (56). While 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 (57) and fatigue (58), 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 due to 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 (59)).

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 (EBV-transformed B cells) between populations (41). Furthermore, we found very little overlap between studies, with only 6 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). While this discrepancy could reflect the differences in the genetic make up of the populations analyzed in the two studies (e.g. different average patterns of response in AAs compared to 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 (60). 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 versus 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” ($p=2.1\times10^{-4}$, FDR<0.083) while genes with population differences in PBMCs were not. Cell-type specificity could also play 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 (i.e. mitogen-activated T cell proliferation). Although other factors are likely to also contribute (e.g. socioeconomic status), together with similar results in a previous study (23), 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 (e.g. *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 prior to 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank T Gajewski and Y Zha at the Human Immunological Monitoring facility for helpful discussions about experimental protocols and study design. JCM was supported by an American Heart Association pre-doctoral fellowship (#11PRE4960001). This work was funded by the NIH Grants DK-56670 and DK-56670-S1 to AD. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

References

1. Verini M, Rossi N, Dalfino T, Verrotti A, Di Gioacchino M, Chiarelli F. Lack of correlation between clinical patterns of asthma and airway obstruction. Allergy Asthma Proc. 2001 Sep-Oct; 22(5):297–302. [PubMed: 11715220]
2. NHLBI. Highlights of the Expert Panel Report 2: Guidelines for Diagnosis and Management of Asthma. Bethesda, MD: NIH Publications; 1997. p. 50
3. Chung, KF.; O'Byrne, P. Pharmacological agents used to treat asthma. In: Fabbri, LM., editor. Asthma. Sheffield: European Respiratory Society Journals; 2003. p. 339-75.
4. Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. Lancet Oncol. Nov; 11(11): 1096–106. [PubMed: 20947430]
5. van der Laan S, Meijer OC. Pharmacology of glucocorticoids: beyond receptors. Eur J Pharmacol. 2008 May 13; 585(2–3):483–91. [PubMed: 18410913]
6. Szefler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. J Allergy Clin Immunol. 2002 Mar; 109(3):410–8. [PubMed: 11897984]
7. Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. Br Med Bull. 2000; 56(4):1054–70. [PubMed: 11359637]
8. Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Pineiro A, Wei LX, et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group. Ann Intern Med. 1999 Mar 16; 130(6):487–95. [PubMed: 10075616]
9. Chan MT, Leung DY, Szefler SJ, Spahn JD. Difficult-to-control asthma: clinical characteristics of steroid-insensitive asthma. J Allergy Clin Immunol. 1998 May; 101(5):594–601. [PubMed: 9600494]
10. Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. Am Rev Respir Dis. 1990 Dec; 142(6 Pt 1):1351–8. [PubMed: 2252253]
11. Hearing SD, Norman M, Smyth C, Foy C, Dayan CM. Wide variation in lymphocyte steroid sensitivity among healthy human volunteers. J Clin Endocrinol Metab. 1999 Nov; 84(11):4149–54. [PubMed: 10566664]
12. Mattano LA Jr, Sather HN, Trigg ME, Nachman JB. Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's Cancer Group. J Clin Oncol. 2000 Sep 15; 18(18):3262–72. [PubMed: 10986059]
13. Weiss ST, Lake SL, Silverman ES, Silverman EK, Richter B, Drazen JM, et al. Asthma steroid pharmacogenetics: a study strategy to identify replicated treatment responses. Proc Am Thorac Soc. 2004; 1(4):364–7. [PubMed: 16113459]
14. Gould W, Peterson EL, Karungi G, Zoratti A, Gaggin J, Toma G, et al. Factors predicting inhaled corticosteroid responsiveness in African American patients with asthma. J Allergy Clin Immunol. Dec; 126(6):1131–8. [PubMed: 20864153]
15. Corrigan CJ, Bungre JK, Assoufi B, Cooper AE, Seddon H, Kay AB. Glucocorticoid resistant asthma: T-lymphocyte steroid metabolism and sensitivity to glucocorticoids and immunosuppressive agents. Eur Respir J. 1996 Oct; 9(10):2077–86. [PubMed: 8902470]
16. Haczku A, Alexander A, Brown P, Assoufi B, Li B, Kay AB, et al. The effect of dexamethasone, cyclosporine, and rapamycin on T-lymphocyte proliferation in vitro: comparison of cells from patients with glucocorticoid-sensitive and glucocorticoid-resistant chronic asthma. J Allergy Clin Immunol. 1994 Feb; 93(2):510–9. [PubMed: 8120277]

17. Alvarez J, Surs W, Leung DY, Ikle D, Gelfand EW, Szefler SJ. Steroid-resistant asthma: immunologic and pharmacologic features. J Allergy Clin Immunol. 1992 Mar; 89(3):714–21. [PubMed: 1545093]
18. Corrigan CJ, Brown PH, Barnes NC, Szefler SJ, Tsai JJ, Frew AJ, et al. Glucocorticoid resistance in chronic asthma. Glucocorticoid pharmacokinetics, glucocorticoid receptor characteristics, and inhibition of peripheral blood T cell proliferation by glucocorticoids in vitro. Am Rev Respir Dis. 1991 Nov; 144(5):1016–25. [PubMed: 1952426]
19. Poznansky MC, Gordon AC, Douglas JG, Krajewski AS, Wyllie AH, Grant IW. Resistance to methylprednisolone in cultures of blood mononuclear cells from glucocorticoid-resistant asthmatic patients. Clin Sci (Lond). 1984 Dec; 67(6):639–45. [PubMed: 6488705]
20. Kirkham BW, Corkill MM, Davison SC, Panayi GS. Response to glucocorticoid treatment in rheumatoid arthritis: in vitro cell mediated immune assay predicts in vivo responses. J Rheumatol. 1991 Jun; 18(6):821–5. [PubMed: 1895263]
21. Seki M, Ushiyama C, Seta N, Abe K, Fukazawa T, Asakawa J, et al. Apoptosis of lymphocytes induced by glucocorticoids and relationship to therapeutic efficacy in patients with systemic lupus erythematosus. Arthritis Rheum. 1998 May; 41(5):823–30. [PubMed: 9588733]
22. Langhoff E, Ladefoged J, Jakobsen BK, Platz P, Ryder LP, Svejgaard A, et al. Recipient lymphocyte sensitivity to methylprednisolone affects cadaver kidney graft survival. Lancet. 1986 Jun 7; 1(8493):1296–7. [PubMed: 2872430]
23. Federico MJ, Covar RA, Brown EE, Leung DY, Spahn JD. Racial differences in T-lymphocyte response to glucocorticoids. Chest. 2005 Feb; 127(2):571–8. [PubMed: 15705998]
24. Akinbami LJ, Schoendorf KC. Trends in childhood asthma: prevalence, health care utilization, and mortality. Pediatrics. 2002 Aug; 110(2 Pt 1):315–22. [PubMed: 12165584]
25. Grant EN, Lyttle CS, Weiss KB. The relation of socioeconomic factors and racial/ethnic differences in US asthma mortality. Am J Public Health. 2000 Dec; 90(12):1923–5. [PubMed: 11111268]
26. Joseph CL, Ownby DR, Peterson EL, Johnson CC. Racial differences in physiologic parameters related to asthma among middle-class children. Chest. 2000 May; 117(5):1336–44. [PubMed: 10807820]
27. Boldizsar F, Talaber G, Szabo M, Bartis D, Palinkas L, Nemeth P, et al. Emerging pathways of non-genomic glucocorticoid (GC) signalling in T cells. Immunobiology. 2010 Jul; 215(7):521–6. [PubMed: 19906460]
28. Stahn C, Buttgereit F. Genomic and nongenomic effects of glucocorticoids. Nat Clin Pract Rheumatol. 2008 Oct; 4(10):525–33. [PubMed: 18762788]
29. Ray A, Prefontaine KE. Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappa B and the glucocorticoid receptor. Proc Natl Acad Sci U S A. 1994 Jan 18; 91(2):752–6. [PubMed: 8290595]
30. Rogatsky I, Ivashkiv LB. Glucocorticoid modulation of cytokine signaling. Tissue Antigens. 2006 Jul; 68(1):1–12. [PubMed: 16774534]
31. Hakonarson H, Bjornsdottir US, Halapi E, Bradfield J, Zink F, Mouy M, et al. Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients. Proc Natl Acad Sci U S A. 2005 Oct 11; 102(41):14789–94. [PubMed: 16203992]
32. Du P, Kibbe WA, Lin SM. lumi: a pipeline for processing Illumina microarray. Bioinformatics. 2008 Jul 1; 24(13):1547–8. [PubMed: 18467348]
33. Lin SM, Du P, Huber W, Kibbe WA. Model-based variance-stabilizing transformation for Illumina microarray data. Nucleic Acids Res. 2008 Feb.36(2):e11. [PubMed: 18178591]
34. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol. 2004; 3:Article3. [PubMed: 16646809]
35. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 2003 Aug 5; 100(16):9440–5. [PubMed: 12883005]
36. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009; 4(1):44–57. [PubMed: 19131956]

37. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 2003; 4(5):P3. [PubMed: 12734009]
38. Ayroldi E, Riccardi C. Glucocorticoid-induced leucine zipper (GILZ): a new important mediator of glucocorticoid action. FASEB J. 2009 Nov; 23(11):3649–58. [PubMed: 19567371]
39. Luca F, Kashyap S, Southard C, Zou M, Witonsky D, Di Rienzo A, et al. Adaptive variation regulates the expression of the human SGK1 gene in response to stress. PLoS Genet. 2009 May. 5(5):e1000489. [PubMed: 19461886]
40. Habib-Agahi M, Phan TT, Searle PF. Co-stimulation with 4-1BB ligand allows extended T-cell proliferation, synergizes with CD80/CD86 and can reactivate anergic T cells. Int Immunol. 2007 Dec; 19(12):1383–94. [PubMed: 17977894]
41. Maranville JC, Luca F, Richards AL, Wen X, Witonsky DB, Baxter S, et al. Interactions between Glucocorticoid Treatment and Cis-Regulatory Polymorphisms Contribute to Cellular Response Phenotypes. PLoS Genet. 2011 Jul.7(7):e1002162. [PubMed: 21750684]
42. Grundberg E, Adoue V, Kwan T, Ge B, Duan QL, Lam KC, et al. Global analysis of the impact of environmental perturbation on cis-regulation of gene expression. PLoS Genet. 2011; 7(1):e1001279. [PubMed: 21283786]
43. Ding J, Gudjonsson JE, Liang L, Stuart PE, Li Y, Chen W, et al. Gene expression in skin and lymphoblastoid cells: Refined statistical method reveals extensive overlap in cis-eQTL signals. Am J Hum Genet. 2010 Dec 10; 87(6):779–89. [PubMed: 21129726]
44. Raychaudhuri S, Stuart JM, Altman RB. Principal components analysis to summarize microarray experiments: application to sporulation time series. Pac Symp Biocomput. 2000:455–66. [PubMed: 10902193]
45. Bisset LR, Schmid-Grendelmeier P. Chemokines and their receptors in the pathogenesis of allergic asthma: progress and perspective. Curr Opin Pulm Med. 2005 Jan; 11(1):35–42. [PubMed: 15591886]
46. Harhaj EW, Dixit VM. Deubiquitinases in the regulation of NF-kappaB signaling. Cell Res. 2011 Jan; 21(1):22–39. [PubMed: 21119682]
47. Hershko DD. Cyclin-dependent kinase inhibitor p27 as a prognostic biomarker and potential cancer therapeutic target. Future Oncol. 2010 Dec; 6(12):1837–47. [PubMed: 21142858]
48. Barnes MJ, Aksoylar H, Krebs P, Bourdeau T, Arnold CN, Xia Y, et al. Loss of T cell and B cell quiescence precedes the onset of microbial flora-dependent wasting disease and intestinal inflammation in Gimap5-deficient mice. J Immunol. 2010 Apr 1; 184(7):3743–54. [PubMed: 20190135]
49. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma ControL study. Am J Respir Crit Care Med. 2004 Oct 15; 170(8):836–44. [PubMed: 15256389]
50. Schwartz JT, Reuling FH, Feinleib M, Garrison RJ, Collie DJ. Twin heritability study of the effect of corticosteroids on intraocular pressure. J Med Genet. 1972 Jun; 9(2):137–43. [PubMed: 5065285]
51. Bray PJ, Cotton RG. Variations of the human glucocorticoid receptor gene (NR3C1): pathological and in vitro mutations and polymorphisms. Hum Mutat. 2003 Jun; 21(6):557–68. [PubMed: 12754700]
52. Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, et al. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab. 1998 Jan; 83(1):144–51. [PubMed: 9435432]
53. Kumsta R, Moser D, Streit F, Koper JW, Meyer J, Wust S. Characterization of a glucocorticoid receptor gene (GR, NR3C1) promoter polymorphism reveals functionality and extends a haplotype with putative clinical relevance. Am J Med Genet B Neuropsychiatr Genet. 2009 Jun 5; 150B(4): 476–82. [PubMed: 18663733]
54. Hawkins GA, Lazarus R, Smith RS, Tantisira KG, Meyers DA, Peters SP, et al. The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. J Allergy Clin Immunol. 2009 Jun; 123(6):1376–83. e7. [PubMed: 19254810]

55. Tantisira KG, Lake S, Silverman ES, Palmer LJ, Lazarus R, Silverman EK, et al. Corticosteroid pharmacogenetics: association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. Hum Mol Genet. 2004 Jul 1; 13(13):1353–9. [PubMed: 15128701]

56. Gould W, Peterson EL, Karungi G, Zoratti A, Gaggin J, Toma G, et al. Factors predicting inhaled corticosteroid responsiveness in African American patients with asthma. J Allergy Clin Immunol. 2010 Dec; 126(6):1131–8. [PubMed: 20864153]

57. Cole SW, Hawkley LC, Arevalo JM, Sung CY, Rose RM, Cacioppo JT. Social regulation of gene expression in human leukocytes. Genome Biol. 2007; 8(9):R189. [PubMed: 17854483]

58. Bower JE, Ganz PA, Irwin MR, Arevalo JM, Cole SW. Fatigue and gene expression in human leukocytes: increased NF-kappaB and decreased glucocorticoid signaling in breast cancer survivors with persistent fatigue. Brain Behav Immun. 2011 Jan; 25(1):147–50. [PubMed: 20854893]

59. Yang JJ, Cheng C, Devidas M, Cao X, Fan Y, Campana D, et al. Ancestry and pharmacogenomics of relapse in acute lymphoblastic leukemia. Nat Genet. 2011 Mar; 43(3):237–41. [PubMed: 21297632]

60. Caliskan M, Cusanovich DA, Ober C, Gilad Y. The effects of EBV transformation on gene expression levels and methylation profiles. Hum Mol Genet. 2011 Apr 15; 20(8):1643–52. [PubMed: 21289059]

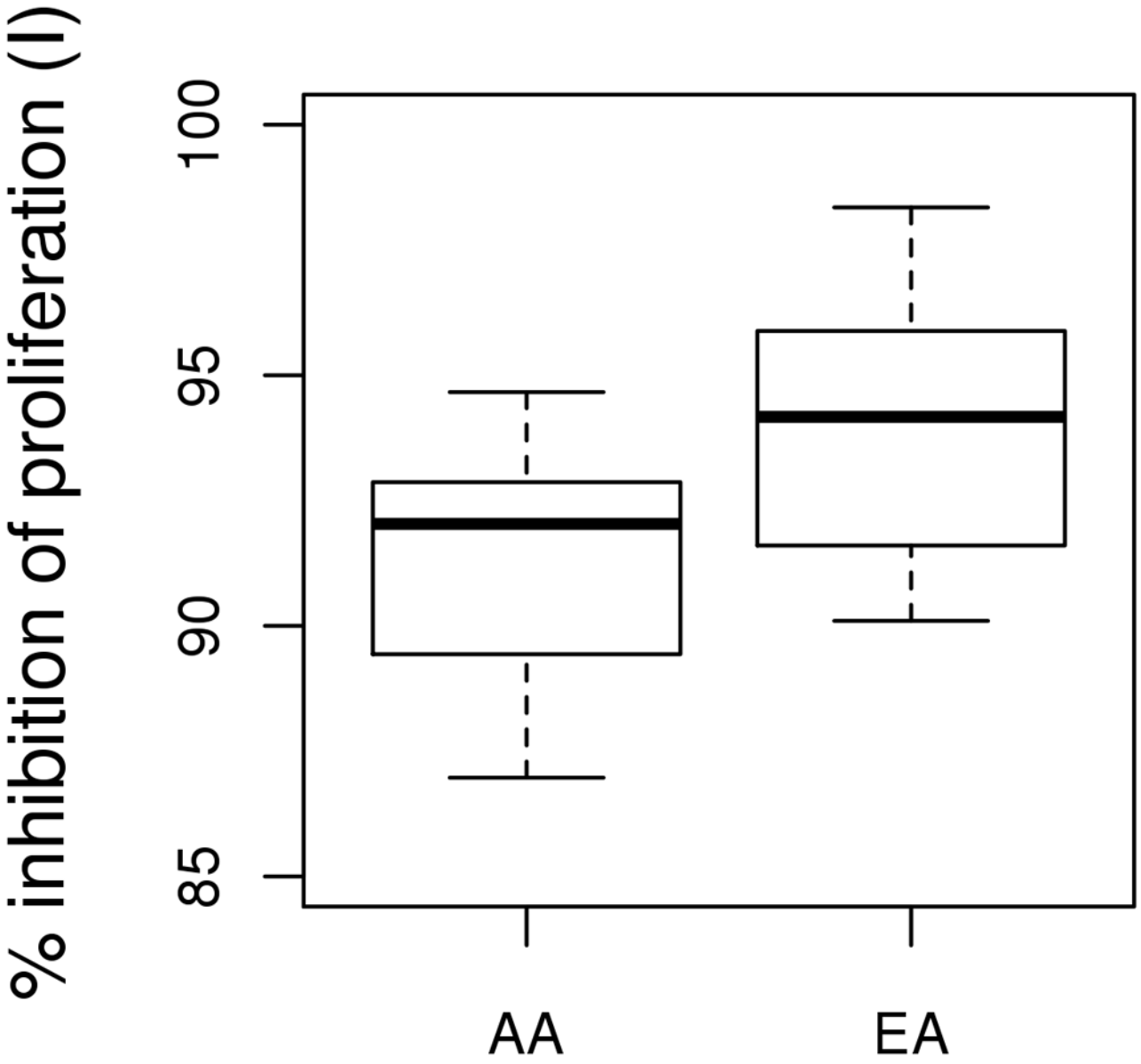

Figure 1.

Boxplots comparing the distribution of %I between populations. PBMCs from AAs show significantly less inhibition of proliferation in response to dex treatment compared to those from EAs ($p = 0.018$).

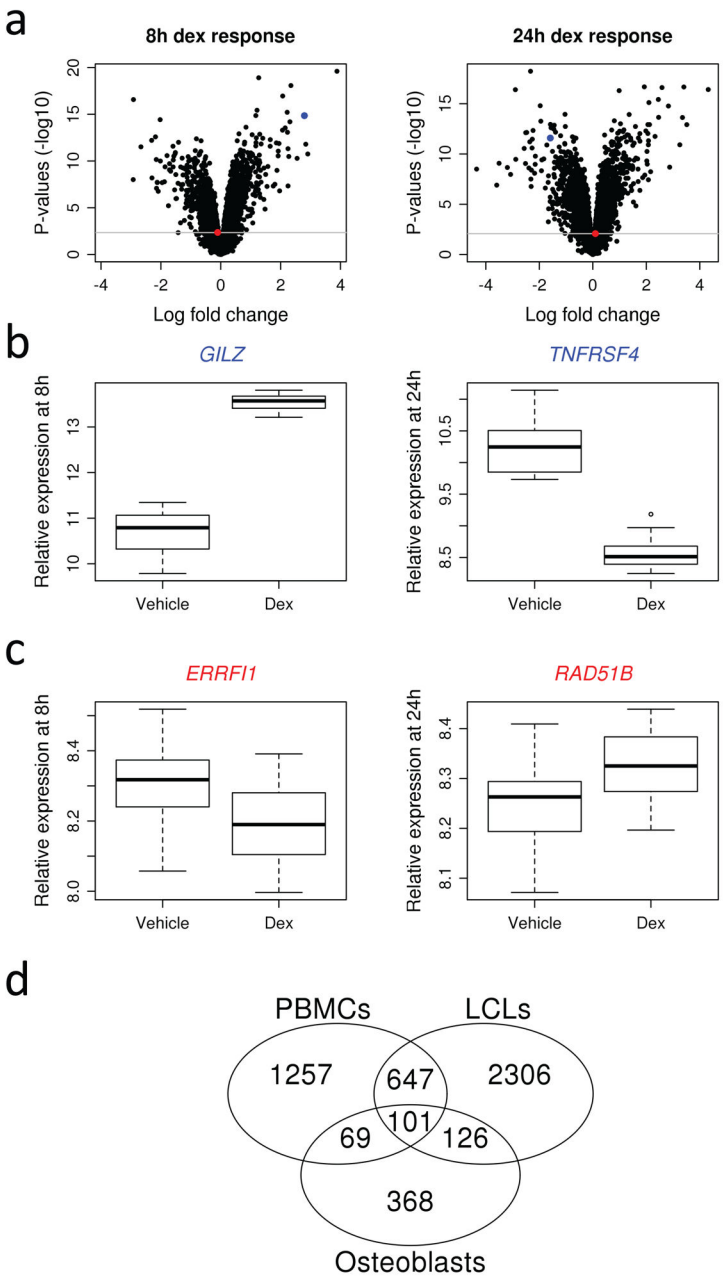

Figure 2.

a) Volcano plots show the mean $\log_2$ 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 8h and 24h are shown separately. Transcriptional response is widespread, includes both up- and down-regulated genes and increases in intensity with duration of treatment. b) Boxplots show examples of genes with large responses to dex treatment, the well characterized GR target glucocorticoid-induced leucine zipper (*GILZ*, $\log_2$ fold change = 2.8, $p = 1.4\times10^{-15}$) and the anti-apoptotic TNF-receptor *TNFRSF4* ($\log_2$ fold change = −1.6, $p = 2.6\times10^{-12}$). Dots in a) corresponding to these genes are colored in blue. c) Boxplots show examles of genes with small, but consistent responses to dex

treatment, the mitogen induced gene *ERRFI1* ($\log_2$ fold change = −0.10, p = 4.4×10^{-3}) and the apoptosis-associated RAD51 family member *RAD51B* ($\log_2$ fold change=0.096, p=8.4×10^{-3}). Dots in a) corresponding to these genes are colored in red. d) A Venn diagram shows the overlap between differentially expressed genes in PBMCs, LCLs and osteoblasts at an FDR<0.01. Counts only reflect genes expressed in all three tissues.

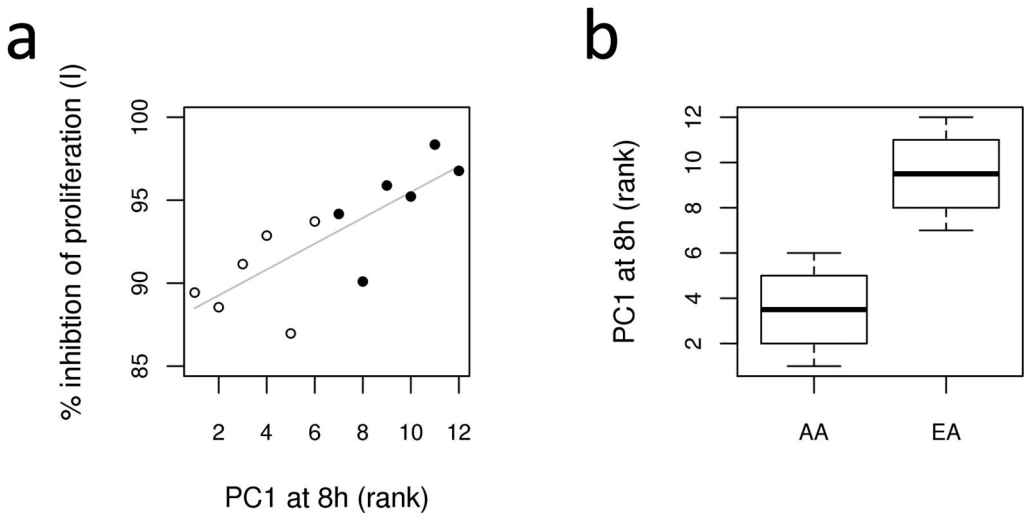

Figure 3.

a) %I is plotted against loadings on PC1 at 8h. AAs are shown as open circles and EAs are shown as closed circles. Transcriptional response (PC1) is correlated with LGS (%I) at 8h (ρ^2=0.68, p=1.7×10^{-3}), even after correcting for population (p=0.039). b) Boxplot shows population differences in $PC1_{8h}$ (p = 4.6×10^{-6}).

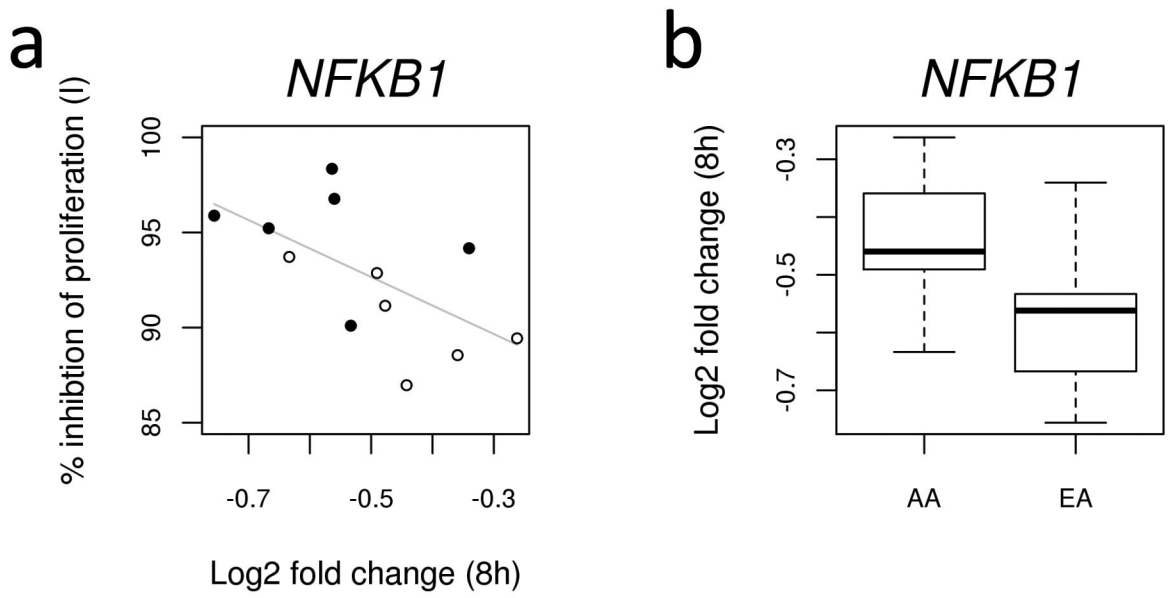

Figure 4.

a) %I is plotted against $\log_2$ fold change at *NFKB1* at 8h. AAs are shown as open circles and EAs are shown as closed circles. b) Boxplots compare the distribution of $\log_2$ fold changes at *NFKB1* between populations.

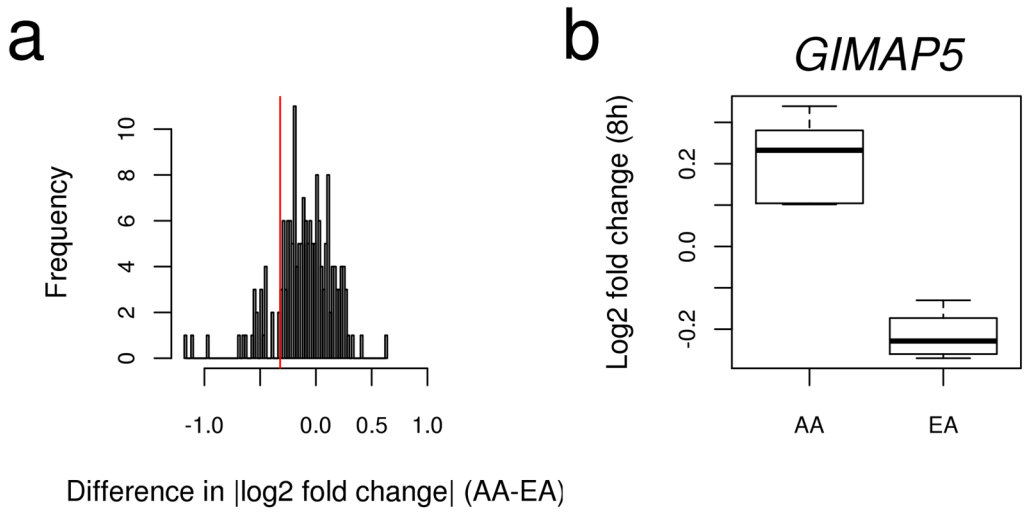

Figure 5.

Population differences in $\log_2$ fold change tend to reflect stronger responses in EA. a) Histogram showing the distribution of population differences in absolute value of the $\log_2$ fold change for the 177 genes with significant inter-ethnic differences. Negative values on the x-axis indicate a stronger response in EAs. The red vertical line marks the position of *GIMAP5* in the distribution. b) As an example of a biologically relevant gene with population differences in response, the distribution of $\log_2$ fold change in EAs and AAs is shown for *GIMAP5*, a known regulator of lymphocyte proliferation.