Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Tantisira KG, Lasky-Su J, Harada M, et al. Genomewide association between *GLCCI1* and response to glucocorticoid therapy in asthma. N Engl J Med 2011;365:1173-83. DOI: 10.1056/NEJMoa0911353.

Genome-wide Association of GLCCI1 with Asthma Steroid Treatment Response

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(Note: In support of this manuscript, this supplemental appendix contains a detailed methods section, three supplemental tables, and five supplemental figures.)

SUPPLEMENTAL ON LINE MATERIALS

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DETAILED METHODS

Study Overview

A flow diagram outlining the methods and study design used is presented in Figure 1.

Screening Study population

CAMP was a clinical trial that followed 1,041 asthmatic children for a mean of 4.6 years and nearly 80% of the original participants for 12 years[1]. Trial design and primary clinical trial outcomes have been published[1, 2]. As part of the clinical trial, individuals were randomly assigned to one of three treatment arms, budesonide (an inhaled corticosteroid), nedocromil, or placebo. A total of 422 non-Hispanic white CAMP subjects and their parents were genotyped on the HumanHap550v3 BeadChip (Illumina, San Diego, CA), as below. 311 CAMP subjects were randomized to the corticosteroid group, including 118 Caucasian probands as part of a parent-child trio. These trios formed the basis of our family-based screening cohort for longitudinal corticosteroid response.

Replication Populations

Three asthmatic clinical trials were used to replicate the initial findings using DNA samples: 1) the common run-in period from the salmeterol or corticosteroids (SOCS)[3] and salmeterol ± inhaled corticosteroids (SLIC)[4] trials, 2) the Adult Study; and 3) the Leukotriene modifier or Corticosteroid or Corticosteroid Salmeterol trial (LOCCS)[5]. Following the initial replication phase, an additional replication limited to the variant associated in each of the populations was performed using two Childhood Asthma Research and Education (CARE) network clinical trials with inhaled corticosteroid response data[6, 7] archived on dbGAP (http://www.ncbi.nlm.nih.gov/gap) within the SNP Health Association Resource (SHARe) Asthma Resource project (SHARP). Three of the four replication populations used adult individuals, which does not pose a problem for the replication since we have previously shown

that the adult and child response to inhaled steroids is similar[8]. A summary of the populations is provided in Table 1. All patients or their legal guardians consented to the study protocol and ancillary genetic testing.

SOCS/SLIC: Two completed trials conducted by the Asthma Clinical Research Network (ACRN), the SOCS[3] and SLIC[4] trials, had a common initial 6-week run-in period utilizing 4 inhalations twice daily of the inhaled corticosteroid triamcinolone prior to separate randomization to one of the two trials. Details regarding the entry criteria, run-in period, and randomization have been published with the primary trial results[3, 4]. Of the 339 subjects eligible for randomization, 336 had DNA available; 66.7% of these were Caucasian, forming the basis of our first replication sample.

Adult Study: The Adult Study was a multicenter 8-week randomized clinical trial comparing the effect of once-daily high-dose inhaled flunisolide vs. standard inhaled corticosteroid therapy. Inclusion criteria were a history of asthma, ≥12% improvement in FEV_1 with albuterol, and using inhaled steroids at randomization. Exclusion criteria were non-asthma pulmonary disease, smoking (≥10 pack-years), and recent asthma exacerbations requiring systemic steroids. Subjects were phoned weekly and had spirometry at 4 and 8 weeks. The 407 Caucasians in this study formed the basis of our second replication cohort.

LOCCS: The completed trials conducted by the American Lung Association Asthma Clinical Research Centers (ALA-ACRC), the Leukotriene modifier or Corticosteroid or Corticosteroid Salmeterol trial (LOCCS)[5] was used as the third replication sample. The LOCCS cohort comprised 500 subjects ≥ 6 yrs old who successfully completed a 4-6 week run-in period of inhaled fluticasone propionate. Subjects were required to have a pre-bronchodilator FEV_1 of ≥ 80% of predicted value, 12% or higher bronchodilator reversibility or PC_{20} of 8 mg/ml or less

within the past two years, and well controlled asthma (Juniper Asthma Control Questionnaire score < 1.5) after the run-in period. Of 280 subjects for whom DNA was available, 191 Caucasians were used for this analysis.

CARE: Two out of five CARE trials included in SHARP enrolled children with asthma with prospective ICS treatment response and were interrogated as a final replication population. Further information on the SHARP project can be found in the database of Genotypes and Phenotypes (dbGaP) (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gap). The two trials have been previously detailed[6, 7]. Medications for these trials were provided by Glaxo Smith Kline[6, 7] and Merck[7]. Since the enrollment criteria, corticosteroid dosage, and clinical centers were similar between the two trials, and a similar follow-up interval for inhaled corticosteroid response was available, data from the two trials was pooled into one CARE analysis to optimize statistical power for replication.

Phenotype

FEV_1 measurements were taken throughout the CAMP trial. In CAMP, the largest observable differences in FEV_1 attributable to budesonide occurred within the first 16 months of the trial[2]. FEV_1 measurements were taken at randomization and months 2, 4, 8, 12, and 16. For each time point after randomization we calculated the difference between the FEV_1 measurement at baseline and at that time point (i.e. $FEV_{1treatment}$ - $FEV_{1baseline}$). We denote these measurements as ΔFEV_1 throughout the remainder of this manuscript. We then generated residuals of this difference after adjusting for known covariates with FEV_1, including age, gender, and height. The ΔFEV_1 measurements were then used in the genome-wide association analysis.

Each replication cohort used a single measure of ΔFEV_1 that was most similar to the CAMP measurements. For the Adult study, the baseline measure of FEV_1 was taken immediately prior to randomization on either a once-daily high-dose inhaled flunisolide or the standard inhaled corticosteroid therapy. The final FEV_1 measurement was taken at the conclusion of the trial and therefore ΔFEV1 measured the change in FEV_1 over an 8 week increment. Because both SOCS/SLIC and LOCCS were clinical trials where individuals were randomized to treatments other than inhaled corticosteroid, the ΔFEV_1 for these studies was measured at the beginning and end of the run-in period; in this period treatment with inhaled glucocorticosteroids was initiated. The CARE trials included children who were either on no active controller medications for at least two weeks prior to randomization or an active two week washout period. FEV_1 change was measured from 6-8 weeks following randomization.

Genotyping and quality control

Genome-wide SNP genotyping was performed in the CAMP sample by Illumina, Inc. (San Diego, CA) on the HumanHap550v3 BeadChip. Genotype reproducibility was assessed by analyzing 4 subjects that were repeated once on each of the 14 genotyping plates; all replicates had at least 99.8% concordance.

The data were cleaned in several steps (Supplemental Table 1). 6,257 markers were removed due to low Illumina clustering scores. An additional 1,329 markers were removed because their flanking sequences did not map to a unique position on the hg17 reference genome sequence. We used PLINK[9] to further QC the remaining markers. All markers had greater than 90% genotyping completion rate, while the average completion rate for each marker was over 99%. 3,790 markers were removed because they were monomorphic in our sample. 2,445 markers were removed due to five or more parent-child genotype inconsistencies. No filtering

was done based on Hardy-Weinberg equilibrium due to ascertainment of the cohort through affected probands. Hardy-Weinberg equilibrium was evaluated on a case-by-case basis in all of the top SNPs that were selected to replicate.

From 561,466 markers present on the BeadChip, 547,645 markers (97.54%) passed quality control metrics. A summary of the genotyping quality control results is provided in Supplemental Table 1. 1169 CAMP subjects were successfully genotyped, including 403 probands and their parents. Of the 1169, 10 were probands without parental DNA, 38 were families of two, including 11 informative single parent-child pairings, 1044 represented 348 complete parent-child trios, and 77 represented 19 families with more than one affected proband. The average genotyping completion rate for each subject was 99.75%.

A total of 13 SNPs with a power ranking in the top 100 and with nominal association p-values less than 0.05 were subsequently genotyped using the Sequenom platform (Sequenom, San Diego, CA) in the three replication cohorts. Upon review of HapMap linkage disequilibrium patterns and our functional results, a single functional SNP, rs37973, was genotyped in CAMP and each of our three replication cohorts using a Taqman assay (Applied Biosystems, Foster City, CA). Average completion rates with the Sequenom iPLEX and Taqman technologies were over 95%. For each assay, 10% of the cohort was chosen at random for duplicate genotyping, and assays with a high discordance rate (>2%) were discarded. A Hardy-Weinberg equilibrium *p*-value was calculated for each assay to insure that the genotyping was accurate and that observed associations were not due to influences other than a real genetic association, such as inaccurate genotyping, non-random mating, mutations, or random genetic drift.

The CARE samples were genotyped using the Affymetrix SNP 6.0 Array as part of SHARP. Since neither rs37972 nor rs37973 are represented on that chip, we imputed HapMap

SNP using CARE data using the program MACH (http://www.sph.umich.edu/csg/abecasis/MACH/index.html). As the fourth replication population identified following the initial replication analysis, the CARE analysis was limited to the rs37972 and rs37973 variants. As expected based on findings in the other populations, the imputed rs37972 and 37973 variants were in perfect linkage disquilibrium, with a correlation coefficient of 1.0.

Statistical Methods

Family-based association testing (FBAT) is a generalization of the TDT, which allows valid testing of association with any phenotype, sampling structure, and pattern of missing marker allele information[10-12]. The FBAT-principal component (FBAT-PC) methodology utilizes the 5 repeated measures of ΔFEV_1 to generate an overall phenotype that has maximum heritability at each SNP (i.e., the overall phenotype which maximizes the potential genetic contribution for each SNP locus)[11]. When constructing the overall phenotype, the FBAT-PC methodology up-weights the time points with heritability evidence for ΔFEV_1 and down-weights the time points with little evidence of heritability, so as to generate a phenotype with maximum heritability. The SNPs with the greatest heritability would, by design, yield the highest power for a subsequent genetic analysis. This composite phenotype is what was used as a phenotype in the analysis. One of the great advantages of family-based methods, such as FBAT-PC, is that the methodology is robust to population stratification; therefore, we are less concerned about observing a spurious result from ancestral differences in our population. We analyzed our GWAS data using the FBAT-PC methodology in the context of the family-based screening algorithm. This algorithm rank orders the SNPs with the most power to detect association in a manner that does not bias the subsequent statistical test of association[13, 14]. It accomplishes this

by using the expected frequency of genotype distributions (based upon parental genotype information) for a proband in place of the actual genotypes of the proband. Since the actual proband genotypes are not being used, the screening algorithm allows for the interrogation of both multiple genetic models and for the determination of the subset of phenotypic measurements (the overall phenotype) that provide the maximal power for each SNP. From this rank ordering, we selected the SNPs that met two criteria to replicate using data from the three clinical trials: 1) they were among the top 100 powered SNPs and 2) the FBAT test statistic was less than 0.05. Thirteen SNPs met these criteria.

Within the replication populations, generalized linear models were used to evaluate the association between each of the selected SNPs and ΔFEV_1 while adjusting for age, sex, and height at baseline using SAS (version 9.1, SAS Institute, Cary, NC. Within the CARE samples, the imputed genotype dosage data was used within the linear models to generate the initial association *p*-values. All genotypes were modeled additively and the genotypic means were calculated using least square means (in the case of CARE, imputed maximal likelihood estimates of the genotype were used for this purpose), adjusted for possible confounders. A replication of the initial finding was defined by having a nominal p-value less than 0.05 in the replication cohort. A p-value that combined evidence across all replication populations was calculated using Liptak's approach[15, 16] and Bonferroni adjusted for multiple testing of the 12 genotyped SNPs in the replication populations. Following the genotyping of the functional variant, a final pooled analysis was performed using all samples in a generalized linear model under the assumption of an additive model, adjusted for age, sex, height, and study. BonfeLogistic regression analysis adjusting for age, sex, and height at baseline was performed to analyze SNP association with lowest vs. highest quartile of $\Delta FEV_{1.}$

Functional Validation

Due to its location just 5' of *GLCCI1,* rs37972, the screened SNP associated with ICS response in each of our four replication populations, was investigated as a potential functional variant. This included resequencing of *GLCCI1* to discover variants in LD with rs37972, analyses of *GLCCI1* expression in various tissue types, analysis of genotypic expression patterns, and allele specific electrophoretic mobility shift (EMSA) and luciferase reporter assays.

***GLCCI1* Resequencing**

Primer pairs flanking the 8 exons, up to 1.2 kb 5' of the *GLCCI1* gene, and the 3'UTR were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). DNA (20ng) extracted from the whole blood of 23 CAMP individuals was used in standard 20µl polymerase chain reactions (PCR) using AmpliTaq Gold polymerase (Applied Biosystems). Amplification was verified by agarose gel electrophoresis prior to sequencing using an ABI3730 DNA analyzer (Applied Biosystems, Foster City, CA). Sequence traces were viewed and analyzed by Consed. Participants varied in their genotypes for rs37972, including 10 individuals with the T/T genotype, 4 heterozygotes and 9 homozygotes for the C allele.

Cells, Reagents and Stimulation

We isolated monocytes from PBMCs of healthy volunteers. PBMCs were prepared by Ficoll-Paque Plus density gradient centrifugation (GE healthcare, Piscataway, NJ). For enrichment of immune cells, we used magnetic activated cell sorting (MACS) system, which is based on MACS MicroBeads, specific monoclonal antibodies conjugated to superparamagnetic particles (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells labeled with MACS Microbeads are retained in the magnetic field within a MACS Column placed in a MACS Separator. For monocytes, we firstly incubated PBMC with CD14 MicroBeads, and cells were then applied to

MACS columns. The purity of $CD14^+$ fraction was >98% by flow cytometry analysis. $CD14^+$ fractions were cultured in plastic dishes (CORNING, Corning, NY) for 2 h at 37°C. After removal of cells non-adherent to dishes, monocytes were collected by treatment with 0.2% EDTA in PBS for 20 min at 4°C. Monocyte-derived dendritic cells were obtained by cultivating the $CD14^+$ fraction with 50 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ) and 100 ng/ml IL-4 (PeproTech). DC expressing $CD11c^+$ were obtained after 6 days culture with a purity of >98%. $CD4^+$ T cells ($CD4^+CD8^-TCR^+$), $CD8^+$ T cells ($CD4^-CD8^+TCR^+$), B cells ($CD3^-CD56^-CD19^+$) and natural killer (NK) cells ($CD3^-CD56^+CD19^-$) were isolated from the $CD14^-$ fractions of PBMCs by using a MoFlo High Speed cell sorter (Dako Cytomation, Carpinteria, CA) with a purity of >98%.

PBMCs, $CD4^+$ T cells, and $CD8^+$ T cells were stimulated by plate-bound anti-CD3 monoclonal antibodies (mAb) (incubated at 1μg/ml, clone number UCHT1) with a soluble anti-CD28 mAb (1μg/ml, clone number CD28.2). IL-4 and IL-12 were purchased from Peprotech EC, Ltd. (London, UK). Other immune cells were stimulated with 5 μg/ml of lipopolysaccharide (LPS) (InvivoGen), 25 μg/ml poly(I:C) (InvivoGen, La Jolla, CA), or macrophage-activating lipopeptide 2 (MALP-2) (Alexis, Lausen, Switzerland). Phosphate-buffered saline (PBS) was used as the vehicle.

Additional B cells were isolated from PBMCs of healthy Japanese volunteers (aged 29 to 47 years) according to the manufacturer's protocol (MACS) (Miltenyi Biotec, Bergisch Gladbach, Germany). PBMCs were prepared by Ficoll-Paque Plus density gradient centrifugation (GE Healthcare, Piscataway, NJ). For enrichment of B cells, we used a B cell isolation kit II. The purity of the B cells fraction was >90% by flow cytometric analysis. Cells were stimulated with 10 ng/ml Phorbol Myristate Acetate (PMA)/200 ng/ml Ionomycin, 8 μg/ml

anti-IgM (mouse F(ab') anti-human IgM) (SouthernBiotech, Birmingham, AL), and 10 ng/ml IL-4. B cells were also cultured with 1 μM dexamethasone (ICN Biomedicals, Costa Mesa, CA).

RNA Isolation and Quantitative Real-time RT-PCR

Total RNA from normal human tissues was purchased from Clontech (Mountain View, CA). Each RNA was reverse transcribed with Superscript III reverse transcriptase and oligo dT primers (Invitrogen, Carlsbad, CA). We used RNAeasy (QIAGEN, Valencia, CA) to extract total RNA, and cDNA was synthesized with random and oligo-dT primers after DNase treatment. The expression of *GLCCI1* in the human tissues and the human immune cells was determined by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) using SYBR® Premix *Ex Taq*™ II (Takara, Shiga, Japan) with an ABI Prism 7000 detection system according to manufacturer's instructions. In all experiments, the amounts of cDNA were standardized by quantification of the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*). RT-PCR primer sets are listed in Supplemental Table 4.

Expression data of EBV-transformed lymphoblastoid cells

We examined the correlation of rs37972 and rs37973 with dexamethasone-mediated changes in *GLCCI1* expression in EBV-transformed lymphoblastoid cell lines derived from 147 subjects within the CAMP cohort who were randomized to the budesonide treatment group. Each of the cell lines was passage zero (i.e. cultured following initial thaw of freshly immortalized cells). Expression was measured following cell culture and stimulation with 10^{-6} M dexamethasone or sham treatment for six hours using the Illumina HumanRef8 v2 BeadChip (Illumina, San Diego, CA), with each sample undergoing both dexamethasone and sham treatment in a paired fashion. *GLCCI1* expression results were validated using RT-PCR in a random subset of 32 of the samples using an ABI TaqMan assay (Applied Biosystems, Foster

City, CA), with *GAPDH* as a housekeeping gene. All BeadChip expression values were background adjusted, log-transformed, and normalized using a variance stablilization algorithm. Genotypic association of *GLCCI1* expression with *GLCCI1* genotype was performed under an additive genetic model, using a generalized linear models framework. *GLCCI1* expression within the CAMP cell lines following dexamethasone treatment was also directly tested for evidence of association with good vs. poor lung function response (i.e. highest vs. lowest quartile of FEV_1 change) in response to budesonide in the same CAMP subjects using a logistic regression model.

We additionally obtained a data set of whole-genome gene expression variation in Epstein-Barr virus (EBV) transformed lymphoblastoid cell lines from 56 CEU (Centre d'Etude du Polymorphisme Humain from Utah) HapMap subjects (www.sanger.ac.uk/humgen/genevar)[17, 18] (Supplemental Figure 4). Correlation between normalized mRNA expression data of *GLCCI1* and genotype were examined.

Electrophoretic Mobility Shift Assay (EMSA)

EMSAs were performed using Gel Shift Assay Systems (Promega, Madison, WI) according to the manufacturer's instructions. GLCCI1 and ZEB1 are expressed in Jurkat, Raji, and THP-1 cells. C/EBP alpha is expressed in Jurkat and THP-1 cells (data not shown). We conducted the EMSA using these cells. Cells (5×10^7) were harvested and then disrupted on ice by hypotonic lysis in 400 µL buffer A (10 mM HEPES-KOH, pH 7.8, 10 mM KCl, 0.1 mM EDTA, pH 8.0, and 0.1 % NP-40). Nuclei were extracted by incubation for 30 min at 4°C in 100 µL buffer C (50 mM HEPES-KOH, pH 7.8, 420 mM KCl, 0.1 mM EDTA, pH 8.0, 5 mM $MgCl_2$, 2 % glycerol) with gentle mixing on a rotator. All buffers included protease inhibitors (1 mM DTT, 0.5 mM PMSF, 2 mg/ml aprotinin, 2 mg/ml pepstain, 2 mg/ml leupeptin). Extracts

were quickly frozen and stored in aliquots at -80 ˚C. The double-stranded oligonucleotides were end-labeled with [γ-^{32}P] ATP using T4 polynucleotide kinase.

Following EMSA, we predicted a potential allelic difference in the cis-acting regulatory function in transcription by use of a bioinformatics approach (TRANSFAC® Professional 10.3) (www.biobase.de/pages/). The sequence containing the A allele of the SNP (rs37973A/G) on the promoter region created a new consensus sequence corresponding to the putative binding element to C/EBP alpha and ZEB1. We then searched cell conditions in which *GLCCI1* is highly induced or the TFs, C/EBP alpha and/or ZEB1, are highly expressed, repeating the EMSA under these conditions.

Luciferase Assay

We constructed luciferase reporter plasmids by cloning the fragments from human genomic DNA amplified by PCR. Products for the enhancer assay were subcloned into the upstream region of the reporter gene pGL4.23-luciferase vector (Promega, Madison, WI). Primer sets used for cloning are detailed in Supplemental Table 4. THP-1 cells and Jurkat cells (5 x 10^4/well) were transfected with these reporter constructs (500ng) and pRL-TK Renilla luciferase vector (10ng) as a normalization control using FuGENE 6 transfection reagent (Roche Diagnostics, Basel, Switzerland) in a 12-well plate. Raji cells (2 x 10^6/well) were transfected with these reporter constructs (0.48 μg) and pRL-TK Renilla luciferase vector (20ng) as a normalization control by electroporation with the nucleofector machine of Amaxa (Lonza, Basel, Switzerland).

Products for the promoter assay were subcloned into the upstream region of the reporter gene pGL3-basic luciferase vector (Promega, Madison, WI). Primer sets used for cloning are detailed in Supplemental Table 4. Raji cells (2 x 10^6/well) were transfected with these reporter

constructs (1.9 μg) and pRL-TK Renilla luciferase vector (40 ng) as a normalization control using Amaxa.

After 24 hours, we lysed the cells and measured firefly and Renilla luciferase activities in a luminometer using the Dual-Luciferase Reporter Assay System (Promega). The relative luciferase activity of the mock and *GLCCI1* reporter constructs was compared as the ratio of the firefly luciferase activity to that of Renilla. The mock transfected average is represented as 1. Comparisons in reporter assays were performed with Student's t-test, and statistical significance was defined at the standard 5% level.

Supplemental Figure Legends

Supplemental Figure 1. Linkage disequilibrium of *GLCCI1* SNPs via resequencing of the 5' UTR, peri-exonic regions, and 3' UTR, as measured via r^2. Of the identified variants, only one, rs37973, is in tight linkage disequilibrium with the initially associated variant, rs37972.

Supplemental Figure 2. *GLCCI1* expression in various human tissues and immune cells. The expression of mRNA of the *GLCCI1* was quantitatively evaluated using quantitative real-time PCR. (**A**) Expression patterns in human tissues. (**B**) Relative expression of *GLCCI1* in immune cells in response to stimuli (plate-bound anti-CD3, 1μg ml-1; soluble anti-CD28, 1μg ml-1; IL-12, 5ng ml-1; IL-4 10ng ml-1; LPS, 5μg ml-1; poly(I:C) 25μg ml-1 ; MALP2 1μg ml-1; Th1, CD3/CD28/IL-12; Th2, CD3/CD28/IL-4). NTC: no template control, no: no stimulation. Immune cells were obtained from three healthy volunteers. Data represent mean of duplicate samples. Two independent experiments were performed with similar results. (**C**) Relative expression in primary B cells. Cells were stimulated with 10 ng/ml PMA/200 ng/ml Ionomycin, 8 μg/ml anti-IgM, and 10 ng/ml IL-4. B cells were cultured with and without 1 μM Dexamethasone. Data show mean ± s.d. of triplicate samples from one experiment done in triplicate. Data were confirmed by three independent experiments. Relative mRNA expression level of B cells without any stimulation was set at 1.0. Similar results were obtained using B cells from three individuals.

Supplemental Figure 3: Expression of *GLCCI1* in CAMP subject cell lines, stratified by dexamethasone exposure status and rs37973 genotype. Boxes within the plot demonstrate median and inter-quartile range of the data, with the maximum and minimum points plotted at

the ends. Cells from subjects homozygous for the wild type (AA, n = 50) demonstrated the highest expression, those from homozygous for the mutant allele (GG, n = 29) the lowest expression, and those heterozygous (n = 68) an intermediate expression value ($p = 0.003$ and 0.002 for sham and dexamethasone, respectively), consistent with the additive model association results. Glucocorticosteroid therapy significantly enhanced the expression compared to sham for each genotype ($p<0.0001$). Therefore, the highest *GLCCI1* expression was noted in cells homozygous wild type treated with glucocorticosteroids and the lowest *GLCCI1* expression noted in untreated homozygous mutant cells. Similar results were obtained for rs37972 (data not shown

Supplemental Figure 4. Expression of *GLCCI1* in the CEPH HapMap samples, stratified by rs37972 genotype. Those homozygous for the major allele demonstrated significantly greater expression compared to those heterozygous or homozygous for the mutant allele (*p*-value = 0.01). Similar results were obtained for rs37973 (data not shown).

Supplemental Figure 5. Luciferase constructs (**A**) and confirmatory comparison of rs37973 allelic variations of *GLCCI1* in relative promoter activity in Raji cells obtained using a pGL3-basic vector analyzed by relative luciferase activity (**B**). Representative data from three experiments done in triplicate. Data represent mean ± s.d. from one experiment done in triplicate. Similar results were obtained by three independent experiments. The data are expressed relative to the luciferase activity of the mock transfectant, which was arbitrarily set at 1. *$P < 0.001$ by Student’s t-test.

Supplemental Table 1: A Summary of the genotyping cleaning procedures for the CAMP GWAS data	
Attribute	**Count (% of 561, 466 Markers on array)**
Low Illumina QC score	6,257 (1.1 %)
Flank sequences do not map to hg17	1,329 (0.2 %)
Monomorphic	3,790 (0.7 %)
Parent-offspring genotype inconsistencies >4	2,445 (0.4 %)
Total number of failed markers	13,821 (2.5 %)
Total number of passed markers	547,645 (97.5 %)
Autosomes	534,290 (95.1 %)
Sex-linked	13,229 (2.4 %)
Mitochondrial genome	126 (0.02 %)

Supplemental Table 2: Association P-values of the Top 100 Powered SNPs in 3 Replication Cohorts

SNP	Power Rank	Model	SOCS/SLIC	LOCCS	Adult Study
rs6993479	5	dominant	0.75	0.47	0.36
rs1320125	26	additive	0.87	0.06	0.12
rs956133	32	additive	0.60	0.48	0.60
rs37972	38	additive	0.03	0.03	0.08
rs10933595	43	additive	0.66	0.28	0.24
rs4282162	49	recessive	0.97	0.94	0.17
rs2804311	61	recessive	0.66	0.08	0.71
rs2644645	83	dominant	0.10	0.50	0.98
rs10496195	92	recessive	0.07	0.08	0.58
rs7498886	93	recessive	0.89	0.37	0.42
rs2172706	95	additive	0.87	0.20	0.50
rs624964	100	dominant	0.26	0.36	0.62

Supplemental Table 3: GLCCI1 Resequencing Results (Peri-exonic, 5', and 3')

SNP	Change	Genomic Location	MAF in Sequenced Cohort(%)
rs37972	C/T	5' of gene	52.2
rs37973	A/G	5' of gene	52.2
rs58145708	A/C	firstEF predicted promoter	2.2
rs7786263	C/G	5'UTR	15.8
Variant5*	C/A	5'UTR	4.3
Variant6*	A/G	intronic	2.2
rs38013	A/G	intronic	41.3
Variant8*	C/T	intronic	2.2
Variant9*	A/G	intronic	6.5
rs10486207	A/G	intronic	37
Variant11*	C/G	intronic	2.2
rs3823840	A/G	intronic	23.9
rs929509	C/T	coding synonymous	26.2
rs12847	A/G	3'UTR	41.3
rs58272960	del/T	3'UTR	28.3
rs4725068	A/G	3'UTR	34.8
rs7795506	G/T	3' of gene	28.3

* Novel variant

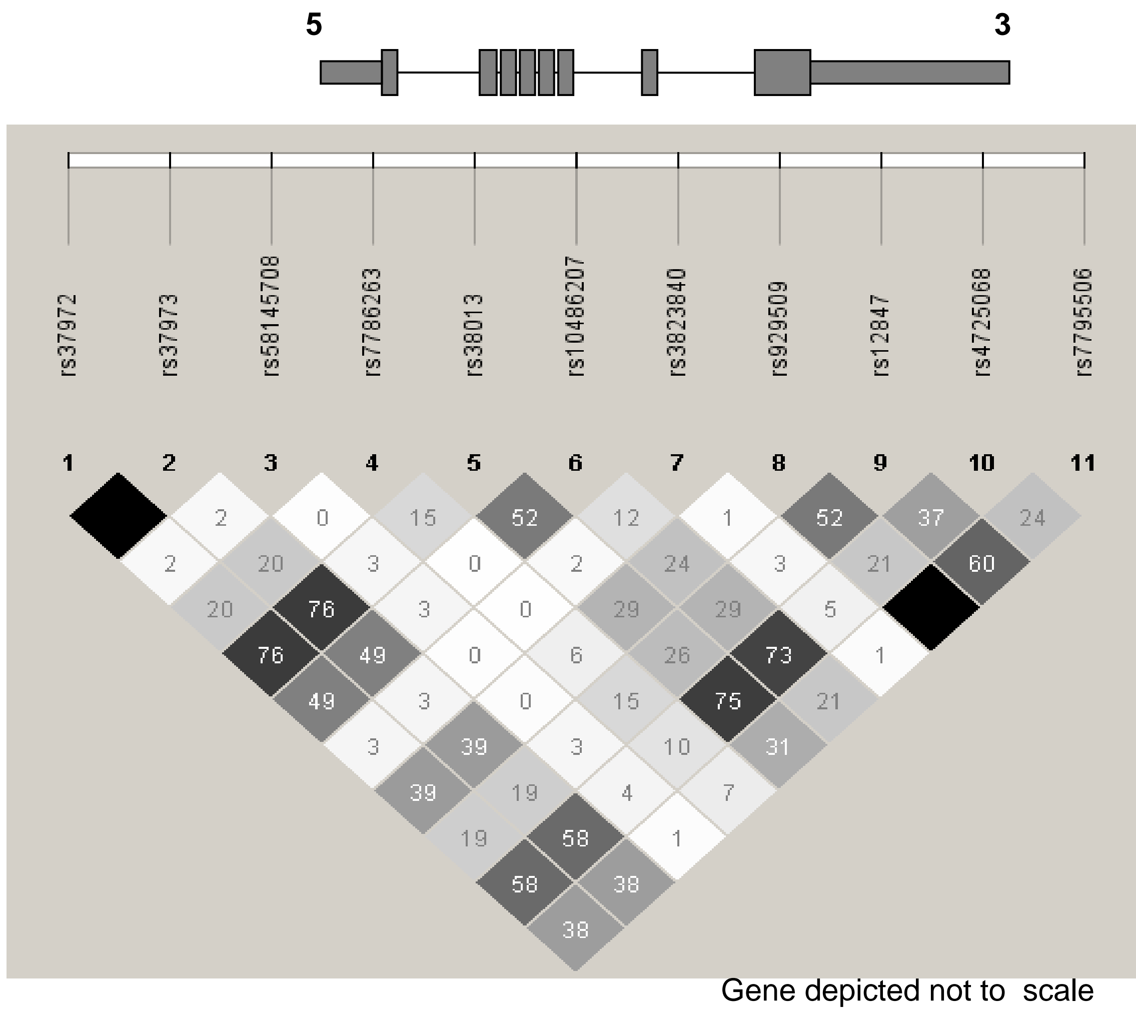
Supplemental Table 4 – Primer Characteristics

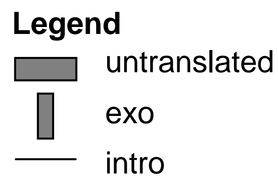
Primer Name	**Sequence (5'-3')**	**Purpose**
GLCCI1 - Forward	GGGAAGGAAGAAGTATCCAAGC	RT-PCR
GLCCI1 - Reverse	GCGAGTACTACTGCTCCGGTA	RT-PCR
GAPDH - Forward	GCACCGTCAAGGCTGAGAAC	RT-PCR
GAPDH - Reverse	ATGGTGGTGAAGACGCCAGT	RT-PCR
rs37972 and rs37973 - Forward	GCATATAGTGCCTGAAATCA	Cloning*
rs37972 and rs37973 - Reverse	ATTTGCAAGTACACCTGCATC	Cloning*
rs37972 - Forward	GCATATAGTGCCTGAAATCA	Cloning*
rs37972 - Reverse	GAACAAAGTAAAATACTCTTAC	Cloning*
rs37973 - Forward	ATGAAGATCCTTACATTAATTGC	Cloning*
rs37973 - Reverse	ATTTGCAAGTACACCTGCATC	Cloning*
rs37973 - Forward	TTGGTACCATGAAGATCCTTACATTAATTG	Cloning†
rs37973 - Reverse	TTGCTAGCTGGCTCTGCGGGCCCTGGA	Cloning†

* Enhancer assay products cloned upstream of pGL4.23 luciferase vector

† Promoter assay products cloned upstream of pGL3-basic luciferase vector

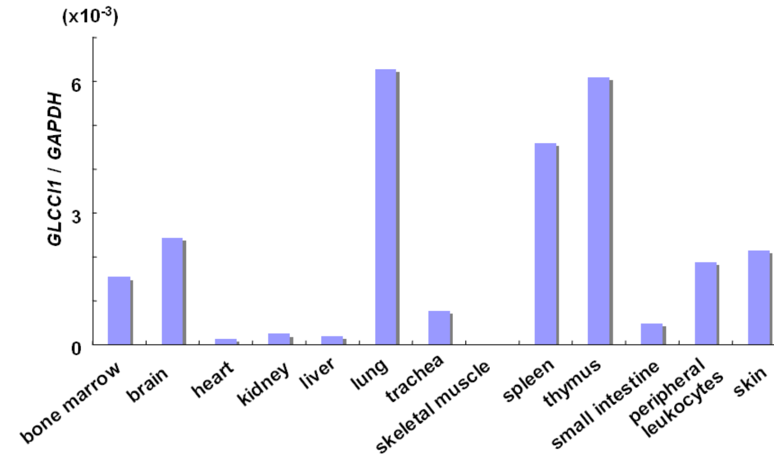
Supplemental Figure 1



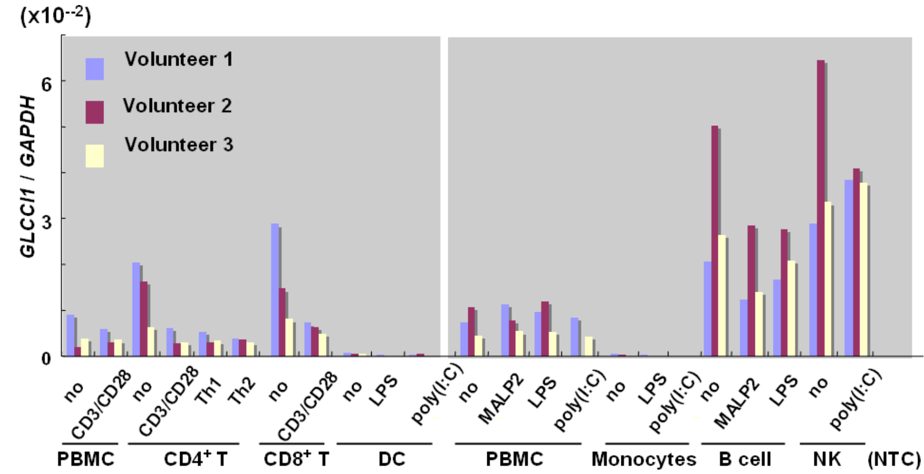


Supplemental Figure 2

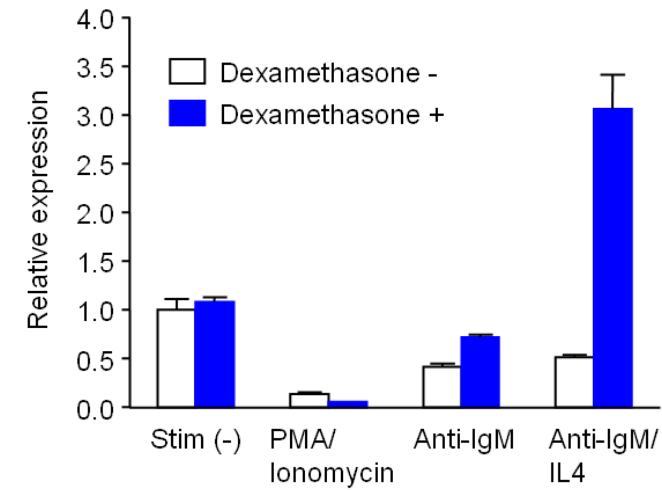
A.



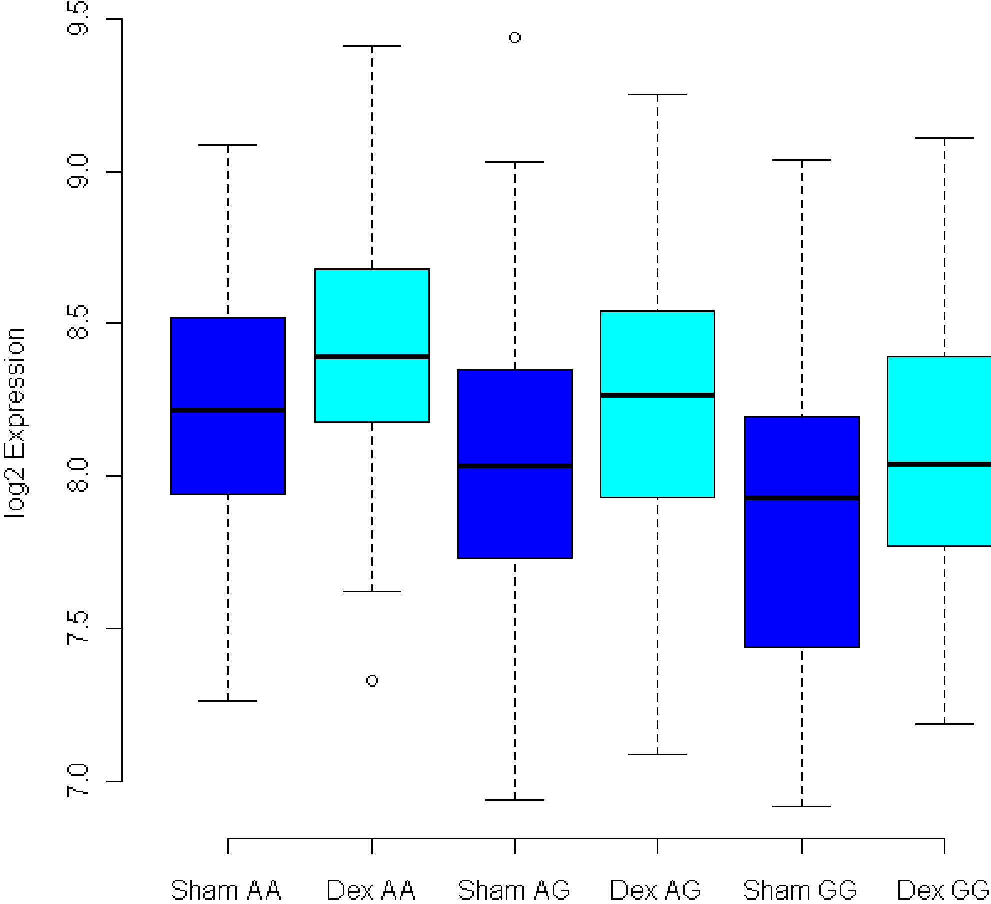
B.



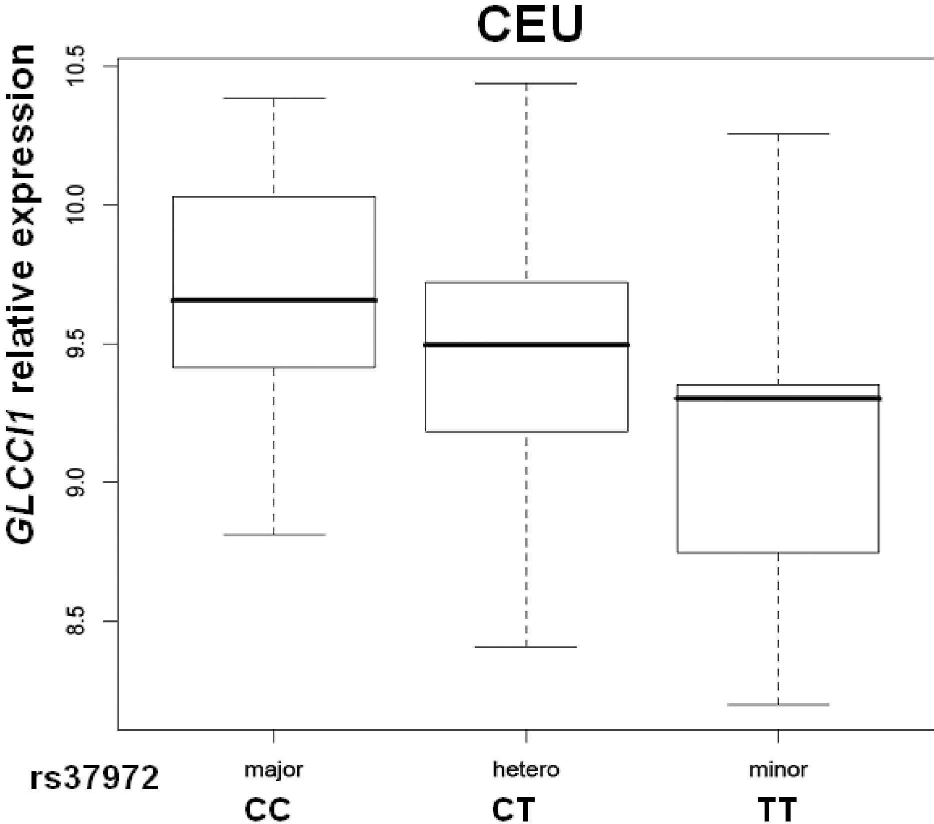
C.



Supplemental Figure 3

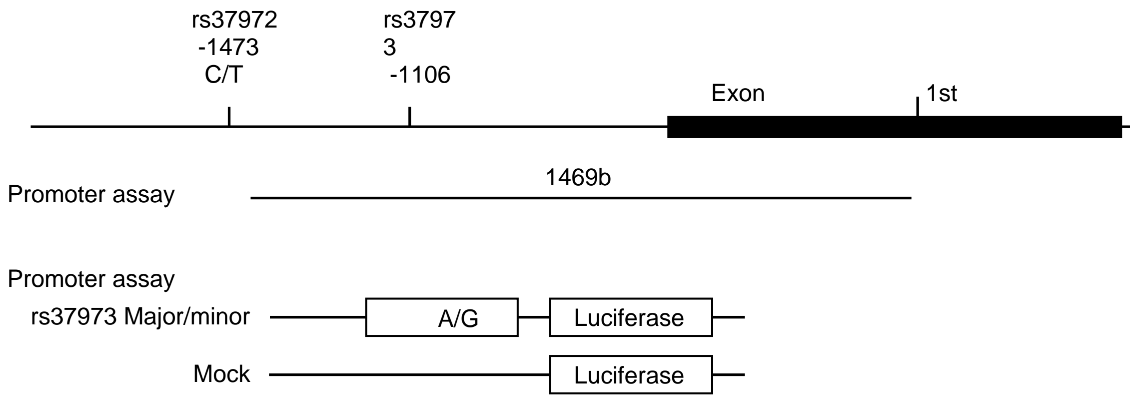


Supplemental Figure 4

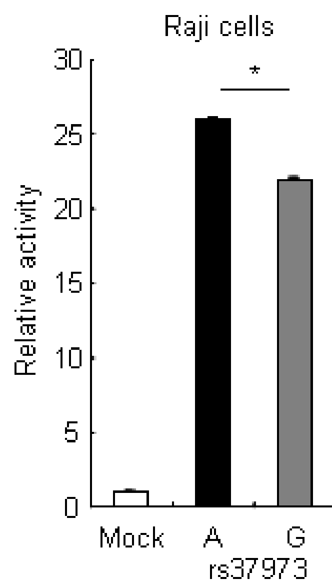


Supplemental Figure 5

A.



B.



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