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Multiomics Analysis Identifies *BIRC3* as a Novel Glucocorticoid Response-Associated Gene

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Abstract

Background: Inhaled corticosteroid (ICS) response among patients with asthma is influenced by genetics, but biologically actionable insights based on associations have not been found. Various glucocorticoid response omics datasets are available to interrogate their biological effects.

Objective: We sought to identify functionally relevant ICS response genetic associations by integrating complementary multiomics datasets.

Methods: Variants with p-values<10^{-4} from a previous ICS response genome-wide association study were re-ranked based on integrative scores determined from: i) glucocorticoid receptor (GR)- and ii) RNA polymerase II (RNAP II)-binding regions inferred from ChIP-Seq data for three airway cell types, iii) glucocorticoid response element (GRE) motifs, iv) differentially expressed genes in response to glucocorticoid exposure according to 20 transcriptomic datasets, and v) expression quantitative trait loci (eQTLs) from GTEx. Candidate variants were tested for association with ICS response and asthma in six independent studies.

Results: Four variants had significant (q-value<0.05) multiomics integrative scores. These variants were in a locus consisting of 52 variants in high LD (r^2≥0.8) near GR-binding sites by the gene *BIRC3*. Variants were also *BIRC3* eQTLs in lung, and two were within/near putative GRE motifs. *BIRC3* had increased RNAP II occupancy and gene expression with glucocorticoid exposure in two ChIP-Seq and 13 transcriptomic datasets. Some *BIRC3* variants in the 52-variant locus were associated (p-value<0.05) with ICS response in three independent studies and others with asthma in one study.

Conclusion: *BIRC3* should be prioritized for further functional studies of ICS response.

Clinical Implication: Genetic variation near *BIRC3* may influence ICS response in people with asthma.

Capsule summary

Multiomics data integration found that genetic variants near the *BIRC3* gene may influence glucocorticoid response in people with asthma by modulating *BIRC3* gene expression via altered GR-binding.

Keywords

Asthma; ChIP-Seq; genome-wide association study; glucocorticoid response; glucocorticoid receptor; inhaled corticosteroids; integrative analysis; multiomics; transcriptomics

Introduction

Inhaled corticosteroids (ICS) are glucocorticoids that are commonly used for the treatment of asthma[1]. Although use of ICS according to clinical guidelines successfully controls symptoms in most patients with persistent asthma, up to 30% of patients have severe asthma that requires additional medications[2]. The inter-individual variability in clinical

response to ICS is not fully understood, but previous pharmacogenetics studies suggest that it is influenced by genetics[3–11]. Genome-wide association studies (GWAS) have yielded reproducible loci associated with many traits, including asthma[12], but their application to the study of ICS response has not yielded consistent findings despite the identification of promising loci such as *GLCCI1*[3]. The lack of large study populations to study ICS response genetics, and the variability in study designs among available studies, including use of different outcomes (e.g., improved lung function, reduced number of asthma exacerbations), are among the issues that have hampered ICS response pharmacogenetics studies. The largest ICS response GWAS to date was based on 2,672 asthma patients treated with fluticasone furoate and fluticasone propionate from clinical studies conducted by GlaxoSmithKline (GSK)[11]. This study, in which response to ICS was measured as the change in forced expiratory volume in one second (FEV_1) from baseline to 8–12 weeks after initiation of ICS treatment, found little evidence that common genetic variants underlie ICS response in asthma patients, as no associations reached the genome-wide significance threshold of p-value$<5\times10^{-8}$. Given the relatively small sample size of this and other ICS response GWAS, some of the nominally significant loci could represent biologically relevant findings.

At a cellular level, glucocorticoids bind to the glucocorticoid receptor (GR), a transcription factor that forms GR-GR dimers in cell nuclei that directly bind to glucocorticoid response elements (GREs) where they induce the transcription of genes encoding anti-inflammatory proteins[13]. GRs also interact with other transcription factors (e.g., nuclear factor kappa B (NF-κB)) to reduce the transcription of genes encoding pro-inflammatory cytokines and cytokine-induced proteins[13]. The binding of GRs in tissues such as airway smooth muscle (ASM), BEAS-2B and A549 cells has been characterized via ChIP-Seq studies to occur at thousands of genomic regions and to change with exposure to glucocorticoids[14–16]. Similarly, the differential expression of thousands of genes in response to glucocorticoid exposure has been measured in various asthma-relevant tissues using gene expression microarrays and RNA-Seq[17,18]. These omics studies have begun to identify cell- and tissue-type specific responses to glucocorticoids that occur at the level of transcription and GR-binding[17,19–21].

The integration of multiomics data has provided insights into pulmonary diseases beyond those available in single-modality studies[22,23]. The combination of two types of omics data has shown promise to 1) prioritize ICS response-related GWAS variants according to transcriptomic data[24], and 2) distinguish primary from secondary targets of the GR via the combination of GR ChIP-Seq and transcriptomic data[15,20]. Although several relevant omics datasets related to glucocorticoid mechanisms of action in asthma-relevant tissues are currently available, including GR ChIP-Seq, glucocorticoid response-related transcriptomics, expression quantitative trait locus (eQTL) and genomic location of GREs, studies have not yet leveraged them to gain insights via their integration. Here, we assigned ICS response GWAS variants with integrative scores based on functional evidence from glucocorticoid response-related multiomics layers to identify ICS response loci whose nominally significant associations were supported by functional evidence.

Methods

Detailed methods are provided in the Online Supplement.

ICS Response GWAS Results

Summary statistics of a previously published GSK ICS response GWAS[11], whose outcome consisted of change in FEV_1 after ICS treatment in 2,672 asthma patients, were obtained. Subjects were primarily of European ancestry (1,787; 67%), and the available summary data included effect sizes and p-values for 10,215,080 variants derived from the hg19 reference genome obtained with an additive model that included relevant covariates. Variants having inconsistent information with 1000 Genomes Project phase 3 (1000G) data were excluded. Among filtered variants, common nominally associated ones with GWAS p-value$<10^{-4}$ were selected. The set of nominally significant variants was expanded to include bi-allelic variants that were in high linkage disequilibrium (LD) with them ($r^2 \geq 0.8$ based on 1000G European (EUR) genotype data). The hg19 coordinates were converted to hg38 coordinates using the UCSC LiftOver tool. Because several GSK subjects were not of European ancestry, an additional panel of variants was selected based on LD according to the 1000G cosmopolitan reference panel[11].

Transcriptomic Data Analysis

Twenty transcriptomic datasets related to glucocorticoid response involving 11 cell types were retrieved from Gene Expression Omnibus (GEO) and analyzed with the RAVED pipeline (https://github.com/HimesGroup/raved)[19]. Differential expression analysis was performed for 21 comparisons of glucocorticoid exposure versus vehicle control *in vitro* and two comparisons of glucocorticoid treatment versus placebo in human studies. Genes/probes tested were considered significant if they had Benjamini-Hochberg adjusted p-values (i.e., q-values) <0.05.

ChIP-Seq Data Analysis

ChIP-Seq datasets involving the GR with dexamethasone exposure for ASM (GSE95632)[14], BEAS-2B (GSE79803)[16] and A549 cell lines (SRP000762)[15] were retrieved from GEO or Sequence Read Archive (SRA) and analyzed with the brocade pipeline (https://github.com/HimesGroup/brocade)[20], which included differential binding analysis comparing GR- or RNA Polymerase II (RNAP II)-occupancy changes in cells exposed to dexamethasone versus vehicle control. Protein-binding sites with Benjamini-Hochberg adjusted p-values (i.e., q-values)<0.05 were considered significant. Target genes were restricted to those having GR-binding sites within 20kb of the gene's transcription start site (TSS), a distance that has been shown to identify well-known glucocorticoid-responsive genes[20], while also having either (1) RNAP II-binding sites within ±5kb to the genes' TSS (i.e. promoter region) in a matched ChIP-Seq dataset, or (2) significant differential expression results in at least one of the transcriptomic datasets. The FIMO tool[25] was used to identify putative GRE motifs within GR-binding sites as determined by sequences having p-values$<10^{-4}$. Protein-coding genes were annotated to protein-DNA binding sites based on GENCODE Human annotation data (v32). Overlap between GWAS variants, GR-binding sites and GRE motifs was performed using the *bedtools intersect* function[26].

eQTL Data

Single-tissue cis-eQTL results for whole blood, lung, and skeletal muscle tissues were obtained from the GTEx V8 release (https://gtexportal.org/home/datasets)[27].

Multiomics Integrative Score

A multiomics integrative score was assigned to each variant based on the sum of scores of evidence from five layers: 1) GR-binding, 2) GRE occurrence, 3) RNAP II-binding, 4) differential expression in responses to glucocorticoids and 5) eQTL. Significance of integrative multiomics scores was determined via permutation and subsequent adjustment using the Benjamini-Hochberg approach. Variants with adjusted p-values (i.e., q-values) <0.05 were considered significant. See Online Supplementary Methods for more detailed description.

Multiomics Analysis of Previously Reported ICS Response-Associated Variants

A secondary analysis was performed to include variants reported by other GWAS[3–10] and candidate gene studies[24,28–31], in which these variants were combined with the GSK GWAS variants. The same criteria as outlined above was applied to expand variant selection based on LD, except that LD structure for variants included was computed using 1000G genotype data that matched the population described in the paper originally describing the association. The multiomics score was computed on this expanded set of variants.

Genetic Association Analysis of ICS Response in Replication Studies

The association analyses between ICS response and 52 *BIRC3* locus variants selected for replication were conducted in the following studies:

GERA cohort.—The Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort[32,33] consisted of 5,710 individuals of European ancestry. The outcome of ICS response was defined as lack of asthma exacerbation while using ICS (i.e. the use of oral corticosteroid (OCS) bursts within 30 days after receiving an ICS prescription).

GALA II and SAGE studies.—The Genes-environments & Admixture in Latino Americans Study (GALA II) consisted of 854 Hispanic/Latino individuals and the Study of African Americans, Asthma, Genes and Environments (SAGE)[10,34,35] consisted of 493 African American individuals. The outcome of ICS response was defined as asthma exacerbation in the prior year while using ICS.

SLOVENIA cohort.—The SLOVENIA cohort[8] consisted of 166 patients of European ancestry. ICS response was defined as a binary outcome of ICS non-responders versus responders based on whether change in FEV_1 in patients after 6 weeks of ICS therapy was less than 8%.

Any variants with p-values<0.05 in a study population were considered significant, as these variants were considered representative of the single *BIRC3* genetic locus identified.

Genetic Association Analysis of Asthma in Replication Studies

We sought to determine whether the ICS-associated variants were also associated with asthma given that differences in ICS response might contribute to asthma development and symptoms and thus, influence the ascertainment of asthma cases in large biobank studies. The association analyses between asthma susceptibility and 52 *BIRC3* locus variants selected for replication were conducted in the following studies:

UK Biobank.—The UK Biobank study consisted of 19,216 individuals with asthma and 162,637 healthy controls of European ancestry from the UK Biobank[36].

EVE Consortium study.—The EVE asthma consortium study[37] included 3,246 cases, 3,385 controls, 1,702 case-parent trios, and 355 family-based cases and 468 family-based controls. Meta-analysis results of asthma GWAS in 3,459 European Americans, 992 African Americans/African Caribbeans, and 1,180 Latino Americans were previously reported[37].

Meta-analysis

We performed meta-analysis using Liptak's method separately for ICS response and asthma studies, where p-value-derived z statistics were weighted based on each study's sample size and a combined p-value for each variant was derived from the resultant z-statistics' distribution[38].

Results

Single Modality Glucocorticoid Response Omics Results

Characteristics of subjects from the previously published GSK ICS response GWAS are in Table I. Of 8,198,689 variants that passed quality control criteria, 6,700,204 (81.7%) common variants (EUR MAF≥0.01) were selected for analyses. No variants reached a genome-wide significance threshold of 5×10^{-8}. The top-ranked associations, with p-values<1×10^{-6}, were in loci near genes *PXDC1*, *C11orf72*, and *RORA* (Fig. E1, Table E1). Based on the 1000G EUR reference panel, a set of 4,468 GWAS variants was selected: 820 variants were nominally associated with ICS response (p-value<10^{-4}) and 3,648 variants were in high LD ($r^2\geq0.8$) with them. Based on the 1000G cosmopolitan reference panel, a set of 2,822 GWAS variants was selected: 883 variants were nominally associated with ICS response and 1,939 variants were in high LD ($r^2\geq0.8$) with them. We next report results based on the 4,468 GWAS variants obtained with the EUR reference panel. Integrative analysis results using the 2,822 variant set corresponding to the 1000G cosmopolitan reference panel can be found in the Online Repository.

Considering the ChIP-Seq data alone, 46,867 GR-binding sites had significantly increased GR occupancy and 7,238 RNAP II-binding sites had significantly different RNAP II occupancy with glucocorticoid exposure in at least one airway cell type (q-value<0.05). Among the many glucocorticoid-responsive GR-binding sites, 2,003 were within 20kb of a gene TSS while also having an associated RNAP II-binding event (RNAP II-binding site within 5kb of the gene's TSS). These 2,003 GR-binding sites with evidence of active

transcription corresponded to 851 genes. Of the 46,867 GR-binding sites, 13,165 (<30%) had putative GRE motifs.

Analysis of 20 transcriptomic datasets (Table E2) identified 13,580 differentially expressed genes (q-value<0.05) in response to glucocorticoids in at least one of the 11 cell types considered. 4,672 differentially expressed genes in airway cells (ASM, A549, BEAS-2B, bronchial epithelial cells) and 2,324 genes in remaining cell types also had nearby GR-binding sites, suggesting that they were primary GR targets. Detailed results of individual studies are available in the web application REALGAR (http://realgar.org/)[39].

1,262,229 cis-eQTLs in lung, 1,277,338 in whole blood and 1,406,351 in skeletal muscle were reported in GTEx.

Intersection of ICS GWAS and Single Modality Omics Results

Of the 4,468 GWAS variants considered, 29 were located within 39 of the 46,867 GR-binding sites (Fig. 1, Table E3). Nine genes (*B4GALT1*, *BIRC3*, *CSF1R*, *EMILIN2*, *GAB1*, *OXSR1*, *PKP1*, *SLC22A14* and *TIMP4*) had GWAS variants within GR-binding sites that were within 20kb of their TSS. Twenty-two of the 39 GR-binding sites had putative GRE motifs, and SNP rs11225201 was the only variant located within a GRE motif in a GR-binding site. 501, 577 and 430 of the 4,468 GWAS variants were cis-eQTLs in lung, whole blood and skeletal muscle, respectively. Among the 29 GWAS variants located within GR-binding sites, eight were eQTLs: four for *BIRC3* in lung, whole blood and/or skeletal muscle tissues; three for *SIL1* in lung; and one for *B4GALT1* in skeletal muscle tissue.

Integrative Analysis of Multiomics Glucocorticoid Response Data Supports a Locus Near *BIRC3* as Involved in ICS Response

Multiomics integrative scores for the 4,468 GWAS variants selected found that four single nuclear polymorphisms (SNPs) near the gene *BIRC3* were significant (score range 8.5 to 12; q-value<0.05) (Fig. 2, Fig. E2, Table E4). These four SNPs were in high LD ($r^2 \geq 0.8$ based on 1000G EUR reference panel) with each other, and SNP rs2846858 had the smallest (4.35×10^{-5}) GWAS p-value among them (Table II). The four SNPs overlapped with six glucocorticoid-responsive GR-binding sites. *BIRC3* had differential RNAP II-binding sites within 5kb of its TSS with exposure to glucocorticoids in BEAS-2B and A549 cells (q-value<0.05), suggesting that GR-binding events led to changes in gene transcription activity (Fig. 3A). The four *BIRC3* SNPs were also eQTLs in lung and blood, and two were additionally *BIRC3* eQTLs in skeletal muscle. SNP rs11225201 was located within a putative GRE motif, and SNP rs17878708 was 231bp from another putative GRE motif. According to transcriptomic data, *BIRC3* was differentially expressed in response to glucocorticoid exposure (q-value<0.05), with increased expression observed in ASM, BEAS-2B, A549, bronchial epithelium, childhood acute lymphoblastic leukemia, lymphoblastoid cells, and U2OS bone osteosarcoma epithelial cells and decreased expression observed in macrophages (Fig. 4). To select variants for replication, we expanded the *BIRC3* region to include all variants in high LD ($r^2 \geq 0.8$ based on 1000G EUR reference panel) with rs2846858 that were within 30kb upstream to 5kb downstream of the *BIRC3* TSS, resulting in a set of 52 variants (GWAS p-values ranged from 2.12×10^{-5} to 5.56×10^{-4};

Table E5). The minor alleles of these variants were associated with decreased change in FEV_1 after ICS treatment, and hence, decreased ICS response; minor alleles were associated with increased gene expression of *BIRC3* in GTEx lung tissues.

Previously Reported ICS Response-Associated Variants Lack Evidence of Altering GR-binding

The set of GSK variants was expanded to 4,271 candidate variants by including 118 variants reported in other ICS response genetics studies[3–10,24,28–31] and 4,153 variants in high LD with them (Table E6). None of the newly included variants overlapped with GSK GWAS variants. Two SNPs from the new set of variants overlapped with GR-binding sites (Table E3): 1) rs3794492, which was within 20kb of the TSS of *LDHAL6B*, was in high LD ($r^2 \geq 0.8$ based on 1000G EUR reference panel) with a GWAS SNP (p-value$<5\times10^{-8}$) reported as associated with dose-dependent response to ICS[6], and 2) rs76390075, which was located 339kb of the TSS of *LTBP1*, was nominally associated (p-value$<10^{-4}$) with asthma exacerbation despite ICS use in a prior study of Hispanic/Latino and African-American children[24]. Neither of these GR-binding sites had evidence of active transcription activity in the corresponding genes, nor were the eQTLs of the genes. Results with this expanded set of variants were generally consistent with those based on the GSK variants alone, and the same four variants near *BIRC3* identified with GSK data remained the only significant ones according to multiomics integrative scores for the 8,739 variants (q-values<0.05) (Fig. E2, Table E6). Of note, multiomics integrative scores of variants in candidate genes *GLCCI1*[3], *CRHR1*[28], *MAPT*[30], *STIP1*[31], and *TBX21*[29] were not greater than those expected by chance (range 0 to 2, q-value≥0.05).

Attempted Replication of *BIRC3* Variant Associations with ICS Response

Characteristics of subjects of four replication studies are in Table I. Forty-three of 52 *BIRC3* variants had results available in these cohorts, and within cohorts, the effect size directions were consistent (Table E5). In the GERA cohort, minor alleles of SNP rs12576911 were associated with increased ICS response (p-value=0.046) (Fig. 3B, Table III): having copies of its minor alleles increased time to a subsequent asthma exacerbation (hazard ratio=0.942 for alternative (i.e., minor) allele compared to the reference (i.e., major) allele). Nine variants in the GALA II study and seven variants in the SAGE study were associated with having an asthma exacerbation in the prior year despite ICS use (p-value<0.05), with SNPs rs2604526 and rs11606268, respectively, having the smallest p-values. Having copies of minor alleles of *BIRC3* locus variants corresponded to decreased asthma exacerbations in GALA II (odds ratio of rs2604526=0.752), and increased asthma exacerbations in SAGE (odds ratio of rs11606268=1.39). In the SLOVENIA cohort, none of the *BIRC3* variants showed significant associations (p-value>0.05). Meta-analysis results found that, of 35 *BIRC3* variants that had results in the five ICS response studies, 33, including the four GR-binding site-overlapping *BIRC3* variants, had p-value<0.05 (Table E5).

BIRC3 Variant Associations with Asthma

Characteristics of UK Biobank participants who comprised an asthma GWAS are in Table I. Forty-eight of 52 *BIRC3* variants had results available, 13 of which were associated with asthma (p-value<0.05), with SNP rs12280343 having the smallest p-value of 0.026 (Fig. 3B,

Table III). Having copies of minor alleles in these variants corresponded to decreased asthma risk (odds ratio of rs12280343=0.974) (Table E5). Characteristics of EVE participants can be found in Table 1 of a previous publication[37]. 15 of 52 variants had results available in at least one EVE racial/ethnic group sub-analysis, but none of the variants had p-value<0.05. Meta-analysis of the UK Biobank and EVE studies found that four of the 15 variants were significant overall (p-value<0.05) (Table E5).

Discussion

The discovery of reproducible glucocorticoid response genetic associations is challenged by the need to collect data on large numbers of patients with consistent and well-defined drug response phenotypes[13]. The largest published GWAS for ICS response that was based on 2,672 asthma patients enrolled in GSK clinical trials lacked statistically significant genetic associations, and its nominally-associated variants have not been the subject of further studies. We reasoned that some of the nominal associations may reflect true associations that did not reach genome-wide significant thresholds due to lack of statistical power stemming from its low sample size. Given that cellular-level glucocorticoid response mechanisms have been studied for many years, and various omics studies have characterized layers of relevant glucocorticoid-response data in an unbiased fashion, designing a biologically principled *ad hoc* integrative score to rank the nominally significant GWAS variants was possible. According to our multiomics integrative score, four GWAS SNPs that were part of a locus near *BIRC3* were identified as having an influence on GR-mediated transcription changes in airway cells that was greater than that expected by chance.

To functionally support SNP-trait associations identified via GWAS, variants are often annotated with information from resources such as GTEx and ENCODE, to determine whether variants are eQTLs or overlap with regulatory elements, respectively. These resources are limited for the study of ICS response because 1) cell type-specific results for prominent asthma tissues such as ASM and airway epithelium are not available, and 2) transcriptomic and DNA-protein binding information for most tissues was measured at baseline. In the case of glucocorticoid responses, richer, trait-specific data can be obtained. We searched the repository GEO, which contains ChIP-Seq and transcriptomic data from published studies related to various cell types and treatment conditions, to identify GR ChIP-Seq datasets corresponding to airway structural cell types treated with equivalent dexamethasone dosage and exposure times, each of which had corresponding RNAP II ChIP-Seq experiments, as well as to obtain a large number of gene expression microarray and RNA-Seq studies involving glucocorticoids. We are limited by the currently available eQTL data in GTEx, which does not include that from airway smooth muscle cells. Additionally, eQTL data derived from people with asthma, and in particular, eQTL data corresponding to the same donors with GWAS and drug response data would enable more precise studies into glucocorticoid response. However, the GTEx eQTL results of asthma-relevant tissues from non-asthma donors we used have been helpful in previous asthma GWAS studies[40,41], and skeletal muscle has been found to have eQTLs that were colocalized with asthma GWAS variants[42], suggesting that the currently used data are of value to study glucocorticoid response in people with asthma. Although our integration results were biased according to the currently available studies in terms of cell types and choice of

glucocorticoid types, dosages and time frames, by integrating all available data, our results were as robust as is currently possible to identify mechanistic hypotheses for consideration.

More broadly, there is an increasing interest in integrative multiomics approaches commensurate with the large amount of omics data being generated. Some of the existing approaches include EUGENE[43], which aggregates GWAS summary statistics from independent eQTLs; PrediXcan[44] and S-PrediXcan[45], which use gene expression prediction models derived from eQTL data to impute gene-level expression in a GWAS dataset; and colocalization methods that identify overlapping GWAS and eQTL and/or methylation quantitative trait locus (mQTL) signals[46,47]. Our method is novel in that it focuses specifically on glucocorticoid responsiveness, and as such, the omics datasets selected for it uniquely address this trait. For example, inclusion of transcription factor binding information has not been used in prior multiomics efforts, but in the case of glucocorticoid response, knowing where the glucocorticoid receptor binds to DNA suggests a direct biological mechanism for DNA variants to influence response to glucocorticoid drugs. We chose to use a binary score for each omics layer based on the presence of evidence in favor of altered GR-binding and/or transcription activity partly because we selected datasets closely related to glucocorticoid response in lung, but a more generalized version of our approach could include a greater number of datasets and/or weight the individual tissues and layers differently based on prior knowledge. For example, the effect sizes of association of individual SNPs could be used as weights to preferentially select SNPs with stronger association signals. As relevant new omics data becomes available (e.g., eQTL for airway smooth muscle and/or people with asthma), the scoring metrics can be adapted to include these data to generate additional hypotheses for mechanistic studies.

The ICS response outcome used in the GSK GWAS, change in FEV_1 after 8–12 weeks of ICS use measured during multicenter clinical trials, is not available in other studies. We nonetheless sought support for genetic associations with ICS response and asthma using independent studies with subjects from diverse populations. In the GSK study, minor alleles of rs2846858 were associated with decreased change in FEV_1 after ICS treatment, and thus, decreased response to ICS. Although rs2846858 was not significantly associated with ICS response in the GERA study, minor alleles of rs12576911, another SNP in the *BIRC3* locus, were associated with increased time to a future asthma exacerbation, a direction of effect that appears opposite to that observed in the GSK study. However, it is possible that the minor alleles of variants in this locus influence change in FEV_1 differently from asthma exacerbations. The SLOVENIA study, which had an outcome that resembled that of the GSK study, did not find any associations in the locus, but we note that its sample size was particularly small (n=166). The outcomes assessed in the GALA II and SAGE studies, namely exacerbations in the prior year while on ICS, resembled those of GERA, but while the direction of effect in GALA II was consistent with GERA, the direction in SAGE was opposite. These contrasting directions of effect may be due to the different racial/ethnic groups represented by these studies—GERA consisted of people of European ancestry, GALA II consisted of Hispanic/Latino subjects, while SAGE consisted of African American subjects. Specifically, the *BIRC3* locus was a distinct region consisting of variants in high LD according to EUR, cosmopolitan and admixed American (AMR) reference panels, but the LD patterns of the variants differed substantially in 1000G data of African populations

(AFR) (Fig. 3B). In terms of asthma, minor alleles of 13 variants within the *BIRC3* locus among UK Biobank participants were associated with decreased asthma risk, indicating a protective role, though this association was not observed among EVE participants.

Our study was limited in its ability to account for differences in genetic ancestry and identify results that may be unique to some racial/ethnic groups. Participants in the original GSK study were of heterogeneous ancestral backgrounds, with 67% of participants identified as having European ancestry and remaining participants as being from other racial/ethnical groups (i.e., 15% were Hispanic, 10% were Asian, 5% were African, and less than 1% were Native American or of mixed ancestry)[11]. We selected GWAS variants based on having high LD ($r^2 \geq 0.8$) with 1000G EUR reference panel and, separately, the 1000G cosmopolitan reference panel, to determine whether our results would remain consistent based on choice of reference panel to identify independent regions of association. We found that our multiomics results did not substantially change: 1) the same four *BIRC3* variants identified with the EUR panel as reference remained the only significant ones when using the 2,822 variants derived from 1000G cosmopolitan reference panel; 2) 40 variants were in high LD ($r^2 \geq 0.8$ based on 1000G cosmopolitan reference panel) with rs2846858—the top-ranked SNP using both reference panels—all of which were among the 52 variants in high LD with rs2846858 using the 1000G EUR reference panel (Table E5); 3) because all 40 variants from the cosmopolitan panel were among the 52 obtained with the EUR panel, variants that were nominally associated with ICS response or asthma among the 40 variants were also replicated when using the EUR reference panel. Future studies are needed to clarify differences in association results within this *BIRC3* locus according to genetic ancestry. Additionally, future primary glucocorticoid response GWAS conducted in a large number of non-European subjects may identify novel ICS response loci that are specific to non-European racial/ethnic groups.

The *BIRC3* gene encodes the protein baculoviral IAP repeat containing 3 (aka. cIAP2), a member of the family of inhibitor of apoptosis (IAP) proteins, which are critical suppressors of cell death and key mediators in inflammatory signaling and immunity via the activation of NF-κB pathways[48]. Prior publications have also reported associations with *BIRC3* variants: 1) a candidate gene study found that a region not in LD with the one presently reported was associated with asthma, eosinophil abundance, and neutrophil abundance (p-value<0.05)[49]; and 2) a GWAS that considered protein expression levels as outcomes[50], found that an intronic *BIRC3* SNP was significantly associated with levels of matrix metalloproteinase-1, a protein linked to airway hyperresponsiveness and asthma severity[51]. Prior studies also support the involvement of BIRC3 in the modulation of asthma-relevant inflammatory pathways. Primary nasal epithelial cells exposed to IFNγ or TNFα had increased BIRC3 protein expression, while BIRC3-depleted cells had increased expression of the apoptosis protein caspase-3 with TNFα exposure, suggesting that BIRC3 protected airway epithelial cells from apoptosis when exposed to pro-inflammatory cytokines[52]. *Birc3*-deficient mice had increased susceptibility to influenza-virus infection and impaired airway epithelium integrity mediated via death-receptor-induced programmed necrosis, suggesting that BIRC3 protected airway cells via maintenance of lung tissue homeostasis and host fitness[53]. Transcriptomic studies reported that *BIRC3* had increased gene expression levels in lymphoblastoid cell lines[54] and airway cells[15,55] when exposed to glucocorticoids, as well

as increased expression levels in asthma patients with higher sputum neutrophils[56]. We found that minor alleles of rs2846858 corresponded to increased *BIRC3* expression in lung according to GTEx results, and that *BIRC3* gene expression increased in response to glucocorticoid exposure in airway cells (Fig. 4), suggesting that minor allele carriers may have increased expression of *BIRC3* in response to glucocorticoid exposure compared to non-carriers. Future studies that capture individual *BIRC3* gene expression across participants can be used to conduct mediation analysis, thereby clarifying the relationship between genetic associations, gene expression and glucocorticoid response outcomes.

In summary, our multiomics integration study identified a novel ICS response-associated locus near *BIRC3* that is worth pursuing in mechanistic studies. Our demonstration that nominally-associated GWAS loci can be effectively prioritized via integration of multiomics data that is tailored to an outcome of interest, is an approach that can be applied to study other complex traits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

1000G	1000 Genomes Project
ASM	airway smooth muscle
ICS	inhaled corticosteroids
eQTL	expression quantitative trait locus
EUR	1000 Genomes European ancestry population
FEV_1	forced expiratory volume in one second
GALA II	Genes-environments & Admixture in Latino Americans Study
GEO	Gene Expression Omnibus

GERA	Genetic Epidemiology Research on Adult Health and Aging
GSK	GlaxoSmithKline
GR	glucocorticoid receptor
GRE	glucocorticoid response element
GWAS	genome-wide association study
LD	linkage disequilibrium
MAF	minor allele frequency
OCS	oral corticosteroid
RNAP II	RNA polymerase II
SAGE	Study of African Americans, Asthma, Genes, and Environments
SNP	single nucleotide polymorphism
SRA	Sequence Read Archive
TSS	transcription start site

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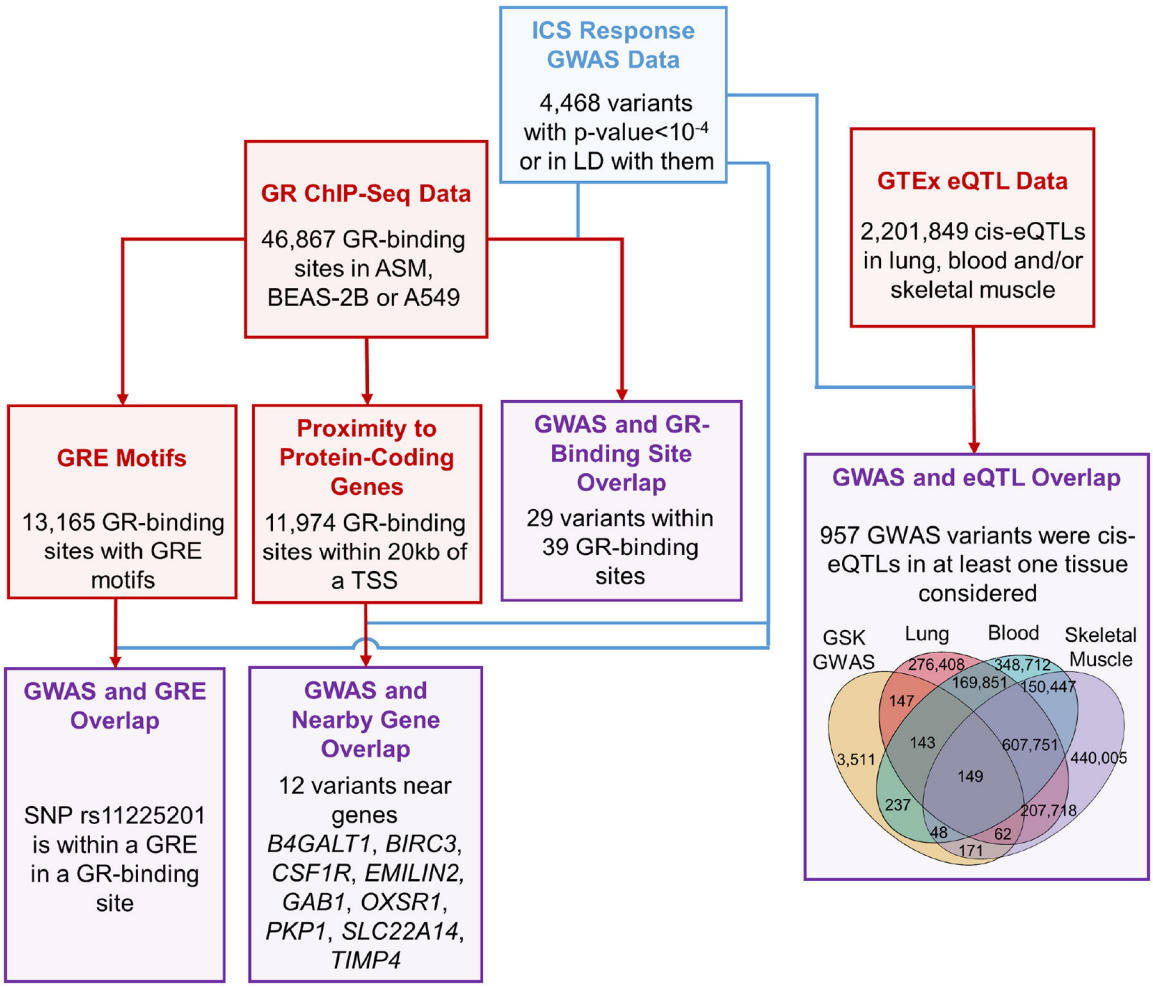
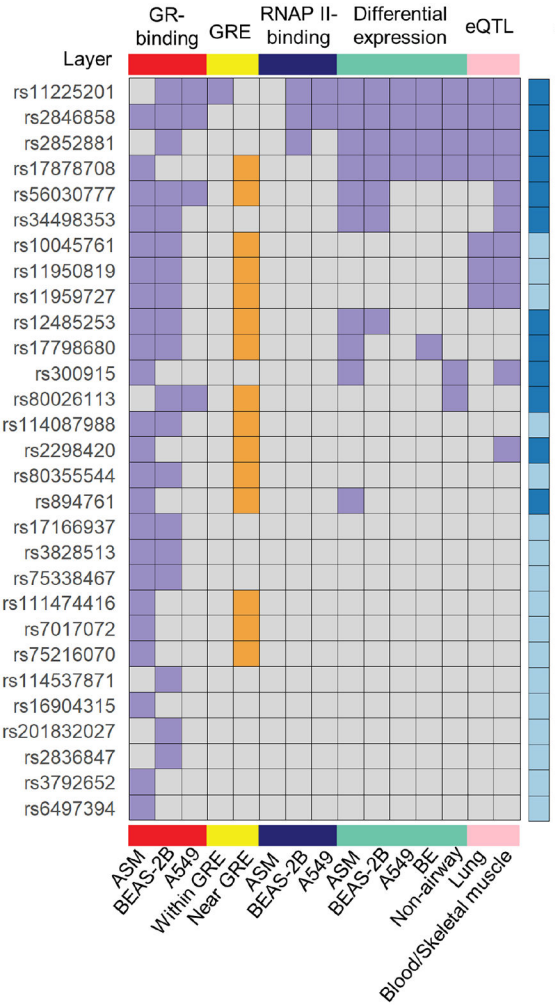


Figure 1.

Intersections between ICS response GWAS and single modality omics results. GWAS results, results from other layers and intersection results are shown in blue, red, and purple boxes, respectively. GR: glucocorticoid receptor; GRE: glucocorticoid response element; TSS: transcription start site.



Chrom:position (hg38)	Observed score	Empirical p-value	BH-adjusted q-value	Annotated genes
11:102301261	12	1.00×10^{-6}	1.49×10^{-3}	*BIRC3*
11:102306442	12	1.00×10^{-6}	1.49×10^{-3}	*BIRC3*
11:102306126	9	1.00×10^{-6}	1.49×10^{-3}	*BIRC3*
11:102320606	8.5	2.00×10^{-6}	2.23×10^{-3}	*BIRC3*
9:33163061	6.5	8.00×10^{-5}	7.15×10^{-2}	*B4GALT1*
3:38168538	5	9.18×10^{-4}	3.99×10^{-1}	*OXSR1*
5:139061600	4.5	9.82×10^{-4}	3.99×10^{-1}	*SIL1*
5:139061411	4.5	9.58×10^{-4}	3.99×10^{-1}	*SIL1*
5:139061276	4.5	9.44×10^{-4}	3.99×10^{-1}	*SIL1*
3:12144055	4.5	9.36×10^{-4}	3.99×10^{-1}	*TIMP4*
5:150108205	4.5	8.55×10^{-4}	3.99×10^{-1}	*CSF1R*
4:143353003	4	1.38×10^{-3}	4.73×10^{-1}	*GAB1*
1:201300202	3.5	1.36×10^{-3}	4.73×10^{-1}	*PKP1*
1:227344694	2.5	2.85×10^{-3}	7.76×10^{-1}	*CDC42BPA*
3:38281697	2.5	2.95×10^{-3}	7.76×10^{-1}	*SLC22A14*
10:20598770	2.5	2.90×10^{-3}	7.76×10^{-1}	*NEBL*
18:2856484	2.5	2.90×10^{-3}	7.76×10^{-1}	*EMILIN2*
7:95550590	2	8.50×10^{-2}	1	*PDK4*
4:143389506	2	8.48×10^{-2}	1	*GAB1*
19:45577422	2	8.53×10^{-2}	1	*OPA3*
15:35394763	1.5	8.57×10^{-2}	1	*DPH6*
8:90883660	1.5	8.61×10^{-2}	1	*NECAB1*
10:20599329	1.5	8.53×10^{-2}	1	*NEBL*
1:227373093	1	2.21×10^{-1}	1	*CDC42BPA*
8:130604647	1	2.23×10^{-1}	1	*ASAP1*
2:173049675	1	2.22×10^{-1}	1	*MAP3K20*
21:39026582	1	2.22×10^{-1}	1	*PSMG1*
4:143390108	1	2.22×10^{-1}	1	*GAB1*
16:19636932	1	2.22×10^{-1}	1	*VPS35L*

Figure 2.

Multiomics integrative scores for 29 GR-binding site-overlapping SNPs. ASM: airway smooth muscle; BE: bronchial epithelium; BH: Benjamini-Hochberg; GR: glucocorticoid receptor; GRE: glucocorticoid response element; RNAP II: RNA polymerase II; TSS: transcription start site.

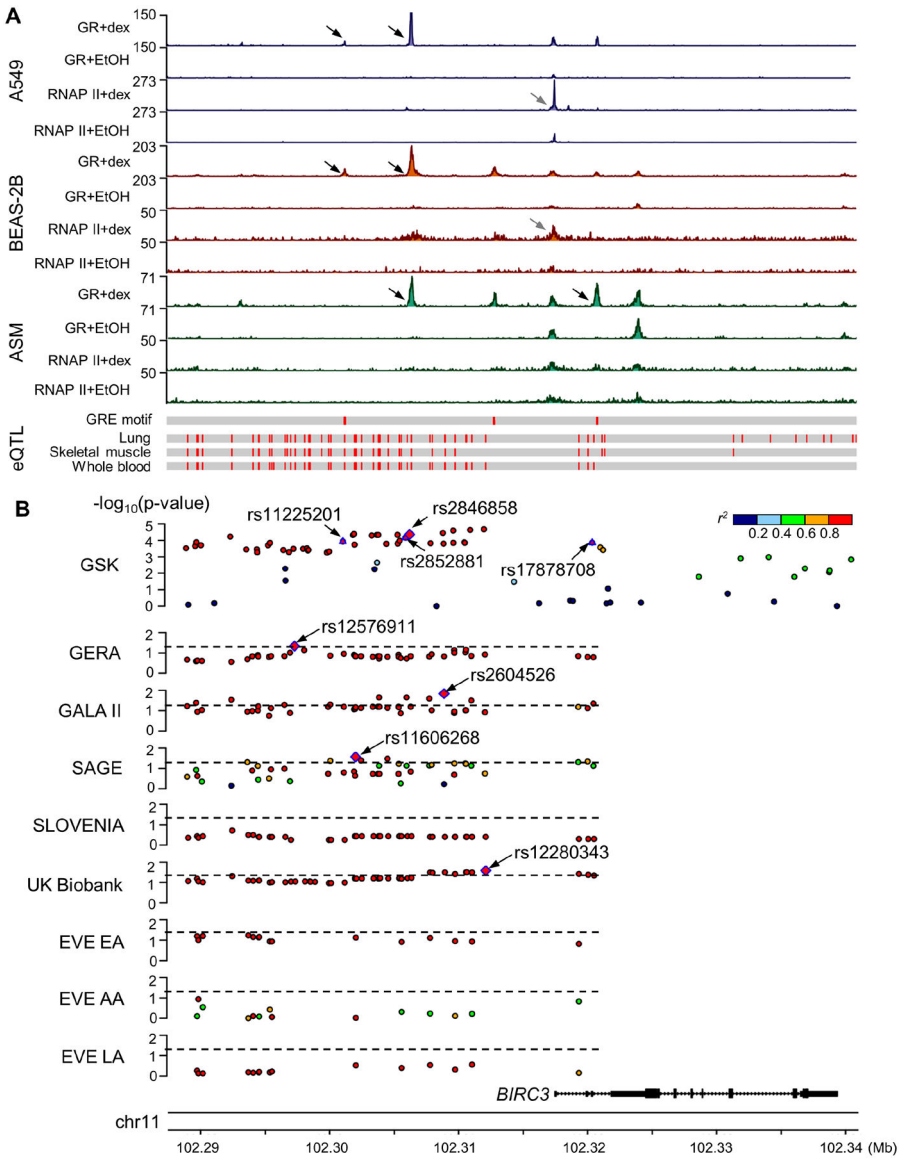


Figure 3.

Multiomics evidence supporting *BIRC3* as a glucocorticoid response-associated gene. A) ChIP-Seq (top), eQTL (bottom) and B) genetic association results in studies of ICS response and asthma susceptibility near the *BIRC3* gene. Black arrows in ChIP-Seq genomic tracks indicate differential binding sites for GR where *BIRC3* variants were located and grey arrows indicate differential binding sites for RNAP II. Pairwise r^2 was computed between rs2846858 and corresponding variants based on 1000G genotype data that matched the subjects' race/ethnicity in each study. Four GR-binding site-overlapping SNPs in the GSK study and the top SNPs with p-value<0.05 in replication studies are annotated. Dashed lines indicate p-value of 0.05. AA: African Americans/African Caribbeans; ASM: airway smooth muscle; Dex: dexamethasone; EA: European Americans; EtOH: ethanol; GR: glucocorticoid receptor; GRE: glucocorticoid response element; LA: Latino Americans; RNAP II: RNA polymerase II.

GEO ID	Tissue	Treatment	Q-value
GSE115830	BEAS-2B	Bud 100nM 2h	9.27E-07
GSE115830	BEAS-2B	Bud 100nM 6h	8.61E-08
GSE115830	BEAS-2B	Bud 100nM 18h	9.56E-08
GSE115830	BEAS-2B	Bud 100nM 1h	4.01E-05
GSE55877	chALL	Dex 100nM 6h	4.80E-02
GSE55876	chALL	Dex 100nM 6h	7.21E-03
GSE17307	A549	Dex 100nM 6h	4.37E-02
GSE1815	BE	Dex 100nM 8h	5.49E-01
GSE3040	LEC	Dex 1μM 16h	5.59E-01
SRP098649	ASM	Bud 100nM 18h	9.12E-22
GSE46448	U2OS	Dex 100nM 4h	2.29E-02
GSE22152	chALL	Dex 100nM 6h	4.15E-03
GSE4917	MCF10A-Myc	Dex 1μM 24h	1.65E-01
GSE4302	BE	Inhaled Flu 1000μg daily	1.20E-01
GSE83233	BE	Inhaled Bud 1600μg once	3.86E-03
GSE34313	ASM	Dex 1μM 24h	8.70E-03
SRP033351	ASM	Dex 1μM 18h	3.09E-01
GSE44248	LCL	Dex 1μM 16h	5.95E-03
GSE13168	ASM	Fluticasone 10nM 0.5h	2.94E-01
GSE84992	myotubes	Prednisolone 1μM 48h	6.79E-01
GSE87000	MACRO	Dex 100nM 6h	5.83E-01
GSE79077	MACRO	Dex 100nM 20h	5.07E-02
GSE61880	MACRO	Dex 100nM 10h	2.16E-02

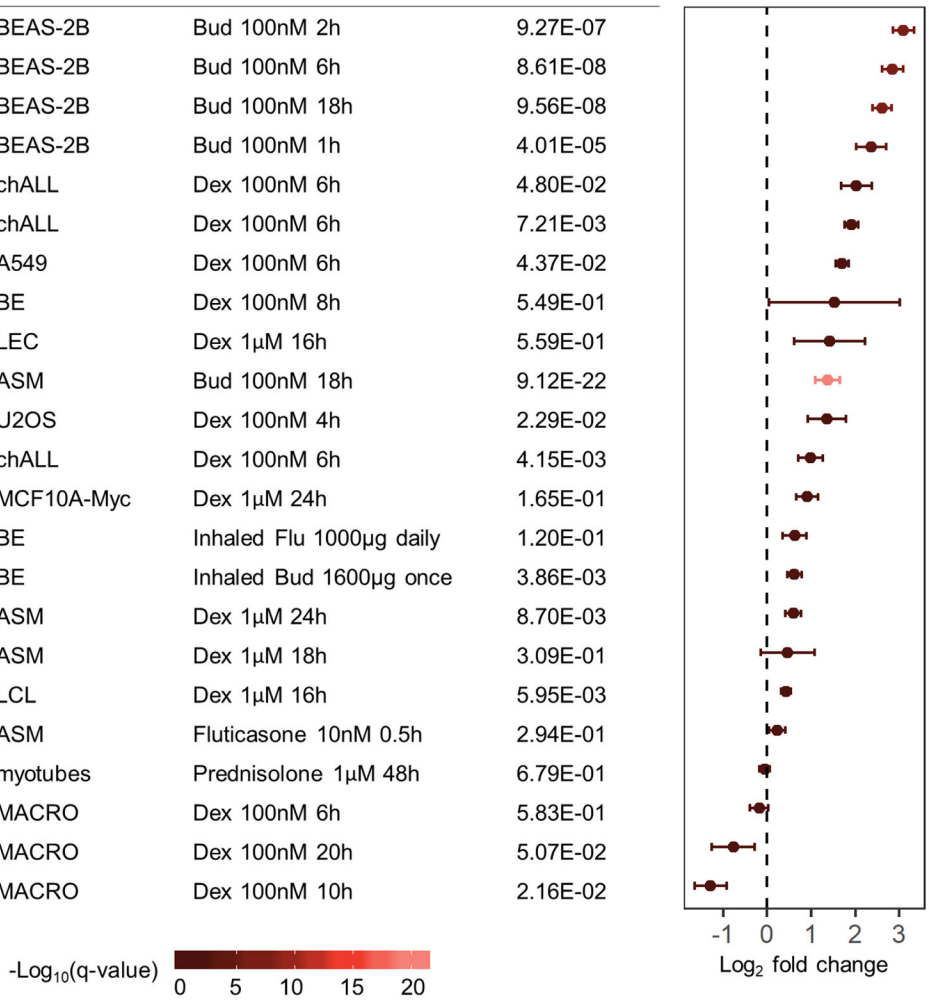


Figure 4.

Differential expression results for *BIRC3* in various tissues exposed to glucocorticoids versus control. ASM: airway smooth muscle; BE: bronchial epithelium; Bud: budesonide; chALL: childhood acute lymphoblastic leukemia; Dex: dexamethasone; LEC: lense epithelial cells; LEL: lymphoblastoid cell line; MACRO: macrophages; MCF10A-Myc: immortalized human mammary epithelial cell line MCF10A overexpressing c-Myc; U2OS: human bone osteosarcoma epithelial cell line.

Table I.

Characteristics of participants in ICS response studies.

A. GSK cohort			
Characteristics	**Phase IIB studies**	**Phase IIIA studies**	**HZA 106837**
Total patients	1,125	679	868
Age (y)	42.1±16.8	44.2±15.6	42.7±14.7
Sex: %female	61.2	58.6	68.6
BMI (kg/m^2)	27.9±6.7	27.9±5.2	27.6±5.9
Height (cm)	167.9±13.4	165.9±7.8	163.9±8.8
Asthma duration (y)	17.7±13.4	14.9±13.0	15.7±11.8
FEV_1 at baseline (% predicted)	69.9±10.1	69.2±10.4	71.7±11.8
FEV_1 change (% predicted)	7.2±13.4	6.9±13.0	6.1±11.8

B. GERA cohort		
Characteristics	**Asthma patients with exacerbation**	**Asthma patients without exacerbation**
Total patients	3,097	2,613
Age (y)	63.8±14.0	63.4±15.2
Sex: %female	2168 (70.0%)	1765 (67.5%)
BMI (kg/m^2)	29.2±6.2)	28.6±6.3
Smoking status: %ever smoker	1378 (45.9%)	1027 (40.9%)
Allergic rhinitis status	1404 (45.3%)	1113 (42.6%)
Gastroesophageal reflux disease	983 (31.7%)	651 (24.9%)
C. GALA II study		
Characteristics	**Asthma patients with exacerbation**	**Asthma patients without exacerbation**
Total patients	567	287
Age (y)	11.9±3.1	12.5±3.3
Sex: %female	246 (43.4%)	119 (41.5%)
D. SAGE study		
Characteristics	**Asthma patients with exacerbation**	**Asthma patients without exacerbation**
Total patients	256	237
Age (y)	13.2±3.4	13.8±3.4
Sex: %female	123 (48.0%)	103 (43.5%)
E. SLOVENIA cohort		
Characteristics	**ICS non-responders**	**ICS responders**
Total patients	94	72
Age (y)	10.7±3.2	11.2±3.5
Sex: %female	35 (37.2%)	33 (45.8%)
F. UK Biobank participants		
Characteristics	**Asthma patients**	**Healthy controls**
Total patients	19,216	162,637

Age (y)	55.8±8.4	57.1±8.3
Sex: %female	62.2	55.4
BMI (kg/m^2)	27.9±5.0	27.1±4.7
Smoking status: %ever smoker	57.4	58.1

Continuous values are shown as mean±standard error. GSK data is derived from Table I by Mosteller *et al*[11].

Table II.

Characteristics of four variants with significant multiomics scores that were in a locus near *BIRC3*.

rsID	Position	Ref	Alt	Alternative allele frequency (EUR/ cosmopolitan)	eQTL Tissue	GSK ICS Response Associations		GR-Binding Site Characteristics					
						Effect size	P-value	Peak start to end	Log_2 Fold change	Q-value	Tissue	Distance to TSS	GRE motif start to end
rs11225201	102301261	T	G	0.330/0.341	L,M,B	−0.043	1.16×10^{-4}	102301030–102301522	5.56	4.70×10^{-8}	BEAS-2B	−15,928	102301253–102301271
								102301132–102301325	3.68	2.85×10^{-3}	A549	−16,125	
rs2852881	102306126	C	T	0.314/0.283	L,B	−0.045	6.82×10^{-5}	102305878–102307125	4.49	1.64×10^{-67}	BEAS-2B	−10,325	-
rs2846858	102306442	C	T	0.315/0.327	L,M,B	−0.046	4.35×10^{-5}	102306146–102306808	4.78	1.05×10^{-7}	ASM	−10,642	-
								102305878–102307125	4.49	1.64×10^{-67}	BEAS-2B	−103,25	
								102306154–102306657	6.32	7.05×10^{-31}	A549	−107,93	
rs17878708	102320606	G	A	0.330/0.291	L,B	−0.044	1.36×10^{-4}	102320563–102321139	7.11	3.83×10^{-10}	ASM	3,113	102320838–102320855

Genomic coordinates are based on hg38 on chromosome 11.

Effect size refers to the alternative allele.

eQTLs in lung (L), whole blood (B), and skeletal muscle (M) tissues are selected.

GR-binding sites containing putative GRE motifs are reflected by presence of information in the column “GRE motif start to end”.

SNP rs11225201 is located within a putative GRE.

Table III.

Replication results of variants within the *BIRC3* locus with p<0.05 in at least one independent study.

GERA ICS Response GWAS							
SNP	**Ref**	**Alt**	**Alt allele frequency**	**Hazard ratio**	**P-value**	**1000G EUR panel based r^2**	**1000G cosmopolitan panel based r^2**
rs12576911	A	G	0.275	0.942	0.046	0.921	0.942
GALA II ICS Response GWAS							
SNP	**Ref**	**Alt**	**Alt allele frequency**	**Odds ratio**	**P-value**	**1000G EUR panel based r^2**	**1000G cosmopolitan panel based r^2**
rs2604526	C	A	0.297	0.752	0.014	0.945	0.687
rs2852880	G	C	0.312	0.769	0.022	0.995	0.809
rs2852881	C	T	0.312	0.769	0.022	0.995	0.813
rs2852882	T	A	0.306	0.771	0.025	0.945	0.792
rs7480738	G	T	0.299	0.775	0.029	0.982	0.685
rs2852891	G	T	0.306	0.779	0.032	0.928	0.786
rs12787745	C	A	0.325	0.790	0.041	0.912	0.759
rs17878708	G	A	0.316	0.793	0.045	0.823	0.738
rs150283650	G	A	0.340	0.803	0.050	0.921	0.889
SAGE ICS Response GWAS							
SNP	**Ref**	**Alt**	**Alt allele frequency**	**Odds ratio**	**P-value**	**1000G EUR panel based r^2**	**1000G cosmopolitan panel based r^2**
rs11606268	C	A	0.316	1.385	0.027	0.995	0.936
rs2846857	G	A	0.314	1.369	0.034	1.000	0.943
rs11600522	A	T	0.318	1.351	0.041	0.995	0.941
rs150283650	G	A	0.311	1.350	0.043	0.921	0.889
rs58277500	C	G	0.328	1.339	0.046	0.823	0.835
rs12098902	G	A	0.322	1.334	0.049	0.921	0.888
rs11602147	C	G	0.421	1.304	0.049	0.824	0.790
UK Biobank Asthma GWAS							
SNP	**Ref**	**Alt**	**Alt allele frequency**	**Odds ratio**	**P-value**	**1000G EUR panel based r^2**	**1000G cosmopolitan panel based r^2**
rs12280343	T	C	0.307	0.974	0.026	0.906	0.880
rs2511293	T	C	0.299	0.975	0.032	0.932	0.882
rs3132831	C	T	0.299	0.975	0.032	0.932	0.886
rs2604526	C	A	0.300	0.975	0.032	0.945	0.687
rs2852891	G	T	0.299	0.975	0.032	0.928	0.786
rs2852882	T	A	0.300	0.975	0.033	0.945	0.792
rs2846849	G	A	0.300	0.975	0.034	0.945	0.894
rs11602147	C	G	0.320	0.976	0.038	0.824	0.790
rs2604527	C	T	0.299	0.976	0.039	0.945	0.916
rs2846848	G	T	0.299	0.976	0.039	0.941	0.891

rs58277500	C	G	0.320	0.977	0.042	0.823	0.835
rs17878708	G	A	0.318	0.977	0.046	0.823	0.738
rs7480738	G	T	0.296	0.977	0.049	0.982	0.685

Hazard ratio and odds ratio use the alternative allele as reference. Pairwise r^2 was computed between rs2846858 and the corresponding variant.