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Combined analysis of transcriptomic and genetic data for the identification of loci involved in glucocorticosteroid response in asthma

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Author contribution

NH-P and MG equally contributed to this work. UP and MP-Y were involved in the conception and design of the study. NH-P, MG, SJ, AC, SJV, VB, MS, LSB, RT, SM, MP, KMV, MK, DBH, ST, CNAP, EGB, AHM-Z, UP, and MP-Y participated in the acquisition of data. NH-P, MG, AJ, LK, SJV, MA-H, MK, LSB, JV, CF, and MP-Y contributed to the analysis and/or interpretation of data. All authors drafted the article and/or participated in the final approval of the manuscript.

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To the Editor,

An increasing number of therapies are available to treat asthma, but inhaled corticosteroids (ICS) are still the most commonly prescribed and effective controller asthma medication[1]. Both environmental and genetic factors are involved in ICS response. However, few biomarkers have been associated with response to asthma treatment with ICS[2]. Here, we aimed to identify novel markers of ICS response combining transcriptomic and genomic data.

A full description of the methods is provided in the Supplementary Methods at the Supporting information and it is also summarized in Figure S1. In the discovery stage, RNA sequencing (RNA-seq) data from airway smooth muscle (ASM) cells isolated from four non-asthmatic donors publicly available at the Sequence Read Archive (SRA, SRP033351) (https://www.ncbi.nlm.nih.gov/sra) were analyzed[3]. Briefly, this study performed RNA-seq of cell lines from four non-asthmatic lung donors treated with control solution or with 1 μM dexamethasone for 18 h[3]. Raw sequencing data were downloaded and processed according to the procedures described in the Supplementary Methods. Differential gene expression of cells treated with glucocorticosteroids (GCs) compared to those treated with control solution was evaluated using linear models. Differentially dysregulated genes were identified after a false discovery rate (FDR) adjustment of 5% (q-value≤0.05).

Differentially expressed genes in the discovery were examined to determine if they also showed changes in peripheral blood mononuclear cells (PBMCs) obtained from ICS non-responders (n=3) and ICS responders (n=3) from the SLOVENIA study[4], defined based on the presence or absence of asthma exacerbations despite ICS therapy. RNA libraries were sequenced using the BGISEQ-500 instrument (BGI Inc.) and data was analyzed following the same methodology as for the ASM cells. Gene expression levels were compared in responders to ICS and ICS non-responder patients, adopting a q-value≤0.05 to declare significance. Validation of significant differentially expressed genes found in the previous

datasets was sought using three additional transcriptomic data of ASM cells after GCs exposure (GSE13168, GSE34313, and SRP098649), which were combined in a random-effects meta-analysis. Evidence of replication was considered for genes with consistent effects and q-value≤0.05.

Genetic variants located within ±100 kilobases (kb) from the genes with significant changes in expression levels across all transcriptomic datasets were examined for association with asthma exacerbations despite ICS. For that, ten studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium were analyzed, including 2,681 European and 1,347 Hispanics/Latinos and African American children and youth treated with ICS with genome-wide genotyping data available (Table S1). Details about each study are described at the Supporting Information and elsewhere[4].

Individuals with asthma exacerbations despite ICS treatment were considered as ICS non-responders and those without asthma exacerbations as ICS responders. Asthma exacerbations were defined by the need for emergency care, hospitalizations, administration of systemic corticosteroids, unscheduled doctor visits, and/or school absences because of asthma (Table S1). Association between imputed single nucleotide polymorphisms (SNPs) and asthma exacerbations despite ICS treatment was tested for each study through logistic regression models including age, sex, and principal components as covariates. Two separate meta-analyses were performed in Europeans and non-Europeans. A Bonferroni-like corrected threshold for significance was obtained for each population as α=0.05/total number of independent variants.

In the discovery, a total of 14,707 genes were analyzed and 4,718 of them were found to be differentially expressed in cells treated with GCs (q-value≤0.05) (Figure 1A). From these genes, 24 showed significant changes in expression levels depending on ICS response status from asthma patients in PBMCs (q-value≤0.05) with consistent alteration across both datasets based on $\log_2$ fold change (FC) values (Figure 1B, Table S2). Moreover, six out of the 24 genes were replicated with a consistent effect of up-regulation in the combined analysis of the three additional transcriptomic datasets of ASM cells treated with GCs: *LTBP1* (q-value=7.46×10^{-4}), *MTURN* (q-value=3.92×10^{-3}), *NAMPT* (q-value=3.77×10^{-7}), *CALD1* (q-value=5.22×10^{-5}), *MMD* (q-value=5.84×10^{-4}), *COL18A1* (q-value=1.93×10^{-3}) (Table S3). The potential implication of these genes on asthma severity was assessed by evaluating their expression in PBMCs according to baseline lung function, showing no significant changes (Table S4). Additionally, only *LTBP1* remained significantly differentially expressed in response to ICS treatment after accounting for baseline lung function (Table S4).

Association of 7,042 SNPs with asthma exacerbations despite ICS use within ±100 kilobases from the six genes validated was tested in Europeans. After applying a Bonferroni-like correction ($p\leq4.96\times10^{-5}$ for 1,007 independent variants tested), the SNP rs11681246 located within *LTBP1* (Figure 1C) was significantly associated with asthma exacerbations despite ICS use in Europeans (OR for G allele: 0.72, 95% CI: 0.63, 0.83, $p=3.28\times10^{-6}$) (Figure 2A–2B). The effect of this association was similar after adjusting by treatment step, as a proxy of disease severity, in a subset of 2,282 patients with complete information about asthma

medication (OR for G allele: 0.74, 95% CI: 0.63, 0.86, p=1.13×10^{-4}). However, this SNP was not replicated in non-Europeans. Nonetheless, we assessed the association of alternative polymorphisms in non-Europeans, revealing the association of six ~~an~~ intronic *LTBP1* variants in high linkage disequilibrium (r^2>0.95), all dependent from the association of rs76390075 (OR for C allele: 0.40, 95% CI: 0.26, 0.63, p=6.76×10^{-5}), after adjustment for 234 independent variants tested (p≤2.14×10^{-4}) (Figure 2C–2D). Moreover, this association was robust to the adjustment by medication step, OR for C allele: 0.41, 95% CI: 0.26, 0.65, p=1.12×10^{-4}. However, the association signals detected in European and admixed populations are not in LD (r^2<0.01). A description of the potential functional implications of these variants is available at the Supporting Information.

This study describes the results of transcriptomic analyses of several datasets revealing *LTBP1* as a gene involved in ICS response among asthma patients. GCs were found to increase *LTBP1* expression levels in four datasets of ASM cells experimentally exposed to different types of GCs. Similar effects were detected in PBMCs obtained from asthma patients that were ICS-responders, but not in non-responders. This suggests that ICS treatment may not induce *LTBP1* expression in PBMCs from non-responders. Additionally, the association of two variants within this gene with asthma exacerbations despite ICS use was found in diverse populations.

LTBP1 encodes a member of the family of latent-transforming growth factor-beta (TGF-β) binding proteins. LTBP1 is involved in the regulation of the TGF-β1 activity, including its activation from a precursor form, folding, secretion out from the cell, and deposition at the extracellular matrix through interactions with fibrillin molecules[5]. Interestingly, TGF-β1 has been proposed to play a key role in cell growth and differentiation, immune response, and airway remodeling[6]. Specifically, increased levels of the active form of TGF-β1 have been detected in asthma patients, which has been suggested to recruit myofibroblasts triggering an increased collagen deposition and the development of subepithelial fibrosis in asthma[7]. *LTBP1* has also been proposed to be involved in allergic diseases and idiopathic pulmonary fibrosis (IPF), where LTBP1 interacts with fibulin 1c (FBLN1), modulating lung remodeling and fibrosis through the regulation of TGF-β1 activation[8]. In fact, the inhibition of FBLN1 binding to LTBP1 has been proposed as a therapeutic strategy to reduce fibrotic processes[8]. Interestingly, genetic variants near or within *LTBP1* are also associated with lung function measurements among participants from the UKBiobank[9].

We acknowledge the limited sample size of the transcriptomic datasets and the lack of functional experiments as major limitations of our study. Therefore, validation of the association in independent populations analyzing larger sample sizes and functional studies are needed. These will provide insights about the biological mechanisms implicating *LTBP1* in ICS response and to test its clinical relevance predicting the treatment response. Additionally, a different gene expression profile would be obtained if the analyses would have been restricted only to ASM cells, as indicated by Kan *et al*[10].

In summary, our study revealed *LTBP1* as a potential novel locus for ICS response in asthma patients. These results indicate that combining publicly available data from different omic

sources could be a powerful approach to provide novel insights about the mechanisms involved in the ICS treatment response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest Statement

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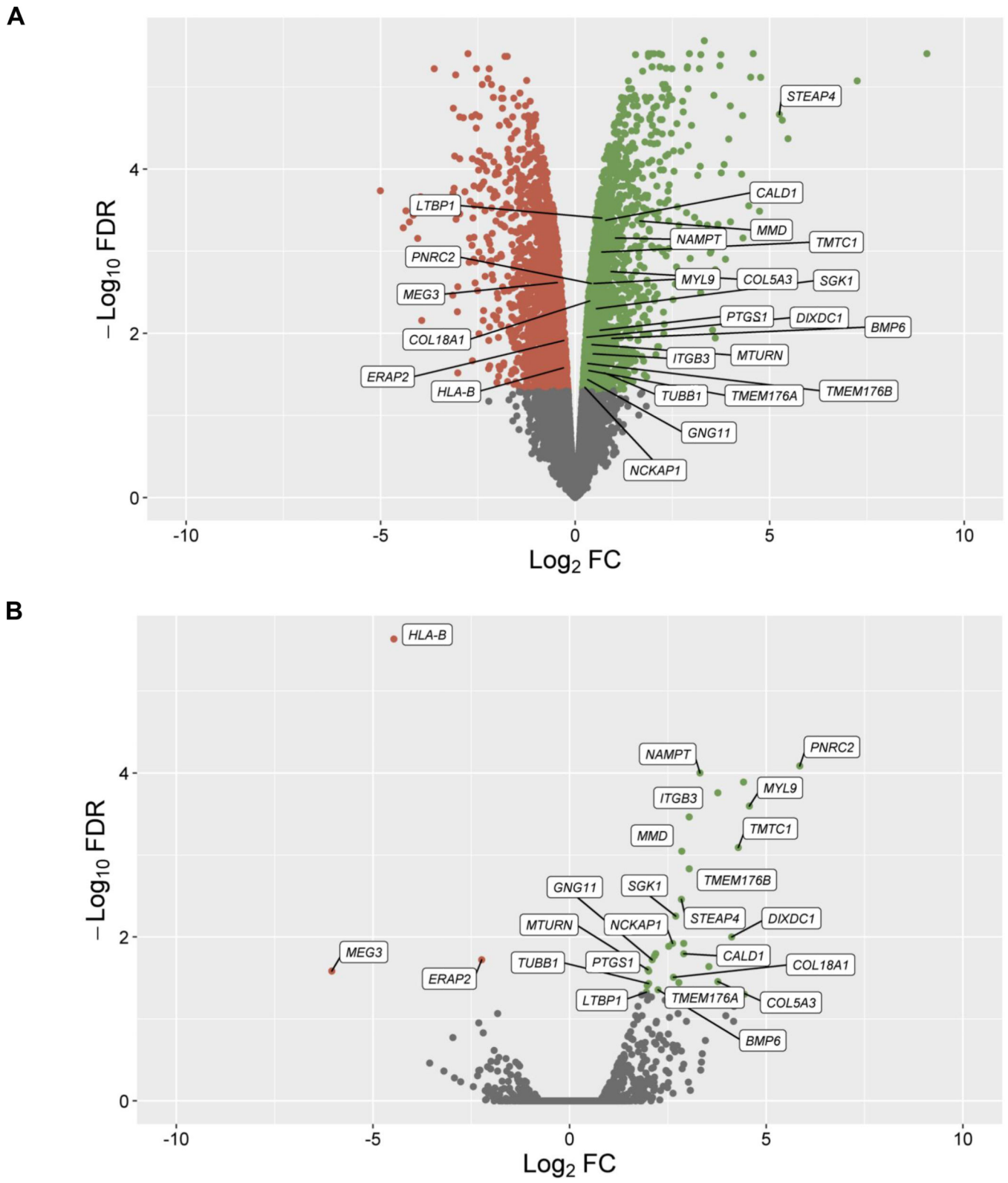

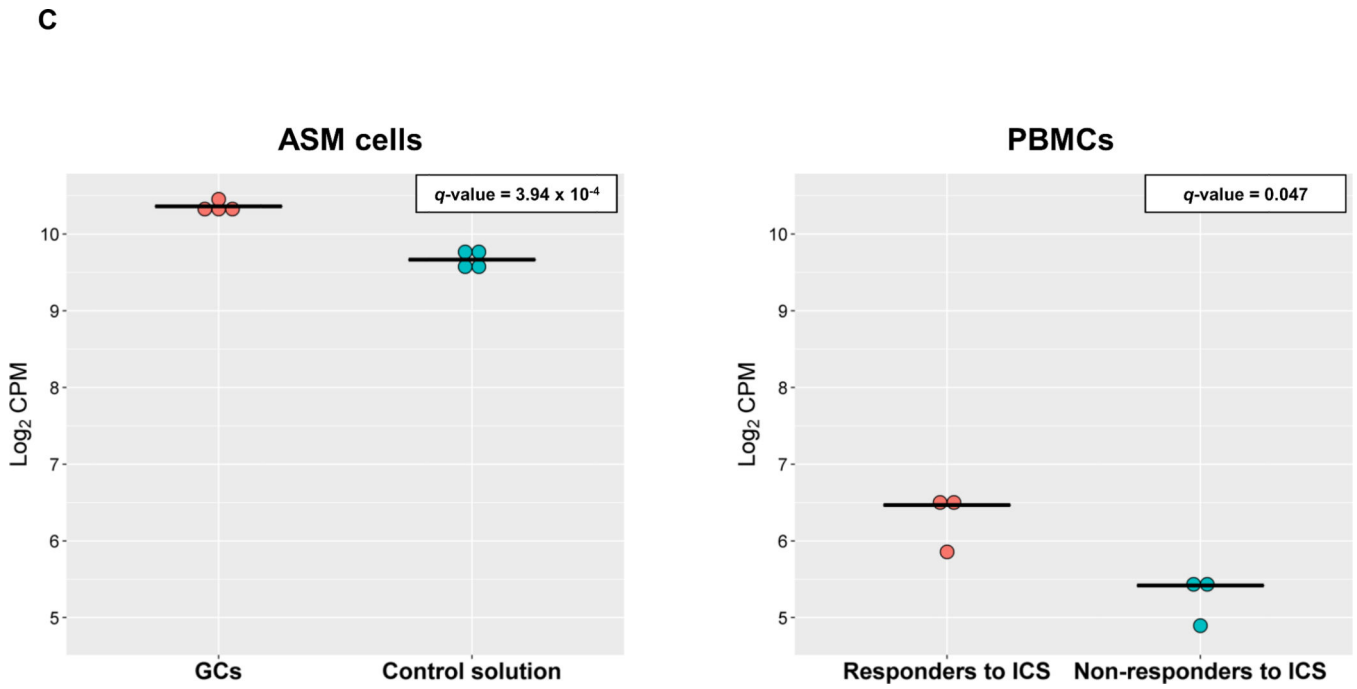

Figure 1. Results of differential expression in response to GCs in ASM cells and PBMCs.

Volcano plot of differential expression results in ASM cells after exposure to GCs compared to a control solution (panel A). Results of differential expression in PBMCs from ICS responders are shown for genes with significant changes in expression levels in ASM cells in response to GCs after adjusting with a false discovery rate (FDR) of 5% (*q*-value≤0.05) (panel B). Results are represented in terms of $\log_2$ fold change ($\log_2$ FC) (*x*-axis) and the logarithmic transformation of FDR ($-\log_{10}$ FDR) (*x*-axis). Genes significantly (*q*-value≤0.05) found to be up-regulated ($\log_2$ FC>0) or downregulated ($\log_2$ FC<0) are represented by means of green or red dots, respectively. Genes with consistent alteration of expression levels in ASM cells and PBMCs are labeled into white boxes.

Dots plots of differential expression for *LTBP1* in ASM cells and PBMCs (panel C). Gene expression levels are represented in terms of $\log_2$ counts per million (CPM) in the *y*-axis as dots for cases (red) and control (blue) samples. The median expression level is represented for each sample group by a black horizontal line. *P*-values adjusted by false discovery rate are shown (*q*-value).

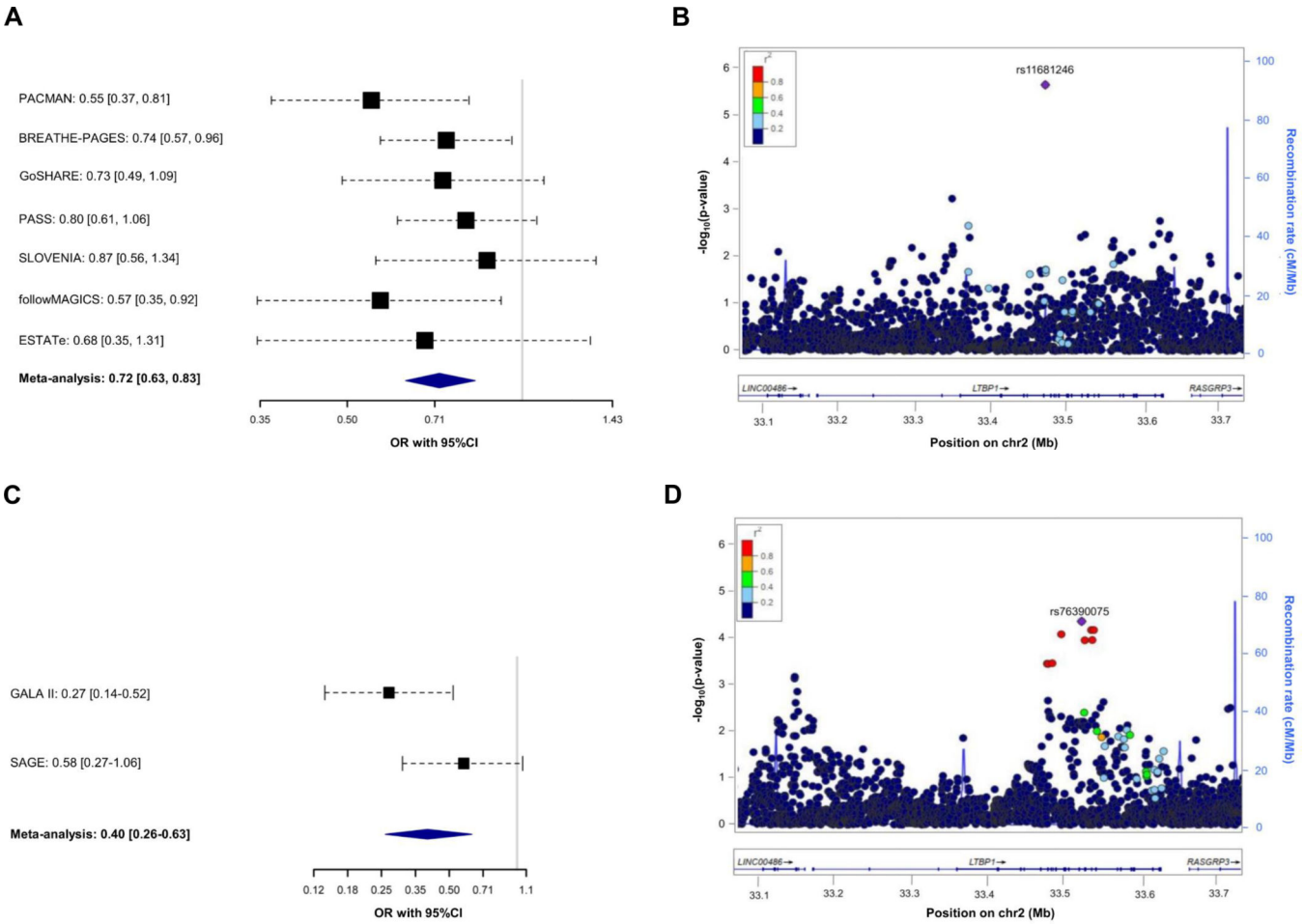

Figure 2. Association results with asthma exacerbations despite ICS treatment for *LTBP1*.

Forest plot of association effects of the SNPs rs11681246 (panel A) across the European studies and rs76390075 (panel C) in non-European studies. The effects are shown in terms of OR for the effect alleles (G and C, respectively) for each study and after performing a meta-analysis of the results by black boxes and a blue diamond, respectively. The 95% CI are represented by black dash lines. Results are not provided for BREATHE since this SNP did not pass quality control checks in this study.

Regional plot of association results with asthma exacerbations despite ICS use among Europeans (panel B) and non-Europeans (panel D). The *y*-axis represents the logarithmic transformation of the association results ($-\log_{10}$ *p*-value) by chromosome position (*x*-axis) for each SNP as a dot. The diamond corresponds to the most significant variant after Bonferroni-like correction; the remaining SNPs are color-coded based on pairwise linkage disequilibrium (r^2 values) with that SNP for Europeans (panel B) or Admixed American populations (panel D) from 1KGP (GRCh37/hg19 build).