Data Supplement

ARG1 is a novel bronchodilator response gene: screening and replication in four asthma cohorts

Augusto A. Litonjua
Jessica Lasky-Su
Kady Schneiter
Kelan G. Tantisira
Ross Lazarus
Barbara Klanderman
John J. Lima
Charles G. Irvin
Stephen P. Peters
John P. Hanrahan
Stephen B. Liggett
Gregory A. Hawkins
Deborah A. Meyers
Eugene R. Bleecker
Christoph Lange
Scott T. Weiss

Methods

Study Populations.

We utilized DNA samples from four clinical trials. All patients or their legal guardians consented to each trial study protocol and ancillary genetic testing. The population we used for the screening algorithm was the Childhood Asthma Management Program (CAMP), a multicenter, randomized, double-blinded clinical trial testing the safety and efficacy of inhaled budesonide vs. nedocromil vs. placebo over a mean of 4.3 years. Trial design and methodology have been published(E1, E2). A total of 418 children were randomized to the placebo group, from which we included the 209 Caucasian probands and their parents as part of a parent-child trio for these analyses. These trios formed the basis of our family-based screening cohort for bronchodilator response (BDR). Only subjects randomized to the placebo group were utilized for the screening analyses to avoid confounding effects of medications (corticosteroids and nedocromil) other than inhaled β-agonist.

The population we used for the first replication study (hereafter called the Asthma Trial) was composed of 432 Caucasian subjects with asthma(E3, E4) who were part of an asthma medication trial conducted by Sepracor, Inc. in the United States. To qualify for inclusion, patients had to be nonsmokers, have no significant comorbid medical conditions, and to have diagnostic findings consistent with moderate to severe asthma according to American Thoracic Society (ATS) criteria(E5). The only medications used by the patients were inhaled beta agonists as needed. Patients were required to have a forced expiratory volume in 1 second (FEV_1) of 40-85% of predicted normal values after at least eight hours without inhaled short-acting β-agonists; oral or inhaled corticosteroids were excluded for six weeks before the study. Reversibility of

airflow obstruction by beta agonists (15% change required) or methacholine sensitivity testing was employed to confirm asthma diagnosis.

Two completed trials conducted by the American Lung Association Asthma Clinical Research Centers (ALA-ACRC), the Leukotriene modifier or Corticosteroid or Corticosteroid Salmeterol trial (LOCCS)(E6) and the Effectiveness of Low Dose Theophylline as Add-on Treatment in Asthma (LODO) trial(E7), were used as the second and third replication samples. 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 PC20 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. The data for BDR for this analysis was determined from spirometry performed after completion of the run-in period. The 166 Caucasian subjects for whom DNA was available were used for this analysis. The LODO cohort comprised 489 participants ≥15 years old with poorly controlled asthma (regardless of baseline treatment regimen) as measured by a score of ≥1.5 on the Juniper asthma control questionnaire. Participants who had smoked within the last 6 months or who had a > 20 pack-year smoking history were excluded. At baseline, asthma severity was rated according to symptoms by the Asthma Symptom Utility Index (ASUI)(E8) and according to lung function by peak flow and spirometry (before and after bronchodilator). The 155 Caucasian subjects for whom DNA was available were used for this analysis.

Genotyping.

We genotyped 844 SNPs in 111 candidate genes: 42 genes involved in beta adrenergic signaling and regulation; 28 genes involved in innate glucocorticoid synthesis and metabolism, cellular receptors, and transcriptional regulators; and 41 genes from prior asthma association studies that had been previously conducted in the CAMP dataset (Supplementary Table 1). Candidate genes in the beta adrenergic and corticosteroid pathways were selected based on prior studies in the literature, their known involvement in metabolic pathways(E9, E10), and on expert opinion (SBL and KGT). Corticosteroid pathway candidate genes were included in this analysis because of the known interactions between beta2 agonists and corticosteroids(E11, E12). Finally, we included the candidate genes that our group had previously genotyped and studied in CAMP, since these were already available and so as to appropriately adjust our current analyses for all prior tests conducted with these genes. SNPs were primarily selected utilizing public databases, although resequencing of several core genes was performed. We over-sampled exonic and promoter regions and attempted coverage of at least one SNP every 10 kb. We emphasized golden-gate validated and LD tag SNPs, where available. Replicate genotyping was performed in the candidate genes powered in CAMP as below.

SNPs were genotyped via an Illumina BeadStation 500G (Illumima Inc., San Diego, CA) and via a SEQUENOM MassARRAY MALDI-TOF mass spectrometer (Sequenom, San Diego, CA). For the Illumina system, primers were created upon the submission of SNPs of interest. For each gene of interest, all SNPs available in the public database (dbSNP) were pulled. The flanking sequences for these SNPs were evaluated with the Illumina Assay Design Tool by the technical support group at Illumina (San Diego, CA). A file was returned that contained, for each SNP, a design score, design rank, and minor allele frequency in all available populations,

functional status, chromosome and position, and validation status (single submitter, multi-submitter, or pre-validated on Illumina platform). Design scores (scale of 0 to 1) indicates the likelihood of the assay design converting to a successful genotyping assay, with higher scores correlating with greater success. The design score is based on an algorithm that takes into account nrepetitive sequence, other SNPs that may interfere with primer placement, and GC content. SNPs with scores above 0.6 were selected for inclusion in the GoldenGate multiplex, with preference given to score above 0.8. Preference was also given to multi-hit or pre-validated assays, and markers with minor allele frequency > 0.5. A semiautomated primer design program (SpectroDESIGNER, Sequenom) was used for Sequenom. Each genotype was checked for percent completion rates and replicate genotyping was performed on a subset of genotypes for quality control. SNPs were also checked for Mendelian inconsistencies and for Hardy-Weinberg Equilibrium.

Statistical Methodology.

The primary outcome measure of the association analyses was acute response to inhaled bronchodilator (BDR), and calculated as the percent difference between the pre- and post-bronchodilator FEV_1 value (BDR = 100 x [post FEV_1 - pre FEV_1/pre FEV_1]). In all analyses, both screening and replication, BDR was treated as a continuous variable. We initially screened the genotypic association with BDR in CAMP using a modified version of the screening algorithm applied to genome-wide association studies as detailed by Van Steen, et al(E13). Our algorithm differed in that we screened to maximize power at the candidate gene level instead of the SNP marker level. This was both to provide a basis for replication at both the SNP and at the locus level, since differences in the LD patterns within our 4 populations were unknown prior to

genotyping. Moreover, we wanted to ascertain a consistent signal across a gene in order to augment our efforts in functional modeling. We used repeated measures of BDR over the four years of the trial in the Placebo group using the FBAT-PC statistic to maximize the heritability of a given marker and thereby maximize power for the screening stage. Only additive genetic models were evaluated for this analysis. We selected the most powerful candidate genes by first ranking the individual SNPs based upon conditional power and then evaluating the median rank of all of the SNPs within a given candidate gene. Conditional power was calculated based on first assigning expected probabilities of genotypes for each proband, based on parental genotypes (i.e. the screening method is blinded to the actual genotypes of the probands), then estimating the effect size for each of the expected proband genotypes. We selected genes whose median SNP ranks for power were within the top 25% of all SNPs genotyped to be taken forward for genotyping in the Asthma Trial population. We evaluated the FBAT-PC statistics for the SNPs for replication for directionality of the association for each SNP, and this allowed us to conduct 1-sided tests in the replication analyses. We selected 99 SNPs from 14 genes for replication in this population.

The analyses of the replicate populations were performed using generalized linear models as incorporated into PROC GLM of the SAS statistical analysis software (version 9.0, SAS Institute, Cary, NC). For all replicate analyses, SNP genotypes were coded for additive models. Only BDR calculated from the pre- and post-bronchodilator spirometric measurements at all baseline visits (prior to randomization) for all the trials were used. All models adjusted for age, gender, height, and baseline FEV_1. Genes with at least one SNP that was at least marginally associated with BDR (one-sided p-value < 0.05) were then genotyped in the final two replicate

populations (these were genotyped together, but analyzed sequentially). We then took the p-values (2-sided) from the original family-based analysis and the 1-sided p-values from the replication cohorts and combined them using a standard Fischer's method to increase statistical efficiency(E14). Prior evaluations of the Asthma Trial cohort has revealed no evidence of population stratification(3, 4). In a separate analysis of a random panel of 160 SNPs across the genome in both the LOCCS and the LODO populations, we found no evidence of population stratification. FBAT testing in CAMP is robust to potential population admixture(E15).

Table E1. 844 SNPs from 111 genes screened in CAMP.

Gene	Chromosome	Role*	rs#	Allele†	PBAT Power‡
ACVRL1	12	candidate	rs706812	A	0.001
ADAM33	20	candidate	rs2280090	G	0.051858291
			rs2280093	C	0.011184686
			rs2485700	T	0.380180012
			rs3918395	G	0.070842636
			rs487377	G	0.255535487
			rs597980	C	0.152426886
			rs615436	A	0.001
			rs630712	A	0.150491812
ADCY7	16	adrenergic	rs1064448	A	0.052175717
			rs1540624	A	0.145272311
			rs1872691	C	0.105425872
			rs2302715	T	0.135980929
			rs4785211	A	0.072756291
			rs7184802	G	0.066152354
ADCY9	16	adrenergic	rs1967309	A	0.073440134
			rs2072341	C	0.130456255
			rs2072342	G	0.051637616
			rs2072346	A	0.145486577
			rs2230739	A	0.073652384
			rs2230742	G	0.201548436
			rs2238436	G	0.169181456
			rs2239313	C	0.197311756
			rs2240735	A	0.094816275
			rs2256156	G	0.149891269
			rs2531977	G	0.170671781
			rs2531990	G	0.103440628
			rs2531992	C	0.250254879
			rs2532001	C	0.022339062
			rs2601790	C	0.001
			rs2601828	G	0.061165768
			rs3730097	G	0.003236582
			rs3730129	G	0.006321889
			rs432166	G	0.05984892
			rs7204987	G	0.149227494
			rs879620	A	0.162428383
ADCYAP1	18	adrenergic	rs2231187	C	0.313085988
			rs928978	G	0.029118168
ADCYAP1R1	**7**	**adrenergic**	**rs1006622**	**T**	**0.256716935**
			rs1468687	**A**	**0.205544836**
			rs2267732	**A**	**0.025170219**
			rs741051	**T**	**0.309872353**
			rs741052	**T**	**0.332164828**
			rs887703	**C**	**0.331598901**
ADRB2	5	adrenergic	rs17287460 (C-709A)	C	0.001
			rs1036173	T	0.17642073
			rs1036174	G	0.281146346
			rs1042713	G	0.106026779

			rs1042714	G	0.132673182
			rs1042718	C	0.444037754
			rs11168070	G	0.306374331
			rs12654778	G	0.055803514
			rs1368277	T	0.044732351
			rs1432626	C	0.004744992
			rs1432628	C	0.454057244
			rs1432630	G	0.02102983
			rs1432631	G	0.030869075
			rs1800888	C	0.001639273
			rs1801704	C	0.186666654
			rs2053044	A	0.160962853
			rs2116717	T	0.191105085
			rs754357	T	0.001278948
AQP5	12	candidate	rs2242357	C	0.224773232
			rs296753	G	0.001
ARG1	**6**	**candidate**	**rs2749935**	**T**	**0.272300521**
			rs2781659	**A**	**0.374627581**
			rs2781663	**A**	**0.320638168**
			rs2781665	**A**	**0.317997759**
			rs3756780	**T**	**0.024807942**
ARRB1	11	adrenergic	rs1676887	C	0.104197828
			rs1789682	A	0.046618146
			rs472112	A	0.330691653
			rs494146	T	0.119123115
			rs508435	C	0.320030629
			rs512797	G	0.208760552
			rs520563	C	0.062436537
			rs529513	C	0.053073291
			rs555031	C	0.051917593
			rs567807	C	0.040410941
			rs576014	G	0.075495613
			rs647630	G	0.040782851
			rs745373	G	0.069142359
			rs746168	T	0.024732639
			rs7929974	G	0.059686764
			rs877711	C	0.034859633
			rs899115	C	0.048020841
ARRB2	17	adrenergic	rs2271167	A	0.093592315
			rs7208257	A	0.164955548
			rs9905578	A	0.166412107
			rs9913156	C	0.066431276
ATP2A2	12	adrenergic	rs1860561	G	0.325541263
			rs3026434	A	0.001
			rs3026446	G	0.003627286
			rs9540	C	0.001
BDKRB1	14	adrenergic	rs10143977	T	0.057200211
			rs10147171	A	0.146053489
			rs11625494	G	0.006929342
			rs12050217	T	0.094172892

			rs2071084	G	0.095907181
			rs885845	G	0.077292011
			rs10220336	A	0.359491693
			rs1046248	G	0.02837315
			rs11627456	C	0.018955238
			rs11847625	C	0.204448735
			rs1800515	C	0.080336103
			rs1959053	A	0.094355506
			rs2069571	C	0.040627941
			rs2069585	C	0.001
			rs2242964	C	0.02075041
			rs4144132	T	0.047567097
			rs4900312	G	0.511285078
			rs4900318	G	0.480240155
			rs4905461	C	0.15200328
			rs4905470	G	0.049482257
			rs5224	C	0.143017742
			rs5225	A	0.119499924
			rs6575577	C	0.132257345
			rs7150828	C	0.276278372
			rs8008168	A	0.309711224
			rs8012552	A	0.514990824
			rs8016905	G	0.108916199
C5	9	candidate	rs17611	T	0.028782159
			rs2300931	T	0.069428559
CCL11	**17**	**candidate**	**rs4795896**	**T**	**0.066454772**
			rs1019109	**G**	**0.339696022**
			rs17809012	**G**	**0.359312003**
			rs1860184	**A**	**0.371290751**
			rs3744508	**G**	**0.351959656**
			rs3815341	**G**	**0.09543791**
			rs4795898	**T**	**0.084612964**
			rs714910	**A**	**0.299029085**
			rs16969415	**C**	**0.085174995**
CD3E	11	steroid	rs1945765	A	0.218305625
CD4	12	steroid	rs1045261	T	0.037378656
			rs1055141	T	0.059973478
			rs3829972	G	0.060567409
CHRM2	**7**	**adrenergic**	**rs6962027**	**A**	**0.296539977**
			rs6963577	**A**	**0.357410007**
			rs6967953	**A**	**0.291108642**
			rs8191992	**A**	**0.284930132**
CHRM3	1	adrenergic	rs10495449	A	0.053451229
			rs12021598	G	0.061688339
			rs12036109	A	0.094461392
			rs2067481	C	0.004729275
			rs4072234	C	0.170240983
			rs4659554	A	0.042674494
			rs6669810	C	0.252467227
			rs6682184	C	0.186652392

			rs7520974	C	0.261688857
COL2A1	**12**	**candidate**	**rs1635536**	**G**	**0.521197672**
			rs1635544	**G**	**0.647647593**
			rs1635546	**G**	**0.592369015**
			rs1793931	**T**	**0.534527587**
			rs1793959	**C**	**0.262988133**
			rs1814231	**G**	**0.001**
			rs2071357	**G**	**0.342896609**
			rs2276454	**A**	**0.41754818**
			rs2276455	**A**	**0.442515171**
			rs2276458	**G**	**0.265763276**
			rs6823	**C**	**0.613177739**
			rs915920	**G**	**0.001**
			rs917055	**G**	**0.625841941**
CPM	**12**	**candidate**	**rs1144960**	**G**	**0.645973616**
			rs1144961	**G**	**0.557243239**
			rs1144963	**C**	**0.297726401**
			rs1908669	**A**	**0.001178004**
			rs2172988	**C**	**0.134677726**
			rs2293637	**G**	**0.277678205**
CREB1	2	adrenergic	rs2254137	A	0.148364189
			rs2551640	T	0.132673716
			rs2551919	C	0.076401059
			rs2551921	A	0.13956091
			rs2709356	C	0.101787864
			rs2709387	G	0.113624623
			rs7369949	T	0.117943003
			rs889895	A	0.111307489
CREB3L2	7	adrenergic	rs273957	C	0.029345962
CREB5	7	adrenergic	rs1008048	A	0.059072291
			rs150607	C	0.280659862
			rs150610	A	0.063282285
			rs160335	G	0.190920131
			rs160337	G	0.143431341
			rs160356	C	0.102925587
			rs160357	A	0.102998686
			rs160369	A	0.063970786
			rs160375	T	0.089178384
			rs177584	G	0.064901588
			rs177590	T	0.053333543
			rs1859020	T	0.191069275
			rs1964240	C	0.120555404
			rs1976489	G	0.095210921
			rs2073537	T	0.086494582
			rs216708	G	0.213137149
			rs216715	T	0.057639682
			rs216730	C	0.025783676
			rs216737	G	0.22533519
			rs216750	T	0.105041981
			rs217515	G	0.280710392

			rs217519	C	0.682008068
			rs2237351	A	0.44483769
			rs2237353	T	0.137142339
			rs2237361	A	0.336778121
			rs2299110	A	0.274981427
			rs2391666	A	0.288084815
			rs2391668	G	0.317802703
			rs310353	A	0.158906021
			rs3757677	G	0.245584528
			rs41304	C	0.129943642
			rs41305	C	0.114963991
			rs41327	A	0.274120017
			rs41333	T	0.055454117
			rs41334	A	0.033857681
			rs41348	C	0.090567667
			rs41351	C	0.385552688
			rs42322	T	0.092975291
			rs4719934	C	0.089746812
			rs4719945	A	0.072538914
			rs4722804	C	0.040961632
			rs4722834	C	0.483247739
			rs6462085	G	0.172519991
			rs6462088	A	0.389057781
			rs6462100	A	0.217025021
			rs6949786	T	0.386471967
			rs6972081	G	0.25586762
			rs6976396	C	0.068462399
			rs740315	A	0.09456921
			rs757980	A	0.314286632
			rs886816	G	0.049193248
			rs989438	T	0.164802879
CREBBP	16	adrenergic	rs129974	G	0.001
			rs2230140	A	0.001
CREBL2	**12**	**adrenergic**	**rs4555**	**A**	**0.254068879**
CREM	**10**	**adrenergic**	**rs1057108**	**A**	**0.244079047**
			rs10827491	**C**	**0.254284554**
			rs10827492	**C**	**0.22569427**
			rs10827493	**C**	**0.283984827**
			rs1148247	**C**	**0.14977395**
			rs11592037	**C**	**0.22461565**
			rs11592356	**T**	**0.336869625**
			rs11597746	**C**	**0.331648519**
			rs1213392	**C**	**0.280511624**
			rs12761675	**T**	**0.258910646**
			rs1545757	**A**	**0.176594992**
			rs2295415	**T**	**0.077870955**
			rs4934535	**C**	**0.146205678**
			rs4934540	**T**	**0.258305323**
			rs4934734	**A**	**0.205964064**
			rs4934736	**G**	**0.244330188**

			rs6481941	**G**	**0.248354291**
			rs7077242	**C**	**0.250529114**
			rs7913615	**A**	**0.249474673**
CRH	8	steroid	rs10105164	C	0.079318914
			rs11997416	C	0.329387244
			rs3176921	A	0.302891618
			rs5030875	T	0.074488853
			rs6472257	C	0.279434243
			rs7835214	T	0.008998522
CRHBP	5	steroid	rs10473984	A	0.040949508
			rs10514082	T	0.09011322
			rs1053989	A	0.010312854
			rs1700676	T	0.088683628
			rs247742	G	0.090868966
			rs3811939	C	0.240640207
			rs7718461	C	0.012410772
CRHR1	17	steroid	rs12150390	A	0.032133224
			rs12950522	T	0.452051696
			rs1396862	T	0.240161625
			rs171440	T	0.513482771
			rs171441	C	0.041119528
			rs173365	A	0.118844745
			rs1876827	A	0.224428224
			rs1876828	A	0.20945287
			rs1876829	A	0.20991795
			rs1876831	G	0.160918441
			rs242925	C	0.366328304
			rs242938	C	0.026690213
			rs242939	A	0.055168716
			rs242941	G	0.322119891
			rs242942	C	0.069707281
			rs242947	C	0.125320551
			rs242949	C	0.301854272
			rs242950	G	0.068102785
			rs4792886	G	0.031563512
			rs4792888	A	0.034166317
			rs7209436	G	0.142123271
			rs739645	G	0.1932213
CRHR2	**7**	**steroid**	**rs1003929**	**C**	**0.287211163**
			rs155100	**T**	**0.510546554**
			rs2014663	**T**	**0.390533615**
			rs2190242	**A**	**0.097135319**
			rs2240403	**C**	**0.071882927**
			rs2251002	**C**	**0.526386461**
			rs2267712	**C**	**0.227704046**
			rs2267713	**G**	**0.489918981**
			rs2267715	**A**	**0.556974612**
			rs2267716	**A**	**0.107541034**
			rs2270007	**G**	**0.206928498**
			rs2270008	**C**	**0.382708948**

			rs2284216	**G**	**0.191034943**
			rs2284217	**G**	**0.43015793**
			rs2284219	**C**	**0.295477331**
			rs2284220	**A**	**0.233274816**
			rs255102	**A**	**0.091497673**
			rs3735430	**C**	**0.002271765**
			rs3779250	**A**	**0.449884297**
			rs4723000	**G**	**0.323052951**
			rs4723002	**A**	**0.055463187**
			rs733453	**A**	**0.503049917**
			rs7793837	**T**	**0.120348962**
			rs8192496	**A**	**0.422070013**
			rs917195	**C**	**0.079008212**
			rs929377	**T**	**0.489698913**
			rs973022	**A**	**0.191183389**
			rs975537	**A**	**0.561840719**
CSK	**15**	**adrenergic**	**rs12439525**	**C**	**0.017997931**
			rs1378942	**A**	**0.267661343**
			rs2168518	**A**	**0.259889193**
			rs2301249	**G**	**0.271375766**
			rs7085	**C**	**0.302556681**
CTSS	**1**	**candidate**	**rs1136774**	**G**	**0.282855819**
CX3CR1	3	adrenergic	rs2669849	A	0.15010471
			rs3732378	C	0.150384071
			rs3732379	G	0.214402095
DEFB1	**8**	**candidate**	**rs2738182**	**A**	**0.710758688**
			rs2741136	**C**	**0.287021919**
			rs5743402	**A**	**0.361865511**
DPP10	2	candidate	rs10208402	C	0.106011773
			rs6737251	T	0.169604702
			rs982213	G	0.783015238
F2R	5	adrenergic	rs153311	C	0.064490777
			rs2227744	A	0.495744636
			rs2227754	A	0.001
			rs2227795	A	0.001
			rs2227817	G	0.001
			rs27135	A	0.337375028
			rs37242	A	0.089913645
F2RL1	5	adrenergic	rs2243010	C	0.100281246
			rs2243072	C	0.003552027
			rs631465	C	0.01209055
			rs6453253	C	0.370624492
F2RL3	19	adrenergic	rs2227349	C	0.060802825
			rs706765	A	0.041701571
			rs7245967	C	0.06717012
			rs773901	A	0.125468343
FBN2	5	candidate	rs154001	T	0.315396916
			rs154003	G	0.213905086
			rs1801167	G	0.001
			rs2042327	T	0.218425441

			rs2307110	A	0.069754958
			rs27855	A	0.18599896
			rs28114	G	0.19433764
			rs32209	T	0.175743397
			rs32216	T	0.277987385
			rs331079	G	0.025259402
			rs3805635	T	0.02299758
			rs3805651	G	0.104419937
			rs3805652	A	0.038489389
			rs468026	T	0.133940325
			rs764371	A	0.105462185
FCER2	19	candidate	rs10415518	G	0.033648218
			rs12610479	G	0.202843348
			rs12611038	A	0.262149529
			rs12980031	A	0.155320675
			rs1990975	C	0.124802901
			rs2277989	T	0.284483267
			rs2277991	T	0.112755888
			rs2287867	T	0.162565659
			rs2287868	T	0.2403883
			rs3760687	C	0.010240238
			rs4804221	A	0.21978839
			rs4804773	A	0.342302546
			rs4996974	T	0.383907392
			rs7249320	G	0.296417825
			rs753733	G	0.06318342
			rs8110128	G	0.120893747
			rs889182	A	0.114907445
GAL	11	adrenergic	rs1042577	T	0.215461261
			rs1546309	A	0.143852595
			rs2513304	A	0.332273463
			rs3136538	A	0.206974022
			rs3136541	C	0.243709464
			rs3136543	A	0.21228777
GATA3	10	candidate	rs10905277	G	0.142840763
			rs1149901	G	0.031664313
			rs1399180	C	0.083082828
			rs2229359	G	0.037732034
			rs2229360	C	0.001
			rs2275806	A	0.178831595
			rs406103	C	0.447198351
			rs485411	G	0.036024832
GRK4	4	adrenergic	rs1024323	C	0.134313057
			rs1056094	G	0.227540918
			rs1801058	C	0.456058127
			rs2471322	A	0.203160852
			rs2471327	C	0.110343217
			rs2471337	A	0.21970918
			rs2471350	A	0.138994039
			rs2488806	T	0.114126711

			rs2488815	G	0.167594142
			rs2960306	G	0.231936649
			rs3021140	C	0.265565148
GRK5	10	adrenergic	rs10886430	A	0.2014091
			rs11198907	C	0.076649799
			rs1268947	G	0.120716409
			rs1473799	C	0.026173123
			rs1475753	C	0.21993736
			rs1537576	C	0.137367549
			rs2085185	G	0.07060072
			rs2230345	T	0.003549978
			rs2230349	C	0.148552534
			rs4752266	A	0.326206526
			rs506657	A	0.190829298
			rs915394	T	0.238084115
			rs928670	A	0.328653439
GRK7	3	adrenergic	rs11921607	T	0.117783275
			rs2138789	A	0.137705936
			rs4234469	G	0.285880035
			rs4337623	A	0.226718476
			rs4683625	C	0.249005544
			rs6806847	A	0.225880364
HAT1	2	steroid	rs10165126	C	0.097389995
			rs10439296	A	0.221910295
			rs10930498	C	0.18045929
			rs11692418	G	0.279534718
			rs1443700	G	0.313639258
			rs1982288	T	0.210954077
			rs3791342	A	0.001
			rs6758494	T	0.280138762
HDAC1	1	steroid	rs6697130	A	0.001
HDAC2	6	steroid	rs10499079	C	0.049945546
			rs10499080	C	0.063810156
			rs2499618	C	0.049536925
			rs3757016	C	0.157203653
			rs6568819	C	0.180301098
			rs9481408	C	0.400636654
HDAC3	**5**	**steroid**	**rs187515**	**C**	**0.234420322**
			rs32956	**C**	**0.284339831**
HDAC5	**17**	**steroid**	**rs228757**	**C**	**0.350353677**
			rs375171	**A**	**0.17927437**
HDAC7A	12	steroid	rs2240106	C	0.107589108
			rs2240108	C	0.070172838
			rs2301783	A	0.125542086
			rs3782908	A	0.408269818
HSPCA	14	steroid	rs2277465	G	0.00129919
			rs2298877	G	0.045349473
			rs4906179	G	0.066133861
			rs8004640	G	0.084642789
IFNG	12	candidate	rs1861494	A	0.054581684

			rs2069727	G	0.087332538
IKBKAP	**9**	**candidate**	**rs10759326**	**A**	**0.454160801**
			rs11791783	**G**	**0.210236129**
IL10	1	candidate	rs1800871	C	0.180983287
			rs1800872	C	0.091547065
			rs1800896	G	0.344973432
			rs3024492	A	0.360132946
			rs3024496	C	0.323327241
			rs3024509	T	0.132081736
IL12B	5	candidate	rs1368439	A	0.229968469
			rs2569253	A	0.084247734
			rs3181216	T	0.283829525
			rs3212219	G	0.120777336
IL13	.	candidate	G+2044A	G	0.179815332
IL18BP	11	candidate	G9772A6	C	0.99999043
			rs1541304	C	0.032313321
			rs1573503	C	0.039641094
			rs1892919	A	0.238324822
			rs2298455	A	0.106359307
			rs3814721	T	0.23974763
			rs949323	G	0.080435917
IL22	12	candidate	rs2227485	C	0.313672938
			rs2227513	A	0.068633788
IRAK3	12	candidate	rs1152888	G	0.140872862
			rs1152909	C	0.234872683
			rs1168774	G	0.194520772
			rs1732887	A	0.024966293
			rs1732888	T	0.055183921
			rs2701653	C	0.080496836
ITPR1	3	adrenergic	rs13079522	C	0.217679473
			rs1389162	A	0.100471669
			rs1866999	A	0.099271793
			rs1994500	A	0.0636528
			rs2054871	C	0.051328803
			rs2119802	A	0.21032721
			rs2291859	C	0.143692665
			rs2291862	C	0.540808322
			rs2306874	C	0.003371338
			rs2306875	A	0.35805047
			rs2306877	G	0.099303681
			rs2306881	G	0.067273526
			rs304011	A	0.050931716
			rs304028	A	0.198055501
			rs304039	G	0.210788702
			rs3792494	A	0.014774918
			rs3792511	A	0.06762596
			rs3804984	C	0.0635552
			rs3804999	C	0.057726513
			rs3805018	A	0.161115835
			rs4510365	T	0.122114757

			rs4685785	A	0.137231681
			rs4685798	C	0.062373573
			rs6442887	A	0.083843588
			rs6442905	G	0.026355986
			rs6768493	G	0.164543294
			rs6786081	G	0.090528733
			rs6786487	G	0.061493027
			rs6802929	G	0.044653673
			rs731915	C	0.188481036
			rs7630009	T	0.153166816
			rs7631664	A	0.072296952
			rs873768	C	0.371844771
			rs901856	G	0.127328929
			rs9815192	A	0.123414069
			rs9876432	T	0.05408341
ITPR2	12	adrenergic	rs1002835	T	0.060631463
			rs1007938	A	0.095725126
			rs1031301	A	0.139870524
			rs10743585	T	0.198398847
			rs10743591	C	0.060719775
			rs10743592	T	0.144525396
			rs10771277	C	0.30996399
			rs10771301	A	0.123089168
			rs10842720	A	0.104445099
			rs10842774	A	0.250039638
			rs10842796	T	0.014244758
			rs10842797	T	0.022274484
			rs10842798	A	0.016915129
			rs11048588	A	0.043933404
			rs12313993	G	0.049222491
			rs12582043	C	0.258806764
			rs1386810	G	0.172520984
			rs1393413	C	0.180434599
			rs1463589	G	0.228349258
			rs1463590	A	0.065708547
			rs1482979	G	0.044832041
			rs1484881	A	0.051299017
			rs1532720	A	0.121026817
			rs1565175	G	0.134385925
			rs1875579	G	0.144365502
			rs2035440	C	0.127456389
			rs2036434	G	0.113219525
			rs2062165	C	0.097108232
			rs2170980	A	0.144964016
			rs2171520	A	0.082148521
			rs2230377	C	0.028316173
			rs2270960	T	0.216454459
			rs2306549	G	0.266559406
			rs2880877	G	0.219108756
			rs3782290	T	0.192903948

			rs3782294	C	0.184991764
			rs3782295	C	0.160719066
			rs3782309	C	0.0462586
			rs3816834	C	0.233113001
			rs4963984	C	0.088346425
			rs4963993	C	0.044787919
			rs4964005	A	0.118411327
			rs7137796	A	0.269343162
			rs728009	A	0.1064405
			rs7297828	A	0.320455494
			rs7306427	C	0.037722993
			rs7309048	C	0.180760705
			rs732149	G	0.230458618
			rs7960020	T	0.175712771
			rs901840	T	0.186409558
			rs984972	C	0.164592686
ITPR3	6	adrenergic	rs10947415	C	0.152634178
			rs10947426	A	0.237369259
			rs10947428	T	0.391017807
			rs1536036	A	0.320025431
			rs1570760	C	0.240397925
			rs2274197	C	0.163313669
			rs2281917	G	0.480273183
			rs2296329	T	0.075356078
			rs2296343	A	0.297529781
			rs2296742	A	0.186150328
			rs3736893	C	0.322833447
			rs3804550	C	0.001
			rs3818526	A	0.499118874
			rs4259245	A	0.383043505
			rs4711332	C	0.121885216
			rs4711336	A	0.137297801
			rs594223	A	0.461252998
			rs6457738	C	0.242242455
			rs6901411	C	0.343627381
			rs6903502	C	0.100210682
			rs6913517	C	0.018516954
			rs6921825	C	0.119025282
			rs9366826	G	0.191381481
			rs9368771	T	0.456901906
			rs942643	C	0.130252086
			rs9469537	C	0.355046527
			rs999943	T	0.196986462
KITLG	12	candidate	ASP210TYR	G	0.001
			rs1472899	T	0.100130794
			rs995030	G	0.039512694
LOX	5	candidate	rs840466	G	0.171182609
			rs840467	G	0.20303067
MAPK1	22	adrenergic	rs13058	A	0.069775933
			rs2266967	C	0.040706842

			rs2266969	G	0.02475453
			rs2283793	A	0.022048352
			rs2283794	A	0.071027449
			rs2298432	A	0.029941269
			rs2298434	G	0.061895455
			rs4821402	C	0.06623561
			rs9610375	C	0.027662652
MAPK3	16	adrenergic	rs12922100	G	0.116702363
MDM2	12	candidate	rs769412	T	0.160677711
MFNG	22	candidate	rs2284052	C	0.021113473
MMP12	11	candidate	rs476391	G	0.110766879
			rs632009	T	0.403426572
			rs651159	A	0.158437354
			rs652438	A	0.097685139
			rs660727	C	0.131074354
MMP19	12	candidate	rs1056784	C	0.001
			rs1056785	T	0.01074801
			rs2242295	A	0.566233124
MS4A2	11	steroid	rs12576889	A	0.035178676
			rs2583476	C	0.126168047
			rs2847655	A	0.152003302
			rs2847668	A	0.128095498
			rs502581	A	0.110095958
			rs512555	G	0.006245678
			rs556917	A	0.048878797
			rs569108	A	0.006262096
			rs574700	C	0.012490966
NCOA1	**2**	**steroid**	**rs2119117**	**C**	**0.264825034**
NCOA2	8	steroid	rs3088092	T	0.056501951
			rs4738070	G	0.021877729
NDFIP1	5	candidate	rs2338820	G	0.108277629
			rs249637	C	0.094152393
			rs249680	A	0.119679119
			rs8378	C	0.178410832
NOS3	**7**	**candidate**	**rs1799983**	**G**	**0.328489109**
NR0B2	1	steroid	rs6659176	C	0.098322665
NR1I2	3	steroid	rs12721607	C	0.035764236
			rs3732360	T	0.062483024
			rs3814055	C	0.064615829
			rs3814058	T	0.105523248
NR3C1	5	steroid	rs10041520	A	0.174877626
			rs10052957	C	0.18220497
			rs10482616	C	0.296096104
			rs10482633	T	0.235748881
			rs10482655	A	0.103998829
			rs1438732	C	0.198131591
			rs2918418	C	0.206929396
			rs2918419	A	0.212746666
			rs2963154	A	0.200220679
			rs33389	C	0.175956395

			rs4986593	A	0.077038757
			rs6188	C	0.157787634
			rs6189	G	0.001
			rs6191	A	0.247702562
			rs6196	T	0.219828574
			rs6198	T	0.232338472
			rs6877893	C	0.279053342
			rs852975	T	0.201812136
			rs852977	A	0.049155217
			rs852978	T	0.265915264
			rs860457	T	0.055273806
			rs9324918	T	0.085377481
P11	12	candidate	rs1233046	G	0.185016658
			rs1235153	C	0.055646268
			rs2074531	G	0.06511118
			rs2074532	C	0.212888695
			rs2285830	T	0.119080516
			rs2285831	G	0.19971427
			rs886431	A	0.182912334
			rs929269	C	0.082260394
PCAF	3	steroid	rs10510499	T	0.049835763
			rs3021408	G	0.08578828
PCDH12	5	candidate	rs10323	A	0.007339409
			rs108042	T	0.134686589
			rs164073	A	0.232223517
			rs164074	G	0.408058635
			rs164079	A	0.344053867
			rs164083	A	0.650156113
			rs164515	T	0.100193843
			rs2434322	C	0.331412767
			rs252108	G	0.128569467
			rs252109	C	0.001
			rs3747717	C	0.173821098
PLCB1	20	adrenergic	rs1033399	A	0.184665341
			rs1033566	A	0.071511799
			rs1040496	C	0.142445409
			rs1047383	C	0.075577025
			rs1237071	A	0.041854043
			rs1883503	C	0.193514331
			rs2076413	G	0.092775988
			rs2142669	A	0.189444068
			rs2223837	A	0.10570851
			rs227130	C	0.122818168
			rs2294259	C	0.023462477
			rs4399790	G	0.231344469
			rs6055578	A	0.199399714
			rs6077332	C	0.083152291
			rs6077414	G	0.184529019
			rs6086473	G	0.131163538
			rs708933	C	0.138629763

			rs737532	T	0.099069903
			rs926506	A	0.086557515
PLCB2	15	adrenergic	rs1869901	T	0.088680831
			rs3784399	C	0.232263337
			rs936212	T	0.001
PLCB3	11	adrenergic	rs2244625	C	0.057438336
			rs915987	C	0.021711042
PLCB4	20	adrenergic	rs1474670	C	0.038934871
			rs2076392	T	0.060691673
			rs6077510	A	0.162075261
			rs742279	G	0.084620533
PLN	6	adrenergic	rs3752581	C	0.484757023
			rs9481825	C	0.133746848
			rs9489435	T	0.034575018
POMC	2	steroid	rs1866146	C	0.3137269
			rs3769671	A	0.028209774
			rs6713532	A	0.098847758
			rs6719226	G	0.026025807
			rs7565877	A	0.153140763
			rs934778	A	0.372722772
PPARG	3	candidate	rs1175542	G	0.252898259
			rs1801282	C	0.130633148
			rs709150	C	0.210913768
			rs709157	A	0.110146665
PTAFR	1	adrenergic	rs313149	A	0.001
			rs313151	G	0.137212086
PTGIR	19	adrenergic	rs1126510	T	0.260201234
			rs11668478	G	0.136345104
RAC2	22	steroid	rs1064498	A	0.116266983
			rs2284038	A	0.119115571
			rs6572	C	0.209685045
			rs739043	C	0.264098091
RAPGEF3	12	adrenergic	rs2072115	A	0.091779575
			rs2074534	G	0.314593596
			rs2238143	G	0.052944401
			rs2238144	C	0.085735385
			rs2239189	C	0.061382451
			rs2240079	G	0.297860091
			rs2240080	T	0.4455054
			rs757282	T	0.27168553
RASGRP4	19	candidate	rs1541919	C	0.184577519
			rs3745962	G	0.060735084
			rs3745963	T	0.036044274
			rs892055	G	0.080856315
			rs919781	C	0.066259284
			RSG4_G165R	G	0.317627516
RGS12	4	adrenergic	rs2269497	A	0.013533329
			rs2281470	C	0.105801661
RGS16	1	adrenergic	rs1144566	C	0.00460986
			rs680431	C	0.02370444

SerpinA6	**14**	**steroid**	**rs1042394**	**G**	**0.285276117**
			rs2228542	**C**	**0.26024923**
			rs3748320	**G**	**0.194064581**
SMARCB1	22	steroid	rs11705032	C	0.014800745
			rs11912715	T	0.0618335
			rs2073387	C	0.062541882
			rs2186364	T	0.058995189
			rs2186370	C	0.039988487
			rs2267034	T	0.04211419
			rs3177244	G	0.030186482
			rs3788362	C	0.042758657
			rs5751738	G	0.062541882
			rs5751745	G	0.037424064
			rs5760045	A	0.041032919
			rs5996620	G	0.05034238
			rs6003904	A	0.025388595
			rs6003909	C	0.038879695
			rs738799	G	0.024756998
			rs8135303	T	0.018951439
			rs9608196	A	0.021897865
			rs9612484	G	0.13489727
SMARCE1	17	steroid	rs1029791	T	0.108284631
			rs1048573	A	0.524267757
			rs1526603	G	0.082081955
			rs2014704	T	0.600549569
			rs3752026	A	0.504680701
			rs757412	G	0.065755506
SPINK5	5	candidate	rs2303067	G	0.189982287
STAT3	17	steroid	rs1053023	A	0.138895101
			rs2230097	T	0.013879767
			rs2293152	G	0.066120383
			rs2306581	T	0.069795483
			rs3198502	A	0.145107281
			rs3744483	A	0.136037536
			rs8075442	G	0.003659108
			rs957971	C	0.037501564
STAT6	12	steroid	rs1057290	G	0.018418786
			rs3024983	C	0.01392359
			rs324015	C	0.09864506
TACR1	2	adrenergic	rs10208860	G	0.166958504
			rs1861457	A	0.052594279
			rs2024512	A	0.038361768
			rs2111378	C	0.092728194
			rs3755456	A	0.079191191
			rs3771809	A	0.063706908
			rs3771827	A	0.138950289
			rs3771836	G	0.1871268
			rs3771859	C	0.097449364
			rs3821318	G	0.279001823
			rs4439987	T	0.147709418

			rs6546951	G	0.038594166
			rs6715729	G	0.028491763
TBX21	17	candidate	CLSNP2_050902	C	0.199320383
			CLSNP3_050902	G	0.171529157
			PRO485PRO	G	0.010442218
			rs11650354	C	0.060939643
			rs1808192	C	0.254876802
			rs2013383	A	0.350882897
			rs2074190	G	0.83533594
			rs2158079	T	0.564666617
			rs2240017	C	0.149997997
			rs2325717	A	0.10240464
			rs4794067	C	0.757533384
			rs7502875	A	0.625420146
			rs9910408	A	0.091129323
			WITBET_10_082902	G	0.037833858
			WITBET_18_082902	C	0.186519107
			WITBET_6	T	0.07041005
			WITBET_8_082902	G	0.06419135
TGFB1	19	candidate	rs1800472	G	0.001499019
TLR10	4	candidate	rs10776482	T	0.278132178
			rs10776483	T	0.353563755
			rs10856839	A	0.082238223
			rs11096955	A	0.189568999
			rs11096956	G	0.310491838
			rs11096957	A	0.194000983
			rs11466617	A	0.103266402
			rs11466657	T	0.029004888
			rs4129009	A	0.105701756
			rs4274855	G	0.111563084
TLR4	9	candidate	rs10759932	T	0.122278817
			rs1927914	G	0.347081488
			rs4986790	A	0.107193733
			rs4986791	C	0.025657056
			rs7866214	C	0.015756465
TNF	6	candidate	rs1800610	G	0.058017131
			rs1800629	G	0.171502513
UCN3	**10**	**steroid**	**rs10795269**	**A**	**0.437990192**
			rs7088971	**C**	**0.601152296**
			rs7478136	**C**	**0.481779856**
VDR	12	candidate	rs10735810	T	0.079955732
			rs1540339	C	0.278656778
			rs2239179	C	0.089538484
			rs3782905	C	0.034802494
			rs731236	G	0.176428429
			rs7975232	A	0.665085079

* Defined as whether the gene was chosen as part of the adrenergic pathway, steroid pathway, or a prior asthma candidate gene. Bolded entries are the genes whose median SNP ranks were in the top quartile of ranks of the 844 SNPs screened.

† The associated allele in an additive genetic model.

‡ Estimated power for replication obtained from the PBAT screening analysis.

References:

E1. 1999. The Childhood Asthma Management Program (CAMP); design, rationale, and methods. Childhood Asthma Management Program Research Group. *Control Clin Trials* 20:91-120.

E2. 2000. Long-term effects of budesonide or nedocromil in children with asthma. Childhood Asthma Management Program Research Group. *N Engl J Med* 343:1054-1063.

E3. Baron, R. M., L. J. Palmer, K. Tantisira, S. Gabriel, L. A. Sonna, L. Le, A. Hallock, T. A. Libermann, J. M. Drazen, S. T. Weiss, and E. S. Silverman. 2002. DNA sequence variants in epithelium-specific ETS-2 and ETS-3 are not associated with asthma. *Am J Respir Crit Care Med* 166(7):927-32.

E4. Silverman, E. S., L. J. Palmer, V. Subramaniam, A. Hallock, S. Mathew, J. Vallone, D. S. Faffe, T. Shikanai, B. A. Raby, S. T. Weiss, and S. A. Shore. 2004. Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. *Am J Respir Crit Care Med* 169(2):214-9.

E5. 1995. ATS Statement: Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 152:s77-s-120.

E6. Peters, S. P., N. Anthonisen, M. Castro, J. T. Holbrook, C. G. Irvin, L. J. Smith, and R. A. Wise. 2007. Randomized comparison of strategies for reducing treatment in mild persistent asthma. *N Engl J Med* 356(20):2027-39.

E7. 2007. American Lung Association Asthma Clinical Research Centers. Clinical trial of low-dose theophylline and montelukast in patients with poorly controlled asthma. *Am J Respir Crit Care Med* 175(3):235-42.

E8. Revicki, D. A., N. K. Leidy, F. Brennan-Diemer, S. Sorensen, and A. Togias. 1998. Integrating patient preferences into health outcomes assessment: the multiattribute Asthma Symptom Utility Index. *Chest* 114(4):998-1007.

E9. Litonjua AA, Thorn CF, Liggett SB. B-agonist and β-blocker pathway. 2004 August 1, 2007 [cited 2008 May 11]. Available from: http://www.pharmgkb.org/do/serve?objId=PA2024&objCls=Pathway.

E10. Weiss ST, Litonjua AA, Tantisira KG, Wong M-L, Thorn CF, Licinio J. Glucocorticoid and inflammatory genes pathway. 2003 August 1, 2007 [cited 2008 May 11]. Available from: http://www.pharmgkb.org/do/serve?objId=PA2026&objCls=Pathway.

E11. Barnes, P. J. 2007. Scientific rationale for using a single inhaler for asthma control. *Eur Respir J* 29(3):587-95.

E12. Johnson, M. 2004. Interactions between corticosteroids and beta2-agonists in asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 1(3):200-6.

E13. Van Steen, K., M. B. McQueen, A. Herbert, B. Raby, H. Lyon, D. L. Demeo, A. Murphy, J. Su, S. Datta, C. Rosenow, M. Christman, E. K. Silverman, N. M. Laird, S. T. Weiss, and C. Lange. 2005. Genomic screening and replication using the same data set in family-based association testing. *Nat Genet* 37(7):683-91.

E14. Skol, A. D., L. J. Scott, G. R. Abecasis, and M. Boehnke. 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 38(2):209-13.

E15. Lange, C., D. DeMeo, E. K. Silverman, S. T. Weiss, and N. M. Laird. 2004. PBAT: tools for family-based association studies. *Am J Hum Genet* 74(2):367-9.