

Letter to the Editor

Polymorphisms in extracellular signal-regulated kinase family influence genetic susceptibility to asthma

To the Editor:

Asthma susceptibility loci determined by genome-wide association studies explain only a fraction of asthma's heritability. Pathway analysis has emerged as a next step toward mining hidden genetic factors in complex diseases. A recent study investigating the association between polymorphisms in Toll-like receptor signaling pathways and asthma revealed that 2 members of the mitogen-activated protein kinase (MAPK) pathway (MAP kinase kinase 3 and extracellular signal-regulated kinase 2 [ERK2]) showed strong association with the disease.¹

ERKs are MAPK family members characterized by the presence of a kinase domain capable of dual phosphorylation of threonine and tyrosine residues of substrates in signaling cascades. Members of the ERK gene family have been shown to play roles in oncogenesis, as well as in immune, metabolic, and endocrine functions (see Fig E1 in this article's Online Repository at www.jacionline.org). Although the role of typical ERKs, such as ERK1 and ERK2, are well studied in the context of asthma and allergic diseases, the knowledge about signaling of the atypical ERKs ERK3 and ERK4 is incomplete. *Erk3*^{-/-} mice exhibit lung developmental defects and neonatal death, whereas *Erk4*^{-/-} mice showed depression-like phenotypes.^{2,3} *Mk5*^{-/-} (or *Mapkapk5*^{-/-}) mice had no LPS tolerance or resistance to endotoxic shock.⁴ These mechanisms might be relevant to asthma pathogenesis, and therefore we extended our genetic analysis beyond ERKs in the Toll-like receptor pathway to a systematic analysis of polymorphisms in all known genes of the ERK family members.

We selected *ERK1*, *ERK2*, *ERK3*, *ERK4*, *ERK5*, *ERK6*, and *MAPKAPK5* (Fig E1, B) and extracted 316 common single nucleotide polymorphisms (SNPs) within these 7 genes and their 5-kb flanking regions from the HapMap phase 2 and 3–merged dataset (release 28), which were represented by 96 tagging SNPs according to linkage disequilibrium analyses (see Table E1 in this article's Online Repository at www.jacionline.org). Genotypes were obtained by using the Illumina Sentrix HumanHap300K BeadChip (n = 47; Illumina, San Diego, Calif), imputation (n = 34), or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (n = 14). Only SNP rs3794897 in *ERK4* could not be genotyped by using any method (see the Methods section and Table E1 in this article's Online Repository at www.jacionline.org). We performed association analyses of tagging SNPs using logistic regression in a population of 651 asthmatic patients and 652 control subjects from Germany and Austria (see the Methods section in this article's Online Repository). SNPs showing an association with asthma were tested for association with atopic and nonatopic asthma status (468 atopic asthmatic patients and 98 nonatopic asthmatic patients against 408 nonatopic nonasthmatic control subjects). We found 9 of 95 tagging SNPs in 5 of 7 genes to be associated with asthma, exceeding the number of associations expected by chance considerably ($n^{\text{expected}} = 5$ SNPs). A complete list of results of asthma association analyses of all the SNPs is presented in Table E2 (in this article's Online Repository at www.jacionline.org). The 9

asthma-associated tagging SNPs represent 57 SNPs within linkage disequilibrium at an r^2 value of greater than 0.8.

In our study the SNP rs4767078 in the gene *MAPKAPK5* showed the strongest association with asthma and atopic asthma. Similarly, SNPs in the genes encoding ERK3 and ERK4, activating MAPKAPK5, were also associated significantly with asthma and atopic asthma (Table I).

ERK3 and ERK4 are classified as atypical MAPKs, and their signaling pathways are not explored sufficiently.⁵ Intriguingly, mice deficient in *Erk3*, *Erk4*, and *Mapkapk5* exhibit lung development defects, depression, a defective response to stress, and lack of endotoxin tolerance.³ Considering our genetic data, it needs to be investigated whether these phenotypes are consequences of defective ERK3/4 signaling and whether their deficiencies predispose to asthma development.

Also, polymorphism rs742184 in *ERK6* was associated with asthma and atopic asthma, but very little is known about the function of this gene. In contrast, ERK2 function is well studied, and 3 ERK2 SNPs (rs8136867, rs7290469, and rs2283792) showed significant associations with asthma (Table I). In a recent study ERK1/2 signaling was found to play a role in T_H2 cell differentiation and experimental asthma development in mice.⁶ Interestingly, ERK1/2 participates in immune, metabolic, endocrine, and oncogenic pathways (Fig E1). To investigate the role of ERK1/2 in asthma pathogenesis might prove worthy but also challenging in future studies, taking the multitude of effects of ERK1/2 involved into account.

Next, we annotated all SNPs within asthma-associated tagging bins based on the information from the SCAN server and SNPper database (see the Methods section in this article's Online Repository). Associated SNPs were mapped to intronic regions (n = 48), the 3' untranslated region (n = 5), and further downstream of the genes (n = 4, see Table E3 in this article's Online Repository at www.jacionline.org). Because many of the SNPs are part of large tagging bins, it remains to be seen whether association effects can be attributed to single SNPs within those bins or alternatively if small effects of many strongly linked SNPs in these bins add up to functional effects.

An electrophoretic mobility shift assay was performed on the most significantly associated SNP, rs4767078, which is located in intron 1 of *MAPKAPK5*. Our results with nuclear protein extracts from the lung epithelial cell line A549 and the T-cell line Jurkat T cells demonstrated specific binding of a yet unknown transcription factor to risk allele A of rs4767078 (Fig 1). A previous study demonstrated that intronic SNPs might affect promoter activity and gene expression by affecting transcription factor binding.⁷ On the basis of these data, we propose a similar role for rs4767078, but further in-depth studies will be needed to evaluate functional properties of ERK pathway SNPs in more detail.

In conclusion, we demonstrate significant associations of polymorphisms in typical and atypical ERK pathway genes with asthma and its subphenotypes for the first time. These results suggest that genetic variation in ERK pathway genes might play a role in asthma development through novel mechanisms, which only recently have begun to be investigated.⁶ We fully acknowledge the explorative nature of this study with limitations of small sample size. The signals are nevertheless strong, and the number of hits in the different genes within the ERK

TABLE I. Selected tagging SNPs significantly associated with asthma and subphenotype analyses for atopic and nonatopic asthma

Genes	Tagging SNP	Minor allele	Asthma		Atopic asthma		Nonatopic asthma	
			OR (L95-U95)	P value	OR (L95-U95)	P value	OR (L95-U95)	P value
ERK2	rs8136867	G	1.24 (1.06-1.45)	.0061	1.18 (0.97-1.43)	.0938	1.11 (0.81-1.53)	.511
	rs7290469	A	0.82 (0.70-0.95)	.0109	0.89 (0.73-1.08)	.2343	0.88 (0.63-1.23)	.458
	rs2283792	T	1.21 (1.03-1.41)	.0166	1.16 (0.95-1.41)	.1378	1.20 (0.87-1.66)	.276
ERK3	rs17612368	G	1.69 (1.04-2.72)	.0322	1.59 (0.90-2.81)	.1090	1.55 (0.63-3.80)	.337
ERK4	rs17742463	G	0.77 (0.64-0.92)	.0037	0.74 (0.60-0.93)	.0088	0.68 (0.47-0.99)	.043
	rs3794901	A	1.32 (1.09-1.62)	.0056	1.32 (1.03-1.69)	.0276	1.33 (0.91-1.96)	.144
	rs4939640	A	0.83 (0.71-0.98)	.0303	0.79 (0.64-0.96)	.0211	0.76 (0.54-1.07)	.118
ERK6	rs742184	T	0.79 (0.67-0.94)	.0076	0.73 (0.59-0.92)	.0060	1.21 (0.86-1.71)	.266
MAPK-APK5	rs4767078	A	1.59 (1.26-2.00)	.000098	1.65 (1.24-2.20)	.0006	1.44 (0.91-2.27)	.122

Odds ratios (OR) within 95% confidence intervals (L95-U95) and P values are presented. Complete analysis results of all the 95 tagging SNPs are available in Table E2.

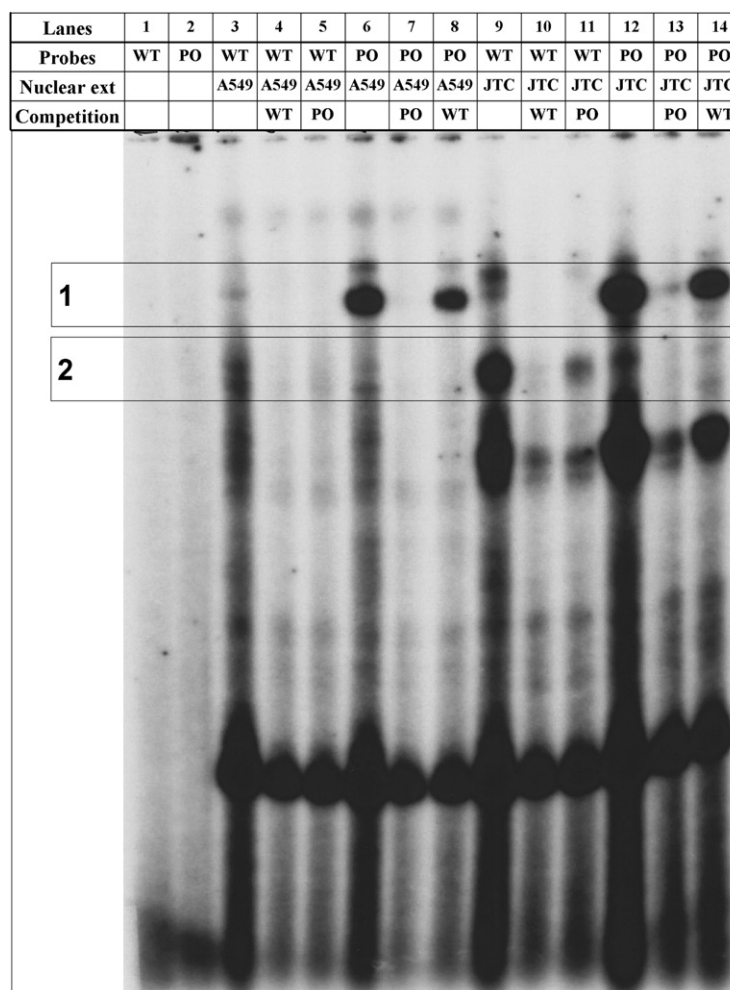


FIG 1. Allele-specific transcription factor binding to MAPKAPK5 SNP rs4767078. Probes containing the wild-type nonrisk allele (WT) T and the risk (PO) allele A of the SNP were tested with nuclear protein extracts from A549 and Jurkat T-cell lines (JTC). PO-specific transcription factor binding is evident in box 1 (lanes 6 and 12), which is supported by competition effects in A549 (lanes 7 and 8) and JTC (lanes 13 and 14). Based on box 2, a quantitative effect in transcription factor binding in JTC might be suggested (lanes 9-14).

family pathways is intriguing. An allele-specific binding of a transcription factor to rs4767078 was observed in electrophoretic mobility shift assay experiments. Replication studies in larger cohorts and further functional assessments are necessary next steps to establish the role in ERKs in asthma development.

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Supported by the German ministry of education and research (BMBF) as part of the National Genome Research Network (NGFN), with grant NGFN 01GS0810, and by the European Commission as part of the GABRIEL (a multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community) contract (no. 018996) under the Integrated Program LSH-2004-1.2.5-1. M.K. is a member of the German Center for Lung Research (DZL). R.C.P. is supported by a research fellowship from the GRK1441 (Allergy) program of Deutsche Forschungsgemeinschaft

(DFG) and was supported by the PhD program "Molecular Medicine," Hannover Biomedical Research School (HBRS).

Disclosure of potential conflict of interest: A. von Berg has received one or more payments for lecturing from or is on the speakers' bureau for the Nestlé Nutrition Institute. E. Rietschel is a board member for MSD. T. Frischer has received one or more payments for lecturing from or is on the speakers' bureau for Abbott. M. Kabesch has been supported by one or more grants from the European Union, the German Ministry of Education and Research, and the German Research Foundation and has received one or more payments for lecturing from or is on the speakers' bureau for the European Respiratory Society, the European Academy of Allergy and Clinical Immunology, the American Thoracic Society, Novartis, and GlaxoSmithKline. The rest of the authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2012.12.675>

Repository/Supplementary materials:

Material and Methods:

Study Populations

Subjects analyzed in this study were recruited from the Multicenter Asthma Genetic in Childhood (MAGIC) study and German participants of the International Study of Asthma and Allergies in Childhood (ISAAC) phase II as previously described (E1-3). Briefly, 1255 children were recruited in the MAGIC study from seven German and Austrian clinical asthma centers between 2001 and 2007. Asthma status was assessed in 865 children (mean age 11 years) by pediatric pulmonologists based on lung function tests, clinical history, physical examination and allergy testing (skin prick testing and serum IgE measurement). Children who were diagnosed as asthmatics and had sufficient high-quality DNA available were included in the GWA study. Allergy testing was done with the CLA[®] Atopy 20 panel (Hitachi Chemical Diagnostics, Inc.) or AllergyScreen (Mediwiss Analytic, Germany) panels and atopy was determined by investigating the response to any of the following common allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoide farinae*, cat epithelium, grass pollen and birch pollen.

The cross-sectional ISAAC phase II study which assessed the prevalence of asthma and allergies with standardized tools around the world was conducted in 1995-1996 and involved school children aged 9–11 years from Munich and Dresden. Rationale and methodology of the ISAAC study has been described in detail earlier (E4). Children whose parents reported a physician's diagnosis of asthma once or recurrent spastic or asthmatic bronchitis more than once were classified as asthmatics. Out of 3099 children enrolled in the ISAAC phase II study, a random subset of 800 age and sex matched German children was selected as reference for the MAGICS asthma case group in the GWA study. Atopy was assessed by skin prick test according to ISAAC criteria to one or more of the following allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, cat dander, mixed grass and tree pollen. In addition, specific serum IgE-based atopy testing was performed in ISAAC II, using the Sx1 *panel* (ImmunoCAP, Phadia, Germany) that tests for the following inhalatory allergens: *D. pteronyssinus*, *Cladosporium herbarum*, local grass pollen, birch pollen, mugwort pollen, rye pollen, cat dander and dog dander.

After genotyping quality control 651 asthmatics were analysed against 652 non-asthmatic controls. For stratification of asthma association by atopy (atopic asthma, non-atopic asthma) in a combined analysis between MAGICS and ISAAC II, results from asthmatics with RAST/CAP class ≥ 3 in MAGICS or equivalent score in SX1 test ≥ 3.5 in ISAAC II were classified as atopic asthmatics. RAST/CAP class $\leq 0/1$ in MAGICS or SPT negative and SX1 test ≤ 0.35 in ISAAC II were defined as non-atopics. Finally, after successful atopy testing and quality control 468 atopic asthmatics and 98 non-atopic asthmatics were available for stratification and analysis against 408 non-atopic non-asthmatic controls. Written informed consent was obtained from the parents of all children from both populations and the study protocols were approved by the ethics committees of each participating center (E2-5).

Gene and SNP selection

We selected *ERK1*, *ERK2*, *ERK3*, *ERK4*, *ERK5*, *ERK6* and *MAPKAPK5* in our study. However, since downstream proteins of ERK1/2 signaling such as RSKs, ELK1 are redundant with other cellular and metabolic pathways, they were not included in the analyses (Figure-1, A). We found that there are 316 common SNPs with minor allele frequency (MAF) $>5\%$ located in within 5kb flanking regions of these genes in HapMap phase-2 & 3 merged (CEU) population (release 28). These SNPs were tagged by 96 tagging SNPs in linkage disequilibrium analyses ($r^2 > 0.8$) using Haploview software (Table-S1).

Genotyping and quality control

Genotyping for GWAS had been performed within the GABRIEL consortium by Illumina Sentrix HumanHap300K BeadChip, as previously described (E2). GWAS chip genotyping covered 47 tagging SNPs in the TLR pathway. Genotypes of an additional 34 tagging SNPs were determined using an imputed dataset derived from Illumina300K Beadchip genotyping and HapMap Phase-II (E3). SNPs which were neither covered by GWAS nor by imputation ($n=14$) were genotyped by MALDI-TOF mass spectrometry (Sequenom Inc., San Diego, USA). Detailed informations are available from the authors upon request. One ERK4 SNP rs3794897 no genotype data could be obtained by any of the methods described above; therefore we performed analyses for 95 tagging SNPs.

Genotyping quality control was performed by excluding data with low call rate (<0.95) and constant deviation from the Hardy–Weinberg Equilibrium (HWE) in the control population after repeated regenotyping.

Statistical analyses

Associations between the tagging SNPs and asthma were tested by PLINK software package (version1.07) to assess additive effects of alleles on the phenotype (E6). Additive effects were statistically modelled by logistic regression and odds ratios (OR), 95% confidence intervals and *P*-values were calculated. Results of asthma associated SNPs were stratified by atopy status in a second analysis.

In silico analyses

We manually annotated the all the SNPs in the asthma associated tagging blocks within the respective genes structures using information from SNPper database (<http://snpper.chip.org/>) and SCAN sever (<http://www.scandb.org/>). For the genes with several known isoforms, the longest isoform was considered as reference for determining the location of the SNPs.

Electrophoretic-mobility shift assay (EMSA)

Nuclear protein extracts from human lung epithelial cell line A549 and human T-cell line Jurkat-T were prepared at 5th passage of the cell culture, as previously described (E7). The protein concentration of nuclear extracts from 14×10^6 cells were measured by BCA kit (www.piercenet.com/) and were stored at -80°C in aliquots. Forward and reverse sequences of oligonucleotides (given below) containing 15 bases flanking the SNP rs4767078 were annealed in a buffer containing 10mM Tris-Cl (pH 8.0), 1mM EDTA and 100mM NaCl at boiling temperature for 5 min followed by slow cooling. Annealed probes were purified from a 15% poly-acrylamide gel with “Freeze n squeeze” columns (www.bio-rad.com/). Purified double stranded probes were labeled at the 5’end with ³²P-ATP using T4-polynucleotide kinase and labeled probes were purified from free radioactivity with mini quick spin oligo columns (www.roche-applied-science.com/). Labeled probes were incubated for 30 minutes, with a binding reaction mix containing nuclear extract (5µg), NaCl (80mM), Glycerol (4%), 2-mercapto-ethanol (1mM), Poly-(dI-dC)(0.25µg) and 1x reaction buffer (10mM Tris-Cl). Binding reactions were resolved on a 5% poly-acrylamide gel in TBE (Tris-Borate-EDTA) buffer at 4°C. For competition assays, 100 fold molar excess of unlabelled probes were used.

Oligonucleotide probes containing SNP rs4767078 (indicated in bold and underlined format):

MK5_WT_Fwd: TACTATCTGATGTAA**TTT**CTCTGTTGATTTT

MK5_WT_Rev: AAAATCAACAGAG**A**AATTACATCAGATAGTA

MK5_PO_Fwd: TACTATCTGATGTAA**A**TTCTCTGTTGATTTT

MK5_PO_Rev: AAAATCAACAGAGAA**TTT**TACATCAGATAGTA

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Supplementary references

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104 **Repository Tables Legends**

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106 **Table-E1**

107 Linkage disequilibrium analyses of SNPs in ERK and MAPKAPK5. Ninety six tagging SNPs tagged
108 316 SNPs ($r^2=0.8$). Genotyping methods are represented by symbols: # - Illumina Chip, § - Imputation,
109 * - MALDI-TOF genotyping.

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111 **Table-E2**

112 Associations of 95 tagging SNPs in ERKs and MAPKAPK5 with asthma. Odds ratio (OR) within 95%
113 confidence (L95-U95) and *P*-values are presented. Associated SNPs are shaded in Gray.

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115 **Table-E3**

116 Annotation of all the SNPs within the asthma associated tagging blocks.

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118 **Table-E1**

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Genes	Tagging SNP		Tagged SNPs
<i>ERK1</i>	§	rs7698	
<i>ERK1</i>	#	rs11865086	
<i>ERK2</i>	#	rs12172554	rs12172554
<i>ERK2</i>	§	rs13515	rs17759598, rs17759796
<i>ERK2</i>	#	rs2283792	rs6928, rs3810610, rs13943, rs5755099, rs2006893, rs743409, rs5999515, rs11704205, rs5999521, rs5999550, rs2266967, rs5749986, rs5749998, rs9607287, rs3788332, rs1892846, rs1892848, rs9610375, rs2283794, rs4821402
<i>ERK2</i>	#	rs2298432	rs2298432
<i>ERK2</i>	§	rs7290469	rs8139376, rs9610271, rs9340, rs1063311, rs2266966, rs2283791, rs9610338, rs2266969, rs2283793, rs9607298, rs9610374, rs5999749, rs5999752, rs2876981, rs11913721
<i>ERK2</i>	§	rs8136867	rs5755694
<i>ERK2</i>	§	rs8140233	
<i>ERK2</i>	#	rs9610417	rs3810606, rs3810608, rs9607272, rs9610470, rs9610487, rs9610496
<i>ERK3</i>	§	rs10851507	
<i>ERK3</i>	#	rs11070877	rs8034167
<i>ERK3</i>	§	rs11070879	
<i>ERK3</i>	§	rs11631522	
<i>ERK3</i>	*	rs11636443	
<i>ERK3</i>	#	rs4627278	rs10518674, rs11070875, rs36046047, rs4775999
<i>ERK3</i>	*	rs17612368	
<i>ERK4</i>	§	rs10432203	rs11662176, rs9953685
<i>ERK4</i>	§	rs10445491	rs4940000
<i>ERK4</i>	#	rs10502909	rs1370482
<i>ERK4</i>	§	rs11660097	
<i>ERK4</i>	#	rs11662719	rs12958523, rs3813087, rs3752087
<i>ERK4</i>	§	rs11662889	
<i>ERK4</i>	§	rs11665303	

ERK4	#	rs11665501	
ERK4	§	rs11665570	
ERK4	§	rs11875289	
ERK4	*	rs12456953	rs12454262
ERK4	§	rs12961853	
ERK4	#	rs1370480	rs2969972, rs4939637, rs10432161, rs10432162, rs1370479, rs11082832
ERK4	#	rs1437648	rs8085534, rs6508014, rs1561261, rs1007484
ERK4	#	rs1545130	rs1545129, rs9949644
ERK4	#	rs16952149	rs12969079, rs12962447
ERK4	#	rs17662557	rs11082834, rs973644, rs3867260
ERK4	*	rs12959952	rs17662955
ERK4	*	rs17662967	
ERK4	*	rs17742463	
ERK4	§	rs17742551	rs17804724, rs12968509, rs1370484
ERK4	#	rs1893488	rs11660924, rs4939996
ERK4	§	rs1893489	rs8097986, rs9955932
ERK4	#	rs1893490	
ERK4	#	rs2051308	
ERK4	#	rs2053248	rs745822, rs8084255, rs1025687, rs1025688, rs9947760, rs3907130
ERK4	#	rs2118370	rs4939994, rs17718895, rs4939641, rs2118369
ERK4	#	rs2969970	rs3853680, rs8093289, rs2338829, rs2969971, rs4939638, rs4245234, rs9951088
ERK4	*	rs3752088	
ERK4		rs3794897	
ERK4	#	rs3794900	
ERK4	#	rs3794901	rs3794898, rs4940006, rs7239019, rs3794902, rs1807382, rs4940007
ERK4	#	rs3813086	rs7235791, rs3911593, rs3911592, rs3752085
ERK4	#	rs3867257	rs1437649, rs4599004, rs745823, rs17742138
ERK4	#	rs3894010	rs8091238
ERK4	§	rs3911848	
ERK4	#	rs4081996	rs2045532, rs4077763, rs11082830, rs6508009, rs4041975

ERK4	#	rs4260159	
ERK4	#	rs4361025	rs8095427, rs7242442, rs4589656, rs2839975
ERK4	#	rs4444423	
ERK4	§	rs4617959	
ERK4	#	rs4939634	rs4441369, rs2045531, rs4939988, rs4939635, rs4939636, rs728682, rs8089517, rs1437654, rs65080, rs1975300, rs11082831, rs1437653, rs736617, rs4322755, rs1020077, rs992595, rs4432342, rs7504876, rs2218003, rs2196642, rs4939639, rs1437650, rs3862671, rs3855
ERK4	#	rs4939640	rs12604601, rs9947567, rs6508020, rs8095563, rs17804574
ERK4	§	rs4939642	
ERK4	#	rs4939992	
ERK4	§	rs4939998	
ERK4	#	rs4939999	
ERK4	§	rs4940002	
ERK4	#	rs4940003	rs9964102, rs894768, rs894765, rs7237543, rs7238170
ERK4	§	rs4940005	
ERK4	§	rs6508017	rs9954381
ERK4	§	rs7227230	
ERK4	§	rs7227542	
ERK4	#	rs7228129	rs4939989
ERK4	*	rs7240429	
ERK4	#	rs751114	rs12607365, rs3892158, rs13381183, rs8095870, rs8097233, rs1893487, rs11664819, rs12962731
ERK4	#	rs753328	rs17741814, rs17718537, rs9962245, rs11873590, rs11873632, rs2338830, rs753327, rs17742008
ERK4	#	rs8094401	rs1469947, rs8091136, rs973645, rs3867259, rs3894011
ERK4	#	rs8096108	rs11660520, rs1370483
ERK4	#	rs893315	
ERK4	#	rs893316	
ERK4	§	rs893320	
ERK4	#	rs930362	rs17804166
ERK4	*	rs9948424	
ERK4	#	rs9949687	rs9960447, rs9960277, rs9304402, rs9959686

<i>ERK4</i>	§	rs9956244	rs9966545
<i>ERK4</i>	#	rs9958610	rs11664092, rs11663892
<i>ERK4</i>	§	rs9962141	rs12604368, rs6508027
<i>ERK5</i>	*	rs1054206	
<i>ERK5</i>	#	rs2048230	
<i>ERK5</i>	§	rs2233072	
<i>ERK6</i>	§	rs1555048	rs2341111, rs5771276, rs5771280, rs1129880
<i>ERK6</i>	*	rs2076139	rs2272857
<i>ERK6</i>	*	rs742184	rs1076649
<i>MAPKAPK5</i>	§	rs16941724	rs12580246
<i>MAPKAPK5</i>	§	rs2097701	rs7314870, rs3177647, rs2879603, rs3213628, rs4767068, rs7980569, rs6489817, rs7976557, rs11066054, rs6489818, rs7297760, rs12426566, rs7294623, rs1981517, rs12423041, rs6489819
<i>MAPKAPK5</i>	*	rs4346023	
<i>MAPKAPK5</i>	#	rs876896	rs7962814, rs12315146
<i>MAPKAPK5</i>	*	rs4767078	

Table-E2

Genes	Tagging SNP	Minor allele	Asthma	
			OR (L95-U95)	P-value
<i>ERK1</i>	rs7698	A	0.95 (0.73-1.24)	0.6936
<i>ERK1</i>	rs11865086	A	1.01 (0.87-1.19)	0.8744
<i>ERK2</i>	rs8136867	G	1.24 (1.06-1.45)	0.00607
<i>ERK2</i>	rs9610417	T	0.90 (0.75-1.09)	0.2872
<i>ERK2</i>	rs12172554	T	0.88 (0.60-1.30)	0.5281
<i>ERK2</i>	rs8140233	T	0.90 (0.72-1.14)	0.3884
<i>ERK2</i>	rs7290469	A	0.82 (0.70-0.95)	0.01089
<i>ERK2</i>	rs2283792	T	1.21 (1.03-1.41)	0.01664
<i>ERK2</i>	rs2298432	A	1.15 (0.98-1.34)	0.08331
<i>ERK2</i>	rs13515	T	0.87 (0.71-1.07)	0.1905
<i>ERK3</i>	rs4627278	G	1.19 (0.91-1.56)	0.2091

<i>ERK3</i>	rs17612368	G	1.69 (1.04-2.72)	0.03216
<i>ERK3</i>	rs11631522	T	1.10 (0.88-1.39)	0.4014
<i>ERK3</i>	rs10851507	G	1.09 (0.84-1.41)	0.5085
<i>ERK3</i>	rs11636443	A	0.87 (0.74-1.01)	0.06138
<i>ERK3</i>	rs11070877	G	1.14 (0.91-1.44)	0.2552
<i>ERK3</i>	rs11070879	G	1.04 (0.86-1.27)	0.6782
<i>ERK4</i>	rs9949687	C	1.06 (0.91-1.24)	0.454
<i>ERK4</i>	rs3867257	G	0.88 (0.74-1.05)	0.1677
<i>ERK4</i>	rs1437648	A	1.06 (0.91-1.25)	0.4479
<i>ERK4</i>	rs2053248	T	1.03 (0.88-1.21)	0.7183
<i>ERK4</i>	rs6508017	C	1.09 (0.91-1.31)	0.3456
<i>ERK4</i>	rs4939992	T	1.09 (0.81-1.46)	0.5883
<i>ERK4</i>	rs7227542	G	1.00 (0.85-1.18)	0.9653
<i>ERK4</i>	rs4939640	A	0.83 (0.71-0.98)	0.03033
<i>ERK4</i>	rs11875289	G	0.80 (0.58-1.10)	0.1706
<i>ERK4</i>	rs1893488	G	1.11 (0.94-1.31)	0.203
<i>ERK4</i>	rs2118370	A	1.06 (0.87-1.31)	0.5526
<i>ERK4</i>	rs12456953	C	1.04 (0.87-1.24)	0.6871
<i>ERK4</i>	rs11660097	C	0.84 (0.68-1.04)	0.1121
<i>ERK4</i>	rs2969970	A	1.00 (0.81-1.22)	0.9872
<i>ERK4</i>	rs8094401	C	1.34 (0.81-2.22)	0.2521
<i>ERK4</i>	rs9958610	A	0.96 (0.80-1.15)	0.6268
<i>ERK4</i>	rs893315	A	1.14 (0.98-1.32)	0.09724
<i>ERK4</i>	rs10502909	T	1.00 (0.85-1.17)	0.9574
<i>ERK4</i>	rs930362	A	1.11 (0.93-1.32)	0.2468
<i>ERK4</i>	rs7228129	G	1.13 (0.92-1.38)	0.2398
<i>ERK4</i>	rs1370480	T	0.84 (0.70-1.01)	0.06196
<i>ERK4</i>	rs753328	C	0.91 (0.77-1.09)	0.3154
<i>ERK4</i>	rs17662557	C	1.12 (0.92-1.37)	0.2732
<i>ERK4</i>	rs11665303	A	0.98 (0.74-1.29)	0.8978
<i>ERK4</i>	rs893320	G	1.18 (0.95-1.45)	0.1319
<i>ERK4</i>	rs4939634	T	0.99 (0.85-1.16)	0.9061
<i>ERK4</i>	rs16952149	A	1.28 (0.98-1.68)	0.06711
<i>ERK4</i>	rs4444423	T	1.02 (0.87-1.21)	0.7675
<i>ERK4</i>	rs4617959	A	1.01 (0.83-1.24)	0.9038
<i>ERK4</i>	rs4081996	C	0.92 (0.79-1.08)	0.2975
<i>ERK4</i>	rs12959952	T	1.03 (0.89-1.21)	0.6703
<i>ERK4</i>	rs1545130	G	0.92 (0.79-1.07)	0.2719
<i>ERK4</i>	rs8096108	C	0.96 (0.75-1.22)	0.7297

ERK4	rs3894010	G	1.14 (0.97-1.34)	0.1058
ERK4	rs4260159	A	0.94 (0.74-1.20)	0.6378
ERK4	rs893316	C	1.11 (0.91-1.35)	0.2905
ERK4	rs9948424	A	0.99 (0.84-1.17)	0.8875
ERK4	rs4939642	A	1.25 (0.65-2.38)	0.5079
ERK4	rs17742551	T	0.93 (0.71-1.22)	0.5883
ERK4	rs11665570	A	1.06 (0.81-1.40)	0.6628
ERK4	rs9962141	A	0.95 (0.80-1.11)	0.4998
ERK4	rs3752088	A	1.05 (0.89-1.24)	0.5409
ERK4	rs7240429	C	1.05 (0.90-1.24)	0.5129
ERK4	rs4940005	A	0.92 (0.75-1.11)	0.3795
ERK4	rs17662967	T	1.24 (0.97-1.58)	0.0825
ERK4	rs3794900	A	0.86 (0.74-1.00)	0.05368
ERK4	rs3794901	A	1.32 (1.09-1.62)	0.00557
ERK4	rs4361025	T	0.86 (0.71-1.06)	0.1532
ERK4	rs11665501	C	0.85 (0.72-1.00)	0.05768
ERK4	rs2051308	T	0.97 (0.83-1.14)	0.7016
ERK4	rs17742463	G	0.77 (0.64-0.92)	0.003747
ERK4	rs7227230	C	0.92 (0.79-1.08)	0.3268
ERK4	rs3911848	A	0.85 (0.58-1.24)	0.3928
ERK4	rs4940003	G	0.91 (0.78-1.07)	0.2499
ERK4	rs4939998	A	1.04 (0.89-1.21)	0.6341
ERK4	rs10432203	C	0.88 (0.74-1.03)	0.1065
ERK4	rs4939999	T	1.09 (0.91-1.30)	0.3391
ERK4	rs3813086	A	1.01 (0.85-1.21)	0.8756
ERK4	rs10445491	C	1.09 (0.86-1.37)	0.4724
ERK4	rs4940002	A	1.05 (0.90-1.23)	0.5467
ERK4	rs9956244	A	1.10 (0.87-1.38)	0.435
ERK4	rs1893489	T	0.99 (0.85-1.15)	0.8737
ERK4	rs1893490	T	0.93 (0.80-1.08)	0.344
ERK4	rs751114	A	0.93 (0.74-1.17)	0.5254
ERK4	rs12961853	C	0.97 (0.79-1.20)	0.7989
ERK4	rs11662889	A	0.93 (0.71-1.22)	0.5877
ERK4	rs11662719	T	1.12 (0.96-1.31)	0.1608
ERK5	rs2233072	T	0.94 (0.78-1.14)	0.5386
ERK5	rs1054206	C	0.89 (0.73-1.09)	0.2677
ERK5	rs2048230	A	1.09 (0.86-1.39)	0.4584
ERK6	rs2076139	T	1.13 (0.95-1.35)	0.1731
ERK6	rs1555048	A	1.09 (0.93-1.30)	0.2911

<i>ERK6</i>	rs742184	T	0.79 (0.67-0.94)	0.007617
<i>MAPKAPK5</i>	rs4346023	C	1.14 (0.96-1.36)	0.1411
<i>MAPKAPK5</i>	rs16941724	G	1.28 (0.95-1.71)	0.1005
<i>MAPKAPK5</i>	rs2097701	G	1.17 (0.95-1.44)	0.1473
<i>MAPKAPK5</i>	rs876896	G	1.07 (0.80-1.44)	0.6394
<i>MAPKAPK5</i>	rs4767078	A	1.59 (1.26-2.00)	0.00009837

Table-E3

Summary:

	Intron	3'UTR	Downstream	Total
<i>ERK2</i>	32	5	2	39
<i>ERK3</i>	0	0	1	1
<i>ERK4</i>	13	0	1	14
<i>ERK6</i>	2	0	0	2
<i>MAPKAPK5</i>	1	0	0	1
Total	48	5	4	57

Full list of SNPs

Genes	Tagging SNP	Tagged SNPs	Location
<i>ERK2</i>	rs8136867	rs8136867	Intron
<i>ERK2</i>		rs5755694	Intron
<i>ERK2</i>	rs7290469	rs7290469	Intron
<i>ERK2</i>		rs8139376	Downstream
<i>ERK2</i>		rs9610271	Downstream
<i>ERK2</i>		rs9340	3'UTR
<i>ERK2</i>		rs1063311	3'UTR
<i>ERK2</i>		rs2266966	Intron
<i>ERK2</i>		rs2283791	Intron
<i>ERK2</i>		rs9610338	Intron
<i>ERK2</i>		rs2266969	Intron
<i>ERK2</i>		rs2283793	Intron
<i>ERK2</i>		rs9607298	Intron
<i>ERK2</i>		rs9610374	Intron
<i>ERK2</i>		rs5999749	Intron
<i>ERK2</i>		rs5999752	Intron
<i>ERK2</i>		rs2876981	Intron
<i>ERK2</i>		rs11913721	Intron
<i>ERK2</i>	rs2283792	rs2283792	Intron

<i>ERK2</i>		rs6928	3'UTR
<i>ERK2</i>		rs3810610	3'UTR
<i>ERK2</i>		rs13943	3'UTR
<i>ERK2</i>		rs5755099	Intron
<i>ERK2</i>		rs2006893	Intron
<i>ERK2</i>		rs743409	Intron
<i>ERK2</i>		rs5999515	Intron
<i>ERK2</i>		rs11704205	Intron
<i>ERK2</i>		rs5999521	Intron
<i>ERK2</i>		rs5999550	Intron
<i>ERK2</i>		rs2266967	Intron
<i>ERK2</i>		rs5749986	Intron
<i>ERK2</i>		rs5749998	Intron
<i>ERK2</i>		rs9607287	Intron
<i>ERK2</i>		rs3788332	Intron
<i>ERK2</i>		rs1892846	Intron
<i>ERK2</i>		rs1892848	Intron
<i>ERK2</i>		rs9610375	Intron
<i>ERK2</i>		rs2283794	Intron
<i>ERK2</i>		rs4821402	Intron
<i>ERK6</i>	rs742184	rs742184	Intron
<i>ERK6</i>		rs1076649	Intron
<i>ERK3</i>	rs17612368	rs17612368	Downstream
<i>ERK4</i>	rs17742463	rs17742463	Intron
<i>ERK4</i>	rs3794901	rs3794901	Intron
<i>ERK4</i>		rs3794898	Intron
<i>ERK4</i>		rs4940006	Intron
<i>ERK4</i>		rs7239019	Intron
<i>ERK4</i>		rs3794902	Intron
<i>ERK4</i>		rs1807382	Intron
<i>ERK4</i>		rs4940007	Downstream
<i>ERK4</i>	rs4939640	rs4939640	Intron
<i>ERK4</i>		rs12604601	Intron
<i>ERK4</i>		rs9947567	Intron
<i>ERK4</i>		rs6508020	Intron
<i>ERK4</i>		rs8095563	Intron
<i>ERK4</i>		rs17804574	Intron
<i>MAPKAPK5</i>	rs4767078	rs4767078	Intron