conditioning regimen might be a reasonable option for most patients, and unconditioned HSCT can be offered for critically ill patients with disseminated infections and end-organ damage. Further studies and long-term follow-up are required to determine the appropriate conditioning regimen.

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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 hereditability. 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 ($n^{expected}$ = 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.

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.

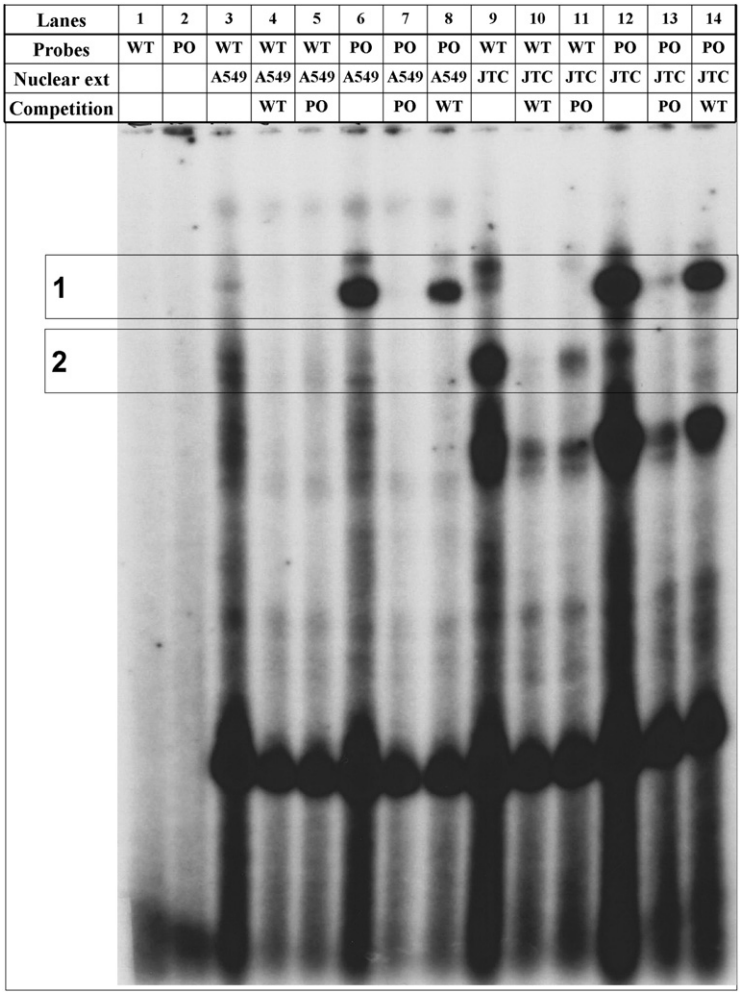

FIG 1. Allele-specific transcription factor binding to *MAPKAPK5* SNP rs4767078. Probes containing the wild-type nonrisk allele *(WT)* T and the risk *(PO)* allele A of the SNP were tested with nuclear protein extracts from A549 and Jurkat T-cell lines *(JTC)*. PO-specific transcription factor binding 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)*.

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 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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Development of skin lesions in filaggrin-deficient mice is dependent on adaptive immunity

To the Editor:

Atopic dermatitis (AD) is an inflammatory skin disease associated with impaired skin barrier function. Mutations in the filaggrin (*FLG*) gene are a risk factor for AD.[1] Filaggrin is expressed in keratinocytes and constitutes a major component of the cornified envelope. Decreased *FLG* expression results in an impaired skin barrier, which facilitates antigen entry via the skin. Loss-of-function mutations of *FLG* in AD are associated with more severe symptoms, earlier onset of the disease, and its persistence into adulthood.[2] Filaggrin-deficient flaky tail (ft) mice ($Flg^{ft/ft}$) harbor a naturally occurring 1 bp deletion (5303delA) in the *Flg* gene, leading to the premature termination of transcription. This loss-of-function mutation mimics an *FLG* mutation found in humans. $Flg^{ft/ft}$ mice on a mixed background develop spontaneous skin inflammation at age 8 weeks and visible skin lesions by 28 weeks.[3,4] Skin inflammation requires T cells in a mouse model of AD induced by epicutaneous sensitization to ovalbumin,[5] but is independent of T and B cells in mice with cutaneous overexpression of thymic stromal lymphopoietin.[6]

To identify the contribution of the adaptive immune system to the skin inflammation in $Flg^{ft/ft}$ mice, we bred them onto the Balb/c background and subsequently intercrossed them with Rag2-deficient ($Rag2^{-/-}$) mice to generate mice that lack functional filaggrin as well as T and B cells ($Rag2^{-/-}/Flg^{ft/ft}$).

By 32 weeks, 15 of the 17 (88%) $Flg^{ft/ft}$ mice had developed skin lesions on the back characterized by fur loss, erythematous scaly