addition, our data reveal that N-Ras and K-Ras, but not H-Ras, are the dominant isoforms in human MCs. We have previously shown that T cells could activate MCs by means of heterotypic adhesion.[1-4] This pattern of activation involves the MAPK5 system and resulted in release of different cytokines. In this study we report that N-Ras is activated downstream of this pathway and is localized to the PM. The question as to which of the 2 GEFs, RasGRP1 or RasGRP4, is principal in MCs is still a matter of debate. Our data support a crucial role for RasGRP1. This work suggests that targeting the Ras pathway might be a possible treatment option for conditions in which MCs interact with T cells, such as sarcoidosis, rheumatoid arthritis, and graft tolerance.

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ORMDL3 variants associated with asthma susceptibility in North Americans of European ancestry

To the Editor:

Asthma is the most common chronic disease in children across all developed countries. Although the cause of the disease remains unknown, it is recognized as a complex genetic disorder with an environmental component.[1,2] As with many other complex diseases, a long list of genes has been associated with asthma through linkage and candidate gene association studies, the majority of which do not replicate.[1] The first genome-wide association study of asthma predisposition was recently published.[3] In that study 317,000 single nucleotide polymorphisms (SNPs) were typed in 994 patients with childhood-onset asthma, resulting in the identification of a novel locus on chromosome 17q12-q21 containing multiple genes and associated markers. Expression analysis in lymphoblastoid cell lines revealed that *ORMDL3* expression was strongly correlated with the asthma-associated variants, leading the authors to conclude that it was the most likely candidate gene at this locus.

ORMDL3 encodes a 4-transmembrane domain–containing protein that is localized to the endoplasmic reticulum membrane.[4] Although current knowledge of *ORMDL3* function is limited, recent studies in yeast suggest the gene product might be involved in protein folding.

To determine whether *ORMDL3* is a genetic risk factor for the development of asthma in North American white subjects, we sought to replicate the association with the 10 most significantly associated SNPs in the study by Moffatt et al[3] in 2 large pediatric asthma cohorts, one comprising patients of Northern European decent and another comprising African American patients. Both cohorts were collected at the Children's Hospital of Philadelphia (CHOP).

This study was approved by the Institutional Review Board at CHOP. Parental informed consent was obtained from all participants in this study for the purpose of DNA collection and genotyping.

All patients and control subjects reported in this study were recruited at the CHOP between 2006 and 2008. All subjects were resident in the Greater Philadelphia area. The study of white subjects included 807 patients with physician-diagnosed asthma and 2583 disease-free control subjects without asthma. The study of African American subjects included 1456 patients with physician-diagnosed asthma and 1973 control subjects without asthma. Both white and African American patients were given diagnoses by CHOP physicians in accordance with the American Thoracic Society criteria[5] and had been prescribed medication to control their asthma. All control samples, both white and African American subjects, had no history of asthma or reactive airway disease and had never been prescribed asthma medications. In addition to self-reported ancestry status, all patients and control subjects were screened at ancestry informative markers using Markov Chain Monte Carlo algorithm, as implemented in STRUCTURE,[6] to reduce the risk of population stratification. Genomic inflation of 1.08 in the white study and 1.1 for the African American study reflected minor background stratification. Mean age of the case cohort was as follows: white subjects, 8.6 years (σ 5.8; 62% male and 38% female); African American subjects, 7.5 years (σ 5.7; 57% male and 43% female). All control subjects were recruited by

TABLE I. Allelic association and odds ratios for the 9 most significantly associated SNPs from the Moffatt et al study[3]

SNP	Base pair	Minor allele	Reference allele	F_A	F_U	CHISQ	*P* value	OR
rs9303277	35229995	T	C	0.5386	0.4979	7.371	.006628	1.176
rs11557467	35282160	T	G	0.5532	0.5113	7.833	.00513	1.183
rs8067378	35304874	G	A	0.5438	0.5012	8.103	.004418	1.186
rs2290400	35319766	G	A	0.5466	0.5052	7.616	.005786	1.179
rs7216389	35323475	C	T	0.5491	0.5089	7.214	.007234	1.174
rs4795405	35341943	T	C	0.5969	0.5607	5.952	.0147	1.162
rs8079416	35346239	T	C	0.5139	0.545	4.33	.03744	0.883
rs3894194	35375519	C	T	0.5266	0.55	2.45	.1175	0.91
rs3859192	35382174	C	T	0.5359	0.5535	1.398	.237	0.928

F_A, Allele frequency in patients; *F_U*, allele frequency in control subjects; *OR*, odds ratio.

CHOP clinicians and nursing staff within the CHOP Health Care Network, including 4 primary care clinics and several group practices and outpatient practices that included well child visits. Mean age in the control cohort was as follows: white subjects, 8.7 years (σ 5.2; 51% male and 49% female); African American subjects, 6.7 years (σ 7.8; 49% male and 51% female).

High-throughput genome-wide SNP genotyping was carried out at the center for applied genomics on the Illumina Infinium II HumanHap550 BeadChip (San Diego, Calif), as previously described.[7]

Of the 10 most highly associated SNPs in the Moffatt et al study,[3] 9 were present on the Illumina HapMap550 BeadChip. The missing SNP, rs4795408, was therefore not included in this study. The remaining 9 SNPs had a call rate of greater than 99% and were in Hardy-Weinberg equilibrium ($P > 10^{-5}$). The results of the allelic association and odds ratios are summarized in Table I.

We detected significant association at 7 of the 9 SNPs tested (Table I). The most strongly associated marker was SNP rs8067378, which was also found to be the most significantly associated in the Moffatt MRC-A familial cohort. The SNP most highly correlated with *ORMDL3* expression that was also the most significantly associated in the Moffatt et al study,[3] rs7216389, was significant in our cohort, with an odds ratio of 1.17 compared with a reported odds ratio of 1.84. The odds ratios for the significantly associated SNPs in our cohort ranged between 1.13 and 1.18, placing them within a similar range as the German cohort examined by Moffatt et al (International Study of Asthma and Allergies in Childhood [ISAAC] II replication study lower 95% CI range, 0.89-1.2). Additionally, the 2 SNPs in the interval, rs3894194 and rs3859192, that were not significantly associated with asthma in our cohort were also not significant in the ISAAC II cohort. We additionally examined for association of these 7 SNPs to asthma in an independent case-control cohort of subjects of African American decent. We detected no evidence for association between these markers and asthma in the 1456 patients and 1973 control subjects examined. None of the SNPs had a *P* value of less than .27 (range: $P = .27$-.96; odds ratio, 0.93-1.1).

This study has replicated the reported association between asthma and variants in and around the *ORMDL3* gene in a cohort of North American white asthmatic subjects. Seven of the 9 SNPs that were tested showed significant association. The remaining 2 SNPs, rs3894194 and rs3859192, might have been in higher linkage disequilibrium with a causal SNP in the British cohort studied by Moffatt et al[3] because the German ISAAC II cohort that was reported in the same study shows the same pattern of association as our data, strongly suggesting that the SNPs are not associated with asthma predisposition. Furthermore, the odds ratios obtained in our cohort are significantly lower than those reported by Moffatt et al, which might be a case of the winner's curse, in which the strength of association is overestimated in initial reports. In contrast, no association was detected between these markers and asthma in African American subjects. Our results are in agreement with another recent study of African American subjects[8] that did not detect association at rs7216389. Galanter et al,[8] did, however, type additional SNPs within *ORMDL3*, identifying association at 2 variants. We cannot therefore exclude the possibility that the white subject–associated SNPs do not tag causal variants that might be present in African American subjects.

In addition to our replication in North American white subjects and the British and German cohorts reported by Moffatt et al,[3] *ORMDL3* has also been replicated in 2 other studies. The first was a study that looked at Mexican, Puerto Rican, and African American subjects,[8] and the second was a study that looked at a Scottish cohort.[9] The weight of evidence across 6 different populations therefore supports the association between variants at the *ORMDL3* locus and asthma.

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The functional activity of basophil granulocytes is modulated by acute mental stress and sympathetic activation *in vivo* and *in vitro*

To the Editor:

Several studies have focused on the effect of acute mental stress with regard to the functional activity of immune cells, including T cells, natural killer cells, and eosinophils, suggesting psychoneuroendocrine mechanisms.[1,2] The effect of acute mental stress on the functional activity of human basophils, however, thus far is not clear.

We therefore aimed to investigate the functional role of acute mental stress on basophil granulocytes, analyzing CD63 surface expression as a basophil activation marker.[3]

In 15 subjects acute mental stress was induced with the Trier Social Stress Test (TSST), including free speech and mental arithmetic in front of an audience (approved by Hannover Medical School Ethics Committee and with written informed consent of the subjects and previously described by us[1,2]). Blood samples were taken 1 hour before stress at baseline, immediately after stress, and 1 hour after stress at follow-up. CD63 surface expression was analyzed with a basophil activation test in 1×10^3 basophils, respectively, and as previously described by us,[3] epinephrine levels were determined with electrochemical detection after HPLC.

Statistical analysis was performed with the Student *t* test with the statistical software package SigmaStat for Windows (Jandel Scientific, Erkrath, Germany). A *P* value of less than .05 was considered statistically significant.

Basophil granulocytes were assessed by means of IgE and CD63 surface expression. Immediately after acute mental stress, we assessed a significantly lower number of $CD63^+$ circulating basophils ($P < .05$; Fig 1, *A*), which increased to baseline values 1 hour later at follow-up ($P < .01$; Fig 1, *A*). More importantly, stimulation of basophils with formyl-methionyl-leucyl-phenylalanine (fMLP) *in vitro* showed a lower upregulation of CD63 when basophils were investigated immediately after mental stress ($P < .001$; Fig 1, *B*). Again, this effect was reversed at follow-up ($P < .001$; Fig 1, *B*).

To confirm our *in vivo* data, we used whole-blood samples of healthy nonatopic subjects (n = 4) who did not participate in the TSST. Basophils of these subjects were incubated either with 100 μL of baseline serum or 100 μL of stress serum derived from subjects who had undergone the TSST (n = 4, unpooled) added to 100 μL of medium for 20 minutes and stimulated with fMLP subsequently. Again, we assessed a significantly lower upregulation of CD63 surface expression after incubation with stress serum ($P < .01$) compared with the C63 surface expression of basophils incubated with baseline serum after fMLP stimulation (Fig 1, *C*).

We hypothesized that increased epinephrine levels might be responsible for the inhibition of CD63 upregulation. Indeed, all 15 subjects who participated in the TSST displayed increased serum levels of epinephrine immediately after acute mental stress compared with baseline and follow-up values ($P < .05$; Fig 1, *D*). Hence we incubated basophils of nonatopic donors (n = 6 who did not participate in the TSST) for 20 minutes with epinephrine (10^{-6} to 10^{-16} mol/L; Aventis Pharma, Bad Soden, Germany) or with stress serum derived from 6 different individuals who did undergo the TSST (unpooled) and stimulated them with fMLP, revealing a significantly lower upregulation of CD63 surface expression ($P < .01$-0.001; Fig 1, *E*). To confirm that these effects were specific for adrenergic modulation, we preincubated these basophils for 20 minutes with butoxamine, a β_2-adrenergic receptor antagonist (10^{-4} to 10^{-8} mol; Sigma Aldrich Pharmaceuticals, Schnelldorf, Germany), for 20 minutes before the incubation with stress serum or epinephrine and fMLP.

Butoxamine significantly inhibited the effect of stress serum and epinephrine, increasing CD63 surface expression in a dose-dependent manner ($P < .01$; Fig 1, *E*), whereas butoxamine on its own had no effect on unstimulated or fMLP-stimulated basophils (Fig 1, *E*).

We also investigated the effect of epinephrine (10^{-10} mol) on basophil fMLP receptor expression (with a fluorescein