B-cell development and function.[5,6] Specifically, the *Pik3cd*$^{-/-}$ and p110δ^{D910A} mice have absent marginal zone B cells and B-1 peritoneal B cells, hypogammaglobulinemia, impaired proliferation to IgM or CD40 ligation, and defective responses to T-independent and T-dependent antigen stimulation. Although p110δ is essential for B-cell function, it does not appear to be essential for the maintenance of total B-cell numbers. Normal B-cell numbers have been found in the *Pik3cd*$^{-/-}$ mice, the previously reported kindred lacking p110δ expression,[3] and our patient with the kinase-dead p110δ^{D853Gfs} mutant. This may reflect the redundancy between p110δ and p110α in the antigen-independent, tonic B-cell receptor signaling important for the development and survival of follicular B cells.[7] In contrast, MZ and B1 cell development depends on antigen-driven BCR signaling that requires intact p110δ.

The p110δ^{D910A} mice as well as 3 of the 4 previously reported patients with LOF variants in *PIK3CD* have susceptibility to colitis, which is also a potential adverse effect associated with p110δ inhibitors used to treat solid and hematopoietic malignancies. This has been attributed to reduced secretion of IL-10 and increased secretion of IL-12/23 from colonic macrophages in response to enteric microbiota.[8] However, colitis is not a universal feature shared by all patients with LOF *PIK3CD* variants or p110δ inhibition, and has not been reported in *Pik3cd*$^{-/-}$ mice. Therefore, the susceptibility to colitis may reflect differences in microbiota, genetic background, or environmental factors.

Our patient experienced no adverse sequelae from live vaccines and had normal T-cell proliferation to anti-CD3+CD28 stimulation. However, the p110δ^{D853Gfs} mutant impaired Ca^{2+} flux in T cells after anti-CD3 crosslinking (Fig 1, *B*). Previous reports of human PI3K deficiency have not assessed Ca^{2+} flux in patient lymphocytes; reduced Ca^{2+} flux has been shown in T cells from p110δ^{D910A} mice. Because the magnitude and frequency of Ca^{2+} flux oscillations in T cells correlates with the strength of downstream TCR signaling,[9] our patient's reduced Ca^{2+} flux reflects a component of impaired cellular immunity in this disease. Of note, opportunistic infections with *Pneumocystic jiroveci* and *Klebsiella aerogenes* were reported in 1 patient with the p110δ^{Q73X1} variant who also had a homozygous mutation in *SKAP*. Two of the 5 reported patients (40%) with LOF *PIK3CD* variants are deceased because of sepsis, indicating the severity of this immunodeficiency.

Precise regulation of p110δ activity is required for the maintenance of host immunity. Our patient demonstrates the essential contribution of the p110δ catalytic domain. Additional patients and studies are needed to determine the outcomes of hematopoietic stem cell transplantation for the treatment of this immunodeficiency.

Sarah Beaussant Cohen, MD[a]
Wayne Bainter, MMSc[a]
Jared L. Johnson, PhD[b]
Ting-Yu Lin, MS[b]
Jenny C. Y. Wong, MS[b]
Jacqueline G. Wallace, BS[a]
Jennifer Jones, BA[a]
Sonia Qureshi, MD[c]
Fatima Mir, MD[c]
Farah Qamar, MD[c]
Lewis C. Cantley, PhD[b]
Raif S. Geha, MD[a]
Janet Chou, MD[a]

From [a]the Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, Mass; [b]Meyer Cancer Center, Weill Cornell Medicine, New York, NY; and [c]the Department of Paediatrics and Child Health, Aga Khan University, Karachi, Pakistan. E-mail: Janet.Chou@childrens.harvard.edu.

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Plasma tryptase elevation during aspirin-induced reactions in aspirin-exacerbated respiratory disease

To the Editor:

Mast cell activation in the respiratory tract is a well-recognized feature of aspirin-induced respiratory reactions in patients with aspirin-exacerbated respiratory disease (AERD). This activation is reflected by release of tryptase and production of lipid mediators, which can result in substantial bronchoconstriction. The use of drugs that target cysteinyl leukotriene (cysLT) synthesis or that block the type 1 cysLT receptor ($CysLT_1R$) can attenuate aspirin-induced bronchoconstriction but generally does not prevent manifestations of nasal congestion, sneezing, and rhinorrhea during formal aspirin challenges.[1,2] Hence, $CysLT_1R$ antagonists are used commonly to increase the safety of diagnostic aspirin challenges and therapeutic desensitization procedures. Nevertheless, some patients with AERD manifest substantial reductions in FEV_1 despite prophylaxis with $CysLT_1R$ antagonists, in some cases associated with extrarespiratory symptoms of gastrointestinal distress, rash,[3,4] and rarely laryngospasm or hypotension. Serum or plasma tryptase levels increase in a subset of patients with AERD during their reaction

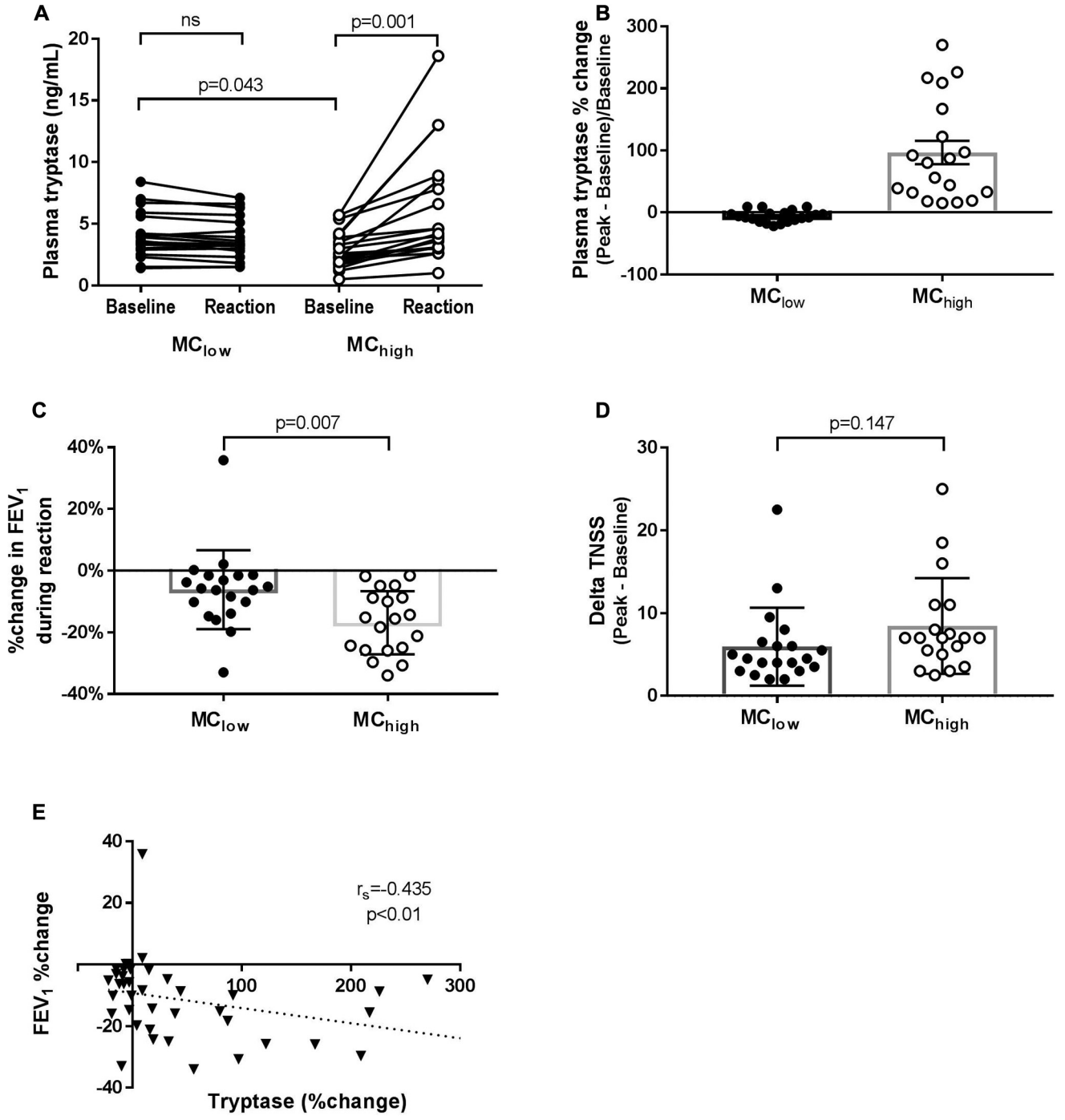

FIG 1. Tryptase, FEV_1, and TNSS stratified by level of plasma tryptase increase during aspirin-induced reaction (MC_{low} = ≥10% increase in plasma tryptase from baseline; MC_{high} = >10% increase in plasma tryptase from baseline). Plasma tryptase change from baseline during aspirin-induced reaction shown as ng/mL tryptase (**A**) and percent change in tryptase (**B**). **C**, Maximal percentage change in FEV_1 during aspirin-induced reaction. **D**, Maximal change in TNSS during aspirin-induced reaction. **E**, Correlation between change in plasma tryptase and change in FEV_1 during aspirin-induced reaction.

to aspirin challenge. We hypothesized that mast cell activation, marked by an increase in plasma tryptase, drives lower airway narrowing (and potentially extrarespiratory symptoms) in subjects with AERD undergoing oral aspirin challenge in the setting of prophylaxis with montelukast, a $CysLT_1R$ antagonist.

To test this hypothesis, subjects with AERD were recruited to undergo a standardized 1-day oral aspirin challenge protocol at the Brigham and Women's Hospital AERD Center. All subjects were pretreated with the $CysLT_1R$ antagonist montelukast for 4 weeks before challenge. All subjects had well-controlled asthma in the previous 6 months with a baseline FEV_1% predicted

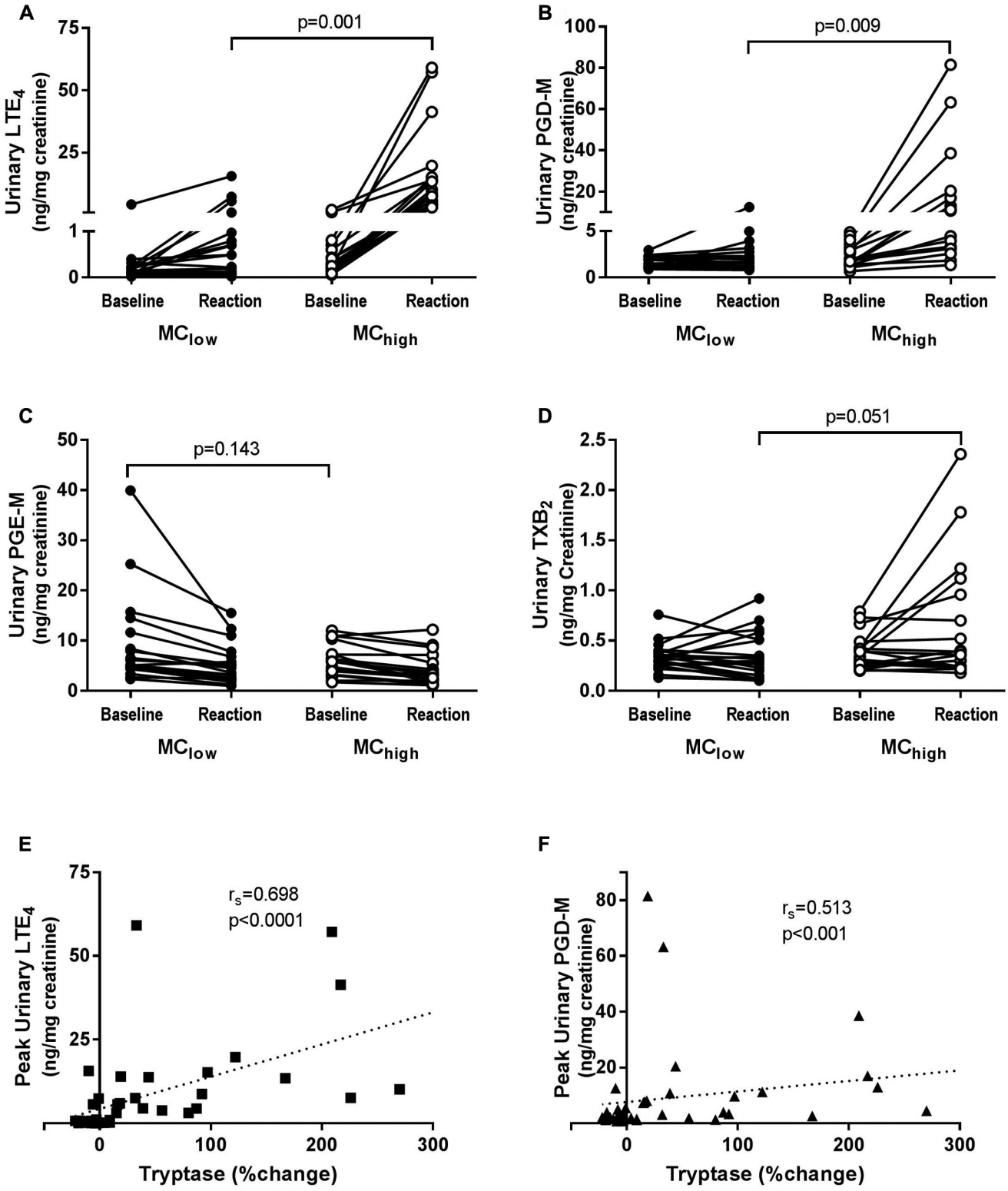

FIG 2. Change in urinary eicosanoids stratified by level of plasma tryptase change during aspirin-induced reaction (MC_{low} = ≤10% increase in plasma tryptase from baseline; MC_{high} = >10% increase in plasma tryptase from baseline). **A**, Baseline and peak LTE_4. **B**, Baseline and peak PGD-M. **C**, Baseline and nadir PGD-M. **D**, Baseline and peak thromboxane B_2 (TXB_2). For *A-D*, n = 19 paired sets for MC_{low} and n = 19 paired sets for MC_{high} subjects. Correlations between change in plasma tryptase and peak urinary LTE_4 (**E**) and PGD-M levels (**F**) are shown.

of greater than or equal to 70%. Aspirin administration started at a dose of 40 mg followed by doubling-dose increases every 90 minutes until the onset of an upper and/or lower respiratory tract reaction. Respiratory reactions were characterized by a decline of greater than or equal to 15% in FEV_1 and/or an increase of 2 or more in nasal symptom scores as assessed by the total nasal symptom score (TNSS).[5] Blood, urine, TNSSs, and lung function were collected at baseline and during the 3-hour period following the onset of a respiratory reaction (see Table E1 in this article's Online Repository at www.jacionline.org). Urinary eicosanoids, platelet-leukocyte aggregates, platelet activation markers, and plasma total tryptase were assessed as previously described.[6] Mast cell activation was defined as an increase in plasma total tryptase of greater than 10% from baseline 1 hour after the onset of a respiratory reaction. Mean and SD are reported and were compared using the Student unpaired or paired *t* test. Correlations were assessed with Spearman correlation coefficient. All analyses were performed using GraphPad Prism version 7.03 for Windows (GraphPad Software, La Jolla, Calif).

Of 39 subjects who completed our oral aspirin challenge protocol and met criteria for an aspirin-induced reaction, 19 demonstrated mast cell activation with a more than 10% increase in serum tryptase 1 hour after the onset of a reaction (denoted as MC_{high}), with the remaining 20 demonstrating less than a 10% rise in tryptase (MC_{low}, Fig 1, *A* and *B*), 16 of whom actually had a decline in tryptase. There were no differences in sex, age, or baseline peripheral blood eosinophil counts, FEV_1% predicted, TNSS, urinary leukotriene E_4 (LTE_4), prostaglandin D metabolite (PGD-M), or thromboxane B_2 levels between the MC_{high} and the MC_{low} groups (see Table E2 in this article's Online Repository at www.jacionline.org). Numbers of platelet-adherent leukocytes and the extent of platelet activation in blood (percent of $CD61^+$ platelets that were $CD62P^+$) also did not differ between groups (data not shown). However, the baseline plasma tryptase level was slightly higher in the MC_{low} subjects than in the MC_{high} subjects (4.1 ± 1.8 vs 3.0 ± 1.5 ng/mL, $P = .043$; Table E2). The MC_{high} subjects with an increase of more than 10% in plasma tryptase exhibited greater declines in FEV_1 than did subjects in the MC_{low} group (−16.8% ± 10.2% vs −6.2% ± 12.8%; Fig 1, *C*) without a corresponding difference in sinonasal symptom scores (Fig 1, *D*). Independent of group assignment, the change in plasma tryptase (%) inversely correlated with the FEV_1% change (Fig 1, *E*). Six subjects reported gastrointestinal symptoms during their aspirin-induced reaction, all of whom were in the MC_{high} group (data not shown). The provocative dose of aspirin needed to elicit symptoms of a reaction did not differ between subjects in the MC_{high} group versus the MC_{low} group.

Compared with subjects in the MC_{low} group, subjects in the MC_{high} group displayed higher peak urinary levels of LTE_4 (1.75 ± 3.80 vs 15.71 ± 17.35 ng/mg creatinine; $P = .001$) and PGD-M (2.69 ± 2.66 vs 16.27 ± 21.80 ng/mg creatinine; $P = .009$), but did not differ in baseline or trough prostaglandin E metabolite or change in thromboxane B_2 (Fig 2, *A-D*). The change in plasma tryptase (%) correlated with the peak urinary LTE_4 and peak PGD-M levels across all 39 subjects (Fig 2, *E*). As we have previously reported,[3] peak urinary LTE_4 and PGD-M levels strongly correlated with each other and with the FEV_1% change observed (data not shown). No difference in platelet-leukocyte aggregates or platelet activation as assessed by %CD62P positive during reaction was observed between groups (data not shown).

In a previous study of 17 subjects with AERD undergoing oral aspirin challenge, Bosso et al[4] reported that 3 subjects displayed increases in serum tryptase, as well as histamine.[4] Notably, all 3 subjects displayed reductions of more than 30% in FEV_1, moderate-to-severe naso-ocular symptoms, and 2 of the 3 developed gastrointestinal symptoms. Eicosanoids were not quantified. Importantly, this study predated the availability of $CysLT_1R$ antagonists, which blunt CysLT-induced bronchoconstriction and effectively "shift" dominant symptoms from the lower to the upper respiratory tract.[1] Our study, conducted with subjects who were all on montelukast prophylaxis, demonstrates that 50% of subjects with AERD display an increase of more than 10% in plasma tryptase using a more sensitive assay than that used in the previous study.[6] The fact that the changes in tryptase correlated inversely with FEV_1% change supports the bronchoconstrictive role of PGD_2 and other smooth muscle-active mediators derived from mast cells, as well as potential functions of cysLTs at receptors other than $CysLT_1R$. A previous study reported that endogenous cysLTs may drive mast cell activation in AERD, based on suppression of tryptase release by treatment with the 5-lipoxygenase inhibitor zileuton during an oral aspirin challenge.[7] It is unclear whether montelukast similarly alters mast cell function, or whether the effect of zileuton reflected involvement of $CysLT_2R$ and/or $CysLT_3R$, as is suggested by murine models.[8] One subject demonstrated a more than 30% fall in FEV_1 without an associated increase in tryptase, urinary LTE4, or PGD-M (Fig 1, *C*), suggesting that additional mast cell–independent pathways are possible. No baseline clinical differences between MC_{high} subjects and MC_{low} subjects were observed, and the only biochemical difference noted at baseline was that MC_{low} subjects had slightly higher plasma tryptase levels (Table E2). This could suggest that the mast cells from the MC_{low} patients actually start out with a baseline level of increased stimulation, though this is not supported by the urinary eicosanoid data, because neither urinary LTE_4 nor PGD-M are increased at baseline in the MC_{low} group. Thus, there are still no biomarkers to date that can prospectively identify those subjects at risk for severe lower respiratory tract and systemic reactions during aspirin challenge.

Although AERD is a highly distinctive syndrome, this work highlights that there are multiple endotypes of AERD. The difference in plasma tryptase may well reflect the extent of mast cell activation in the lungs (and in some instances in the intestine), which comprise much larger mucosal surfaces (and many more mast cells) than the sinonasal mucosa. This difference in mast cell distribution and burden in the lungs versus the sinonasal mucosa may account for why the change in tryptase was more closely correlated to the change in FEV_1 in our study. These differences provide one explanation for why some have respiratory tract–restricted disease and others demonstrate a systemic disease, and for why some exhibit lower respiratory tract involvement despite $CysLT_1R$ antagonist prophylaxis. How and whether these differences impact therapeutic response to daily high-dose aspirin therapy and novel biologic therapies available and in development for the treatment of moderate-to-severe asthma and nasal polyposis

is not known. As we seek precision medicine, the contribution of mast cells needs to be considered.

Katherine N. Cahill, MD[a,b]*
Katherine Murphy, BA[a]
Joseph Singer, BA[a]
Elliot Israel, MD[a,b,c]
Joshua A. Boyce, MD[a,b]
Tanya M. Laidlaw, MD[a,b]

From [a]Brigham and Women's Hospital, Jeff and Penny Vinik Center for Allergic Diseases Research, Division of Rheumatology, Immunology, and Allergy, Building for Transformative Medicine, [b]Harvard Medical School, and [c]Brigham and Women's Hospital, Division of Pulmonary and Critical Care Medicine, Boston, Mass. E-mail: tlaidlaw@bwh.harvard.edu.

*Katherine N. Cahill, MD, is currently affiliated with the Department of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University, Nashville, Tenn.

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Validation of the maximum symptom day among children with asthma

To the Editor:

The maximum symptom day (MSD) is a composite measure of asthma symptomatology that integrates 3 questions related to daytime and nighttime asthma symptoms. It is the largest value of the number of days in the previous 2 weeks that a participant reports (1) cough, wheezing, or shortness of breath, (2) slowed activities due to these symptoms, or (3) nocturnal awakening due to these symptoms. Originally defined in the pivotal National Cooperative Inner City Asthma Study, the MSD has been used as a primary end point in numerous studies of inner-city asthma (some of which are mentioned in the "Studies Using the MSD" section in this article's Online Repository at www.jacionline.org). Surprisingly, since the first publication citing this outcome almost 20 years ago, it has never been validated.[1]

The standardization of outcomes is important in conducting comparable and reproducible research. A necessary requirement is the use of outcomes that have been contextually validated. Because this has not performed for the MSD, it was classified as "emerging" by the 2010 NIH/AHRQ Asthma Outcomes Workshop, where its core use in asthma research was not supported. Additional evidence reviews have criticized outcomes such as the MSD on this basis.[2,3]

Because a large body of research relies on the MSD, its validation is important, not only to support inferences in these past and future studies but also to address the disparity that arises by the prioritization of, and reliance on, an unvalidated outcome for research within minority populations. The unintended consequence is that the strength of evidence from these studies, which are specifically aimed at understanding and intervening upon asthma within especially vulnerable populations, may be downgraded, stifling development and dissemination of effective interventions.

Our objective was to assess the reliability, validity, responsiveness, and predictive characteristics of the MSD. We analyzed the Mouse Allergen and Asthma Intervention Trial, a parallel-design randomized clinical trial testing a pest management intervention for allergic asthma.[4] A total of 350 participants recruited from Baltimore, Maryland, and Boston, Massachusetts, were followed every 3 months for 1 year (5 encounters). Outcomes recorded at each encounter included the MSD, days of rescue inhaler use in the previous 2 weeks, unanticipated asthma-related

TABLE I. Internal consistency reliability and criterion validity

	Reliability	Criterion validity Spearman correlation between MSD and criteria					
Encounter	Cronbach α	C-ACT	ACT	ATAQ-Control	SABA days	FEV_1pp	FEV_1/FVC
Baseline	0.74	−0.51	−0.68	0.35	0.50	−0.13*	−0.11*
3 mo	0.81			0.60	0.64		
6 mo	0.82	−0.57	−0.75	0.56	0.59	−0.20†	−0.23
9 mo	0.74			0.60	0.74		
12 mo	0.82	−0.49	−0.69	0.56	0.64	−0.20†	−0.30
Average	0.79	−0.52	−0.71	0.53	0.62	−0.18	−0.21

SABA, Short-acting beta agonist.
All cells are statistically significant at a P value of $< .001$ unless noted otherwise.
*Not statistically significant ($P > .05$).
†$P < .05$.

TABLE E1. Study sample collection plan

	Reaction							
Sample	**Baseline**	**Onset**	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
Blood	×			×				×
Urine	×	×			×			×
Spirometry	×	×		×		×		×
Nasal symptom score	×	×	×	×	×	×	×	×

TABLE E2. Study population baseline characteristics

Characteristic	MC_{low}	MC_{high}	*P* value
Sample size (n)	20	19	
Sex: female, %	55	52.6	
Age (y)	49.3 ± 8.4	43.5 ± 10.0	
White race, n	18	19	
Hispanic ethnicity, n	1	1	
FEV_1% predicted	92.5 ± 15.2	93.2 ± 10.2	.866
TNSS	3.4 ± 2.4	5.1 ± 5.5	.240
Plasma tryptase (ng/mL)	4.1 ± 1.8	3.0 ± 1.5	.043
Absolute blood eosinophils (/μL)	490 ± 310	560 ± 430	.562
Urinary eicosanoids (ng/mL)			
LTE_4	0.35 ± 0.94	0.44 ± 0.51	.708
PGD-M	2.22 ± 1.66	2.32 ± 1.20	.827
PGE-M	9.16 ± 9.10	5.94 ± 3.10	.148
TXB_2	0.34 ± 0.14	0.39 ± 0.17	.797

Mean and SD reported unless otherwise noted.
PGE-M, Prostaglandin E metabolite; *TXB_2*, thromboxane B_2.