

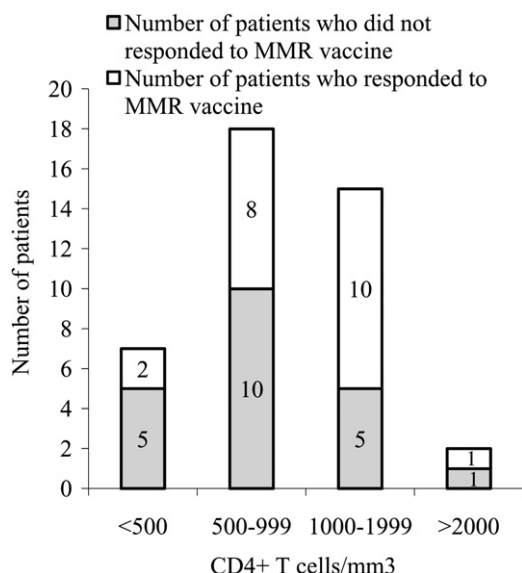


FIG 2. Distribution of CD4⁺ T cells in 40 patients with 22q11 microdeletion at the time of MMR antibody assessment.

Indeed, among 7 of the 22q11 microdeletion patients with less than 500 CD4⁺ cells/mm³, 5 patients did not have antibodies to MMR compared with only 16 patients who did not have antibodies to MMR among the 35 patients with 500 or more CD4⁺ cells/mm³ (Fig 2); however, this difference was not statistically significant ($P = .205$, Fisher exact test).

In summary, we have demonstrated here that MMR vaccination is safe in patients with 22q11.2 microdeletion who have at least near or more than 500 circulating CD4⁺ cells/mm³. However, sustaining long-term protective MMR antibody levels, even after repeat vaccination, appear far lower than those in age-matched control subjects. On the basis of this study, we propose that patients with 22q11.2 microdeletion should be periodically assessed for antibody levels and reimmunized accordingly.

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Methacholine is more sensitive than mannitol for evaluation of bronchial hyperresponsiveness in children with asthma

To the Editor:

Asthma is characterized by bronchial hyperresponsiveness (BHR). In the laboratory, BHR is most commonly assessed by using direct bronchoconstrictor stimuli such as methacholine or histamine, which act via effector cells such as airway smooth muscle and endothelial cells. In contrast, BHR can be assessed by using indirect stimuli¹ such as AMP, exercise, eucapnic hyperventilation, and inhaled mannitol, a stimulus agent that has recently been introduced.² Bronchoconstriction induced by indirect stimulus occurs after mast cell stimulation, triggering the release of inflammatory mediators. For this reason, it has been proposed as a more useful test for both evaluating asthma and predicting the effect of anti-inflammatory treatment than directly acting agents. However, the sensitivity shown by indirect stimuli is lower than that of direct methods.³ Sverril et al⁴ recently showed that in an unselected sample of young adults, bronchial provocation with inhaled dry-powder mannitol demonstrated high diagnostic specificity for the diagnosis of asthma, although their results were not compared against other methods of assessing BHR.

The aim of this study was to examine the diagnostic properties of methacholine and mannitol challenge for assessment of BHR in a pediatric population presenting with asthma symptoms.

Thirty children under the age of 15 who had current (on previous 3 weeks) asthma-related symptoms (chest tightness, cough, wheezing, episodic breathlessness, exercise-induced cough, or dyspnea) were enrolled in the study during their first visit to our clinic.

At the clinic, all participants underwent skin prick testing with common aeroallergens, fraction of exhaled nitric oxide (FENO; NIOX MINO, Aerocrine, Sweden) and spirometry. Bronchial provocation testing with methacholine was performed on the first day, and inhaled mannitol was used on other days. Tests were performed between 9:00 AM and noon.

TABLE I. Characteristics and results of the participants

Age (y)	Sex	% Predicted FEV ₁	Methacholine test	Mannitol test	PC ₂₀ methacholine		PD ₁₅ Mannitol (mg)	FENO (ppb)	Previous treatment	Atopy
					(mg/mL)					
9	F	118	Positive	Negative	0.29		>635	89	No	Yes
11	M	102	Positive	Positive	5.14		448.7	58	No	Yes
15	F	84	Positive	Negative	1.17		>635	19	No	No
6	M	115	Positive	Negative	1.57		>635	9	No	Yes
14	F	110	Positive	Positive	4.7		588	15	No	Yes
14	M	100	Positive	Negative	2.42		>635	25	No	Yes
9	M	90	Positive	Positive	0.29		159.1	28	No	No
7	M	87	Positive	Negative	0.14		>635	17	No	Yes
8	M	94	Positive	Negative	0.29		>635	38	ICS	Yes
6	F	105	Positive	Negative	3.95		>635	9	No	No
15	F	90	Positive	Positive	4.7		449.7	8	No	Yes
10	M	107	Positive	Negative	0.14		>635	27	No	Yes
12	M	91	Positive	Positive	0.09		341.6	45	No	Yes
10	M	112	Positive	Negative	3.45		>635	15	No	Yes
10	F	96	Negative	Positive	>16		475	12	No	Yes
8	M	92	Negative	Negative	9.6		>635	55	No	Yes
13	M	109	Negative	Negative	8.59		>635	48	No	Yes
4	M	89	Positive	Negative	0.26		>635	12	No	Yes
9	M	95	Positive	Negative	0.43		>635	64	No	Yes
12	M	100	Positive	Negative	6.36		>635	16	No	Yes
11	M	97	Positive	Negative	0.93		>635	18	No	Yes
8	M	90	Positive	Positive	0.19		134.7	30	No	Yes
11	F	99	Positive	Positive	1.01		448.7	52	No	Yes
6	M	126	Positive	Positive	0.27		28.7	13	No	No
11	F	114	Positive	Positive	0.65		68.7	14	No	No
9	F	109	Positive	Negative	0.35		>635	8	No	No
9	M	92	Positive	Positive	6.6		89.9	26	No	Yes
9	F	77.1	Positive	Positive	0.25		478	19	No	No
11	M	84	Positive	Positive	0.34		18.4	36	No	Yes
11	F	99	Positive	Negative	0.94		>635	22	No	Yes

F, Female; ICS, inhaled corticosteroid; M, male; PD₁₅, Provocative dose that decreases FEV₁ 15%.

Dry-powder mannitol (Aridol; Pharmaxis, Sydney, Australia) was administered according to the manufacturer's recommendations. FEV₁ recorded after inhalation of a 0-mg placebo capsule constituted baseline lung function. The challenge was discontinued when FEV₁ decreased by 15% or more with respect to baseline values or once the maximum cumulative dose of 635 mg had been administered. On a different day (minimum 2 days, maximum 4 days), a methacholine test was performed by using the tidal volume technique as previously described.⁵ Asthma drugs were withheld for the generally recommended interval (use of short-acting and long-acting inhaled β_2 -adrenergic agents was discontinued for at least 8 hours and 48 hours before the challenge, respectively), leukotriene modifiers were not taken for 7 days, and patients abstained from using inhaled steroids for at least 2 weeks.

Thirty patients with a mean age of 9.89 ± 2.76 years (range, 6–15 years) were analyzed. Table I displays participant characteristics and test results. Withdrawal of inhaled steroids was impossible in only 1 patient. Twenty-three patients showed atopic status with at least 1 positive skin prick test. Response to methacholine (PC₂₀ < 8 mg/mL) was positive in 27 patients (90%), with geometric mean PC₂₀ of 0.92 (95% CI, 0.5–1.6) mg/mL; only 13 patients (43%) had positive results to mannitol (PD₁₅ [provocative dose that decreases FEV₁ 15%] < 635 mg/mL), with a geometric mean PD₁₅ of 184.5 mg (95% CI, 91.3–373.3). No significant correlation was found between the results of both tests. In 2 patients,

both tests produced negative results (methacholine 8.59 and 9.6 mg/mL), with high FENO levels (55 and 48 ppb). In another, response to methacholine was negative but was positive to mannitol (PD₁₅ = 475 mg/mL) despite normal FENO levels. In 12 patients, both tests were positive. Presence of atopy, FENO, and predicted FEV₁% were not associated with positive results to any of the tests.

Our results confirm that bronchoprovocation with a directly acting stimulus such as methacholine is extremely sensitive, demonstrating BHR as a result of the presence of current asthma symptoms more reliably than mannitol in this population of children with asthma. Therefore, methacholine is a poor predictor of hyperresponsiveness to mannitol. However, indirect stimulus may be a clinically relevant marker for assessing the clinical course of asthma, leading us to conclude that the 2 tests are complementary but not interchangeable in clinical practice.

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PDE11A associations with asthma: Results of a genome-wide association scan

To the Editor:

The results of 4 successful genome-wide association studies have been reported recently, yielding 4 distinct loci, *ORMDL3*, *CHI3L1*, *PDE4D*, and *DENND1B*,¹⁻⁴ with the disparity in the results likely caused by differences in the phenotypes studied. In an attempt to dissect further the genetic network in complex asthmatic phenotypes, we investigated allergic asthma specifically defined in children at age 6 years. Cases were patients with allergic asthma with early-onset persistent asthma defined as having physician diagnosed asthma and wheeze at ages 1 and 6 years and asthma medication use and allergies as reported by the mother; controls were defined as those having no diagnosis of asthma or reactive airway disease and no symptoms at ages 1 or 6 years and no asthma medication use and no allergies (see Definition of Cases and Controls section in the Online Repository at www.jacionline.org for additional recruitment details and Table E1 for demographic and phenotype data). Sixty-six cases and 42 controls were successfully genotyped and analyzed by Affymetrix Genome-Wide Human Single Nucleotide Polymorphism (SNP) Array 5.0 (Affymetrix, Santa Clara, Calif) after whole-genome amplification. To test for potential population stratification, we calculated a genomic inflation factor for this study of 1.03 by using EIGENSTRAT,⁵ indicating only minimal background stratification (see Statistical Analysis section in the Online Repository at www.jacionline.org).

No SNP achieved genome-wide significance using strict Bonferroni criteria for multiple testing ($0.05/396,207 = 1.26 \times 10^{-7}$) as the threshold of significance (see this article's Fig E1 in the Online Repository at www.jacionline.org). Overall, there were 39 SNPs with P values $< 1 \times 10^{-4}$ for either the allelic or genotypic test, with 11 of these 39 SNPs forming 3 clusters (2 or more SNPs) in 3 discrete coding regions (Table I; see this article's Table E2 in the Online Repository at www.jacionline.org). Of the 5 SNPs selected, 4 of them were successfully genotyped in the larger collection of case ($n = 104$) and control ($n = 503$) samples

(All Perinatal Risk of Asthma in Infants with Asthmatic Mothers [All PRAM]) that included the original case ($n = 66$) and control ($n = 42$) samples. The P values for these SNPs did not change substantially from their initial significance levels, with all P values within an order of magnitude from their initial value from the genome-wide association study (GWAS; Tables I and E2).

Notably, 1 SNP, rs11684634, achieved a P value of 8.9×10^{-7} at the first stage GWAS under the allelic association test (by Fisher exact test), which was reduced to 3.82×10^{-5} on resequencing of this SNP on the same subjects by using unamplified DNA via a TaqMan assay. Several inconsistent genotype calls were observed between the 2 processes (see TaqMan Genotyping section in the Online Repository at www.jacionline.org). Nevertheless, judging from the result in the extended cohort ($P = 3.4 \times 10^{-2}$; All PRAM minus the samples in the original GWAS), we regarded this SNP as potentially interesting. SNP rs11684634 is located on chromosome 2 in an intron within the *PDE11A* gene (Entrez Gene ID 50940). We resequenced all unique exons of the 4 isoforms of *PDE11A* in a subset of the GWAS cases ($n = 50$) on the basis of their rs11684634 genotype and all controls ($n = 42$). We identified 123 variants (114 SNPs and 9 insertions/deletions; see this article's Table E3 in the Online Repository at www.jacionline.org) in the 48 fragments that were sequenced to cover *PDE11A*. Four of the variants, located in 2 regions of *PDE11A*, were potentially in a high degree of linkage disequilibrium with rs11684634. Two of the 4 variants were then successfully genotyped in the extended cohort (Table I).

To attempt to replicate the association to *PDE11A*, we took a gene-centric approach⁶ and queried 5 existing GWASs of asthma phenotypes for evidence for association with SNPs within *PDE11A*. Three of the 5 datasets had at least 1 SNP with a P value $< .05$ within *PDE11A* (Table II), with only the MRC-A/UK-C dataset not yielding a nominally significant SNP. When rs11684634 was imputed for the British 1958 Birth Cohort (B58C) dataset, a nominally significant result ($P = .046$; Table II) was obtained for this cohort among cases with asthma with onset ≥ 17 years of age and had the same direction of association as in the PRAM cohort. SNP rs11685634 was not significant among childhood-onset patients with asthma in the B58C cohort (Table II). The combined P value for rs11684634 for All PRAM and B58C adult-onset cases by using the Fisher method was 6.0×10^{-5} .

The report of an association with asthma to *PDE4D*³ lends credence to our finding of an association within *PDE11A*, another member of the phosphodiesterase superfamily of genes. Furthermore, the same cohorts exhibiting an association to *PDE4D* also show nominal associations to SNPs within *PDE11A*, suggesting that multiple loci throughout this superfamily of genes may contribute to the susceptibility of asthma. Several phosphodiesterase (PDE)-4 inhibitors have been found to be effective in suppressing inflammation and have been developed as potential therapies for chronic obstructive pulmonary disease and asthma.⁷ *PDE3* and *PDE7* are also expressed in inflammatory cells, and *PDE5* was initially used to treat patients with exercise-induced asthma and showed bronchodilator effects.^{8,9}

One major limitation of the current study is the fact that the initial discovery sample used for the GWAS is underpowered to detect loci with small effect size. We have attempted to address this issue by validating the *PDE11A* association in a larger set of cases and controls, albeit from the same study population, as well as compare our case allele frequencies to additional sets of publicly available control samples (see Table E4 in the Online