Letter to the Editor

Genetic variation in T_H17 pathway genes, childhood asthma, and total serum IgE levels

To the Editor:

The role of T_H17 cells and T_H17-associated cytokines in autoimmune diseases and chronic inflammation is widely recognized.[1] In children with atopic asthma, T_H17 cells in peripheral blood were found to be increased and inversely correlated with asthma control.[2] The cytokine milieu has a decisive effect on the balance between developing immunosuppressive regulatory T cells or proinflammatory T_H17 cells. Dysregulation of the cytokine balance can therefore contribute to autoimmunity and chronic inflammation.[3] IL-17A and IL-17F are signature cytokines secreted by T_H17 cells and potent inducers of inflammation. Increased levels of these cytokines were observed in airways of patients with asthma,[4] and first candidate gene studies suggested single nucleotide polymorphisms (SNPs) in *IL17A* and *IL17F* to be associated with asthma.[5,6]

This study investigated whether genetic variants in the T_H17 pathway influence asthma and total serum IgE levels during childhood. We analyzed genes involved in the differentiation and maintenance of T_H17 cells and genes coding for T_H17-related effector cytokines (Fig 1; see the Methods section in this article's Online Repository at www.jacionline.org). The relevance of associations in T_H17 pathway genes was ranked with an algorithm considering *P* values, effect sizes, and multiple testing.

Subjects (651 with asthma and 652 without asthma as controls) for association analyses with asthma and total serum IgE levels were derived from the Multicentre Asthma Genetics In Childhood Study (MAGICS, cases) and the International Study of Asthma and Allergy in Childhood, phase II (ISAAC II, reference population). Both populations were of German origin and genetically homogeneous, and the studies were performed with very similar tools and definitions (see the Methods section; also see, Fig E1 in the Online Repository at www.jacionline.org).

All 17 genes of the T_H17 pathway were investigated in a systematic tagging SNP approach based on HapMap CEU data. In total, 203 tagging SNPs ($r^2 \geq 0.8$; minor allele frequency ≥0.03) captured all essential genetic information of 404 polymorphisms present in the T_H17 pathway genes including ±10 kilobase pairs flanking sequences (see Table E1 in this article's Online Repository at www.jacionline.org). Tagging SNPs (n = 17) were genotyped if not covered by previous genome-wide chip genotyping (n = 91) or imputation (n = 94). Only rs3136558 located in *IL1B* could neither be genotyped nor imputed and had to be excluded from further analysis. Imputed tagging SNPs showing associations ($P < .05$) with asthma were genotyped for validation (see this article's Methods section).

All 202 tagging SNPs were analyzed by using logistic regression to determine additive effects of SNPs on asthma status. Significant associations ($P < .05$) with asthma were observed for 11 tagging SNPs (see Table E2, *C* in this article's Online Repository at www.jacionline.org). While 11 associations do not considerably exceed stochastic expectations (202 SNPs × 0.05 ≈ 10 SNPs), these associations clustered to specific parts of the T_H17 pathway, suggesting a nonrandom distribution of association results. To address multiple testing without overcorrecting (such as with Bonferroni correction) and the risk to miss true associations, a ranking algorithm was applied as previously published.[7] The algorithm accounts for the maximal *P* value and effect size per gene in relationship to the number of SNPs tested per gene (see this article's Methods section). This allows one to investigate clustering effects in disease-associated pathways. By applying this approach, the top ranked gene showing asthma association was found to be *IL23A* followed by *RORγt*, *IL17F, IL23R*, *IL17A*, *TGFB1*, and *IL22* (Table I).

Associations with atopic and nonatopic asthma were investigated in 468 atopic asthmatic patients, 98 nonatopic asthmatic patients, and 408 nonasthmatic nonatopic controls (see this article's Methods section and Fig E1). Atopic asthma was again associated with *IL17A*, *IL23A*, *RORγt*, *IL23R*, and *IL17F* though

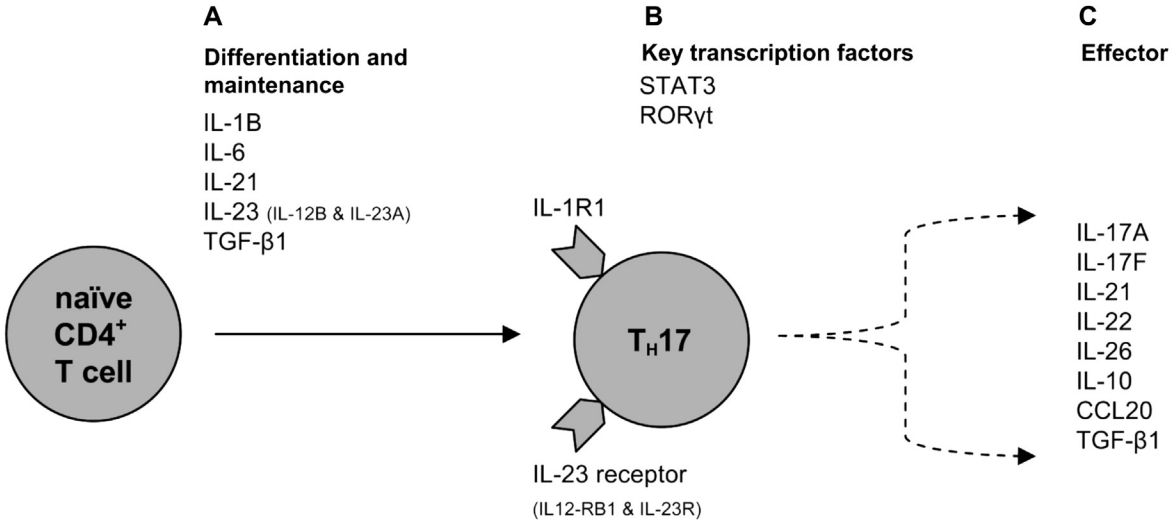

FIG 1. Genes associated with the T_H17 pathway. The PubMed database was reviewed for molecules involved in the differentiation of naive $CD4^+$ T cells into the T_H17 lineage or maintenance of the T_H17 phenotype **(A)**, key transcription factors **(B)**, and T_H17 effector cytokines **(C)**. All 17 genes were selected for genetic association analyses with asthma phenotypes and total serum IgE levels.

TABLE I. Ranking of T_H17 pathway genes associated with asthma, atopic or nonatopic asthma, and total serum IgE

Asthma

Gene	P_{min}	OR_{max}	*n*	*N*	Ranking score
IL23A	.0276	1.38	1	1	4.30
RORγt	.0064	1.24	3	16	3.23
IL17F	.0115	1.29	1	10	2.75
IL23R	.0138	1.30	3	23	2.73
IL17A	.0186	1.21	1	10	2.31
TGFB1	.0287	1.24	1	10	2.10
IL22	.0465	1.20	1	15	1.70

Atopic asthma

Gene	P_{min}	OR_{max}	*n*	*N*	Ranking score
IL17A	.0001	1.47	2	10	6.80
IL23A	.0142	1.57	1	1	5.80
RORγt	.0007	1.41	4	16	5.62
IL23R	.0245	1.24	1	23	2.09
IL17F	.0457	1.27	2	10	2.04

Nonatopic asthma

Gene	P_{min}	OR_{max}	*n*	*N*	Ranking score
IL17F	.0212	2.49	4	10	5.84
IL23R	.0152	2.95	2	23	5.82
IL22	.0477	3.35	1	15	4.72
IL12RB1	.0112	1.73	2	11	3.99
IL17A	.0111	1.51	2	10	3.55
IL6	.0395	1.39	1	14	2.09
IL12B	.0446	1.44	1	13	2.09

Total serum IgE (ISAAC II)

Gene	P_{min}	OR_{max}*	*n*	*N*	Ranking score
IL21	.0009	1.80	2	7	7.01
IL12B	.0139	1.28	4	13	3.11
IL17A	.0201	1.41	2	10	2.87
IL1R1	.0173	1.32	2	25	2.51
IL22	.0341	1.33	1	15	2.09
IL23R	.0311	1.25	1	23	1.97
IL26	.0336	1.17	1	14	1.85

Total serum IgE (MAGICS/ISAAC II)

Gene	P_{min}	OR_{max}*	*n*	*N*	Ranking score
IL21	.0001	1.64	2	7	8.13
IL12B	.0007	1.24	8	13	6.35
IL17A	.0056	1.36	2	10	3.68
IL1R1	.0125	1.22	2	25	2.51

Tagging SNP association data were analyzed with a ranking algorithm to systematically assess the significance of single genes on the T_H17 pathway. The function accounts for the strength of tagging SNP associations, effect sizes, and multiple testing. The tables are arranged according to the final ranking score of the gene.

n, Number of tagging SNPs with $P < .05$; *N*, total number of tagging SNPs; OR_{max}, strongest *odds ratio* for the risk allele among SNPs with $P < .05$; P_{min}, most significant *P* value among tagging SNPs.

*Estimates have been transformed into *OR*s.

in a different ranking order (Table I). In nonatopic asthma, the order of associated genes was *IL17F*, *IL23R*, *IL22*, *IL12RB1*, *IL17A*, *IL6*, and *IL12B* (Table I).

Effects on total serum IgE levels were analyzed by applying linear regression adjusted for age, sex, and asthma status. IgE level measurements were available for 1137 subjects (452 MAGICS and 685 ISAAC II). Associations were first explored in the cross-sectional ISAAC II population (Table I). Analyses in the combined MAGICS/ISAAC II data set confirmed associations and ranking of *IL21*, *IL12B*, *IL17A*, and *IL1R1* (Table I). *IL21* was strongly associated with IgE levels but not with asthma. This suggests a distinct role of IL-21 in total serum IgE levels and the development of allergy.

While we acknowledge the explorative character of our study with small sample sizes, it is intriguing that in all phenotypes at least 1 molecule involved in IL-23 signaling (*IL23A*, *IL23R*, *IL12B*, or *IL12RB1*) obtained a high-ranking score and that the proinflammatory cytokine *IL17A* was consistently associated. In addition, all asthma phenotypes were associated with *IL17F*. The importance of the IL-23/IL-17 axis in chronic inflammation has recently been highlighted by a haplotype analysis in Crohn disease.[8] Associated haplotypes also contained polymorphisms identified in our study (rs2275913 and rs10484879 in *IL17A*, rs375947 in *IL12RB1*, and rs3212227 in *IL12B*). *RORγt*, another part of the IL-23/IL-17 axis, also gained high-ranking scores in asthma and atopic asthma, emphasizing its prominent role in T_H17 differentiation and the induction of proinflammatory cytokines such as IL-17A and IL-17F.[9]

Functional relevance of associated polymorphisms in *IL17A* (rs2275913) and *IL23R* (rs7517847, rs790631, and rs10889675) has recently been shown. These polymorphisms correlate with the T_H17 cell marker and cytokine expression in human cord blood.[10] Early effects of T_H17 polymorphisms on the cytokine milieu indicate that SNPs in the T_H17 pathway may contribute to asthma development at a very early stage. Further promising targets for functional analyses exist: Polymorphisms rs11209026 (*IL23R*) and rs375947 (*IL12RB1*) lead to amino acid changes. Both SNPs were associated with nonatopic asthma and had been linked to inflammatory diseases before (see Table E3 in this article's Online Repository at www.jacionline.org). Additional fine mapping of associated genes may identify further causal mutations in the T_H17 pathway.

This study indicates that genetic variation in the IL-23/IL-17 axis is involved in the development of childhood asthma and total serum IgE levels. In addition, IgE levels seem to be affected by mutations in *IL21* and *IL1R1*. This information can be useful when targeting T_H17 genes and T_H17-associated mechanisms for therapeutic purposes in different diseases in the future.

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REFERENCES

1. Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 cells: new players in asthma pathogenesis. Allergy 2011;66:989-98.
2. Kerzel S, Dehne J, Rogosch T, Schaub B, Maier RF, Zemlin M. T(H)17 cell frequency in peripheral blood from children with allergic asthma correlates with the level of asthma control. J Pediatr 2012;161:1172-4.
3. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006;441:235-8.
4. Silverpil E, Linden A. IL-17 in human asthma. Expert Rev Respir Med 2012;6:173-86.
5. Chen J, Deng Y, Zhao J, Luo Z, Peng W, Yang J, et al. The polymorphism of IL-17 G-152A was associated with childhood asthma and bacterial colonization of the hypopharynx in bronchiolitis. J Clin Immunol 2010;30:539-45.
6. Kawaguchi M, Takahashi D, Hizawa N, Suzuki S, Matsukura S, Kokubu F, et al. IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. J Allergy Clin Immunol 2006;117:795-801.
7. Pandey RC, Michel S, Tesse R, Binia A, Schedel M, Liang L, et al. Genetic variation in the Toll-like receptor signaling pathway is associated with childhood asthma. J Allergy Clin Immunol 2013;131:602-5.
8. McGovern DP, Rotter JI, Mei L, Haritunians T, Landers C, Derkowski C, et al. Genetic epistasis of IL23/IL17 pathway genes in Crohn's disease. Inflamm Bowel Dis 2009;15:883-9.
9. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17$^+$ T helper cells. Cell 2006;126:1121-33.
10. Lluis A, Ballenberger N, Illi S, Schieck M, Kabesch M, Illig T, et al. Regulation of Th17 markers early in life through maternal farm exposure. J Allergy Clin Immunol 2013 [In press].

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