

Letter to the Editor

Genetic variants in the *GATA3* gene are not associated with asthma and atopic diseases in German children

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In asthma and atopy a disturbed regulation of T cells can lead to an imbalance between T_H1 and T_H2 cells and toward an increased T_H2 response. The transcription factor GATA3 is necessary and sufficient for the differentiation of naive T cells into T_H2 cells and also the maintenance of T_H2 cytokine expression (IL-4, IL-5, and IL-13) in already differentiated T_H2 cells.¹ GATA3 is not only involved in T-cell regulation but directly influences asthma symptoms and development. Selectively silencing

GATA3, Sel et al² recently demonstrated the essential involvement of GATA3 in the development of asthma in a murine model of acute experimental allergic asthma.

Thus the aim of the present study was to investigate whether polymorphisms in the *GATA3* gene exist that might affect T_H cell differentiation and influence the pathogenesis of asthma and allergy.

By using the HapMap database, *GATA3* polymorphisms (minor allele frequency $\geq 3\%$) were identified and genotyped by means of matrix-assisted laser desorption/ionization time of flight mass spectrometry in German children from Munich (n = 1159) and Dresden (n = 1940) recruited in the cross-sectional International Study of Asthma and Allergy in Childhood phase II³ and children

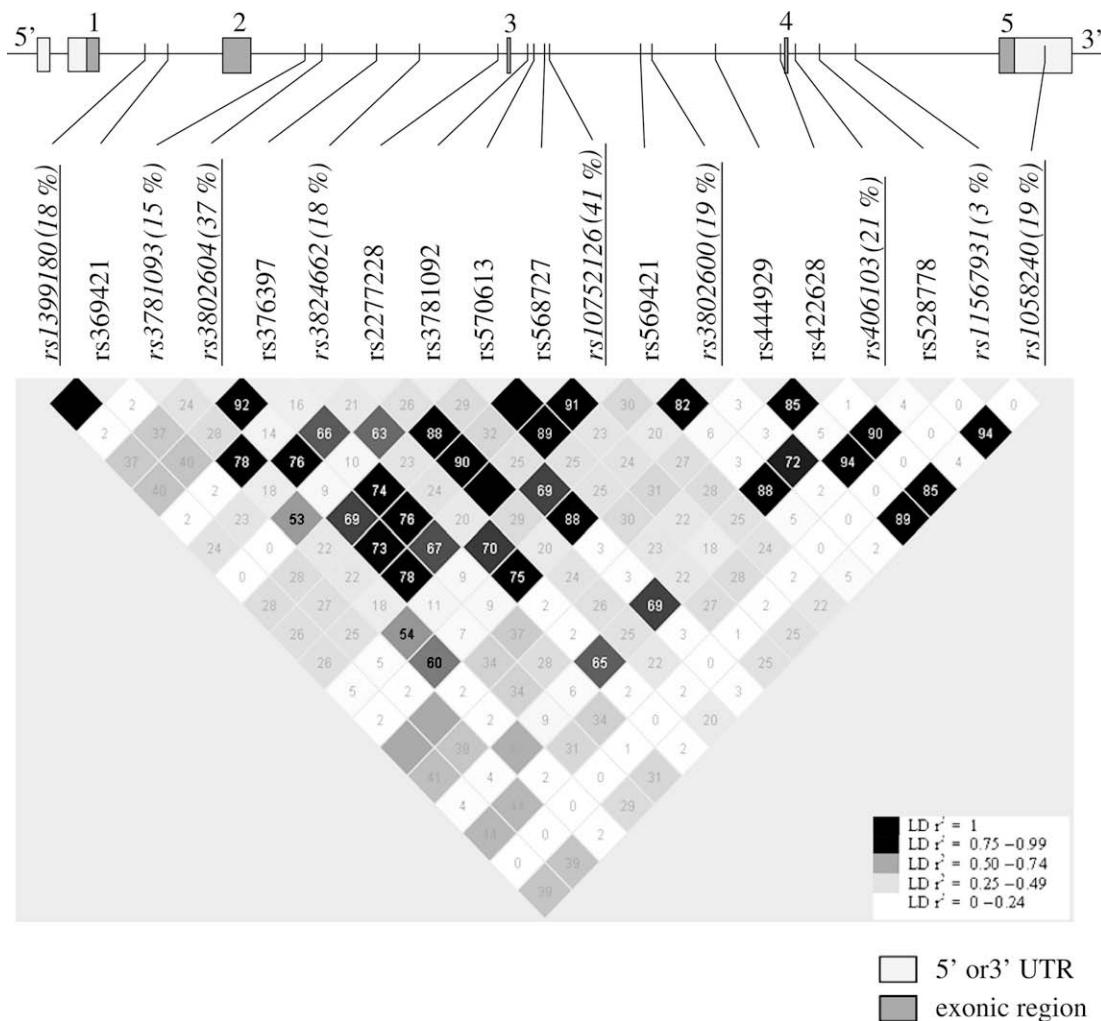


FIG 1. Description of the *GATA3* gene structure on chromosome 10p15, identified *GATA3* polymorphisms, linkage disequilibrium (LD; r^2 plot), tagging SNPs, and minor allele frequencies of genotyped *GATA3* polymorphisms. *GATA3* SNPs with a minor allele frequency of at least 3% were identified with the HapMap database. Linkage disequilibrium between *GATA3* polymorphisms was determined by using the information of the HapMap database. Color coding for the linkage disequilibrium plot is given by Haploview: white ($r^2 = 0$), shades of gray ($0 < r^2 < 1$), and black ($r^2 = 1$). *GATA3* tagging SNPs are underlined. Minor allele frequencies are shown in brackets and are based on the genotyping in the pooled cross-sectional study population (total n = 4264). Genotyped *GATA3* polymorphisms are shown in italics. UTR, Untranslated region.

TABLE I. Tests for associations and equivalence tests for all *GATA3* SNPs with asthma, hay fever, eczema, and atopy in the pooled cross-sectional study population from Dresden, Leipzig, and Munich (total n = 4264)

		CLs for the OR			<i>P</i> value, χ^2 test	Equivalence limits (using an OR of 0.8-1.25)		Equivalence decision	
		OR	Lower CL	Upper CL		C1(s)	C2(s)	X	
Asthma	rs1399180	1.18	0.97	1.44	.0954	114	123	134	No
	rs3781093	0.97	0.78	1.22	.8061	97	104	98	Yes
	rs3802604	1.03	0.87	1.21	.7457	233	256	248	Yes
	rs3824662	1.03	0.83	1.26	.8171	114	123	120	Yes
	rs10752126	1.00	0.85	1.18	.9881	262	288	275	Yes
	rs3802600	1.02	0.84	1.25	.8222	120	131	127	Yes
	rs406103	0.97	0.80	1.18	.7505	132	144	134	Yes
	rs11567931	1.06	0.69	1.63	.7812	22	23	24	No
	rs1058240	1.12	0.92	1.36	.2589	125	136	141	No
Hay fever	rs1399180	1.02	0.83	1.26	.8614	110	119	116	Yes
	rs3781093	1.04	0.83	1.30	.7475	94	101	100	Yes
	rs3802604	1.02	0.87	1.21	.7790	225	247	239	Yes
	rs3824662	1.12	0.92	1.38	.2637	110	118	124	No
	rs10752126	1.03	0.87	1.21	.7240	250	274	266	Yes
	rs3802600	1.08	0.88	1.32	.4800	116	126	127	No
	rs406103	0.97	0.80	1.19	.7983	126	138	129	Yes
	rs11567931	1.29	0.87	1.93	.2073	22	23	28	No
	rs1058240	1.06	0.87	1.29	.5772	121	131	131	Yes
Eczema*	rs1399180	0.89	0.75	1.04	.1354	216	245	211	No
	rs3781093	1.04	0.88	1.22	.6857	188	212	204	Yes
	rs3802604	0.90	0.80	1.02	.1152	450	509	454	Yes
	rs3824662	1.08	0.92	1.26	.3427	218	247	244	Yes
	rs10752126	0.92	0.82	1.05	.2078	501	564	512	Yes
	rs3802600	1.00	0.86	1.17	.9872	230	261	245	Yes
	rs406103	0.93	0.80	1.08	.3328	252	287	256	Yes
	rs11567931	1.34	0.99	1.82	.0545	44	46	56	No
	rs1058240	1.01	0.87	1.18	.8691	239	272	257	Yes
Atopy	rs1399180	1.00	0.88	1.14	.9994	333	381	356	Yes
	rs3781093	1.00	0.86	1.15	.9455	289	330	308	Yes
	rs3802604	1.02	0.92	1.13	.7161	691	784	744	Yes
	rs3824662	1.04	0.91	1.19	.5575	336	385	368	Yes
	rs10752126	1.05	0.95	1.16	.3742	778	877	844	Yes
	rs3802600	1.03	0.91	1.17	.6568	357	410	389	Yes
	rs406103	1.01	0.89	1.14	.8940	389	447	419	Yes
	rs11567931	1.10	0.84	1.45	.5007	67	70	73	No
	rs1058240	1.12	0.99	1.27	.0705	366	420	420	Yes

CL, Confidence limit.

*Eczema is a pooled variable: physician's diagnosis of atopic dermatitis in Munich and Dresden and ever eczema plus ever itchy dermal changes in Leipzig.

from Leipzig (n = 1165) phenotyped with a similar protocol.⁴ The prevalence of asthma and allergies was assessed by means of self-administered questionnaires and objective measurements, such as lung function tests, skin prick tests, and serum IgE measurements. Further information on primer sequences, technical details, or population characteristics are available from the corresponding author on request.

Based on the linkage disequilibrium structure of the *GATA3* gene obtained from the HapMap database, 3 single nucleotide polymorphisms (SNPs; rs3781093, rs3824662, and rs11567931) and 6 tagging SNPs (rs1399180, rs3802604, rs10752126, rs3802600, rs406103, and rs1058240) capturing the genetic information of the entire gene locus were genotyped. As indicated by Fig 1, all identified polymorphisms were located in intronic regions of the gene, with the exception of SNP rs1058240, which was located in the 3' untranslated region of the gene.

The associations between the genotyped SNPs and asthma, hay fever, eczema, and atopy were investigated by using pooled data from Dresden, Munich, and Leipzig (total n = 4264, Table I).

None of the SNPs showed any significant associations with these phenotypes in an allelic model.⁵ An equivalence test was performed to test for statistical significance of the absence of associations between *GATA3* SNPs and these atopic phenotypes (Table I).⁶ By using these tests, it can be measured how likely it is that an effect falls within a certain predefined odds ratio (OR) range near a null effect or is absent. For our study, this range was defined with an OR from 0.8 to 1.25. For most of the tested *GATA3* polymorphisms, the equivalence test rejected the hypothesis of association, even excluding relatively small contributions of *GATA3* to disease development defined in the set range. Because the allelic model captures more or less the information of a dominant model, we also investigated *GATA3* SNP effects using a recessive model. By using this approach, SNP rs3824662 showed borderline nominal significant associations with eczema (OR, 1.61; 95% CI, 1.07-2.42; *P* = .0214) and hay fever (OR, 1.69; 95% CI, 1.00-2.84; *P* = .0474), not withstanding correction for multiple testing. Additionally, haplotype analyses of the 9 SNPs were conducted, but no associations with asthma or atopy could be detected (data not shown).

Polymorphisms in the *GATA3* gene locus were also specifically investigated in an additional sample of 728 asthmatic children from the Multicenter Asthma Genetic in Childhood study and 694 reference children (randomly selected from the Munich and Dresden International Study of Asthma and Allergy in Childhood populations), as previously published in a genome-wide association study (GWA), to further strengthen the analysis.⁷ Eight of the 9 *GATA3* SNPs investigated in the present study had also been genotyped in the GWA study (rs1399180, rs3781093, rs3802604, rs3824662, rs570613 [in linkage disequilibrium with tagging SNP rs10752126], rs3802600, rs569421 [in linkage disequilibrium with tagging SNP rs406103], and rs1058240). By using information of the allelic model, none of these *GATA3* SNPs showed a significant association with asthma in the GWA study, confirming our negative results. Thus we could exclude major contributions of *GATA3* SNPs to asthma development in our study population. When we tried to replicate previous associations from Finnish populations⁸ between *GATA3* polymorphisms and highly increased total serum IgE levels in our populations, rs1399180 (OR, 1.27; 95% CI, 1.06-1.52; $P = .0085$) and rs11567931 (OR, 1.55; 95% CI, 1.10-2.19; $P = .0114$) were found to be associated with total serum IgE levels beyond the 90% percentile (>457 IU/mL), not withstanding correction for multiple testing with the Bonferroni correction.

Interestingly, effects on asthma were previously observed in genetic studies of the major counterparts of *GATA3* in T-cell regulation: T-box expressed in T cells (*TBX21*) and H. 20-like homeobox 1 (*HLX1*). Polymorphisms in these genes significantly modify asthma risk in our studies^{9,10} and other's studies.¹¹⁻¹³ These results suggest that relatively small genetic variation in T_H1 signals affect asthma development, whereas genetic alterations in T_H2 -related signals show no such consequences. Support for this hypothesis derives from previous animal models, in which it was shown that *TBX21* transgenic mice had an asthmatic phenotype very similar to that of mice with a complete loss of *TBX21* already when *TBX21* expression is reduced in mice heterozygous for *TBX21*.¹⁴ Furthermore, it was recently proposed by Usui et al¹⁵ that *GATA3* directs naive T cells toward T_H2 differentiation only until its effects are overruled by *TBX21*. In that regard it is intriguing that a recent twin study suggested the T_H1 response to be predominantly genetically determined, whereas this could not be shown for the initiation of the T_H2 response.¹⁶

Taken together, it might be speculated that genetic variations in the major T_H2 -associated transcription factor *GATA3* have no major influence on the origin of asthma because an increased T_H2 response is initiated by a weak or absent T_H1 signal rather than by an augmented T_H2 signal.

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