

11. Kool M, Willart MA, van Nimwegen M, et al. An unexpected role for uric acid as an inducer of T helper 2 cell immunity to inhaled antigens and inflammatory mediator of allergic asthma. *Immunity*. 2011;34:527-540.

SUPPORTING INFORMATION

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Functional CTLA-4 variants associate to both allergic asthma and rhinitis potentially by modulating naïve regulatory T cells

To the Editor,

A genome-wide linkage study previously indicated the chromosome 2q region containing the cytotoxic T-lymphocyte protein 4 (CTLA-4) gene might be a candidate asthma locus.¹ However, prior reports of associations between CTLA-4 genetic variants and asthma are conflicting and inconclusive.²⁻⁴ This study aimed to determine the role of CTLA-4 single nucleotide polymorphisms (SNPs) on allergy risk by carrying out genetic association and functional analyses. Detailed information on the cohorts, sample processing and experimental methods have been provided in Appendix S2.

We identified 5 tag-SNPs (rs733618, rs4553808, rs16840252, rs231775, and rs3087243) in CTLA-4 from the Hapmap Chinese Han population (CHB, Figure S1). These tag-SNPs were genotyped in a cohort of 1703 Singapore Chinese adults (age: 22.2 ± 5.6 , 42% male, Table S1). Of these 5 CTLA-4 tag-SNPs genotyped, rs3087243 has the highest significance level of associations with AR without asthma ($p = 4.69 \times 10^{-3}$, OR = 1.38), asthma without AR ($p = 3.49 \times 10^{-4}$, OR = 1.58), and AR with asthma ($p = 6.67 \times 10^{-4}$, OR = 1.52, Table 1).

Next, we determined if rs3087243 has a functional effect on CTLA-4. We extracted CTLA-4 mRNA expression data from 31,300 whole blood samples constituting 36 different cohorts collected by the eQTLgen consortium. In these cohorts, meta-analysis showed a strong correlation between allele "A" of rs3087243 and increasing CTLA-4 mRNA expression (meta p -value = 2.67×10^{-69} , combined Z-score = 17.60, Figure 1A). From the HapMap CHB data, tag-SNP rs3087243 is in a strong linkage disequilibrium ($r^2 = 1$) with rs11571316 that is located ~1.4 kb upstream of the CTLA-4 gene (Figure S1). We cloned -1686bp to +110bp of the CTLA-4 gene to conduct an in vitro luciferase assay and showed a significantly higher CTLA-4 promoter activity associated with allele "A," as compared to allele "G" of rs3087243 ($p < .01$, Figure 1B,C). This suggests rs11571316 might represent a functional SNP that influences the CTLA-4 promoter activity and causes the apparent genetic association between the tag-SNP rs3087243 and respiratory allergies.

Lastly, when analyzing the CTLA-4 surface expression of T cells by flow cytometry, we demonstrated a functional effect of

TABLE 1 Genetic association of CTLA-4 tag SNPs to allergic rhinitis and asthma in Singapore Chinese population

Reference/Phenotype		AR without asthma (N = 455) vs. Nonatopic Nonallergic controls (N = 525)		AR with asthma (N = 338) vs. Nonatopic Nonallergic controls (N = 525)		Asthma without AR (N = 379) vs. Nonatopic Nonallergic controls (N = 525)	
SNP	Allele	Logistic p	OR	Logistic p	OR	Logistic p	OR
rs733618	G/A	3.04E-03*	0.76 (0.63-0.91)	5.64E-02	0.82 (0.66-1.01)	4.27E-02*	0.81 (0.67-0.99)
rs4553808	G/A	9.08E-01	0.98 (0.74-1.31)	2.33E-01	0.82 (0.58-1.14)	1.92E-01	0.81 (0.60-1.11)
rs16840252	T/C	7.96E-01	0.96 (0.73-1.28)	2.34E-01	0.82 (0.59-1.14)	2.00E-01	0.82 (0.60-1.11)
rs231775	A/G	9.24E-03*	1.30 (1.07-1.57)	2.86E-02*	1.29 (1.03-1.61)	4.91E-02*	1.24 (1.00-1.54)
rs3087243	A/G	4.69E-03*	1.38 (1.10-1.73)	3.49E-04*	1.58 (1.23-2.02)	6.67E-04*	1.52 (1.19-1.93)

Note: Logistic regression test was performed adjusting for age and gender.

Abbreviations: CI, confidence interval; Logistic p, adjusted logistic regression p -value; OR, adjusted odds ratio, using minor allele as the reference category.

*Logistic $p < .05$ is considered significant association.

rs11571316 on protein levels in T regulatory (Treg) cells. On peripheral blood mononuclear cell (PBMC) samples from a Singapore Chinese cohort ($n = 201$, age: 21.0 ± 2.3 , 58% male), we observed robust CTLA-4 expression on Treg cells (gated for CD4+ CD25+ Foxp3+), while only lower amounts of the protein were detected on CD25-Foxp3- T effector (Teff) cells (Figure S2). Compared to memory-type Treg (CD45RA-), the basal levels in naïve-type Treg cells (CD45RA+) were also lower. However, in this subset we observed a dosage effect of increasing CTLA-4 protein levels from the "CC" to "TT" genotype of rs11571316 (ANOVA $p = 8.5 \times 10^{-10}$, Figure 1D). Besides this strong effect on naïve Treg, nominal association between rs11571316 genotype and CTLA-4 protein levels was only observed in T effector (Teff) cells (ANOVA $p = .011$, Figure S3). This suggests that rs11571316 might regulate CTLA-4 protein levels mainly in naïve Treg cells and contribute to the development of allergy. In agreement with this, prior reports have also demonstrated an important role of Treg cells in the control of airway inflammation in asthmatic patients.⁵ In our study however, there lack of distinction between the asthma positive and negative group regarding the rs3087243 genotype, which needs to be validated in future studies. However, a separate linkage block in 2q was previously reported to modulate the mRNA expression of ICOS among Treg and Teff subsets and associated with the risk of atopy and asthma.⁶ Therefore,

we speculated an interplay between ICOS and CTLA-4 in these T cell subsets might contribute to the pro-inflammatory condition that underlies allergic sensitization and manifestation of respiratory symptoms (Figure S4). Furthermore the lack of association to CTLA-4 SNPs to respiratory allergies in other populations could be due to variability in disease definition. Our data highlights a strong association of both CTLA-4 and ICOS SNPs to the allergic underpinning of both asthma and rhinitis. Hence follow-up studies in other cohorts with allergic sensitization and clinical manifestation might highlight the role of these candidate genes in their populations.

In conclusion, this study identified a functional genetic signal that was associated with allergy via modulating CTLA-4 expression in Treg cell subsets. Our data highlights a possible mechanism for how a targeted immune dysregulation can affect the risk for multiple disease phenotypes. The naïve Treg phenotype warrants further investigation as a potential target for therapeutic intervention in affected patients by harnessing strategies to attenuate CTLA-4 expression on these cells.

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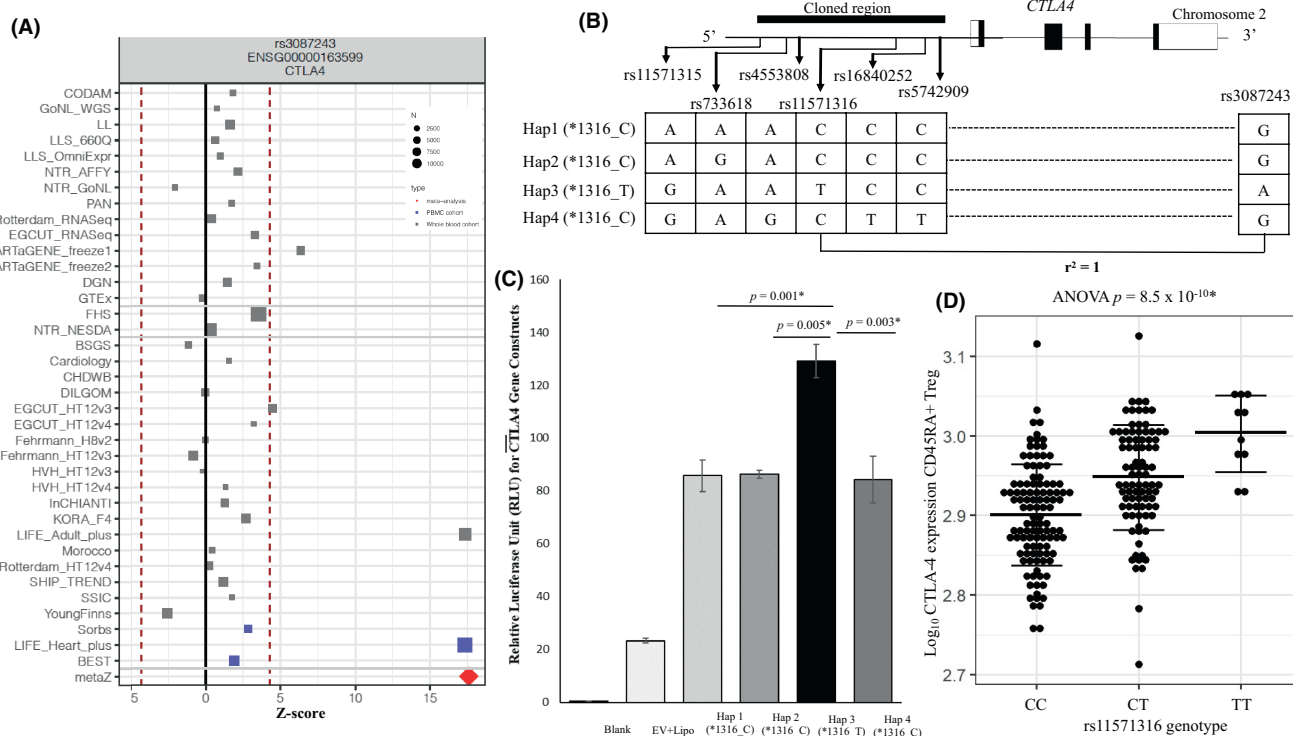


FIGURE 1 Functional association between 2q variants and CTLA-4 expression. (A) Meta-analysis of whole blood eQTL association between CTLA-4 mRNA expression and rs3087243 genotypes, using 31,300 samples constituting 36 different cohorts from the eQTLgen consortium. (B) Schematic illustration of the CTLA-4 gene region with the relative location of SNPs. The cloned region and haplotypes used for the in vitro luciferase assay were also indicated. (C) Relative luciferase units (RLUs) of the CTLA-4 promoter construct were compared across haplotypes. Promoter-less luciferase plasmid pGL4.10 (empty vector, EV) was used as a background control. Lipo: Lipofectamine transfection reagent. (D) Protein expression of CTLA-4 in naïve-type CD45RA+ T regulatory (Treg) cells was compared across rs11571316 genotypes. An independent t-test was conducted to determine statistical significance in (C) and (D) that was defined as p -value $< .05$

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CONFLICT OF INTEREST

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REFERENCES

1. Wjst M, Fischer G, Immervoll T, et al. A genome-wide search for linkage to asthma. German Asthma Genetics Group. *Genomics*. 1999;58:1-8.
2. Munthe-Kaas MC, Håkon Carlsen K, Helms PJ, et al. CTLA-4 polymorphisms in allergy and asthma and the TH1/ TH2 paradigm. *J Allergy Clin Immunol*. 2004;114:280-287.
3. Howard TD, Postma DS, Hawkins GA, et al. Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol*. 2002;110:743-751.
4. Hizawa N, Yamaguchi E, Jinushi E, Konno S, Kawakami Y, Nishimura M. Increased total serum IgE levels in patients with asthma and promoter polymorphisms at CTLA4 and FCER1B. *J Allergy Clin Immunol*. 2001;108:74-79.
5. Jansen K, Satitsuksanoa P, Wirz OF, et al. T regulatory cells from atopic asthmatic individuals show a Th2-like phenotype. *Allergy*. 2021;77(4):1320-1324.
6. Andiappan AK, Narayanan S, Myers RA, et al. Genetic variants of inducible costimulator are associated with allergic asthma susceptibility. *J Allergy Clin Immunol*. 2015;135:556-558.

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