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ORIGINAL ARTICLE

Toll-like receptor 1 N248S polymorphism affects T helper 1 cytokine production and is associated with serum immunoglobulin E levels in Taiwanese allergic patients

Chin-An Yang ^a, Bor-Luen Chiang ^{a,b,*}^a Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan^b Medical Research, National Taiwan University Hospital, Taipei, Taiwan

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KEYWORDS

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Background/purpose: This study was carried out to investigate whether toll-like receptor-1 (TLR1) rs4833095 (N248S) variant, common in the Taiwanese population, contributes to pathogenesis of allergy.

Methods: TLR2/1 ligand Pam3CSK4-stimulated cytokine production in peripheral blood mononuclear cells and monocyte-derived dendritic cells of different genotypes were measured via enzyme-linked immunosorbent assay. Ninety-three Taiwanese allergic patients (with asthma, allergic rhinitis, or atopic dermatitis) and 76 controls were recruited for genotyping. Serum immunoglobulin E (IgE) levels were evaluated in 60 allergic patients.

Results: The homozygous TLR1 C variant allele carrier had increased Pam3CSK4-induced tumor necrosis factor- α and interleukin-12 responses in peripheral blood mononuclear cells and monocyte-derived dendritic cells, respectively. Furthermore, although the C/C genotype was not associated with susceptibility to atopic diseases, it was correlated with lower total IgE levels in sera of allergic patients.

Conclusion: Our data suggest that the TLR1 N248S polymorphism might play a role in Th1/Th2 differentiation, and the determination of serum IgE levels. However, interactions with other genetic and environmental factors might be required to contribute to risk of allergic diseases in our population.

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* Corresponding author. Department of Pediatrics, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei 100, Taiwan.
E-mail address: gicmbor@ntu.edu.tw (B.-L. Chiang).

Introduction

Toll-like receptor (TLR) drives innate immune response and subsequent immune regulations when encountering microbes. In accordance with the hygiene hypothesis, allergic diseases were found to be less frequent in mycobacterium-endemic areas.¹ Furthermore, in small case-control studies, single nucleotide polymorphisms (SNPs) in TLR2, TLR4, TLR6, TLR10, and CD14, a co-receptor with TLR4 for detection of bacterial lipopolysaccharide (LPS), have been suggested to contribute to atopic disease susceptibilities.² It is conceivable that SNPs affecting TLR functions might influence the production of T helper (Th) 1/Th2 cytokines, and thus alter the risk of infection or allergy.

TLR1 SNPs rs4833095 (N248S) and rs5743618 (I602S) had been reported to be highly associated with differential whole blood IL-6, tumor necrosis factor (TNF)- α , and interleukin (IL)-1 β productions in response to TLR2/1 ligand Pam3CSK4 stimulation.³ The hyper-responsive variant of rs5743618 SNP have been shown to confer risk to *Helicobacter pylori*-mediated gastric disease, which is of Th1 pathology.⁴ By contrast, the hyporesponsive TLR1 602S variant was reported to be associated with protection against mycobacterial diseases.⁵ For allergy, the hyper-responsive TLR1 rs5743595 SNP, together with rs4833095 was found to reduce the risk of childhood asthma in a European population.⁶

Of note, there is great ethnical difference in the prevalence of TLR1 SNPs.⁷ The rs5743618 variant allele is almost absent in East Asians,⁸ but the rs4833095 polymorphism is common in our population. Furthermore, a recent meta-analysis showed that while TLR1 1805T (rs5743595) increased susceptibility to pulmonary tuberculosis in African and American Hispanic individuals, TLR2 2258A enhanced risks of tuberculosis in Asians.⁹ The functional impact and correlation with disease susceptibility of TLR1 N248S (rs4833095) in Chinese is still unclear. Since dendritic cell is essential in determining Th1/Th2 differentiation,¹⁰ we aimed to determine if the prevalent TLR1 N248S SNP affects Th1 cytokine production in DCs and if it plays a role in allergic diseases in Taiwanese.

Methods

Participants

We recruited 93 patients who were diagnosed and treated with allergic diseases at National Taiwan University Hospital Allergy Clinic (35 asthma, 32 allergic rhinitis, 26 atopic dermatitis, mean $\pm$ standard deviation age = 10.06 $\pm$ 7.3 years, 46 male, 47 female). The patients were grouped according to their main diagnosis. Thirteen of 35 asthma patients and eight of 26 atopic dermatitis patients also had allergic rhinitis. Another two of the atopic dermatitis patients had histories of allergic rhinitis and asthma. Retrospective medical chart review was done to identify serum total immunoglobulin E (IgE) levels and specific serum IgE titer against the mite antigen *Dermatophagoides pteronyssinus* (Dp). For TLR1 rs4833095 genotyping, 76 age- and sex-matched controls without history of atopic allergy and whose serum total IgE level $\leq$ 100 kU/L were recruited.

Blood was further taken from 20 genotyped control volunteers for peripheral blood mononuclear cell (PBMC) isolation and subsequent cell stimulation assays. All participants were resident Taiwanese of Han Chinese ancestry; the aboriginal Taiwanese population was not included. The study was approved by the Institutional Ethic Committee (Institute Review Board number: 201105059RC) and informed consent was obtained from each individual (informed consent sheet was signed by parents if age was < 15 years).

Genotyping

Genomic DNA was extracted and genotyped for TLR1 rs4833095 polymorphism via PCR-melting curve analysis on a LightCycler (Roche Diagnostics, Mannheim, Germany) as previously described.¹¹ The primers and hybridization probes were purchased from TIB MOLBIOL (Berlin, Germany). Primers rs4833095 forward: TTggATgTgTCAGTCAAgACTgTAg, reverse: CTCTgAAgTCCAgCTgACC; probes sensor: TTAAGgTAAgACTTgATAACTTTg-FL, and anchor: 640-gTTTgAAgTTTCgCCAgAATACTTAgg-p.

Cell stimulation

PBMCs were isolated via Ficoll Paque gradient centrifugation. In some experiments, monocytes were further isolated via Dynabeads (Invitrogen, Carlsbad, CA, USA) and cultured for 7 days in complete RPMI medium with IL-4 20 ng/mL and GM-CSF 50 ng/mL to generate monocyte-derived dendritic cells (MoDCs). Both IL-4 and GM-CSF were added to the medium immediately after monocyte isolation. PBMCs and MoDCs were then stimulated with TLR2/1-ligand Pam3CSK4 (Invivogen, San Diego, CA, USA) at 100 ng/mL for 4 hours or 24 hours and cytokine production in the supernatants was measured by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA). For comparison, MoDCs were also stimulated with a non-TLR2/1 ligand LPS (a TLR4 ligand) at 100 ng/mL for 24 hours.

Statistical analysis

Fisher's exact test was applied to compare the percentage of rs4833095 C/C genotype between allergic patients and healthy controls. Mann-Whitney *U* test was used to compare the Pam3CSK4-stimulated cytokine amounts and serum IgE levels between the homozygous and non-homozygous SNP carriers. All statistical tests were performed on GraphPad Prism version 5., GraphPad Software, Inc., La Jolla, CA, USA.

Results

Influence of rs4833095 SNP on TLR2/1-stimulated cytokine production in PBMCs

We first evaluated TLR2/1 ligand Pam3CSK4-induced cytokine secretion in PBMCs derived from healthy individuals with different rs4833095 genotypes. The homozygous rs4833095 C allele carriers were found to have increased PBMC TNF- α responses as compared with wild type allele

carriers (median TNF- α in C/C vs. T/T+T/C = 301.8 pg/mL vs. 113 pg/mL, Mann–Whitney *U* test, $p = 0.04$, Fig. 1A). However, the Pam3CSK4-stimulated IL-10 production in PBMC was similar among TLR1 genotypes (Fig. 1B). Because the Pam3CSK4-induced IL-4 and IL-13 were undetectable in PBMC supernatant (data not shown), we analyzed baseline IL-4 level in plasma derived from various genotype carriers. No difference in plasma IL-4 level was detected (Fig. 1C).

Influence of rs4833095 SNP on TLR2/1-stimulated cytokine production in MoDCs

Second, we derived DCs from monocytes of different rs4833095 genotypes. Similarly, individuals with C/C genotype showed higher IL-12 response to Pam3CSK4 stimulation in MoDCs, as compared with wild type allele carriers (median IL-12 in C/C vs. T/T+T/C = 286.7 pg/mL vs. 51.8 pg/mL, Mann–Whitney *U* test, $p = 0.02$, Fig. 2A). The Pam3CSK4-induced IL-10 response in MoDCs of rs4833095 C/C carrier did not differ from the responses of wild type allele carriers significantly, although a mild trend of higher IL-10 production was noted ($p = 0.05$, Fig. 2B). In addition, the IL-12 and IL-10 responses to the non-TLR2/1 ligand LPS in MoDCs showed no difference between the homozygous and non-homozygous C allele carriers (Figs. 2C and 2D).

Association of rs4833095 SNP with susceptibility to allergic diseases and serum IgE levels

In order to investigate whether the enhanced TLR2/1-induced TNF- α and IL-12 responses contribute to protection of allergic diseases in our population, we further genotyped 93 patients (35 asthma, 32 allergic rhinitis, 26 atopic dermatitis) and 76 controls. However, we did not detect an association of the homozygous C allele carrier with susceptibility to allergic diseases (Table 1).

Furthermore, since IgE titers have been reported to correlate with severity of some allergic symptoms,^{12,13} we analyzed the association of TLR1 N248S with serum IgE levels. The rs4833095 C/C genotype carrier was found to

have lower total serum IgE levels as compared with T/C and T/T genotypes in the allergic patient group (median total serum IgE of C/C vs. T/T+T/C = 232 vs. 669 IU/mL, Mann–Whitney *U* test $p = 0.001$, Fig. 3A). Although the difference was not as prominent as shown in Fig. 3A, we also observed a trend in the control group (serum IgE ≤ 100 KU/l) that the rs4833095 C/C genotype carriers who showed higher TLR2/1-induced TNF- α and IL-12 cell responses had lower serum total IgE level as compared with T/T+T/C genotypes (data not shown).

As for the titer of Dp-specific IgE in the allergic patient group, the rs4833094 C/C genotype only showed a trend of lower specific IgE titer as compared with T/T+T/C group [$p = 0.08$, calculated by Fisher's exact test comparing the C/C genotype percentage between patients whose Dp-specific IgE titer was < Class 3 and those having Dp-specific IgE titer $\geq$ Class 3, 5/9 (55.5%) vs. 4/15 (21.1%), Fig. 3B].

Discussion

Consistent with a previous report,³ we showed that the TLR1 N248S homozygous variant is functional in our population, which could confer stronger Th1 inflammatory response. It has been reported in a European asthmatic population that another TLR1 SNP, rs5743595, could not only augment Th1 cytokine expression, but also reduce Pam3CSK4-mediated IL-4 secretion.⁶ However, we could not detect a significant amount of Pam3CSK4-induced IL-4 and IL-13 in PBMCs of healthy controls. It is possible that Pam3CSK4-triggered PBMC Th2 cytokine responses might require *in vivo* presensitization. Although the MoDCs of homozygous rs4833095 variant had a trend of higher IL-10 secretion after TLR2/1 stimulation, the direct influence of TLR1 N248S on Th2 cytokine production still needs further investigations.

It has been proposed that, in early life, encountering certain antigens with pathogens capable of activating TLR will polarize the immune response toward Th1 and thus suppressing Th2 responses.¹⁴ Since atopic allergy is a Th2-predominant disease, the enhanced Th1 response to

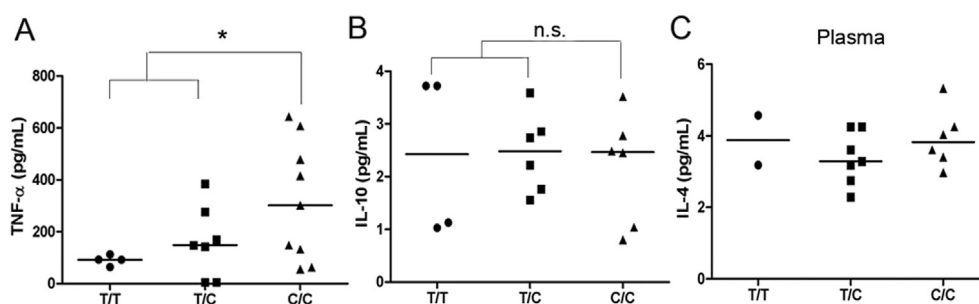


Figure 1. Influence of toll-like receptor (TLR)1 rs4833095 C/C homozygosity on peripheral blood mononuclear cell (PBMC) cytokine production in control individuals. (A) Tumor necrosis factor- α production in PBMCs after 4 hours of 100 ng Pam3CSK4 (Pam3) stimulation. T/T, $n = 4$; T/C, $n = 7$; and C/C, $n = 9$. Tumor necrosis factor- α levels detected in medium only had been subtracted. (B) Interleukin (IL)-10 production in PBMCs after 24 hours of 100 ng Pam3CSK4 stimulation. T/T, $n = 4$; T/C, $n = 6$; and C/C, $n = 6$. Baseline IL-10 level had been subtracted. (C) Baseline plasma IL-4 level in various rs4833095 genotypes. Lines represent medians. T/T, $n = 2$; T/C, $n = 7$; and C/C, $n = 6$. * $p < 0.05$ by Mann–Whitney *U* test comparing cytokine levels of T/T+T/C with C/C genotype. n.s. = not statistically significant by Mann–Whitney *U* test. T/T = the T/T genotype for rs4833095 (TLR1 N248S); T/C = the T/C genotype for rs4833095; C/C = the C/C genotype for rs4833095.

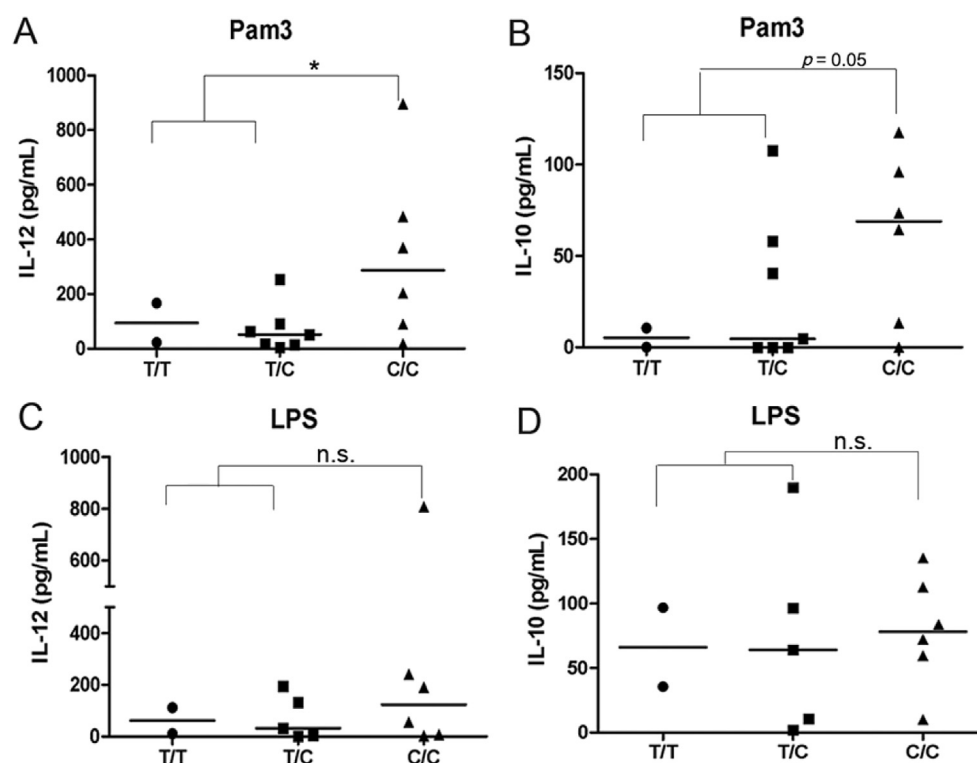


Figure 2. Influence of toll-like receptor (TLR)1 rs4833095 C/C homozygosity on monocyte-derived dendritic cell (MoDC) cytokine production in controls. Pam3CSK4 (100 ng) induced (A) Interleukin (IL)-12 and (B) IL-10 production in MoDCs of different genotypes. T/T, $n = 2$; T/C, $n = 7$; and C/C, $n = 6$. Lipopolysaccharide (100 ng) induced (C) IL-12 and (D) IL-10 production in MoDCs of different genotypes. MoDCs were stimulated for 24 hours. Cytokine levels detected in medium only were subtracted. Lines represent medians. T/T, $n = 2$; T/C, $n = 5$; C/C, $n = 6$. * $p < 0.05$ by Mann-Whitney U test comparing cytokine levels of T/T+T/C with C/C genotype. T/T = the T/T genotype for rs4833095 (TLR1 N248S); T/C = the T/C genotype for rs4833095; C/C = the C/C genotype for rs4833095.

Table 1 Association of TLR1N248S with susceptibility to allergic diseases^a

rs4833095	<i>n</i>	Genotype (%)			Variant allele C (%)	Odds ratio (95%CI)	<i>p</i>
		T/T	T/C	C/C			
Asthma	35	6 (17)	16 (46)	13 (37)	42 (60)		0.35
Allergic rhinitis	32	3 (9)	17 (53)	12 (38)	41 (64)		0.31
Atopic dermatitis	26	5 (19)	13 (50)	8 (31)	29 (56)		0.57
Total patients	93	14 (15)	46 (50)	33 (35)	112 (60)	1.19 (0.62–2.27)	0.29
Controls	76	22 (29)	30 (39)	24 (32)	78 (51)		

^a Genotype % is calculated by dividing the number of patients with T/T, T/C, or C/C genotype by total patient number of each group. Frequency of TLR1 rs4833095 C allele (variant C allele) in every allergic disease is calculated by dividing the number of C alleles with total C allele + T allele number (2N) in each group. The p value is calculated by Fisher's exact test comparing percentages of C/C between allergic patients and controls.

microbes might protect from allergy development.^{6,15} However, we could not detect an association of the homozygous rs4833095 C variant, which confers a stronger Th1 response, with protection from allergic diseases. It is likely that other factors during Th2 cell differentiation contribute to the pathogenesis of allergy. Consistent with this, a recent genome-wide association meta-analysis of self-reported allergy identified susceptibility loci including not only 4p14 near the TLR1/TLR6/TLR10 region, but also regions involving T helper cell differentiation and HLA types.¹⁶

Our results could be limited by the relatively small sample size and the heterogeneity of our patients. Different allergic diseases might be caused by different triggers and maintenance factors in the environment, thus modulating the effect of a single TLR1 SNP. For example, the impact of TLR1 SNP might be evident under the interaction of other environmental factors, as reported in several asthma susceptibility genes.^{17,18} Moreover, the effect of TLR1 N248S variant could be confounded by gene–gene interactions. It is known that another functional TLR1 noncoding SNP, rs5743595, is also common in our population. Studies need to

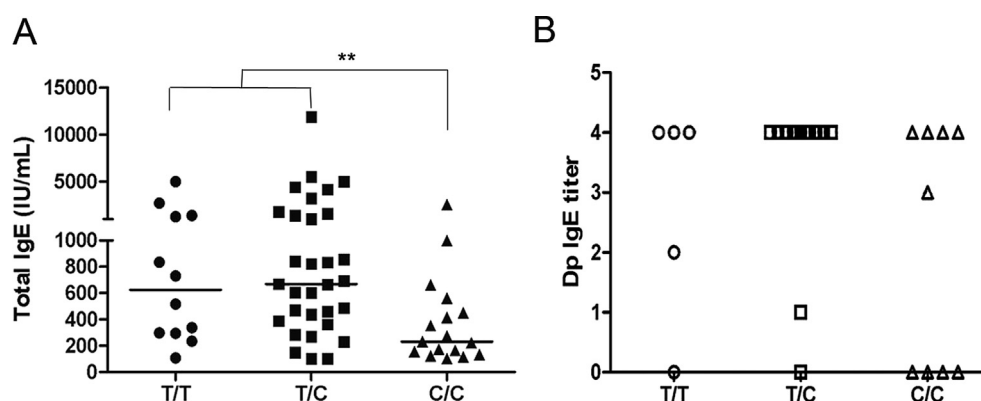


Figure 3. Influence of toll-like receptor (TLR)1 rs4833095 C/C homozygosity on serum immunoglobulin E (IgE) levels in allergic patients. (A) Total serum IgE levels in allergic patients with different genotypes. T/T, $n = 12$; T/C, $n = 31$; and C/C $n = 17$, lines represent medians. $**p < 0.01$ by Mann–Whitney U test comparing IgE levels of T/T+T/C with C/C genotype. (B) Specific serum IgE titer against the mite antigen *Dermatophagoides pteronyssinus* (Dp; measured by multiple allergosorbent chemiluminescent assay Class 0–4) in allergic patients of various rs4833095 genotypes. T/T, $n = 5$; T/C, $n = 14$; and C/C $n = 9$. T/T = the T/T genotype for rs4833095 (TLR1 N248S); T/C = the T/C genotype for rs4833095; C/C = the C/C genotype for rs4833095.

be done to elucidate if the combined presence of rs4833095 and rs5743595 variants exerts stronger influence on allergy development in Taiwanese.

Although we did not detect an association of rs4833095 TLR1 C/C genotype with susceptibility to atopic diseases, it was correlated with lower IgE levels in sera of allergic patients. Serum levels of IgE have been reported to correlate with the severity of allergic diseases and helminth infections.^{19,20} TLR1 N248S heterozygosity had been shown to associate with placental malaria infection in an African population.²¹ Although polymorphisms on molecules of TLR pathways have been reported to alter serum IgE levels,^{22,23} our study is the first to demonstrate the association of TLR1 rs4833095 C/C genotype with lower total serum IgE levels. Investigations on detecting TLR2/1-induced Th2 cytokine secretion in allergic patients of different genotypes, and analyzing the association of allergic disease severity with TLR1 SNP variants in a larger cohort might provide further roles of TLR1 rs4833095 on allergy pathogenesis in our population.

In conclusion, our study shows the functional impact of TLR1 N248S on Th1 cytokine production and serum IgE levels. Our results could be also applied to research on the susceptibility to Th1-predominant diseases, such as *Mycobacterium tuberculosis*, an endemic disease in Taiwan.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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