

ORIGINAL ARTICLE

Allelic variants of CD40 and CD40L genes interact to promote antibiotic-induced cutaneous allergic reactions

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Summary

Background The danger hypothesis provides a new perspective of the mechanisms underlying drug allergy. In this study, we evaluated associations between variations in the genes involved in danger signal pathways and antibiotic-induced cutaneous allergic reactions (AICARs).

Methods Two hundred cases with urticaria, angio-oedema, maculopapular rash, and erythema multiforme caused by antibiotics were extracted from the database of the Adverse Drug Reaction Research Group in Korea. All cases were confirmed by an allergy specialist.

Causative antibiotics included penicillin, cephalosporin, quinolone, and others (approximately 40 different types). Ten single nucleotide polymorphisms (SNPs) in seven genes (–318C > T, +49A > G, and +6230G > A in *CTLA4*, IVS+17T > C in *CD28*, –3479T > G and I170V in *CD86*, –1C > T in *CD40*, –3458A > G in *CD40LG*, –308G > A in *TNF*, and –31T > C in *IL1B*) were scored for cases and for healthy subjects without a history of AICARs. **Results** Our analysis failed to reveal differences in the distribution of the 10 SNPs between cases and controls. However, we could find a gene–gene interaction between –1C > T in *CD40* and –3458A > G in *CD40L* using multifactor dimensionality reduction analysis. Subjects with minor alleles of both SNPs showed a significant risk for developing AICARs [$P = 0.017$, odds ratio (OR) (95% confidence interval) = 2.93 (1.20–7.97)].

Conclusion Our findings suggest that a genetic interaction between *CD40* and *CD40L* favours the development of AICARs.

Keywords antibiotics, CD40, CD40 ligand, drug hypersensitivity

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Introduction

The danger hypothesis proposed by Matzinger [1] essentially focuses on the primary driving forces to protect against danger that will result in full-blown immune responses. This hypothesis was proposed to explain the events responsible for various immunopathologic reactions, and currently, even drug-induced allergic reactions are being reevaluated in the context of this new perspective [2–4]. There are many examples in which specific situations that can mediate a danger signal are associated with an increase in the occurrence of drug-induced allergic reactions. It is well known that when ampicillin is given to patients with mononucleosis, there is a marked increase in the risk of an allergic reaction [5]. In addition,

HIV-positive patients have a much greater risk of developing an allergic reaction to sulphonamides [6], and furthermore, open-heart surgery, which should clearly produce some type of danger signal, appears to increase the risk of procainamide-induced agranulocytosis [7].

The nature of such danger signals has not been fully defined, although it has been postulated that endogenous materials and signals released by necrotic or apoptotic cells could act in this manner [3, 8–11], and in line with this theory, injured and dead cells have been found to up-regulate co-stimulatory factors such as B7 and CD40 on antigen-presenting cells and eventually to activate T cells [4]. Cytokines like TNF- α and IL-1 β are also thought to be important danger signals [9].

Infectious conditions are invariably associated with tissue damage, and thus are sources of danger signals. In the present study, we assumed that the genetic susceptibility also contributes as danger signals, and may trigger an unwanted allergic reaction to innocent antibiotics used to treat infectious conditions. To explore this possibility, we performed a genetic association study in patients with delayed cutaneous allergic reactions attributed to immunological disorders associated with antibiotics administration [12].

Methods

Study subjects and phenotype definitions

Case subjects were extracted from the database of the Adverse Drug Reaction Research Group in Korea, members of which are drawn from five large hospitals (Seoul National University Hospital, Ajou University Hospital, Dankook University Hospital, Seoul National University Bundang Hospital, and Eulji University Hospital). All cases were identified by allergy specialists at the participating hospitals by history taking, reviewing medical records, and in some cases, by performing specific challenge tests with suspected antibiotics. Documented antibiotic-induced cutaneous allergic reactions (AICARs) were urticaria, angio-oedema, acute generalized exanthematous pustulosis, maculopapular rash, and erythema multiforme. Control subjects were recruited from among the healthy controls that we enrolled in our previous study [13]. No controls had a history of an allergic reaction to any antibiotic, as determined by an interview with an allergy specialist and a supporting questionnaire (a. *Have you been told by a doctor that you are allergic to antibiotics?* b. *Have you ever been prescribed antibiotics? And if so, did you experience any adverse reaction to the antibiotics prescribed?*). All subjects enrolled in this study provided written informed consent, and the study protocol was approved by the institutional review board at each of the above-mentioned hospitals.

Selection of genes and single nucleotide polymorphisms

Based on the previous reviews [1–4], seven candidate genes coding danger signals and co-stimulatory molecules were selected: *CTLA4* (cytotoxic T lymphocyte-associated protein 4), *CD28* (CD28 molecule), *CD86* (CD86 molecule), *CD40* (CD40 molecule), *CD40LG* (CD40 ligand), *TNF α* , and *IL1B*. Ten single nucleotide polymorphisms (SNPs) on those genes (Table 1) were then scored with respect to the previously reported association with various immunologic diseases and their functional relevance (except for IVS+17T>C on *CD28*; see supplementary Table S1). Scoring was performed by the high-throughput single base-pair extension method (SNP-ITTM

Table 1. Genes and SNPs evaluated in this study

Gene	SNP	rs number	Minor allele frequency*
<i>CTLA4</i> [†]	–318C>T	rs5742909	12.9%
	+49A>G	rs231775	31.8%
	+6230G>A	rs3087243	17.8%
<i>CD28</i>	IVS3+17T>C	rs3116496	7.5%
<i>CD86</i>	–3479T>G	rs2715267	24.6%
	I179V	rs2681417	0.3%
<i>CD40</i>	–1C>T	rs1883832	30.8%
<i>CD40LG</i>	–3458A>G	rs3092952	6.0%
<i>TNF</i>	–308G>A	rs1800629	8.5%
<i>IL1B</i>	–31T>C	rs1143627	47.4%

*In the control group.

[†]Cytotoxic T lymphocyte-associated protein 4.

assay) using an SNPstream25K system, which was customized to automatically genotype DNA samples in 384-well plates and provide a colorimetric readout (Orchid Biosciences, Princeton, NJ, USA).

Statistical analysis

Association analysis was performed for each SNP using a minor allele-dominant model, i.e. AA vs. AB+BB, where A is the major allele and B the minor allele, which assesses the elevated risk of disease development in individuals homozygous and heterozygous for the risk allele. *P*-values and odds ratios (ORs) were determined by logistic regression analysis controlled for age and sex. Compliance with the Hardy–Weinberg equilibrium was determined using 2×2 tests. To reveal the gene–gene interaction, multifactor dimensionality reduction (MDR) analysis was used in this study (version 0.6.2; Computational Genetics Laboratory, Dartmouth Medical School, Hanover, NH; <http://www.epistasis.org>) as described previously [14]. All statistical analyses were performed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA), and *P*-values of <0.05 were regarded as significant.

Results

A total of 200 subjects with AICARs were enrolled in the present study. The case subjects' characteristics and the names of causative antibiotics are summarized in Table 2. One hundred and eighty-five subjects were enrolled as controls (mean age±SD, 47.8±12.2) and 54.9% were male. All 10 SNPs examined in the present study were in Hardy–Weinberg equilibrium and no significant association was found between any individual SNP and the development of AICARs (see supplementary Table S2). Table 3 summarizes, for each factor evaluated, the average cross-validation (CV) consistency, average prediction error, and the *P*-value obtained by the MDR analysis. One two-factor model (*CD40*–1C>T and *CD40LG*–3458A>G) had a

Table 2. Characteristics of patients with antibiotic-induced cutaneous allergic reactions (*N* = 200)

Characteristics	Number (%)
Age, mean ($\pm$ SD)	37.2 ($\pm$ 24.2)
Male	97 (48.5%)
Causative drugs	
Penicillin*	36 (18.0%)
Cephalosporin [†]	102 (51.0%)
Quinolone [‡]	29 (14.5%)
Others [§]	33 (16.5%)
Cutaneous allergic reactions	
Urticaria	57 (28.5%)
Angio-oedema	12 (6.0%)
Urticaria with angio-oedema	25 (12.5%)
Acute generalized exanthematous pustulosis	18 (9.0%)
Maculopapular rash	73 (36.5%)
Erythema multiforme	15 (7.5%)

*Including amoxicillin (20 cases) and ampicillin (4 cases).

[†]Including cefaclor (18 cases), ceftriaxone (14 cases), cefotaxime (13 cases), cefuroxime (10 cases), and cefazolin (9 cases).

[‡]Including ciprofloxacin (11 cases) and levofloxacin (9 cases).

[§]Including macrolide (5 cases) and vancomycin (5 cases).

Table 3. A summary of MDR

No. of factors	Combination	CV consistency	Prediction error (%)	<i>P</i> -value
2	–1C>T (<i>CD40</i>), –3458A>G (<i>CD40LG</i>)	10/10	27.2	0.001
3	–1C>T (<i>CD40</i>), –3458A>G (<i>CD40LG</i>), –318C>T (<i>CTLA4</i>)	9/10	36.5	0.055
4	–1C>T (<i>CD40</i>), –3458A>G (<i>CD40LG</i>), –318C>T (<i>CTLA4</i>), –31T>C (<i>IL1B</i>)	8/10	41.7	0.623
5	–1C>T (<i>CD40</i>), –3458A>G (<i>CD40LG</i>), –318C>T (<i>CTLA4</i>), –31T>C (<i>IL1B</i>), +49A>G (<i>CTLA4</i>)	6/10	43.7	0.828

CV, cross-validation.

maximum CV consistency (10 out of 10) and a minimum prediction error (27.2%) ($P = 0.001$). Moreover, these could be reproduced by logistic regression analysis. As shown in Table 4, the combined effect of *CD40*–1C>T and *CD40LG*–3458A>G was greater than their individual effects on the development of AICARs. Subjects with the variant alleles of both genes (CT or TT genotype of *CD40*–1C>T and AG or GG genotype of *CD40LG*–3458A>G) were significantly more likely to develop AICARs than those with the wild alleles of both genes (CC genotype of *CD40*–1C>T and CC genotype of *CD40LG*–3458A>G) [$P = 0.017$, OR (95% confidence interval) = 2.93 (1.20–7.97)].

Discussion

This study demonstrates that a genetic interaction between *CD40* and *CD40LG* confers susceptibility to the development of AICARs. This is the first report to show that genetic components influence drug allergy in the context of the danger hypothesis. So far, the hapten hypothesis and the pharmacological interaction hypotheses have prevailed in terms of conceptualizing drug allergy. We are not of the opinion that the danger hypothesis entirely explains the mechanisms underlying drug allergy, but rather prefer to view it as a complementary concept. Haptens can bind covalently to cellular proteins or toll-like receptors [15] and stimulate cells of the innate immune system; for example, they up-regulate CD40 on dendritic cells [15, 16]. Alternatively, the uptake and intracellular processing of haptens may activate a cell and, if it is an antigen-presenting cell, it might up-regulate costimulatory molecules like CD40 [16]. Pharmacologic interactions explain well the drug adverse reactions induced by chemically inert drugs that are incapable of conjugating with carrier proteins and thus cannot be antigens [17]. However, this does not address the issue of what controls the level of signal 2 [18]. It has been well established that Signal 2 must be provided to T cells to enable their activation, and thus is an important component of self/non-self differentiation [19]. Collectively, the danger hypothesis is an additive but essential concept in understanding the mechanism of drug allergy.

It was reported that the CD40/CD40 ligand is highly expressed in the skin during Stevens–Johnson syndrome or during toxic epidermal necrolysis caused by drug exposure [20]. However, the role of CD40 signalling in AICARs has not been investigated. The release of cell contents from necrotic cells, which is a ubiquitous feature of infectious conditions, up-regulates CD40 expression [21, 22], and in addition, it is evident that some reactive metabolites of antibiotics can also cause cell damage or stress [18, 23]. Furthermore, a certain drug, itself, can induce increased expression of CD40 on dendritic cells [24]. All these findings suggest that the CD40/CD40L system participates in the development of AICARs.

One of the interesting findings of the present study is that the individual SNP on *CD40* and *CD40LG* showed no significant association but their combined effect may be relevant. Drug allergy is a complex trait and thus the evaluation of genetic influences on drug allergy should be conducted in the context of gene–gene interactions. For this reason, in the present study, we evaluated interactions between individual SNPs using MDR analysis. This is the first report to show evidence for a synergistic interaction between *CD40*–1C>T and *CD40LG*–3458A>G. –1C>T on the 5'UTR region of *CD40*, and its genotype affects the efficiency of CD40 translation, leading to differential expression of CD40 protein [25]. –3458A>G on *CD40LG*

Table 4. Combined genetic effects on antibiotic-induced cutaneous allergic reactions

Genotype		Frequency		P-value	aOR (95% CI)*
Gene 1 (SNP)	Gene 2 (SNP)	Case, n (%)	Control, n (%)		
<i>CD40</i> (–1C>T)	<i>CD40LG</i> (–3458A>G)				
CC	AA	73 (52.9%)	65 (42.7%)	1.00	
CC	AG+GG	14 (53.8%)	12 (46.2%)	0.929	1.04 (0.45–2.37)
CT+TT	AA	85 (52.5%)	77 (47.5%)	0.947	0.98 (0.62–1.55)
CT+TT	AG+GG	23 (76.7%)	7 (23.3%)	0.017	2.93 (1.20–7.97)

*Logistic regression analysis adjusted for age and gender.

Bold values represent the significant results.

SNP, single nucleotide polymorphism; CI, confidence interval; aOR, adjusted odds ratio.

was proven to be a regulator of serum CD40L levels [26]. The functional relevance of both SNPs strongly supports our results.

Surely, the case subjects enrolled in the present study had co-morbid diseases beyond allergy to antibiotics, which could be a relevant confounding factor. However, various co-morbid diseases and comparison with apparently healthy controls might dilute the genetic effect originating from co-morbid diseases. In addition, to generalize our results, three points should be considered. Our definition of control subjects totally depended on self-reported questionnaires, the comparability of the case and control subjects was not fully demonstrated, and we could not completely remove the risk that spurious interactions might be identified, although our results were confirmed by MDR analysis based on permutation. We sincerely hope that our results are confirmed in a successive and large-scale study.

In summary, the present study shows that allelic variants of *CD40* and *CD40LG* genes interact to increase the likelihood of AICARs significantly. Despite the recent enthusiasm, we believe that the danger hypothesis does not entirely explain drug allergy. Nevertheless, our results encourage us to re-explore the danger hypothesis in the context of drug allergy.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Functional and clinical relevances of the SNPs examined in the present study and appropriate references.

Table S2. Genetic effects of individual SNPs on antibiotic-induced cutaneous allergic reactions.

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SUPPLEMENTARY MATERIAL

Table S1. Functional and clinical relevances of the SNPs examined in the present study and appropriate references

Gene	SNP	Clinical relevancy	Functional relevancy
<i>CTLA4</i> [†]	-318C>T	Yes ^{1,2}	Yes ³
	+49A>G	Yes ^{2,4}	Yes ⁵
	6230G>A	Yes ⁶	Yes ⁷
<i>CD28</i>	IVS3+17T>C	Yes ⁸	No
<i>CD86</i>	-3479T>G	Yes ⁹	Yes ⁹
	I179V	Yes ¹⁰	Yes ¹⁰
<i>CD40</i>	-1C>T	Yes ^{11,12}	Yes ¹¹
<i>CD40L</i>	-3458A>G	Yes ¹³	Yes ¹³
<i>TNF</i>	-308G>A	Yes ¹⁴	Yes ¹⁵
<i>IL1B</i>	-31T>C	Yes ¹⁶	Yes ¹⁷

[†] *CTLA4*: gene coding cytotoxic T-lymphocyte-antigen 4

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Table S2. Genetic effects of individual SNPs on antibiotic-induced cutaneous allergic reactions

Gene (SNPs)	Genotype frequency				P value*	aOR (95% C.I.) [†]
	Case, n (%)		Control, n (%)			
<i>CTLA4</i>						
-318C>T	CC	CT + TT	CC	CT + TT	0.394	1.24 (0.75-2.03)
	145 (74.4%)	50 (24.6%)	128 (75.3%)	42 (24.7%)		
+49A>G	AA	AG + GG	AA	AG + GG	0.594	1.12 (0.73-1.74)
	84 (42.4%)	114 (57.6%)	74 (44.8%)	91 (55.2%)		
+6230G>A	GG	GA + AA	GG	GA + AA	0.455	1.18 (0.76-1.85)
	121 (61.4%)	76 (38.6%)	117 (67.2%)	57 (32.8%)		
<i>CD28</i>						
IVS3+17T>C	TT	TC + CC	TT	TC + CC	0.667	1.13 (0.63-2.05)
	168 (85.3%)	29 (14.7%)	154 (85.1%)	27 (14.9%)		
<i>CD86</i>						
-3479T>C	TT	TC + CC	TT	TC + CC	0.617	1.11 (0.733-1.70)
	102 (52.3%)	93 (47.7%)	95 (54.3%)	80 (45.7%)		
I179V	II	IV + VV	II	IV + VV	0.980	-
	198 (100.0%)	0 (0%)	181 (99.5%)	1 (0.5%)		
<i>CD40</i>						
-1C>T	CC	CT + TT	CC	CT + TT	0.835	1.05 (0.68-1.62)
	87 (44.6%)	108 (55.4%)	79 (47.3%)	88 (52.7%)		
-580G>A	GG	GA + AA	GG	GA + AA	0.060	0.66 (0.43-1.02)
	105 (53.0%)	93 (47.0%)	77 (44.3%)	97 (55.7%)		

CD40L

-3458A>G	AA	AG + GG	AA	AG + GG		
	162 (81.4%)	37 (18.6%)	154 (88.5%)	20 (11.5%)	0.255	1.43 (0.77-2.63)

TNF

-308G>A	GG	GA + AA	GG	GA + AA		
	163 (82.7%)	34 (17.3%)	148 (83.6%)	29 (16.4%)	0.910	1.03 (0.59-1.81)

IL1B

-31T>C	TT	TC + CC	TT	TC + CC		
	49 (25.0%)	147 (75.0%)	35 (20.1%)	139 (79.9%)	0.212	0.72 (0.43-1.20)

* Minor allele dominant model (AA vs. AB+BB, where A is the major frequency allele and B is the minor frequency allele)

† Logistic regression analysis adjusted for age and gender