

Association of *FANCC* polymorphisms with FEV1 decline in aspirin exacerbated respiratory disease

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Abstract Aspirin exacerbated respiratory disease (AERD) is a clinical condition characterized by severe decline in forced expiratory volume in one second (FEV1) following the ingestion of non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin. The exacerbated inflammatory response in *Fancc*-deficient mice has been reported to be associated with hemopoietic responses that are also related to AERD pathogenesis. To investigate associations of *FANCC* polymorphisms with AERD and related phenotypes, this study genotyped 25 common single nucleotide polymorphisms (SNPs) in a total of 592 Korean asthmatics including 163 AERD and 429 aspirin-tolerant asthma (ATA) subjects. Logistic analysis revealed that genetic polymorphisms of the *FANCC* gene might not be directly related to AERD development and nasal polyposis ($P > 0.05$).

However, the FEV1 decline by aspirin provocation showed significant associations with *FANCC* polymorphisms ($P = 0.006$ – 0.04) and a haplotype (unique to *rs4647416G > A*, $P = 0.01$ under co-dominant, $P = 0.006$ under recessive model). In silico analysis showed that the “A” allele of *rs4647376C > A*, which was more prevalent in AERD than in ATA, could act as a potential branch point (BP) site for alternative splicing (BP score = 4.16). Although replications in independent cohorts and further functional evaluations are still needed, our preliminary findings suggest that *FANCC* polymorphisms might be associated with the obstructive symptoms in allergic diseases.

Keywords Aspirin exacerbated respiratory disease (AERD) · *FANCC* · Single nucleotide polymorphism · Haplotype

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Introduction

Aspirin as a non-steroidal anti-inflammatory drug (NSAID) is one of the most frequently used analgesics, and is used as a medication to treat inflammation. The prevalence estimate of aspirin hypersensitivity is about 0.6–2.5% in the general population and increases up to 5–10% in asthmatic patients [1, 2]. Aspirin exacerbated respiratory disease (AERD) is characterized by a severe drop in forced expiratory volume in one second (FEV1), a standard test used to measure airflow in the airways, following the ingestion of NSAIDs including aspirin [3, 4]. The clinical symptom of AERD is the so-called aspirin triad consisting of aspirin hypersensitivity, bronchial asthma and chronic rhinosinusitis with nasal polyposis [2, 4]. Although pathogenesis of AERD is not yet fully understood, over expressions of pro-inflammatory cysteinyl leukotrienes (CysLTs) and CysLT receptors have been proposed as a major factor in AERD development [5].

Associations of genetic polymorphisms on multiple leukotriene pathways and CysLT receptor genes, such as *5-lipoxygenase* (*ALOX5*) [6], *leukotriene C4 synthase* (*LTC4S*) [7], and cysteinyl leukotriene receptors (*CYSLTR1* and *CYSLTR2*) [8, 9], with AERD have been widely studied. However, genes on other inflammatory and immune pathways have also provided new insights on AERD [10, 11]. Candidate gene approaches that study other potential disease mechanisms may thus offer a more detailed explanation of aspirin-exacerbated respiratory pathogenesis.

The human *Fanconi anemia, complementation group C* (*FANCC*) gene on chromosome 9q22.3 has a 219 kb length composed of 15 exons. Mutations in the *FANCC* gene underlie Fanconi anemia [12], a genetic disease with complex symptoms that include acute myelogenous leukemia, as well as asthma and allergies as previously

observed in one patient [13]. Recently, *Fancc*-deficient mice have been shown to experience an exacerbated inflammatory response through hemopoietic suppression [14]. Furthermore, the significant increase in hemopoietic progenitors in aspirin-sensitive asthmatics suggests that bronchial obstruction due to aspirin hypersensitivity might involve systemic responses and could be associated with mobilization of hemopoietic progenitors [15]. Based on these previous reports, we hypothesized that polymorphisms of *FANCC* might be associated with aspirin-exacerbated complications of asthma.

Materials and methods

Study subjects

Study subjects were recruited from the following Korean hospitals: Soonchunhyang, Chungbuk National, Chonnam National, Seoul National, and Chung-Ang Universities. Study protocols were approved by the Institutional Review Board of each hospital. All subjects were Korean, and provided written informed consent. All patients showed clinical symptoms in accordance with the definition of asthma set forth in the Global Initiative for Asthma (GINA) guidelines. Evaluation of asthma included histories of dyspnea and wheezing during the previous 12 months, together with one of the following: (1) a >15% increase in FEV1 or a >12% increase in FEV1 plus 200 ml follow-up inhalation of a short-acting bronchodilator, (2) <10 mg/ml PC20 methacholine, or (3) >20% increase in FEV1 following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators. Total IgE was assessed using the CAP system (Pharmacia Diagnostics, Sweden). The oral aspirin provocation test was performed with slight modifications in increasing doses [16], following the

Table 1 Clinical profiles of study subjects

Clinical profile	All asthmatics (<i>n</i> = 592)	AERD (<i>n</i> = 163)	ATA (<i>n</i> = 429)
Age of study subjects [year, mean (range)]	46.15 (15.40–77.88)	43.13 (17.22–72.73)*	47.30 (15.40–77.88)
Sex (<i>n</i> , male/female)	206/386	59/104	147/282
Total smoker (current smoker; ex-smoker) (%)	27.70 (12.50; 15.20)	21.47 (12.88; 8.59)*	30.07 (12.35; 17.72)
Body mass index (kg/m ²)	24.24 ± 3.39	23.39 ± 3.25*	24.58 ± 3.39
Decline of FEV1 by aspirin provocation (%)	9.27 ± 13.24	24.63 ± 16.11**	3.54 ± 4.85
Blood eosinophil (%)	6.01 ± 5.73	5.96 ± 5.21	6.03 ± 5.92
PC20 methacholine (mg/ml)	6.43 ± 8.67	5.02 ± 7.83*	6.91 ± 8.90
Positive rate of skin test (%)	56.42	52.76	57.81
Total IgE (IU/ml)	357.65 ± 604.09	348.60 ± 596.44	361.00 ± 607.56

Each clinical profile of AERD is compared to the respective ATA controls. Age indicates first medical examination

AERD aspirin exacerbated respiratory disease, ATA aspirin-tolerant asthma

* *P* < 0.05, ** *P* < 0.0001

guidelines from EAACI/GA2LEN [17]. Based on the oral aspirin provocation test, subjects were stratified into two groups as follows: patients who showed 20% or greater decrease in FEV1 or 15–19% decrease in FEV1 with naso-ocular or cutaneous reactions were classified as AERD cases, whereas asthmatics who showed less than 15% decreases in FEV1 without naso-ocular or cutaneous reactions were grouped as ATA controls.

SNP selection and genotyping

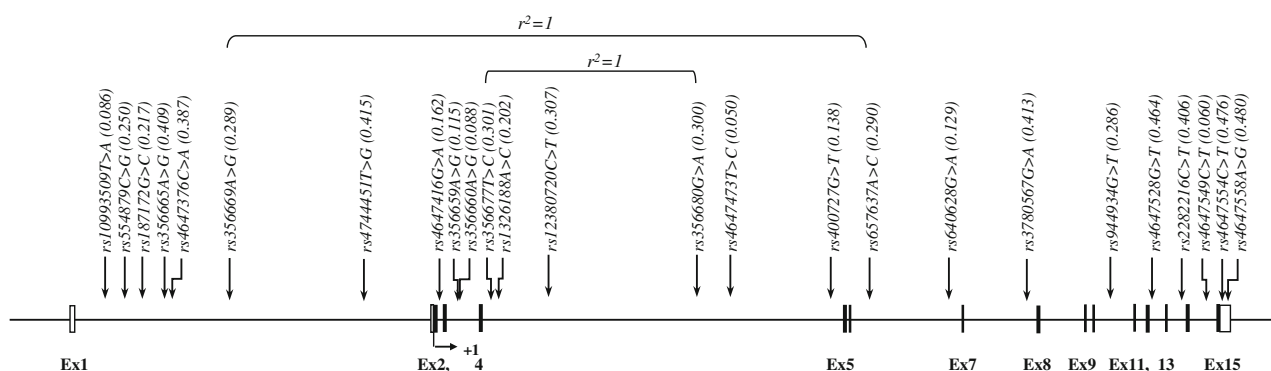
A total of 25 common SNPs with a minor allele frequency (MAF) >0.05 were selected for genotyping based on the Asian population in the released version #21 from the International HapMap Project database (<http://hapmap.ncbi.nlm.nih.gov/index.html.en>). Genotyping was performed for a total of 592 asthmatics, including 163 AERD patients and 429 ATA controls, using the TaqMan assay and the ABI prism 7900HT sequence detection system

(Applied Biosystems, USA), with assessment of data quality by duplicate DNA ($n = 10$ per reaction, 2.7% of total samples). SNPs that did not satisfy the following criteria were excluded from the study: (1) a minimum call rate of 95%, (2) no duplication error, (3) Hardy–Weinberg equilibrium greater than $P > 0.05$.

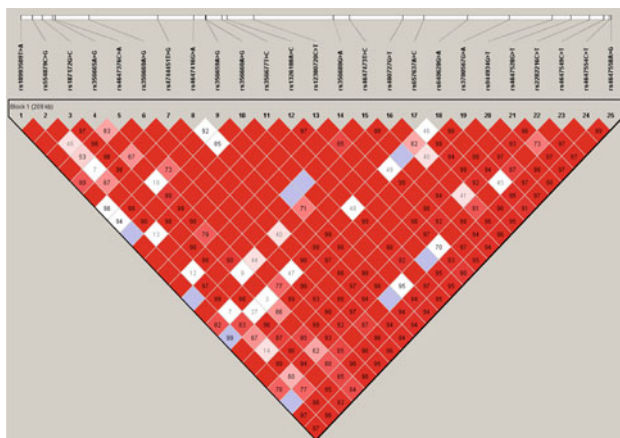
Statistics

The Haploview v4.1 software downloaded from the Broad Institute (<http://www.broadinstitute.org/mpg/haploview>) was used to analyze linkage disequilibrium (LD) of *FANCC* polymorphisms [18]. LD coefficients ($|D'|$ and r^2) between all pairs of biallelic loci were used to determine LD among the SNPs. Haplotypes were estimated using the PHASE algorithm (ver. 2.0) [19]. Logistic analysis was used to assess the association of each genotype and haplotype on the *FANCC* gene with AERD and FEV1 decline by aspirin provocation, with adjustments for age, gender,

A Map of *FANCC* (Fanconi anemia, complementation group C) on chromosome 9q22.3 (219Kb)



B LDs of *FANCC* polymorphisms



C Haplotypes of *FANCC* polymorphisms

Hap.	rs109935097>A	rs554879C>G	rs1871272G>C	rs356665A>G	rs4647376C>A	rs356669A>G	rs474451T>G	rs4647416G>A	rs356659A>G	rs356660A>G	rs356677T>C	rs1326188A>C	rs12380720C>T	rs356680G>A	rs464743T>C	rs400727G>T	rs657637A>C	rs640628G>A	rs3780567G>A	rs944934G>T	rs4647528G>T	rs228216C>T	rs4647549C>T	rs4647554C>T	rs4647558A>G	Freq.
ht1	T	C	G	A	C	A	T	G	A	A	T	A	T	G	T	G	A	G	G	G	C	C	C	A	0.253	
ht2	T	C	G	A	C	A	T	G	A	A	T	C	C	G	T	G	A	G	G	G	C	C	C	A	0.193	
ht3	T	C	C	G	A	G	G	A	A	C	A	C	A	T	G	C	A	A	T	T	T	C	T	G	0.157	
ht4	T	G	G	A	A	G	G	G	T	A	C	G	T	T	A	A	A	G	T	T	C	T	G	0.079		
ht5	A	G	G	A	G	G	G	A	A	C	A	C	A	T	G	C	G	A	T	T	T	C	T	G	0.063	
ht6	T	G	G	C	A	T	G	A	A	T	A	C	G	C	T	A	G	G	G	T	C	T	T	G	0.048	
ht7	T	C	C	G	A	G	G	A	A	C	A	C	A	T	G	C	G	A	T	T	T	C	T	G	0.040	
ht8	T	C	G	G	C	A	T	G	A	A	T	A	T	G	T	G	A	G	G	G	C	C	A	0.027		
ht9	T	G	G	C	A	G	G	A	T	A	C	G	T	G	A	A	A	G	T	T	C	T	G	0.026		
ht10	T	C	G	A	C	A	T	G	A	A	T	A	C	G	T	G	A	G	G	G	C	C	A	0.025		
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.090	

Fig. 1 Physical map, LDs, and haplotypes of the *FANCC* gene. **a** Physical map of *FANCC* and its SNPs genotyped in this study. Complete LD is denoted as $r^2 = 1$. The coding exons are represented by black blocks, and 5'-untranslated region (UTR) and 3'UTR by

white blocks. **b** LDs plot of *FANCC* polymorphisms. **c** Haplotypes of the *FANCC* gene. Haplotypes with frequency > 0.05 (denoted by *italics*) were used for association analyses

smoking status, atopy, and body mass index as covariates, using the Statistical Analysis System (SAS). To better ascertain the genetic effects of genotypes on the disease, this study employed three different genetic models (co-dominant, dominant, and recessive) in the association analysis.

Results

Characteristics of study subjects

Demographic characteristics of study subjects were such that the female–male ratio was ~1.9:1 for asthmatics and ~1.8:1 for AERD patients, as expected with gender differences in adult asthma according to the Global Allergy and Asthma European Network (GA2LEN). About a 7-fold increase in the mean FEV1 decline by aspirin provocation in AERD patients compared to ATA controls was observed ($P < 0.0001$, Table 1). In addition, the mean age of aspirin challenge, smoking status, body mass index, and PC20 methacholine level in AERD patients were significantly lower than those in ATA controls ($P < 0.05$).

FANCC polymorphisms and no association with the risk of AERD

A total of 25 common polymorphisms (minor allele frequency > 0.05) were selected and successfully genotyped in a total of 592 Korean asthmatics including 163 AERD, and 429 ATA subjects (Fig. 1a; Table 2). All SNPs showed no deviation from Hardy–Weinberg Equilibrium ($P > 0.05$, Table 2). Most SNPs are located at the intronic regions, except for *rs4647554C > T* and *rs4647558A > G* which are located in the 3'-untranslated region (3'UTR). Complete LDs ($r^2 = 1$) were found between *rs356669A > G* and *rs657637A > C*, and between *rs356677T > C* and *rs356680G > A* (Fig. 1a). Pair-wise comparisons revealed one tight LD block (Fig. 1b), and its haplotypes were inferred using PHASE software (Fig. 1c). Only common haplotypes with frequency greater than 0.05 were used for the haplotype association analysis. Results of logistic analyses using multiple models revealed no statistically significant associations of polymorphisms and haplotypes in the *FANCC* gene with AERD development ($P > 0.05$, Supplementary Table 1).

Table 2 List of *FANCC* SNPs genotyped in this study

SNP ID	Position	MAF	HWE*
<i>rs10993509T > A</i>	Intron1	0.085	0.188
<i>rs554879C > G</i>	Intron1	0.250	0.651
<i>rs187172G > C</i>	Intron1	0.217	0.025
<i>rs356665A > G</i>	Intron1	0.409	0.204
<i>rs4647376C > A</i>	Intron1	0.387	0.960
<i>rs356669A > G</i>	Intron1	0.289	0.218
<i>rs4744451T > G</i>	Intron1	0.415	0.216
<i>rs4647416G > A</i>	Intron2	0.162	0.190
<i>rs356659A > G</i>	Intron3	0.115	0.908
<i>rs356660A > G</i>	Intron3	0.088	0.933
<i>rs356677T > C</i>	Intron4	0.301	0.199
<i>rs1326188A > C</i>	Intron4	0.202	0.914
<i>rs12380720C > T</i>	Intron4	0.307	0.054
<i>rs356680G > A</i>	Intron4	0.300	0.234
<i>rs4647473T > C</i>	Intron4	0.050	0.625
<i>rs400727G > T</i>	Intron4	0.138	0.512
<i>rs657637A > C</i>	Intron6	0.290	0.212
<i>rs640628G > A</i>	Intron6	0.129	0.854
<i>rs3780567G > A</i>	Intron7	0.413	0.284
<i>rs944934G > T</i>	Intron10	0.286	0.160
<i>rs4647528G > T</i>	Intron12	0.464	0.475
<i>rs2282216C > T</i>	Intron13	0.406	0.250
<i>rs4647549C > T</i>	Intron14	0.060	0.111
<i>rs4647554C > T</i>	3'UTR	0.476	0.950
<i>rs4647558A > G</i>	3'UTR	0.480	0.522

Minor allele frequency (MAF) indicates that of total asthmatics in Korean cohort

* P -values of deviation from Hardy–Weinberg equilibrium (HWE)

Significant association of FANCC polymorphisms and haplotype with decline of FEV1 by aspirin provocation

Since the decline of FEV1 is a marker of airflow obstruction following an aspirin challenge, additional association analysis with decline of FEV1 by aspirin provocation was performed. Interestingly, 12 SNPs ($P = 0.006–0.04$) and haplotype *FANCC_h13* ($P = 0.01$ and 0.006 under the co-dominant and dominant model, respectively), which is unique to *rs4647416G > A*, were significantly associated with the decline of FEV1 by aspirin provocation in all the asthma patients (Table 3). The strength of association with polymorphisms was increased in the AERD patients group

($P = 0.002–0.04$), whereas only *rs640628G > A* and *FANCC_h14* showed nominal signals in the case of ATA controls (Table 4).

Potential branch point (BP) site for alternative splicing by *rs4647376C > A*

In silico analysis using EMBL-EBI splice site prediction (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>) revealed that the ATA[C/A]C sequence containing the “A” allele of *rs4647376C > A* in the first intron was estimated to be a potential BP site for alternative splicing, with a BP score of 4.16 (Supplementary Fig. 1). When the subjects ratio and genotypes (CC, CA, and AA) of *rs4647376C > A* were

Table 3 Associations of polymorphisms and haplotypes of *FANCC* with the decline on FEV1 in total asthmatics

SNP or Haplotype	Position	C/C	C/R	R/R	P_a	P_b	P_c
<i>rs10993509T > A</i>	Intron1	491 (8.91 ± 12.74)	99 (10.92 ± 15.43)	2 (6.15 ± 4.03)	0.19	0.15	0.65
<i>rs554879C > G</i>	Intron1	331 (9.23 ± 13.49)	225 (8.35 ± 11.53)	36 (14.77 ± 18.64)	0.39	0.83	0.008
<i>rs187172G > C</i>	Intron1	356 (8.29 ± 11.98)	216 (10.82 ± 15.14)	20 (8.88 ± 10.81)	0.09	0.05	0.85
<i>rs356665A > G</i>	Intron1	199 (8.08 ± 11.81)	300 (9.72 ± 14.02)	93 (10.14 ± 13.38)	0.18	0.16	0.51
<i>rs4647376C > A</i>	Intron1	221 (7.57 ± 10.60)	283 (9.71 ± 14.20)	87 (11.97 ± 15.38)	0.009	0.03	0.04
<i>rs356669A > G</i>	Intron1	293 (7.93 ± 11.05)	256 (10.26 ± 15.11)	43 (12.02 ± 14.06)	0.02	0.03	0.17
<i>rs4744451T > G</i>	Intron1	208 (7.59 ± 10.78)	274 (9.53 ± 14.02)	108 (11.29 ± 14.36)	0.02	0.04	0.09
<i>rs4647416G > A</i>	Intron2	412 (8.17 ± 11.74)	168 (11.96 ± 16.16)	12 (7.57 ± 10.56)	0.02	0.006	0.80
<i>rs356659A > G</i>	Intron3	463 (9.09 ± 13.18)	120 (8.88 ± 12.33)	8 (16.61 ± 17.48)	0.67	0.99	0.10
<i>rs356660A > G</i>	Intron3	494 (9.10 ± 13.07)	93 (9.51 ± 13.58)	5 (17.78 ± 20.50)	0.47	0.69	0.12
<i>rs356677T > C</i>	Intron4	282 (7.92 ± 10.97)	263 (10.22 ± 15.10)	47 (11.59 ± 13.79)	0.02	0.03	0.22
<i>rs1326188A > C</i>	Intron4	374 (9.52 ± 13.72)	194 (8.73 ± 11.79)	24 (8.82 ± 16.33)	0.80	0.75	0.94
<i>rs12380720C > T</i>	Intron4	295 (10.23 ± 14.17)	228 (8.41 ± 12.06)	68 (6.97 ± 10.94)	0.02	0.03	0.13
<i>rs356680G > A</i>	Intron4	283 (7.90 ± 10.96)	261 (10.31 ± 15.11)	47 (11.59 ± 13.79)	0.02	0.02	0.22
<i>rs4647473T > C</i>	Intron4	534 (9.39 ± 13.43)	57 (8.00 ± 11.11)	1 (−4.00)	0.26	0.31	0.26
<i>rs400727G > T</i>	Intron4	440 (9.27 ± 13.31)	142 (8.88 ± 12.67)	10 (12.85 ± 17.24)	0.98	0.76	0.35
<i>rs657637A > C</i>	Intron6	292 (7.94 ± 11.07)	256 (10.13 ± 15.02)	43 (12.02 ± 14.06)	0.02	0.03	0.16
<i>rs640628G > A</i>	Intron6	448 (9.12 ± 13.34)	134 (9.19 ± 12.61)	10 (14.82 ± 15.92)	0.64	0.90	0.20
<i>rs3780567G > A</i>	Intron7	209 (7.57 ± 10.76)	276 (9.49 ± 13.98)	106 (11.39 ± 14.47)	0.02	0.04	0.07
<i>rs944934G > T</i>	Intron10	295 (7.87 ± 10.79)	256 (10.39 ± 15.27)	41 (11.79 ± 14.53)	0.01	0.02	0.19
<i>rs4647528G > T</i>	Intron12	174 (7.59 ± 11.14)	286 (9.34 ± 13.51)	131 (10.81 ± 14.32)	0.05	0.11	0.12
<i>rs2282216C > T</i>	Intron13	215 (7.70 ± 10.98)	273 (9.52 ± 14.38)	104 (11.66 ± 13.96)	0.02	0.05	0.07
<i>rs4647549C > T</i>	Intron14	521 (9.53 ± 13.52)	71 (7.07 ± 10.60)	–	0.12	0.12	–
<i>rs4647554C > T</i>	3'UTR	161 (7.72 ± 11.45)	295 (9.19 ± 13.23)	135 (10.78 ± 14.40)	0.07	0.17	0.12
<i>rs4647558A > G</i>	3'UTR	162 (7.73 ± 11.39)	288 (9.01 ± 13.26)	141 (11.06 ± 14.32)	0.05	0.18	0.06
<i>FANCC_h1</i>		344 (10.03 ± 13.93)	197 (8.30 ± 12.23)	51 (7.44 ± 11.76)	0.05	0.05	0.30
<i>FANCC_h2</i>		386 (9.42 ± 13.56)	185 (8.78 ± 11.99)	21 (9.75 ± 17.24)	1.00	0.87	0.67
<i>FANCC_h3</i>		417 (8.20 ± 11.77)	166 (11.86 ± 16.13)	9 (8.86 ± 11.96)	0.01	0.006	0.96
<i>FANCC_h4</i>		502 (9.17 ± 13.19)	86 (9.05 ± 12.86)	4 (20.63 ± 22.50)	0.60	0.90	0.06
<i>FANCC_h5</i>		518 (9.11 ± 13.25)	72 (10.22 ± 13.20)	2 (6.15 ± 4.03)	0.63	0.56	0.65

P -values are adjusted for initial diagnosis age, sex, smoking, atopy, and body mass index. C/C, C/R and R/R indicate the homozygote of the common allele, and the heterozygote and homozygote of the rare allele, respectively. P_a , P_b and P_c indicate the P -value of co-dominant, dominant, and recessive models, respectively

Bold values indicate $P < 0.05$

Table 4 Relation of *FANCC* SNPs to the decline on FEV1 in AERD and in ATA

	SNP/Haplotype	Position	C/C		C/R		R/R		<i>P_a</i>	<i>P_b</i>	<i>P_c</i>
			<i>n</i>	Decline of FEV1 (%)	<i>n</i>	Decline of FEV1 (%)	<i>n</i>	Decline of FEV1 (%)			
In AERD	<i>rs10993509T > A</i>	Intron1	129	24.14 ± 15.40	31	26.65 ± 18.93	0	–	0.33	0.33	–
	<i>rs554879C > G</i>	Intron1	87	25.88 ± 15.83	61	20.99 ± 14.48	12	34.00 ± 21.61	0.93	0.29	0.03
	<i>rs187172G > C</i>	Intron1	91	21.88 ± 15.65	64	28.49 ± 16.53	5	25.08 ± 9.50	0.03	0.01	0.98
	<i>rs356665A > G</i>	Intron1	49	21.82 ± 15.21	85	25.50 ± 17.10	26	27.04 ± 14.11	0.13	0.12	0.39
	<i>rs4647376C > A</i>	Intron1	53	20.33 ± 13.34	80	26.20 ± 16.65	27	28.39 ± 18.12	0.01	0.02	0.13
	<i>rs356669A > G</i>	Intron1	71	21.18 ± 14.05	77	26.79 ± 17.75	12	31.15 ± 12.71	0.008	0.01	0.09
	<i>rs4744451T > G</i>	Intron1	51	20.29 ± 13.59	75	26.09 ± 16.71	33	26.99 ± 16.73	0.03	0.02	0.24
	<i>rs4647416G > A</i>	Intron2	102	21.88 ± 15.35	56	29.55 ± 16.57	2	27.00 ± 15.56	0.006	0.004	0.76
	<i>rs356659A > G</i>	Intron3	124	24.82 ± 15.73	32	22.17 ± 16.49	3	31.83 ± 21.76	0.71	0.47	0.38
	<i>rs356660A > G</i>	Intron3	132	24.78 ± 15.57	26	23.24 ± 18.32	2	32.25 ± 30.76	0.94	0.77	0.48
	<i>rs356677T > C</i>	Intron4	69	20.89 ± 13.93	78	26.95 ± 17.80	13	30.53 ± 12.37	0.006	0.009	0.10
	<i>rs1326188A > C</i>	Intron4	104	24.94 ± 16.81	51	23.12 ± 13.89	5	33.38 ± 22.35	0.95	0.67	0.17
	<i>rs12380720C > T</i>	Intron4	85	26.31 ± 16.84	57	23.18 ± 15.12	17	19.05 ± 12.86	0.06	0.09	0.16
	<i>rs356680G > A</i>	Intron4	69	20.89 ± 13.93	78	26.95 ± 17.80	13	30.53 ± 12.37	0.006	0.009	0.10
	<i>rs4647473T > C</i>	Intron4	144	25.16 ± 16.28	16	19.80 ± 14.03	0	–	0.20	0.20	–
	<i>rs400727G > T</i>	Intron4	117	25.52 ± 15.64	40	21.48 ± 17.01	3	31.83 ± 21.76	0.40	0.24	0.47
	<i>rs657637A > C</i>	Intron6	71	21.18 ± 14.05	76	26.65 ± 17.83	12	31.15 ± 12.71	0.008	0.02	0.09
	<i>rs640628G > A</i>	Intron6	120	25.07 ± 15.91	37	22.59 ± 16.54	3	31.83 ± 21.76	0.68	0.45	0.38
	<i>rs3780567G > A</i>	Intron7	51	20.29 ± 13.59	75	26.09 ± 16.71	33	26.99 ± 16.73	0.03	0.02	0.24
	<i>rs944934G > T</i>	Intron10	71	20.71 ± 13.78	78	27.08 ± 17.72	11	32.50 ± 12.46	0.002	0.005	0.04
	<i>rs4647528G > T</i>	Intron12	41	21.23 ± 14.01	79	24.92 ± 16.49	39	26.75 ± 16.56	0.10	0.12	0.26
	<i>rs2282216C > T</i>	Intron13	54	20.58 ± 13.66	73	26.42 ± 17.91	33	27.27 ± 14.71	0.03	0.02	0.24
	<i>rs4647549C > T</i>	Intron14	142	25.27 ± 16.36	18	19.54 ± 13.29	0	–	0.16	0.16	–
	<i>rs4647554C > T</i>	3'UTR	40	21.21 ± 14.19	78	24.86 ± 16.50	41	26.70 ± 16.35	0.10	0.12	0.26
	<i>rs4647558A > G</i>	3'UTR	40	21.21 ± 14.19	75	24.82 ± 16.75	44	26.64 ± 15.91	0.11	0.12	0.26
	<i>FANCC_h1</i>		96	26.36 ± 16.51	50	22.75 ± 15.71	14	19.42 ± 13.68	0.07	0.09	0.23
	<i>FANCC_h2</i>		106	24.80 ± 16.69	49	23.35 ± 14.10	5	33.38 ± 22.35	0.86	0.77	0.17
	<i>FANCC_h3</i>		103	22.02 ± 15.34	55	29.41 ± 16.69	2	27.00 ± 15.56	0.009	0.007	0.76
	<i>FANCC_h4</i>		137	24.70 ± 15.71	21	23.42 ± 18.18	2	32.25 ± 30.76	0.95	0.88	0.48
	<i>FANCC_h5</i>		138	24.77 ± 16.16	22	23.74 ± 16.14	0	–	0.93	0.93	–

Table 4 continued

	SNP/Haplotype	Position	C/C		C/R		R/R		<i>P_a</i>	<i>P_b</i>	<i>P_c</i>
			<i>n</i>	Decline of FEV1 (%)	<i>n</i>	Decline of FEV1 (%)	<i>n</i>	Decline of FEV1 (%)			
In ATA	<i>rs10993509T > A</i>	Intron1	359	3.49 ± 4.91	68	3.75 ± 4.57	2	6.15 ± 4.03	0.40	0.45	0.53
	<i>rs554879C > G</i>	Intron1	242	3.30 ± 4.91	163	3.67 ± 4.84	24	5.15 ± 4.02	0.09	0.20	0.09
	<i>rs187172G > C</i>	Intron1	264	3.62 ± 4.90	150	3.41 ± 4.94	15	3.49 ± 2.82	0.52	0.49	0.89
	<i>rs356665A > G</i>	Intron1	150	3.59 ± 5.32	212	3.50 ± 4.70	67	3.58 ± 4.23	0.93	0.90	1.00
	<i>rs4647376C > A</i>	Intron1	167	3.54 ± 4.98	202	3.23 ± 4.79	59	4.65 ± 4.59	0.38	1.00	0.08
	<i>rs356669A > G</i>	Intron1	221	3.69 ± 4.94	177	3.17 ± 4.87	31	4.61 ± 3.79	0.93	0.50	0.28
	<i>rs4744451T > G</i>	Intron1	156	3.46 ± 5.05	198	3.30 ± 4.86	74	4.43 ± 4.34	0.28	0.75	0.11
	<i>rs4647416G > A</i>	Intron2	309	3.66 ± 4.91	110	3.21 ± 4.82	10	3.68 ± 2.97	0.39	0.31	0.91
	<i>rs356659A > G</i>	Intron3	337	3.34 ± 4.86	87	4.09 ± 4.75	5	7.48 ± 4.48	0.05	0.10	0.08
	<i>rs356660A > G</i>	Intron3	360	3.38 ± 4.77	66	4.23 ± 5.18	3	8.13 ± 4.27	0.07	0.11	0.13
	<i>rs356677T > C</i>	Intron4	212	3.72 ± 5.00	183	3.19 ± 4.85	34	4.34 ± 3.74	0.80	0.44	0.41
	<i>rs1326188A > C</i>	Intron4	267	3.59 ± 5.07	143	3.60 ± 4.49	19	2.35 ± 4.26	0.53	0.73	0.34
	<i>rs12380720C > T</i>	Intron4	209	3.73 ± 4.57	170	3.50 ± 4.63	50	2.90 ± 6.48	0.34	0.49	0.35
	<i>rs356680G > A</i>	Intron4	213	3.70 ± 4.99	181	3.25 ± 4.83	34	4.34 ± 3.74	0.89	0.54	0.43
	<i>rs4647473T > C</i>	Intron4	387	3.58 ± 4.86	41	3.40 ± 4.66	1	—	0.44	0.57	0.17
	<i>rs400727G > T</i>	Intron4	321	3.38 ± 4.79	101	3.97 ± 5.00	7	4.72 ± 5.52	0.25	0.27	0.54
	<i>rs657637A > C</i>	Intron6	220	3.68 ± 4.95	178	3.19 ± 4.86	31	4.61 ± 3.79	0.96	0.53	0.28
	<i>rs640628G > A</i>	Intron6	326	3.29 ± 4.86	96	4.12 ± 4.73	7	7.53 ± 3.96	0.02	0.04	0.03
	<i>rs3780567G > A</i>	Intron7	157	3.46 ± 5.03	200	3.30 ± 4.84	72	4.40 ± 4.39	0.31	0.78	0.13
	<i>rs944934G > T</i>	Intron10	223	3.80 ± 4.99	176	3.11 ± 4.79	30	4.20 ± 3.93	0.49	0.24	0.54
	<i>rs4647528G > T</i>	Intron12	132	3.37 ± 5.25	206	3.41 ± 4.58	91	4.09 ± 4.84	0.38	0.68	0.28
	<i>rs2282216C > T</i>	Intron13	160	3.38 ± 4.99	199	3.36 ± 4.86	70	4.45 ± 4.42	0.23	0.59	0.11
	<i>rs4647549C > T</i>	Intron14	377	3.65 ± 4.87	52	2.80 ± 4.66	0	—	0.21	0.21	—
	<i>rs4647554C > T</i>	3'UTR	121	3.26 ± 5.33	215	3.56 ± 4.57	93	3.87 ± 4.84	0.41	0.46	0.54
	<i>rs4647558A > G</i>	3'UTR	122	3.31 ± 5.28	211	3.45 ± 4.55	96	4.03 ± 4.91	0.33	0.54	0.31
	<i>FANCC_h1</i>		246	3.72 ± 4.56	146	3.41 ± 4.71	37	2.90 ± 6.89	0.31	0.36	0.46
	<i>FANCC_h2</i>		277	3.62 ± 5.03	136	3.53 ± 4.52	16	2.37 ± 4.34	0.45	0.62	0.34
	<i>FANCC_h3</i>		313	3.66 ± 4.89	109	3.21 ± 4.82	7	3.67 ± 3.04	0.37	0.31	0.94
	<i>FANCC_h4</i>		363	3.35 ± 4.76	64	4.47 ± 5.17	2	9.00 ± 5.66	0.03	0.05	0.12
	<i>FANCC_h5</i>		377	3.43 ± 4.86	50	4.27 ± 4.75	2	6.15 ± 4.03	0.13	0.14	0.53

P-values are adjusted for initial diagnosis age, sex, smoking, atopy, and body mass index. *C/C*, *C/R* and *R/R* indicate the homozygote of the common allele, and the heterozygote and homozygote of the rare allele, respectively. *P_a*, *P_b* and *P_c* indicate the *P*-value of co-dominant, dominant, and recessive models, respectively

Bold values indicate *P* < 0.05

also compared with FEV1 decline by aspirin provocation (Fig. 2), the allele dose effect of the variation on FEV1 decline was found under the co-dominant model, with an increasing pattern of FEV1 decline induced by the “A” allele ($P = 0.009$).

Discussion

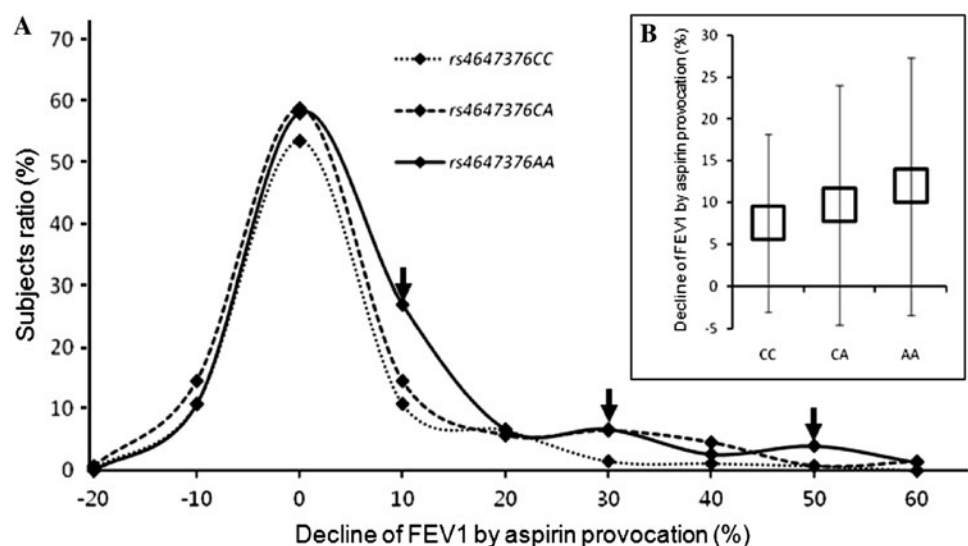
Considering the fact that many alternative transcripts of the *FANCC* gene have been discovered according to the Ensembl genome browser (<http://www.ensembl.org/index.html>) (Supplementary Fig. 1), the *rs4647376C > A* variant in the first intron may also be a potential BP site for alternative splicing. Recently, in silico analyses for splice site prediction have correctly predicted the direction of microarray and polymerase chain reaction (PCR) results [20], suggesting that the extent of cis-regulated alternative splicing among individuals may be greater than previously expected. In our extended in silico analysis of whether the *rs4647376C > A* polymorphism could be a putative binding site for regulators using the Signal Scan program (<http://www.bimas.cit.nih.gov/molbio/signal/>), the AGATAA sequence including the “A” allele of *rs4647376C > A* was thought to be an element for the binding of GATA-2 factor, whereas the CCACC including the “C” allele of the SNP was observed to be an element for the binding of CAC-binding_pro factor. This result suggests that the *rs4647376C > A* polymorphism may not only be a potential BP site for alternative splicing, but also a potential allele-specific binding site for a transcription factor related to the phenotype association.

Nasal polyposis is a chronic inflammatory condition that constitutes one of the symptoms of the so-called aspirin triad. The expression of several genes, including

cyclooxygenase-2 (COX-2) and its polymorphisms, have been reported to be involved in the pathogenesis of nasal polyposis in AERD, suggesting an interplay of genetic mechanisms [21, 22]. Given these facts, additional associations of *FANCC* polymorphisms and haplotypes with the presence of nasal polyps in AERD subjects were investigated. However, no significant relations between *FANCC* polymorphisms and nasal polyposis in AERD were found ($P > 0.05$, Supplementary Table 2), even among all asthmatic subjects ($P > 0.05$, Supplementary Table 3). Although further comparisons with normal healthy controls are needed, our preliminary results suggest that the *FANCC* gene may play a role in the obstructive phenotype of asthma, rather than in other phenotypes.

FEV1, as an airway function marker, accounts for the classification of asthma severity, and has been implicated in risk of asthma attacks in a pediatric population [23, 24]. One study has suggested an association of the *FANCC* gene with the physiologically relevant stress of hypoxia-reoxygenation transition on which premature senescence has been observed in *Fancc*^{−/−} bone marrow hematopoietic cells [25]. The *FANCC* gene may also be related to other extrabronchial symptoms such as aspirin-induced urticaria/angioedema [26]. In addition, potential associations with other diseases also could be derived from previous evidence on alterations of FEV1 decline in patients with cystic fibrosis, an autosomal recessive disease that is characterized by a progressive deterioration in lung function [27, 28]. Slow rates of FEV1 also have been found in patients with cystic fibrosis who are treated with ibuprofen [29]. Moreover, polymorphisms of the *pyrin domain containing 3 (NLRP3)* have been reported to be significantly associated with food-induced anaphylaxis and aspirin-induced asthma [30], suggesting a possible relationship between FEV1 decline and anaphylaxis. Taken together, despite the

Fig. 2 Allele dose effect of *rs4647376C > A* in *FANCC* on FEV1 decline by aspirin provocation. **a** Homozygote of the rare allele (AA) of *rs4647376C > A* in the *FANCC* gene shows a more significant increase of subjects ratio of FEV1 decline than other genotypes from 10% FEV1 decline as indicated by arrows. **b** Allele dose effect of “A” variant of *rs4647376C > A* on FEV1 decline. Distributions present the mean $\pm$ SE values of FEV1 decline (%) by aspirin provocation in each genotype



need for further studies, these previous reports and our findings suggest a potential association of *FANCC* gene with obstructive diseases.

Along with the overproduction of pro-inflammatory cysteinyl leukotrienes in aspirin hypersensitivity [3], AERD development has been known to be accompanied by activation of inflammatory cells including eosinophils and mast cells [31]. Furthermore, allergen-sensitive asthmatic patients have been reported to show an increase of hemopoietic progenitors in bone marrow [32, 33]. Indeed, deficiency of the *Fancc* gene in mice has shown defects in bone marrow, exacerbated inflammatory response, and hypersensitivity to septic shock by lipopolysaccharide (LPS) [14]. This evidence suggests that the *FANCC* gene may play a potential role in the aspirin-induced airway remodeling associated with hemopoietic progenitors, and therefore, further replication and functional studies are needed.

In conclusion, the present study is the first to investigate the genetic associations of common *FANCC* polymorphisms with aspirin hypersensitivity and the related phenotypes in asthma. Although further functional evaluations are required and a few limitations such as insufficient sample size of AERD patients and no healthy controls have been observed in the study, our preliminary findings suggest that *FANCC* polymorphisms and the *FANCC*_{ht3} haplotype (unique to *rs4647416G > A*) could be susceptible markers for the decline of FEV1 in AERD patients.

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