

The evidence of genetic polymorphisms of genes involved in the *P2RX7* signaling pathway as predictive biomarkers for response and loss of response to infliximab against Crohn's disease

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Abstract

Infliximab (IFX) is a chimeric anti-tumor necrosis factor- α monoclonal antibody exerting the therapeutic effect for Crohn's disease (CD). To identify certain genes related to the effect of IFX and biomarkers to predict the effect of IFX, we examined an association study between 35 tag single nucleotide polymorphisms (SNPs) in six candidate genes involved in the *P2RX7* signaling pathway and response to IFX after 10 weeks, 1 year, and 2 years of treatment for Japanese CD patients. A total of 127 CD patients were divided into two groups, including responders and non-responders, at each period of IFX treatment. The frequencies of alleles and genotyped at each tag SNP between responders and non-responders were compared in three different inheritance models at each period of treatment. Statistical analyses indicated that polymorphism of rs11670259 in *CARD8* contributed to response and primary non-response to IFX after 10 weeks of treatment, and that polymorphisms of *P2RX7*, *CARD8*, and *CASP1* independently contributed to response and secondary loss of response to IFX after 1 year of treatment. Subsequently, using the associated tag SNPs as a biomarker, genetic test revealed that the polymorphism of *CARD8* and the combination polymorphisms of *P2RX7* and *CASP1* were useful as a biomarker to predict response to IFX after 10 weeks and 1 year of treatment, respectively. Therefore, *CARD8* is an IFX-related gene after 10 weeks of treatment, and *P2RX7*, *CARD8*, and *CASP1* are IFX-related genes after 1 year of treatment for Japanese CD patients. These genes in the *P2RX7* signaling pathway could therefore be potential targets for new therapeutic drugs to combat primary non-response and secondary loss of response to IFX for CD patients.

Abbreviations: ASC: apoptosis-associated speck-like protein containing a card; ATI: antibodies to IFX; CARD8: caspase recruitment domain-containing protein 8; CASP1: caspase 1; CD: Crohn's disease; CDAI: Crohn's disease activity index; HWE: Hardy-Weinberg equilibrium; IFX: infliximab; IL: interleukin; NF- κ B: nuclear factor kappa-B; NLRP3: NLR family pyrin domain-containing 3; P2RX7: purinergic receptor P2X, ligand-gated ion channel, 7; SNPs: single nucleotide polymorphisms; TNF- α anti-tumor necrosis factor-alpha; TNFR: TNF receptor

Introduction

Crohn's disease (CD) is pathophysiologically characterized by granulomatous inflammation in the gastrointestinal tract with the dysfunction of both the mucosal immune system and inflammatory response. Although the etiology of CD is still unknown, many genetic and environmental factors contribute to the onset of CD, because this disorder is one of the multifactorial disorders [1-3]. Since no fundamental therapies for CD have yet been established, CD treatment is determined according to the present site of the lesions, the degree of inflammation, response to the past treatment, and the presence or absence of complications in order to induce remission as early as possible [4].

Infliximab (IFX) is a chimeric anti-tumor necrosis factor- α (TNF- α) monoclonal antibody, which is used for the CD patients with moderate to severe disease activity [4-6]. A randomized clinical trial using a 5 mg/kg intravenous infusion of IFX (ACCENT I) has revealed that 58% of all patients showed good response to IFX after 2 weeks of treatment. However, among the responders after 2 weeks, 22% of the patients discontinued maintenance treatment by 54 weeks of the treatment period [7]. Therefore, response to the therapeutic drugs for CD as well as susceptibility to the onset of CD are involved as a multifactorial complex.

Some association studies using single nucleotide polymorphisms

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(SNPs) have shown the possible IFX-related genes for rheumatoid arthritis, such as TNF receptor (TNFR) superfamily member 1B [8], Fc gamma receptors IIA and IIIA [9], AFF3 [10], CD226 [10], protein tyrosine phosphatase receptor type C [11], and p38 mitogen-activated protein kinase [12]. While, our previous study has reported that the polymorphisms of *IL17F* and *TRAF3IP2* are associated with response to IFX after 1 year of treatment for Japanese CD patients [13]. As the results, we next focused on the purinergic receptor P2X, ligand-gated ion channel, 7 (*P2RX7/P2RX7*) signaling pathway, which is related to intestinal inflammation through another non-TNF-dependent inflammation signaling pathway.

P2RX7 is a ligand-gated membrane ion channel which plays a crucial role in many cellular functions, such as vascular reactivity, apoptosis, cytokine secretion, and tissue inflammation [14]. This purinergic receptor is expressed in peripheral macrophages, mast cells, lymphocytes, erythrocytes, fibroblasts, microglia, astrocytes, Schwann cells, and dendritic cells, and is activated by high concentrations of extracellular ATP [15], thus resulting in the opening of ion channels and subsequently leading to the influx of Ca^{2+} and the efflux of K^+ . The increase in cytosolic Ca^{2+} and subsequent decrease in intracellular K^+ activate the assembly of caspase recruitment domain-containing protein 8 (*CARD8/CARD8*), NLR family pyrin domain-containing 3 (*NLRP3/NLRP3*), apoptosis-associated speck-like protein containing a card (*ASC/PYCARD*), and caspase 1 (*CASP1/CASP1*) [16], thus resulting in the release of inflammatory cytokines including interleukin (IL)-1 β (*IL1B/IL1B*) [16,17], IL-2, IL-4, IL-6, IL-13, and IL-18 (*IL18*) [17], and other inflammatory mediators, such as nitric oxide synthase, cyclooxygenase-2, TNF- α [18], phospholipase-D, phospholipase A_2 , nuclear factor kappa-B (NF- κ B) [19], and mitogen activated protein kinases. Since the *P2RX7* signaling pathway regulates inflammation through the secretion of these cytokines and mediators, *P2RX7* antagonists can be used in the treatment of inflammatory bowel disease [20]. In addition, the production of IL-1 β was found to decrease in *caspase1*-deficient mice as well as in *P2RX7*-deficient mice [21-23]. Moreover, *NLRP3* and *CARD8* are susceptibility genes for the onset of CD [24]. The number of mast cells expressing *P2RX7* have been reported to increase in the colon of CD patients and intestinal inflammation is inhibited by the treatment of anti-*P2RX7* antibody [25].

We therefore hypothesized that not only the activation of the TNFR signaling pathway, but also the activation of the *P2RX7* signaling pathway may contribute to inflammation of the intestines in CD patients. The elevated production of inflammatory cytokines and mediators through the activation of the *P2RX7* signaling pathway in the genetic background may lead to the perpetuation of the chronic intestinal inflammatory process and might thereby result in loss of response to IFX. Therefore, we examined an association study between polymorphisms of six target genes (*P2RX7*, *CARD8*, *PYCARD*, *CASP1*, *IL1B*, and *IL18*) involved in the *P2RX7* signaling pathway and the therapeutic effect of IFX at the short, middle, or long period of treatment for Japanese CD patients. Another purpose of this study was to investigate whether the associated polymorphisms can be used as new genetic biomarkers for predicting either response or loss of response to IFX at each period by genetic testing.

Patients and methods

Patients

In this study, 127 unrelated Japanese CD patients were enrolled and treated with IFX at three general hospitals, namely Oita Red Cross Hospital, Nagasaki Harbor Medical Center City Hospital, or Nagasaki

University Hospital from 2004 to 2012.

The study protocol was approved by the Ethics Committee dealing with Human Genome and Gene Analysis at Oita Red Cross Hospital, Nagasaki Harbor Medical Center City Hospital, and Nagasaki University. Written informed consent was obtained from all patients.

Definition of the therapeutic effect of IFX: Since a higher Crohn's disease activity index (CDAI) of more than 150 is regarded as active-phase CD patients [26], responders to IFX were defined as those showing a decrease in CDAI of less than 150 and an improvement in clinical manifestations, laboratory data, and/or endoscopic findings at each period of treatment. Non-responders to IFX were defined as those showing no change in the CDAI value or any exacerbation of the disease activity.

Study design

All enrolled patients were analyzed after 10 weeks of treatment. Out of the 127 CD patients, 116 patients were subsequently analyzed after 1 year of treatment because they showed response to IFX after 10 weeks of treatment. Thus, 11 patients indicated primary non-response to IFX. Likewise, out of 116 patients, 97 patients, who had shown response to IFX after 1 year of treatment, were subjected to an analysis after 2 years of treatment. Therefore, 19 patients indicated secondary loss of response to IFX after 1 year of treatment. The clinical characteristics of the patients in each group at the end of this study are shown in Table 1.

Selection of tag SNPs in the candidate genes: The selected six candidate genes involved in the *P2RX7* signaling pathway included *P2RX7* (OMIM #602566) located at 12q24.31; *CARD8* (OMIM #609051) located at 19q13.33; *PYCARD* (OMIM #606838) located at 16p11.2; *CASP1* (OMIM #147678) located at 11q22.3; *IL18* (OMIM #600953) located at 11q23.1, and *IL1B* (OMIM #147720) located at 2q14.1.

Obtaining information on SNPs in the target genes, selecting the candidate tag SNPs, and determining the genotyped tag SNPs were carried out according to the same methods as reported previously [13,27,28]. The gene structures and positions of the genotyped tag SNPs in the candidate genes are shown in Figure 1.

Genotyping of tag SNPs in candidate genes: Genetic analyses for the genomic DNA extracted from each patient and genotyping of 35 tag SNPs in 6 genes by PCR-restriction fragment length polymorphism, PCR-direct DNA sequencing, or probe-based high resolution melting method were done according to the same method as reported

Table 1. Comparison of the characteristics between responders and non-responders to IFX in CD patients. (IFX, infliximab; CD, Crohn's disease; SD, standard deviation).

Characteristics	Number (%) of CD patients	
	Responders	Non-responders
10 weeks (<i>n</i> = 127)		
Number (%)	116 (91.3)	11 (8.7)
Age, mean \pm SD (years)	35.2 \pm 11.8	35.3 \pm 11.8
Male/female (%)	67/49 (57.8/42.2)	10/1 (90.9/9.1)
1 year (<i>n</i> = 116)		
Number (%)	97 (83.6)	19 (16.4)
Age, mean \pm SD (years)	35.2 \pm 11.9	35.6 \pm 11.7
Male/female (%)	56/41 (57.7/42.3)	11/8 (57.9/42.1)
2 years (<i>n</i> = 97)		
Number (%)	82 (84.5)	15 (15.5)
Age, mean \pm SD (years)	35.1 \pm 11.9	35.3 \pm 11.8
Male/female (%)	48/34 (58.5/41.5)	8/7 (53.3/46.7)

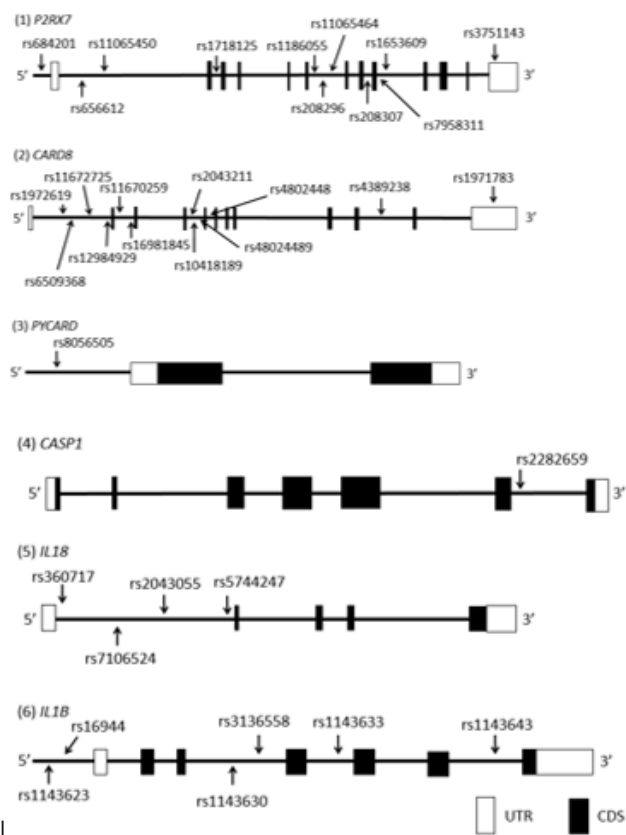


Figure 1. Gene structures and locations of the genotyped tag SNPs in each gene. The horizontal bars indicate the genomic sequences of the candidate genes. Full boxes represent exons in each gene, and open boxes show the untranslated regions. The arrows indicate the positions of the genotyped tag SNP sites and their names are presented above each site.

previously [13,27,28]. Information on the sequences of primer sets, annealing temperature, cycle number, restriction enzymes, and genetic analytic methods for each SNP is shown in Table 2.

Statistical analysis

A comparison of the clinical characteristics, the significance of deviation from the Hardy-Weinberg equilibrium (HWE), and the frequencies of alleles and genotypes, subsequent multivariate regression analysis, and the statistical value of genetic tests were analyzed by the same methods as reported previously [13,27,28].

Results

Comparison of the clinical characteristics between responders and non-responders to IFX in each group

Comparison analyses of the clinical characteristics between responders and non-responders to IFX in each group revealed a significant difference in the gender after 10 weeks of treatment. The percentage of males in non-responders to IFX was higher than that in responders (90.9% vs. 57.8%, $P = 0.049$; Table 1), thereby indicating that male gender showed loss of response to IFX after 10 weeks of treatment. Conversely, females indicated response to IFX after 10 weeks of treatment. However, no significant differences in the mean age and gender were observed between responders and non-responders to IFX in other groups.

Association of tag SNPs with response to IFX after 10 weeks of treatment

The frequencies and distributions of minor alleles and genotypes at tag SNPs in each gene were identified and compared between responders and non-responders to IFX after 10 weeks of treatment (Table 3). The two tag SNPs, rs2043211 and rs1972619 in *CARD8*, were excluded from the subsequent analyses because they were not in HWE.

The frequencies of a heterozygous C/T genotype and a minor homozygous T/T genotype of rs11670259 in *CARD8* in the minor allele dominant model were significantly decreased in responders in comparison to those in non-responders (31.9% vs. 63.6%, $P = 0.047$, OR = 0.268; Table 3), thereby indicating ~3.7-fold loss of response to IFX after 10 weeks of treatment. Conversely, the possession of a major homozygous C/C genotype of rs11670259 in *CARD8* indicated ~3.7-fold response to IFX.

Moreover, the frequencies of a heterozygous G/C genotype and a minor homozygous C/C genotype of rs1143623 in *IL1B* in the minor allele dominant model were significantly decreased in responders in comparison to those in non-responders (55.2% vs. 90.9%, $P = 0.025$, OR = 0.123; Tables 3), indicating that this genotype is associated with ~8.1-fold loss of response to IFX. Conversely, the possession of a major homozygous G/G genotype of rs1143623 in *IL1B* indicated ~8.1-fold response to IFX after 10 weeks of treatment.

There were no significant differences in the frequencies of any other alleles and genotypes at tag SNPs between responders and non-responders after 10 weeks of treatment.

Association of tag SNPs with response to IFX after 1 year of treatment

The frequencies and distributions of minor alleles and genotypes at tag SNPs in each gene were identified and compared between responders and non-responders to IFX after 1 year of treatment (Table 4).

With regard to rs3751143 in *P2RX7*, the frequency of a minor homozygous G/G genotype in the minor allele recessive model were significantly lower in responders in comparison to those in non-responders (6.2% vs. 31.6%, $P = 0.001$, OR = 0.143; Table 4). This result implied that ~7.0-fold loss of response to IFX after 1 year of treatment. Conversely, the possession of a major homozygous T/T genotype or a heterozygous T/G genotype of rs3751143 indicated ~7.0-fold response to IFX.

Moreover, the frequencies of a heterozygous C/T genotype and a minor homozygous T/T genotype of rs4389238 in *CARD8* in the minor allele dominant model were significantly decreased in responders in comparison to those in non-responders (46.4% vs. 78.9%, $P = 0.012$, OR = 0.231; Tables 4), indicating that these genotypes are associated with ~4.3-fold loss of response to IFX. Conversely, the possession of a major homozygous C/C genotype of rs4389238 in *CARD8* indicated ~4.3-fold response to IFX after 1 year of treatment.

In addition, the frequencies of a heterozygous A/G genotype and a minor homozygous G/G genotype of rs2282659 in *CASP1* in the minor allele dominant model were significantly increased in responders in comparison to those in non-responders (55.7% vs. 15.8%, $P = 0.002$, OR = 6.698; Tables 4), indicating that these genotypes are associated with ~6.7-fold response to IFX. Conversely, possessing a major homozygous A/A genotype of rs2282659 in *CASP1* indicated ~6.7-fold loss of response to IFX after 1 year of treatment.

Table 2. Information on genotyping of tag SNPs in the candidate genes (SNP, single nucleotide polymorphism; 3'-UTR, 3'-untranslated region; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; HRM, high resolution melting).

Gene	tag SNP	Major > Minor	Sequence of primer (5' to 3')		Annealing temperature (°C)	Cycle number	Analytic method (Restriction enzyme)	
			Forward	Reverse				
<i>P2RX7</i>	rs684201	G > A	ATCTGATTTCCTCCACCAAC	GACTGGGAGCTTCCATTATGC	55	30	PCR-RFLP (<i>Bsr</i> D I)	
	rs656612	A > C	GCAATTGCTGACCCCTATT	CAACAGCTTGGTGGTCACAG	56	30	PCR-RFLP (<i>Cac</i> 8 I)	
	rs11065450	C > A	GTGCCGGTCTCTGTTCTTAG	GCTTGGGTCTCCTGTTGTGT	57	50	PCR-HRM	
	rs1718125	G > A	GGCTGGTCTCTTGGTAGA	GGGTGAGATCCAGGAGATGA	57	30	PCR-RFLP (<i>Bsh</i> 1236 I)	
	rs1186055	G > T	GAAGATCTGGGGGAAGGAAG	GCTTGGCACAACCTAGTATCTCTGG	56	50	PCR-HRM	
	rs208296	C > T	AGCCATTTCCCAAGGACAC	GGGGGAAGGAAGTTTCTCA	55	30	PCR-RFLP (<i>Xsp</i> I)	
	rs11065464	C > A	GGCAAGCAACTCCCTGAACT	GGCATTACTGTGGCACCTC	58	30	PCR-RFLP (<i>Mae</i> III)	
	rs208307	G > C	TGGGACACTGTGGATTCTGA	AGGAGGCAGTGATCATTGG	55	30	PCR-RFLP (<i>Hpy</i> CH4 V)	
	rs7958311	G > A	AACGGTGTGTGTCCAGAC	CCACTGGGTGAGTTGACCT	57	30	PCR-RFLP (<i>Fok</i> I)	
	rs1653609	A > C	CACCCATAATCCCCTACCC	AGAATTTGAGTGGCTGTGG	54	30	PCR-RFLP (<i>Mbo</i> I)	
<i>CARD8</i>	rs3751143	T > G	TGTCTGGACAGGACCGCTT	TTCTTGGACAACAGAGGAG	59	30	PCR-RFLP (<i>Hha</i> I)	
	rs1971783	T > C	CCAATAGCTTATATGCCAGAAGG	CAGGTGAACACTCCAGCAAAT	55	30	PCR-RFLP (<i>Bsa</i> A I)	
	rs4389238	C > T	AATAGGGTTCGCGCTCTAC	TGCCCAGGAAGAAGGACATA	57	30	PCR-RFLP (<i>Fau</i> I)	
	rs4802448	G > A	ACAGGTGCTGTTGGGATACAG	TCCAGTCTCAGCAAATGGT	57	50	PCR-HRM	
	rs4802449	G > A	ACTTGACCACACTGGGAAG	GCTCCAGCAACCATAGCAA	57	30	PCR-RFLP (<i>Dde</i> I)	
	rs10418189	G > A	ACAGCGCATCCAGATCAT	ACCTCAAGGGCATAGACTGCT	57	30	PCR-direct DNA sequencing	
	rs2043211	A > T	CCCTGAGTTCGATGAAAAA	TAGGGGCTGAGGAATGACT	53	30	PCR-RFLP (<i>Mbo</i> I)	
	rs16981845	T > C	CTCAGACTCCATGACTCTTTCTT	TACTAATGCCCTGCAATCC	55	30	PCR RFLP (<i>Hsp</i> 92 II)	
	rs11670259	C > T	CCATGGGAGTTTCCCTTCA	CCGAGACGGTGTGAGTACTTT	56	50	PCR-HRM	
	rs12984929	G > T	CCAACCTGACAGAGGCAAAA	GAGGGTGGACACATACATTG	56	30	PCR-RFLP (<i>Hinc</i> II)	
<i>IL18</i>	rs11672725	C > T	CCCTGGCCAGAGAAATAAT	CGCAGGCTGTGAGAAGCATA	56	50	PCR-HRM	
	rs6509368	G > A	TCAACCAGCTTATCTGAATGTTAGC	GTTGACCATGTTCCGCAA	55	30	PCR-RFLP (<i>Afa</i> I)	
	rs1972619	G > A	TGGAAGCATTTGTGTGTGTG	ACTGTGTTTGGGGAGCAGAC	56	30	PCR-RFLP (<i>Hpy</i> 8 I)	
	rs8056505	T > C	AGGGGAGCCAGAATTTGATC	GCTCCACCACACACAGCTAT	56	30	PCR-RFLP (<i>Hpy</i> CH4 IV)	
	<i>CASP1</i>	rs2282659	A > G	GGAGCGGGGTGAAACTAAAT	CCACAATGGGCTCTGTTTTT	54	30	PCR-RFLP (<i>Ban</i> II)
	<i>IL18</i>	rs5744247	C > G	GAGAAAGAGGGCCACCAAAT	TGGCTAGTACTGAAGTGAATC	54	30	PCR-RFLP (<i>Ple</i> I)
		rs2043055	A > G	CCTCCACCTGAAAGCCAAT	TCCTGGGAGGATCTTCTGA	55	30	PCR-RFLP (<i>Rsa</i> I)
		rs7106524	G > A	GTGGCTGAACTCACCACAGA	CTTTCAGGCCAGGTGACTA	57	50	PCR-HRM
		rs360717	C > G	CCATGGCTGACTTTCCAAAT	GCACAGAGCCCAACTTTTA	54	30	PCR-RFLP (<i>Aci</i> I)
	<i>IL1B</i>	rs1143643	G > A	AAAAGCCCTGGAAACTAGG	CACATGATCAGGAGCCAGAC	54	50	PCR-HRM
	rs1143633	A > G	TGTCTTAGCCACCCACTC	TCCATATCCTGCTCCCTGGAG	56	30	PCR-RFLP (<i>Fun</i> 4H I)	
	rs3136558	T > C	GGTGTGCAAAAGCCACATT	GAGGAGGAAAGGGCTTGA	55	30	PCR-RFLP (<i>Mbo</i> I)	
	rs1143630	C > A	AGTGGGGCATGTACAAAAA	AGATTATCCCTCTGTAAGCTC	54	50	PCR-HRM	
	rs16944	G > A	GCCCTCCCTGTCTGTATTGA	AGGCACCTTGTGCTGCTC	56	30	PCR-RFLP (<i>Ava</i> I)	
	rs1143623	G > C	ATCAGAAGGCTGCTTGGAGA	AAACCTTGCTCCTCTGGTT	56	30	PCR-RFLP (<i>Bse</i> D I)	

Table 3. Allele and genotype comparisons in three inheritance models between responders and non-responders to IFX after 10 weeks of treatment for CD patients.

Gene	Tag SNP (Major > Minor)	Genotype	Number (%) of CD patients		Inheritance model*	P value	OR	95% CI	
			Responders (n = 116)	Non-responders (n = 11)					
<i>P2RX7</i>	rs684201	MAF	0.211	0.182	Allele	1.000	1.205	0.390-3.724	
		G > A	G/G	72 (62.1)	7 (63.6)	Dominant	1.000	1.069	0.296-3.864
		G/A	39 (33.6)	4 (36.4)	Recessive	1.000	1.135	0.059-21.87	
	rs656612	MAF	0.306	0.409	Allele	0.320	0.637	0.260-1.558	
		A > C	A/A	52 (48.8)	3 (27.3)	Dominant	0.347	0.462	0.117-1.828
		A/C	57 (49.1)	7 (63.6)	Recessive	0.526	0.642	0.072-5.757	
	rs11065450	MAF	0.332	0.409	Allele	0.465	0.718	0.294-1.752	
		C > A	C/C	51 (44.0)	3 (27.3)	Dominant	0.352	0.478	0.121-1.894
		C/A	53 (45.7)	7 (63.6)	Recessive	1.000	1.154	0.136-9.814	
	rs1718125	MAF	0.263	0.273	Allele	0.921	0.951	0.356-2.541	
G > A		G/G	63 (54.3)	5 (45.5)	Dominant	0.754	0.701	0.203-2.427	
G/A		45 (38.8)	6 (54.5)	Recessive	1.000	1.802	0.097-33.31		
rs1186055	MAF	0.392	0.590	Allele	0.070	0.447	0.184-1.088		
	G > T	G/G	42 (36.2)	1 (9.1)	Dominant	0.097	0.176	0.022-1.425	
	G/T	57 (49.1)	7 (63.6)	Recessive	0.378	0.458	0.110-1.900		
rs208296	MAF	0.319	0.318	Allele	0.994	1.004	0.393-2.566		

	C > T	C/C	51 (44.0)	4 (36.4)				
		C/T	56 (48.3)	7 (63.6)	Dominant	0.756	0.728	0.202-2.625
		T/T	9 (7.8)	0 (0.0)	Recessive	1.000	2.033	0.111-37.27
rs11065464		MAF	0.237	0.045	Allele	0.055	6.527	0.858-49.51
	C > A	C/C	67 (57.8)	10 (90.9)				
		C/A	43 (37.1)	1 (9.1)	Dominant	0.049	7.315	0.906-59.17
		A/A	6 (5.2)	0 (0.0)	Recessive	1.000	1.353	0.071-25.61
rs208307		MAF	0.147	0.318	Allele	0.205	0.536	0.201-1.425
	G > C	G/G	86 (74.1)	6 (54.5)				
		G/C	26 (22.4)	3 (27.3)	Dominant	0.174	0.419	0.119-1.472
		C/C	4 (3.4)	2 (18.2)	Recessive	0.085	0.161	0.026-1.000
rs7958311		MAF	0.349	0.182	Allele	0.156	2.414	0.790-7.375
	G > A	G/G	53 (45.7)	8 (72.7)				
		G/A	45 (38.8)	2 (18.2)	Dominant	0.117	3.170	0.800-12.55
		A/A	18 (15.5)	1 (9.1)	Recessive	1.000	1.837	0.221-15.24
rs1653609		MAF	0.280	0.364	Allele	0.408	0.681	0.273-1.700
	A > C	A/A	59 (50.9)	4 (36.4)				
		A/C	49 (42.2)	6 (54.5)	Dominant	0.530	0.552	0.153-1.988
		C/C	8 (6.9)	1 (9.1)	Recessive	0.570	0.741	0.084-6.536
rs3751143		MAF	0.319	0.455	Allele	0.196	0.562	0.232-1.361
	T > G	T/T	54 (46.6)	2 (27.3)				
		T/G	50 (43.1)	6 (54.5)	Dominant	0.343	0.431	0.109-1.705
		G/G	12 (10.3)	2 (18.2)	Recessive	0.348	0.519	0.100-2.689
<i>CARD8</i>	rs1971783	MAF	0.478	0.455	Allele	0.830	1.101	0.458-2.648
	T > C	T/T	28 (24.1)	3 (27.3)				
		T/C	65 (56.0)	6 (54.5)	Dominant	0.729	1.179	0.293-4.748
		C/C	23 (19.8)	2 (18.2)	Recessive	1.000	1.113	0.225-5.507
rs4389238		MAF	0.297	0.182	Allele	0.328	1.905	0.622-5.834
	C > T	C/C	56 (48.3)	7 (63.6)				
		C/T	51 (44.0)	4 (36.4)	Dominant	0.364	1.875	0.521-6.752
		T/T	9 (7.8)	0 (0)	Recessive	1.000	2.033	0.111-37.03
rs4802448		MAF	0.310	0.227	Allele	0.478	1.530	0.543-4.308
	G > A	G/G	58 (50.0)	7 (63.6)				
		G/A	44 (37.9)	3 (27.3)	Dominant	0.531	1.750	0.486-6.301
		A/A	14 (12.1)	1 (9.1)	Recessive	1.000	1.372	0.163-11.55
rs4802449		MAF	0.220	0.455	Allele	0.014	0.338	0.138-0.827
	G > A	G/G	70 (60.3)	3 (27.3)				
		G/A	41 (35.3)	6 (54.5)	Dominant	0.053	0.246	0.062-0.978
		A/A	5 (4.3)	2 (18.2)	Recessive	0.113	0.203	0.034-1.196
rs10418189		MAF	0.474	0.318	Allele	0.161	1.932	0.760-4.914
	G > A	G/G	31 (26.7)	5 (45.5)				
		G/A	60 (51.7)	5 (45.5)	Dominant	0.291	2.285	0.651-8.026
		A/A	25 (21.6)	1 (9.1)	Recessive	0.459	2.747	0.335-22.47
rs16981845		MAF	0.034	0.136	Allele	0.001	0.032	0.007-0.130
	T > C	T/T	108 (93.1)	8 (72.7)				
		T/C	8 (6.9)	3 (27.3)	Dominant	0.055	0.198	0.044-0.894
		C/C	0 (0)	0 (0)	Recessive	-	-	-
rs11670259		MAF	0.172	0.364	Allele	0.029	0.365	0.143-0.927
	C > T	C/C	79 (68.1)	4 (36.4)				
		C/T	34 (29.3)	6 (54.5)	Dominant	0.047	0.268	0.074-0.971
		T/T	3 (2.6)	1 (9.1)	Recessive	0.307	0.265	0.025-2.794
rs12984929		MAF	0.349	0.500	Allele	0.159	0.536	0.223-1.291
	G > T	G/G	52 (44.8)	2 (18.2)				
		G/T	47 (40.5)	7 (63.6)	Dominant	0.115	0.274	0.057-1.322
		T/T	17 (14.7)	2 (18.2)	Recessive	0.669	0.773	0.153-3.890
rs11672725		MAF	0.190	0.318	Allele	0.150	0.502	0.193-1.304
	C > T	C/C	74 (63.8)	5 (45.5)				
		C/T	40 (34.5)	5 (45.5)	Dominant	0.330	0.473	0.136-1.644
		T/T	2 (1.7)	1 (9.1)	Recessive	0.240	0.175	0.015-2.107
rs6509368		MAF	0.388	0.273	Allele	0.287	1.690	0.638-4.480
	G > A	G/G	47 (40.5)	5 (45.5)				
		G/A	48 (41.4)	6 (54.5)	Dominant	0.758	1.223	0.353-4.243

		A/A	21 (18.1)	0 (0)	Recessive	0.209	5.178	0.293-91.37
<i>PYCARD</i>	rs8056505	MAF	0.159	0.136	Allele	1.000	1.202	0.338-4.268
		T > C	82 (70.2)	8 (72.7)				
		T/T	31 (26.7)	3 (27.3)	Dominant	1.000	1.106	0.277-4.421
		T/C	3 (2.6)	0 (0)	Recessive	1.000	0.709	0.034-14.61
<i>CASPI</i>	rs2282659	MAF	0.273	0.364	Allele	0.358	0.652	0.261-1.630
		A > G	59 (50.9)	3 (27.3)				
		A/A	51 (44.0)	8 (72.7)	Dominant	0.207	0.362	0.092-1.434
		A/G	6 (5.2)	0 (0)	Recessive	1.000	1.353	0.070-25.61
<i>IL18</i>	rs5744247	MAF	0.440	0.364	Allele	0.492	1.373	0.555-3.399
		C > G	34 (29.3)	6 (54.5)				
		C/C	62 (53.4)	2 (18.2)	Dominant	0.099	2.894	0.827-10.12
		C/G	20 (17.2)	3 (27.3)	Recessive	0.418	0.556	0.135-2.279
	rs2043055	MAF	0.414	0.364	Allele	0.648	1.235	0.499-3.060
		A > G	39 (33.6)	5 (45.5)				
		A/A	58 (50.0)	4 (36.4)	Dominant	0.512	1.645	0.472-5.731
		A/G	19 (16.4)	2 (18.2)	Recessive	1.000	0.881	0.176-4.405
	rs7106524	MAF	0.414	0.364	Allele	0.648	1.235	0.499-3.060
		G > A	38 (32.8)	4 (36.4)				
		G/A	60 (51.7)	6 (54.5)	Dominant	0.752	1.173	0.323-4.254
		A/A	18 (15.5)	1 (9.1)	Recessive	1.000	1.837	0.221-15.24
	rs360717	MAF	0.121	0.227	Allele	0.179	0.467	0.160-1.364
		C > G	89 (76.7)	6 (54.5)				
		C/C	26 (22.4)	5 (45.5)	Dominant	0.143	0.364	0.103-1.287
		C/G	1 (0.9)	0 (0)	Recessive	1.000	0.299	0.011-7.768
<i>IL1B</i>	rs1143643	MAF	0.422	0.545	Allele	0.266	0.609	0.253-1.468
		G > A	38 (32.8)	1 (9.1)				
		G/A	58 (50.0)	8 (72.7)	Dominant	0.171	0.205	0.025-1.663
		A/A	20 (17.2)	2 (18.2)	Recessive	1.000	0.937	0.188-4.673
	rs1143633	MAF	0.405	0.545	Allele	0.202	0.568	0.236-1.367
		A > G	38 (32.8)	2 (18.2)				
		A/A	62 (53.4)	6 (54.5)	Dominant	0.500	0.456	0.094-2.216
		A/G	16 (13.8)	3 (27.3)	Recessive	0.213	0.427	0.102-1.779
	rs3136558	MAF	0.491	0.364	Allele	0.252	1.691	0.683-4.184
		T > C	27 (23.3)	4 (36.4)				
		T/C	64 (55.2)	6 (54.5)	Dominant	0.461	1.884	0.512-6.925
		C/C	25 (21.6)	1 (9.1)	Recessive	0.459	2.747	0.335-22.47
	rs1143630	MAF	0.151	0.273	Allele	0.138	0.474	0.173-1.294
		C > A	84 (72.4)	7 (63.6)				
		C/A	29 (25.0)	2 (18.2)	Dominant	0.505	0.667	0.183-2.432
		A/A	3 (2.6)	2 (18.2)	Recessive	0.059	0.119	0.018-0.810
	rs16944	MAF	0.427	0.682	Allele	0.022	0.347	0.136-0.884
		G > A	38 (32.8)	0 (0)				
		G/A	57 (49.1)	7 (63.6)	Dominant	0.033	0.089	0.005-1.545
		A/A	21 (18.1)	4 (36.4)	Recessive	0.225	0.387	0.104-1.443
	rs1143623	MAF	0.336	0.636	Allele	0.005	0.289	0.116-0.719
		G > C	52 (44.8)	1 (9.1)				
		G/C	50 (43.1)	6 (54.5)	Dominant	0.025	0.123	0.015-0.993
		C/C	14 (12.1)	4 (36.4)	Recessive	0.050	0.240	0.062-0.926

*Allele: allele model; Dominant: the minor allele dominant model; Recessive: the minor allele recessive model. (IFX, infliximab; CD, Crohn's disease; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency).

There were no significant differences in the frequencies of any other alleles and genotypes at tag SNPs between responders and non-responders after 1 year of treatment.

Association of tag SNPs with response to IFX after 2 years of treatment

The frequencies and distributions of minor alleles and genotypes at tag SNPs in each gene were identified and compared between responders and non-responders to IFX after 2 years of treatment (Table 5).

No significant differences in the frequencies of alleles and genotypes at tag SNPs were observed between responders and non-responders after 2 years of treatment (Table 5).

The interaction of genetic and environmental factors in response to IFX after 10 weeks of treatment

Univariate analyses of the differences in the frequencies of the genotypes between responders and non-responders indicated that genetic factors, the C/C genotype of rs11670259 in *CARD8* and the G/G genotype of rs1143623 in *IL1B*, as well as the environmental factor

Table 4. Allele and genotype comparisons in three inheritance models between responders and non-responders to IFX after 1 year of treatment for CD patients.

Gene	Tag SNP (Major > Minor)	Genotype	Number (%) of CD patients		Inheritance model*	P value	OR	95% CI
			Responders (n = 97)	Non-responders (n = 19)				
<i>P2RX7</i>	rs684201 G > A	MAF	0.222	0.158	Allele	0.379	1.519	0.596-3.870
		G/G	59 (60.8)	13 (68.4)				
		G/A	33 (34.0)	6 (31.6)	Dominant	0.533	1.395	0.488-3.987
	rs656612 A > C	A/A	5 (5.2)	0 (0)	Recessive	0.590	2.319	0.123-43.72
		MAF	0.320	0.237	Allele	0.312	1.514	0.676-3.390
		A/A	41 (42.3)	11 (57.9)				
	rs11065450 C > A	A/C	50 (51.5)	7 (36.8)	Dominant	0.210	1.878	0.694-5.084
		C/C	6 (6.2)	1 (5.3)	Recessive	1.000	1.187	0.135-10.46
		MAF	0.361	0.184	Allele	0.035	2.500	1.046-5.974
	rs1718125 G > A	C/C	39 (40.2)	12 (63.2)				
		C/A	46 (47.4)	7 (36.8)	Dominant	0.065	2.550	0.922-7.047
		A/A	12 (12.4)	0 (0)	Recessive	0.211	5.702	0.323-100.6
rs1186055 G > T	MAF	0.273	0.211	Allele	0.422	1.410	0.608-3.270	
	G/G	51 (52.6)	12 (63.2)					
	G/A	39 (40.2)	6 (31.6)	Dominant	0.397	1.546	0.561-4.261	
rs208296 C > T	A/A	7 (7.2)	1 (5.3)	Recessive	1.000	1.400	0.162-12.09	
	MAF	0.392	0.395	Allele	0.973	0.988	0.485-2.012	
	G/G	36 (37.1)	6 (31.6)					
rs11065464 C > A	G/T	46 (47.4)	11 (57.9)	Dominant	0.646	0.782	0.273-2.238	
	T/T	15 (15.5)	2 (10.5)	Recessive	0.735	1.555	0.325-7.435	
	MAF	0.340	0.211	Allele	0.117	1.933	0.839-4.454	
rs208307 G > C	C/C	40 (41.2)	11 (57.9)					
	C/T	48 (49.5)	8 (42.1)	Dominant	0.181	1.959	0.723-5.308	
	T/T	9 (9.3)	0 (0)	Recessive	0.352	4.186	0.234-75.06	
rs7958311 G > A	MAF	0.247	0.184	Allele	0.402	1.456	0.602-3.520	
	C/C	54 (55.7)	13 (68.4)					
	C/A	38 (39.2)	5 (26.3)	Dominant	0.304	1.725	0.606-4.916	
rs1653609 A > C	A/A	5 (5.2)	1 (5.3)	Recessive	1.000	0.978	0.108-8.881	
	MAF	0.155	0.105	Allele	0.616	1.555	0.514-4.704	
	G/G	70 (72.2)	16 (84.2)					
rs3751143 T > G	G/C	24 (24.7)	2 (10.5)	Dominant	0.393	2.057	0.555-7.628	
	C/C	3 (3.1)	1 (5.3)	Recessive	0.516	0.574	0.057-5.838	
	MAF	0.361	0.289	Allele	0.399	1.386	0.648-2.962	
rs4389238 C > T	G/G	41 (42.3)	12 (63.2)					
	G/A	42 (43.3)	3 (15.8)	Dominant	0.095	2.341	0.848-6.464	
	A/A	14 (14.4)	4 (21.1)	Recessive	0.492	0.633	0.183-2.185	
rs4802448 G > A	MAF	0.294	0.211	Allele	0.296	1.560	0.674-3.610	
	A/A	46 (47.4)	13 (68.4)					
	A/C	45 (46.4)	4 (21.1)	Dominant	0.094	2.402	0.844-6.840	
rs4802449 G > A	C/C	6 (6.2)	2 (10.5)	Recessive	0.616	0.560	0.104-3.013	
	MAF	0.289	0.474	Allele	0.025	0.451	0.222-0.916	
	T/T	47 (48.5)	7 (36.8)					
<i>CARD8</i>	T/G	44 (45.5)	6 (31.6)	Dominant	0.354	0.621	0.225-1.710	
	G/G	6 (6.2)	6 (31.6)	Recessive	0.001	0.143	0.040-0.510	
	MAF	0.490	0.421	Allele	0.439	1.319	0.653-2.665	
rs4802448 G > A	T/T	21 (21.6)	7 (36.8)					
	T/C	57 (58.8)	8 (42.1)	Dominant	0.157	2.111	0.739-6.031	
	C/C	19 (19.6)	4 (21.1)	Recessive	1.000	0.913	0.272-3.068	
rs4802448 G > A	MAF	0.268	0.447	Allele	0.027	0.452	0.222-0.924	
	C/C	52 (53.6)	4 (21.1)					
	C/T	38 (39.2)	13 (68.4)	Dominant	0.012	0.231	0.071-0.746	
rs4802448 G > A	T/T	7 (7.2)	2 (10.5)	Recessive	0.640	0.661	0.126-3.459	
	MAF	0.309	0.316	Allele	0.937	0.970	0.459-2.051	
	G/G	48 (49.5)	10 (52.6)					
rs4802449 G > A	G/A	38 (39.2)	6 (31.6)	Dominant	0.802	1.134	0.424-3.036	
	A/A	11 (11.3)	3 (15.8)	Recessive	0.699	0.682	0.171-2.722	
	MAF	0.216	0.237	Allele	0.782	0.890	0.391-2.026	
rs4802449 G > A	G/G	60 (61.9)	10 (52.6)					

		G/A	32 (33.0)	9 (47.4)	Dominant	0.452	0.685	0.255-1.843
		A/A	5 (5.2)	0 (0)	Recessive	0.590	2.319	0.123-43.72
	rs10418189	MAF	0.479	0.447	Allele	0.718	1.137	0.566-2.288
	G > A	G/G	25 (25.8)	6 (31.6)				
		G/A	51 (52.6)	9 (47.4)	Dominant	0.601	1.329	0.456-3.871
		A/A	21 (21.6)	4 (21.1)	Recessive	1.000	1.036	0.311-3.454
	rs16981845	MAF	0.036	0.026	Allele	1.000	1.385	0.165-11.49
	T > C	T/T	90 (92.8)	18 (94.7)				
		T/C	7 (7.2)	1 (5.3)	Dominant	1.000	0.714	0.083-6.169
		C/C	0 (0)	0 (0)	Recessive	-	-	-
	rs11670259	MAF	0.170	0.184	Allele	0.833	0.908	0.368-2.236
	C > T	C/C	67 (69.1)	12 (63.2)				
		C/T	27 (27.8)	7 (36.8)	Dominant	0.613	0.768	0.275-2.143
		T/T	3 (3.1)	0 (0)	Recessive	1.000	1.444	0.071-29.41
	rs12984929	MAF	0.351	0.342	Allele	0.921	1.038	0.499-2.158
	G > T	G/G	44 (45.4)	8 (42.1)				
		G/T	38 (39.2)	9 (47.4)	Dominant	0.794	0.876	0.324-2.369
		T/T	15 (15.5)	2 (10.5)	Recessive	0.735	1.555	0.325-7.435
	rs11672725	MAF	0.186	0.211	Allele	0.720	0.854	0.362-2.019
	C > T	C/C	63 (64.9)	11 (57.9)				
		C/T	32 (33.0)	8 (42.1)	Dominant	0.559	0.742	0.273-2.021
		T/T	2 (2.1)	0 (0)	Recessive	1.000	1.021	0.047-22.22
	rs6509368	MAF	0.376	0.447	Allele	0.411	0.745	0.369-1.504
	G > A	G/G	41 (42.3)	6 (31.6)				
		G/A	49 (40.2)	9 (47.4)	Dominant	0.386	0.630	0.221-1.798
		A/A	17 (17.5)	4 (21.1)	Recessive	0.747	0.797	0.235-2.701
<i>PYCARD</i>	rs8056505	MAF	0.165	0.132	Allele	0.809	1.304	0.473-3.595
	T > C	T/T	68 (70.1)	14 (73.7)				
		T/C	26 (26.8)	5 (26.3)	Dominant	1.000	1.194	0.394-3.623
		C/C	3 (3.1)	0 (0)	Recessive	1.000	1.444	0.072-29.12
<i>CASPI</i>	rs2282659	MAF	0.309	0.079	Allele	0.003	5.225	1.546-17.67
	A > G	A/A	43 (44.3)	16 (84.2)				
		A/G	48 (49.5)	3 (15.8)	Dominant	0.002	6.698	1.832-24.51
		G/G	6 (6.2)	0 (0)	Recessive	0.587	2.770	0.150-51.29
<i>IL18</i>	rs5744247	MAF	0.443	0.421	Allele	0.801	1.095	0.542-2.213
	C > G	C/C	29 (29.9)	5 (26.3)				
		C/G	50 (51.5)	12 (63.2)	Dominant	1.000	0.837	0.276-2.541
		G/G	18 (18.6)	2 (10.5)	Recessive	0.521	1.937	0.410-9.141
	rs2043055	MAF	0.407	0.447	Allele	0.646	0.849	0.421-1.710
	A > G	A/A	35 (36.1)	4 (21.1)				
		A/G	45 (46.4)	13 (68.4)	Dominant	0.290	0.472	0.145-1.535
		G/G	17 (17.5)	2 (10.5)	Recessive	0.735	1.806	0.381-8.562
	rs7106524	MAF	0.402	0.474	Allele	0.412	0.747	0.372-1.502
	G > A	G/G	34 (35.1)	4 (21.1)				
		G/A	48 (49.1)	12 (63.2)	Dominant	0.293	0.494	0.152-1.607
		A/A	15 (15.5)	3 (15.8)	Recessive	1.000	0.976	0.253-3.765
	rs360717	MAF	0.119	0.132	Allele	0.788	0.888	0.315-2.503
	C > G	C/C	75 (77.3)	14 (73.7)				
		C/G	21 (21.6)	5 (26.3)	Dominant	0.769	0.821	0.266-2.533
		G/G	1 (1.0)	0 (0)	Recessive	1.000	0.606	0.024-15.45
<i>IL1B</i>	rs1143643	MAF	0.428	0.395	Allele	0.706	1.147	0.564-2.331
	G > A	G/G	31 (32.0)	7 (36.8)				
		G/A	49 (50.5)	9 (47.4)	Dominant	0.678	1.242	0.446-3.463
		A/A	17 (17.5)	3 (15.8)	Recessive	1.000	1.133	0.297-4.327
	rs1143633	MAF	0.421	0.368	Allele	0.614	1.203	0.586-2.468
	A > G	A/A	31 (32.0)	7 (36.8)				
		A/G	52 (53.6)	10 (52.6)	Dominant	0.678	1.242	0.446-3.463
		G/G	14 (14.4)	2 (10.5)	Recessive	1.000	1.434	0.298-6.897
	rs3136558	MAF	0.469	0.605	Allele	0.125	0.576	0.284-1.171
	T > C	T/T	25 (25.8)	2 (10.5)				
		T/C	53 (54.6)	11 (57.9)	Dominant	0.235	0.339	0.073-1.571
		C/C	19 (19.6)	6 (31.6)	Recessive	0.245	0.528	0.178-1.569

	rs1143630	MAF	0.144	0.184	Allele	0.530	0.747	0.300-1.861
	C > A	C/C	72 (74.2)	12 (63.2)				
		C/A	22 (22.7)	7 (36.8)	Dominant	0.324	0.595	0.211-1.680
		A/A	3 (3.1)	0 (0)	Recessive	1.000	1.444	0.072-29.12
	rs16944	MAF	0.448	0.316	Allele	0.131	1.762	0.840-3.693
	G > A	G/G	30 (30.9)	8 (42.1)				
		G/A	47 (48.5)	10 (52.6)	Dominant	0.343	1.624	0.593-4.448
		A/A	20 (20.6)	1 (5.3)	Recessive	0.190	4.675	0.588-37.18
	rs1143623	MAF	0.351	0.263	Allele	0.297	1.511	0.693-3.296
	G > C	G/G	42 (43.3)	10 (52.6)				
		G/C	42 (43.3)	8 (42.1)	Dominant	0.455	1.455	0.543-3.900
		C/C	13 (13.4)	1 (5.3)	Recessive	0.461	2.786	0.342-22.68

* Allele: allele model; Dominant: the minor allele dominant model; Recessive: the minor allele recessive model. (IFX, infliximab; CD, Crohn's disease; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency).

Table 5. Allele and genotype comparisons in three inheritance models between responders and non-responders to IFX after 2 years of treatment for CD patients.

Gene	Tag SNP (Major > Minor)	Genotype	Number (%) of CD patients		Inheritance model*	P value	OR	95% CI
			Responders (n = 82)	Non-responders (n = 15)				
<i>P2RX7</i>	rs684201	MAF	0.244	0.100	Allele	0.096	2.904	0.836-10.08
	G > A	G/G	47 (57.3)	12 (80.0)				
		G/A	30 (36.6)	3 (20.0)	Dominant	0.150	2.979	0.781-11.36
		A/A	5 (6.1)	0 (0)	Recessive	1.000	2.200	0.116-41.89
	rs656612	MAF	0.317	0.333	Allele	0.861	0.929	0.406-2.124
	A > C	A/A	36 (43.9)	5 (33.3)				
		A/C	40 (48.8)	10 (66.7)	Dominant	0.574	0.639	0.201-2.035
		C/C	6 (7.3)	0 (0)	Recessive	0.586	2.634	0.141-49.26
	rs11065450	MAF	0.372	0.300	Allele	0.451	1.382	0.595-3.209
	C > A	C/C	33 (40.2)	6 (40.0)				
		C/A	37 (45.1)	9 (60.0)	Dominant	0.986	0.990	0.322-3.044
		A/A	12 (14.6)	0 (0)	Recessive	0.203	5.496	0.308-97.95
	rs1718125	MAF	0.299	0.133	Allele	0.075	2.769	0.918-8.361
	G > A	G/G	40 (48.8)	11 (73.3)				
		G/A	35 (42.7)	4 (26.7)	Dominant	0.097	2.888	0.849-9.814
		A/A	7 (8.5)	0 (0)	Recessive	0.591	3.079	0.167-56.82
	rs1186055	MAF	0.396	0.367	Allele	0.760	1.134	0.507-2.539
	G > T	G/G	31 (37.8)	5 (33.3)				
		G/T	37 (45.1)	9 (60.0)	Dominant	1.000	0.823	0.257-2.631
		T/T	14 (17.1)	1 (6.7)	Recessive	0.454	2.883	0.350-23.75
	rs208296	MAF	0.335	0.367	Allele	0.739	0.872	0.388-1.960
	C > T	C/C	35 (42.7)	5 (33.3)				
		C/T	39 (47.6)	9 (60.0)	Dominant	0.578	0.671	0.211-2.140
		T/T	8 (9.8)	1 (6.7)	Recessive	1.000	1.514	0.175-13.07
	rs11065464	MAF	0.256	0.200	Allele	0.513	1.377	0.527-3.600
	C > A	C/C	44 (53.7)	10 (66.7)				
		C/A	34 (41.5)	4 (26.7)	Dominant	0.408	1.727	0.543-5.498
		A/A	4 (4.9)	1 (6.7)	Recessive	0.577	0.718	0.075-6.906
	rs208307	MAF	0.140	0.233	Allele	0.195	0.536	0.206-1.391
	G > C	G/G	62 (75.6)	8 (53.3)				
		G/C	17 (20.7)	7 (46.7)	Dominant	0.077	0.369	0.119-1.144
		C/C	3 (3.7)	0 (0)	Recessive	1.000	1.365	0.067-27.78
	rs7958311	MAF	0.366	0.333	Allele	0.733	1.154	0.507-2.627
	G > A	G/G	34 (41.5)	7 (46.7)				
		G/A	36 (43.9)	6 (40.0)	Dominant	0.708	1.235	0.409-3.731
		A/A	12 (14.6)	2 (13.3)	Recessive	1.000	1.114	0.223-5.574
	rs1653609	MAF	0.293	0.300	Allele	0.936	0.966	0.413-2.259
	A > C	A/A	40 (48.8)	6 (40.0)				
		A/C	36 (43.9)	9 (60.0)	Dominant	0.531	0.700	0.228-2.145
		C/C	6 (7.3)	0 (0)	Recessive	0.586	2.634	0.141-49.26
	rs3751143	MAF	0.262	0.433	Allele	0.057	0.465	0.209-1.036
	T > G	T/T	43 (52.4)	4 (26.7)				

		T/G	35 (42.7)	9 (60.0)	Dominant	0.092	0.330	0.097-1.121
		G/G	4 (4.9)	2 (13.3)	Recessive	0.232	0.333	0.055-2.009
<i>CARD8</i>	rs1971783	MAF	0.512	0.367	Allele	0.143	1.814	0.812-4.049
	T > C	T/T	17 (20.7)	4 (26.7)				
		T/C	46 (56.1)	11 (73.3)	Dominant	0.733	1.390	0.393-4.916
		C/C	19 (23.2)	0 (0)	Recessive	0.037	0.105	0.006-1.838
	rs4389238	MAF	0.274	0.233	Allele	0.641	1.243	0.499-3.096
	C > T	C/C	43 (52.4)	9 (60.0)				
		C/T	33 (40.2)	5 (33.3)	Dominant	0.589	1.361	0.444-4.170
		T/T	6 (7.3)	1 (6.7)	Recessive	1.000	1.105	0.123-9.901
	rs4802448	MAF	0.299	0.367	Allele	0.460	0.736	0.326-1.662
	G > A	G/G	41 (50.0)	7 (46.7)				
		G/A	33 (40.2)	5 (33.3)	Dominant	0.812	0.875	0.290-2.636
		A/A	8 (9.8)	3 (20.0)	Recessive	0.368	0.432	0.100-1.863
	rs4802449	MAF	0.201	0.300	Allele	0.227	0.588	0.246-1.402
	G > A	G/G	52 (63.4)	8 (53.3)				
		G/A	27 (32.9)	5 (33.3)	Dominant	0.460	0.659	0.217-2.000
		A/A	3 (3.7)	2 (13.3)	Recessive	0.170	0.247	0.038-1.622
	rs10418189	MAF	0.506	0.333	Allele	0.082	2.049	0.904-4.647
	G > A	G/G	20 (24.4)	5 (33.3)				
		G/A	41 (50.0)	10 (66.7)	Dominant	0.525	1.550	0.473-5.074
		A/A	21 (25.6)	0 (0)	Recessive	0.036	10.840	0.621-189.0
	rs16981845	MAF	0.037	0.033	Allele	1.000	1.101	0.128-9.524
	T > C	T/T	76 (92.7)	14 (93.3)				
		T/C	6 (7.3)	1 (6.7)	Dominant	1.000	0.905	0.101-8.108
		C/C	0 (0)	0 (0)	Recessive	-	-	-
	rs11670259	MAF	0.152	0.267	Allele	0.126	0.495	0.198-1.234
	C > T	C/C	59 (72.0)	8 (53.3)				
		C/T	21 (25.6)	6 (40.0)	Dominant	0.152	0.446	0.145-1.369
		T/T	2 (2.4)	1 (6.7)	Recessive	0.399	0.350	0.030-4.124
	rs12984929	MAF	0.341	0.400	Allele	0.537	0.778	0.350-1.729
	G > T	G/G	38 (46.3)	6 (40.0)				
		G/T	32 (39.0)	6 (40.0)	Dominant	0.650	0.772	0.252-2.367
		T/T	12 (14.6)	3 (20.0)	Recessive	0.697	0.686	0.168-2.796
	rs11672725	MAF	0.171	0.267	Allele	0.214	0.566	0.229-1.401
	C > T	C/C	55 (67.1)	8 (53.3)				
		C/T	26 (31.7)	6 (40.0)	Dominant	0.305	0.561	0.184-1.709
		T/T	1 (1.2)	1 (6.7)	Recessive	0.287	0.173	0.010-2.927
	rs6509368	MAF	0.372	0.400	Allele	0.771	0.888	0.401-1.969
	G > A	G/G	35 (42.7)	6 (40.0)				
		G/A	33 (40.2)	6 (40.0)	Dominant	0.847	0.895	0.292-2.749
		A/A	14 (17.1)	3 (20.0)	Recessive	0.723	0.824	0.205-3.306
	<i>PYCARD</i>	MAF	0.177	0.100	Allele	0.424	1.933	0.549-6.807
	T > C	T/T	56 (68.3)	12 (80.0)				
		T/C	23 (28.0)	2 (20.0)	Dominant	0.542	1.857	0.482-7.148
		C/C	3 (3.7)	0 (0)	Recessive	1.000	1.365	0.067-27.78
	rs2282659	MAF	0.317	0.267	Allele	0.583	1.277	0.533-3.058
	A > G	A/A	35 (42.7)	8 (53.3)				
		A/G	42 (51.2)	6 (40.0)	Dominant	0.445	1.535	0.508-4.632
		G/G	5 (6.1)	1 (6.7)	Recessive	1.000	0.909	0.099-8.382
	rs5744247	MAF	0.457	0.367	Allele	0.358	1.456	0.652-3.251
	C > G	C/C	24 (29.3)	5 (33.3)				
		C/G	41 (50.0)	9 (60.0)	Dominant	0.765	1.208	0.373-3.909
		G/G	17 (20.7)	1 (6.7)	Recessive	0.290	3.662	0.449-29.85
	rs2043055	MAF	0.409	0.400	Allele	0.930	1.036	0.468-2.292
	A > G	A/A	30 (36.6)	5 (33.3)				
		A/G	37 (45.1)	8 (53.3)	Dominant	1.000	0.867	0.271-2.775
		G/G	15 (18.3)	2 (13.3)	Recessive	1.000	1.455	0.297-7.138
	rs7106524	MAF	0.396	0.433	Allele	0.704	0.859	0.391-1.886
	G > A	G/G	29 (35.4)	5 (33.3)				
		G/A	41 (50.0)	7 (46.7)	Dominant	1.000	0.914	0.285-2.930
		A/A	12 (14.6)	3 (20.0)	Recessive	0.697	0.686	0.168-2.796

	rs360717	MAF	0.104	0.200	Allele	0.133	0.463	0.166-1.290
	C > G	C/C	66 (80.5)	9 (60.0)				
		C/G	15 (18.3)	6 (40.0)	Dominant	0.082	0.364	0.113-1.170
		G/G	1 (1.2)	0 (0)	Recessive	1.000	0.571	0.022-14.67
<i>IL1B</i>	rs1143643	MAF	0.415	0.500	Allele	0.385	0.708	0.325-1.546
	G > A	G/G	27 (32.9)	4 (26.7)				
		G/A	42 (51.2)	7 (46.7)	Dominant	0.768	0.741	0.216-2.543
		A/A	13 (15.9)	4 (26.7)	Recessive	0.293	0.518	0.143-1.880
	rs1143633	MAF	0.396	0.500	Allele	0.289	0.657	0.301-1.434
	A > G	A/A	27 (32.9)	4 (26.7)				
		A/G	45 (54.9)	7 (46.7)	Dominant	0.768	0.741	0.216-2.543
		G/G	10 (12.2)	4 (26.7)	Recessive	0.222	0.382	0.102-1.432
	rs3136558	MAF	0.470	0.467	Allele	0.977	1.012	0.464-2.207
	T > C	T/T	21 (25.6)	4 (26.7)				
		T/C	45 (54.9)	8 (53.3)	Dominant	1.000	1.056	0.303-3.676
		C/C	16 (19.5)	3 (20.0)	Recessive	1.000	0.970	0.244-3.846
	rs1143630	MAF	0.152	0.100	Allele	0.580	1.619	0.456-5.744
	C > A	C/C	60 (73.2)	12 (80.0)				
		C/A	19 (23.2)	3 (20.0)	Dominant	0.753	1.467	0.378-5.692
		A/A	3 (3.7)	0 (0)	Recessive	1.000	1.365	0.067-27.780
	rs16944	MAF	0.445	0.467	Allele	0.827	0.917	0.420-2.001
	G > A	G/G	26 (31.7)	4 (26.7)				
		G/A	29 (47.6)	8 (53.3)	Dominant	0.772	0.783	0.228-2.694
		A/A	17 (20.7)	3 (20.0)	Recessive	1.000	1.046	0.265-4.131
	rs1143623	MAF	0.354	0.333	Allele	0.830	1.094	0.480-2.494
	G > C	G/G	35 (42.7)	7 (46.7)				
		G/C	36 (43.9)	6 (40.0)	Dominant	0.775	1.175	0.389-3.547
		C/C	11 (13.4)	2 (13.3)	Recessive	1.000	1.007	0.200-5.081

* Allele: allele model; Dominant: the minor allele dominant model; Recessive: the minor allele recessive model. (IFX, infliximab; CD, Crohn's disease; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency).

of female gender showed response to IFX after 10 weeks of treatment. Subsequently, multivariate logistic regression analysis revealed that only the C/C genotype of rs11670259 in *CARD8* independently contributed to response to IFX ($P = 0.017$, OR = 5.391; Table 6). Conversely, the C/T or T/T genotype of rs11670259 in *CARD8* contributed to primary non-response to IFX after 10 weeks of treatment.

The gene-gene interaction in response to IFX after 1 year of treatment

Likewise, in order to investigate the influence of the interaction of associated genetic factors on response to IFX after 1 year of treatment, multivariate logistic regression analysis indicated that three genetic factors, the T/T or T/G genotype of rs3751143 in *P2RX7*, the C/C genotype of rs4389238 in *CARD8*, and the A/G or G/G genotype of rs2282659 in *CASP1*, independently contributed to response to IFX ($P = 0.012$, OR = 6.379, $P = 0.013$, OR = 5.114, $P = 0.004$, OR = 7.803, respectively; Table 7).

Conversely, the G/G genotype of rs3751143 in *P2RX7*, the C/T or T/T genotype of rs4389238 in *CARD8*, and the A/A genotype of rs2282659 in *CASP1* independently contributed to loss of response to IFX after 1 year of treatment.

Verification of genetic test to predict response to IFX after 10 weeks of treatment

In order to predict response to IFX for CD patients after 10 weeks of treatment, genetic test was carried out using an independent genetic factor, the C/C genotype of rs11670259 in *CARD8*, as a biomarker (Table 8). This test indicated that the sensitivity, specificity, positive

predictive value, and negative predictive value were estimated to be at 68.1%, 63.6%, 95.2%, and 15.9%, respectively (Table 8).

Verification of genetic test to predict response to IFX after 1 year of treatment

Likewise, we performed genetic test with a combination of the three independent genetic factors as biomarkers to better predict response to IFX for CD patients after 1 year of treatment, indicating that the best combination of marker 6 (T/T or T/G genotype of rs3751143 in *P2RX7* and A/G or G/G genotype of rs2282659 in *CASP1*) was useful as a biomarker with the values of the highest scores of the P value, OR, sensitivity, specificity, and positive predictive value (Table 9).

Discussion

This study is the first demonstration to report that the polymorphisms of *CARD8*, *P2RX7*, and *CASP1* independently contribute to the therapeutic effect of IFX for CD patients.

From the pathophysiological perspective at 10 weeks after the start of IFX administration, the C/C genotype at rs11670259 in *CARD8* may decrease the function of *CARD8* in the genetic background, thereby leading to the diminution of the production of inflammatory cytokines as well as inflammatory mediators through the *P2RX7* signaling pathway [14-19]. Therefore, not only the suppression of the TNFR signaling pathway due to IFX, but also the diminution of the *P2RX7* signaling pathway due to this polymorphism may show good response to IFX after 10 weeks of treatment (Figure 2).

In contrast, the C/T or T/T genotype of rs11670259 in *CARD8* may increase the function of *CARD8* in the genetic background,

Table 6. The interaction of genetic and environmental factors for response to IFX after 10 weeks of treatment for CD patients.

Factor	OR (95% CI)	P value*
C/C genotype of rs11670259 in <i>CARD8</i>	5.391 (1.352 - 21.49)	0.017
G/G genotype of rs1143623 in <i>IL1B</i>	8.293 (0.966 - 71.21)	0.054
Female	7.364 (0.852 - 63.62)	0.070

* Allele: allele model; Dominant: the minor allele dominant model; Recessive: the minor allele recessive model. (IFX, infliximab; CD, Crohn's disease; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency).

Table 7. Gene-gene interaction among *P2RX7*, *CARD8*, and *CASP1* genotypes for response to IFX after 1 year of treatment for CD patients.

Factor	OR (95% CI)	P value*
T/T or T/G genotype of rs3751143 in <i>P2RX7</i>	6.379 (1.498 - 27.17)	0.012
C/C genotype of rs4389238 in <i>CARD8</i>	5.114 (1.416 - 18.48)	0.013
A/G or G/G genotype of rs2282659 in <i>CASP1</i>	7.803 (1.938 - 31.42)	0.004

*Factors were statistically analyzed by multivariate logistic regression analysis. (IFX, infliximab; CD, Crohn's disease; OR, odds ratio; CI, confidence interval).

Table 8. Genetic factor determined by genetic test for response to IFX after 10 weeks of treatment for CD patients.

Biomarker	Statistical results		Genetic diagnosis			
	OR (95% CI)	P value*	sensitivity	specificity	PPV	NPV
C/C genotype of rs11670259 in <i>CARD8</i>	3.736 (1.029 - 13.57)	0.047	68.1	63.6	95.2	15.9

*Factors were statistically analyzed by multivariate logistic regression analysis. (IFX, infliximab; CD, Crohn's disease; OR, odds ratio; CI, confidence interval).

Table 9. Combination of genetic factors determined by genetic test for response to IFX after 1 year of treatment for CD patients. *Factors were statistically analyzed by Fisher's exact test. (IFX, infliximab; CD, crohn's disease; OR, odds ratio; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value)

Biomarker	<i>P2RX7</i>	<i>CARD8</i>	<i>CASP1</i>	Statistical results		Genetic diagnosis			
	rs3751143	rs4389238	rs2282659	OR (95% CI)	P value*	sensitivity	specificity	PPV	NPV
marker 1	T/T or T/G	-	-	7.000 (1.961 - 24.99)	0.004	93.8	31.6	87.5	50.0
marker 2	-	C/C	-	4.333 (1.341 - 14.01)	0.012	53.6	78.9	92.9	25.0
marker 3	-	-	A/G or G/G	6.698 (1.831 - 24.50)	0.002	55.7	84.2	94.7	27.1
marker 4	T/T or T/G	C/C	-	5.444 (1.490 - 19.90)	0.006	50.5	84.2	94.2	25.0
marker 5	-	C/C	A/G or G/G	6.592 (0.837 - 51.91)	0.071	26.8	94.7	96.3	20.2
marker 6	T/T or T/G	-	A/G or G/G	9.424 (2.064 - 43.04)	0.001	52.6	89.5	96.2	27.0
marker 7	T/T or T/G	C/C	A/G or G/G	6.250 (0.792 - 49.28)	0.069	25.8	94.7	96.2	20.0

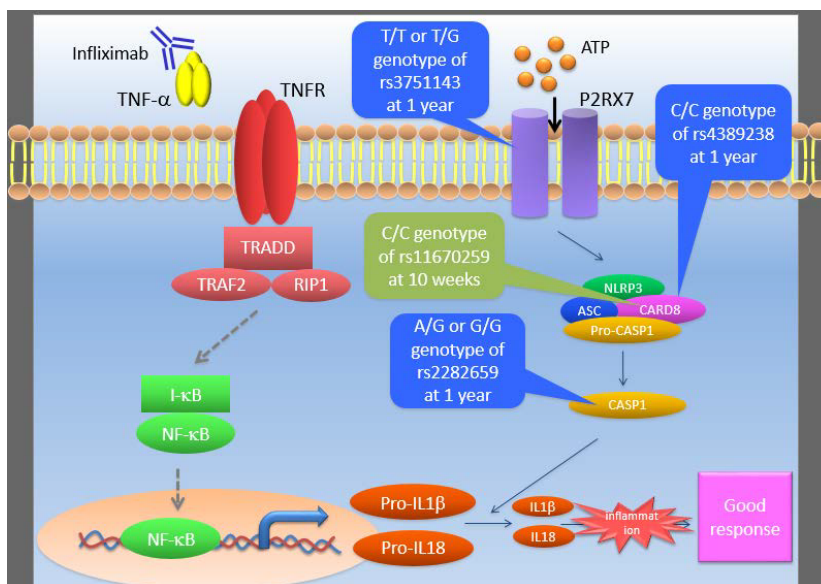


Figure 2. The putative mechanism of good response to infliximab for CD patients.

A schematic representation indicates the putative mechanism of good response to infliximab after 10 weeks and 1 year of treatment. Ligands, TNF- α and ATP, activate its receptors, TNFR and P2RX7, respectively. Subsequently the downstream signals in each signaling pathway induce the production of inflammatory cytokines including IL-1 β and IL-18. In the CD patients with the present polymorphisms of *P2RX7*, *CARD8*, and *CASP1*, the signals in the *P2RX7* pathway may be diminished in the genetic background. Whereas, the signals in the TNFR pathway can be suppressed by infliximab.

Abbreviations: TNF, tumor necrosis factor; TNFR, TNF receptor; TRADD, tumor necrosis factor receptor type 1-associated DEATH domain protein; TRAF2, TNF receptor-associated factor 2; RIP1, receptor-interacting serine/threonine-protein kinase 1; I- κ B, inhibitor of kappa B; NF- κ B, nuclear factor-kappa B; ATP, adenosine triphosphate; P2RX7, purinergic receptor P2X, ligand gated ion channel, 7; NLRP3, NLR family pyrin domain containing 3; CARD8, caspase recruitment domain family member 8; ASC, apoptosis-associated speck-like protein containing a carboxy-terminal CARD; CASP1, caspase 1; IL-1 β , interleukin 1 beta; IL-18, interleukin 18.

thereby leading to the acceleration of the production of inflammatory cytokines and mediators through the *P2RX7* signaling pathway. The condition with the elevated production of inflammatory cytokines and mediators through the activation of the *P2RX7* signaling pathway due to this polymorphism may predominate over that with the suppressed production of inflammatory cytokines and mediators through the TNFR signaling pathway due to IFX. Therefore, the CD patients under such conditions may eventually result in primary non-response after 10 weeks of treatment (Figure 3).

At 1 year after the start of IFX administration, the T/T or T/G genotype of rs3751143 in *P2RX7*, the C/C genotype of rs4389238 in *CARD8*, or A/G or G/G genotype at rs2282659 in *CASP1* may slightly reduce the function of *P2RX7*, *CARD8*, and *CASP1* in the genetic background, thereby leading to a decrease in the production of inflammatory cytokines and mediators through the *P2RX7* signaling pathway. As similar to the mechanism observed after 10 weeks of treatment, in these CD patients, both the diminution of the *P2RX7* signaling pathway due to these polymorphisms and the suppression of the TNFR signaling pathway due to IFX may show good response to IFX after 1 year of treatment (Figure 2).

Conversely, the G/G genotype of rs3751143 in *P2RX7*, the C/T or T/T genotype of rs4389238 in *CARD8*, and A/A genotype at rs2282659 in *CASP1* may slightly accelerate the function of *P2RX7*, *CARD8*, and *CASP1* in the genetic background, thereby leading to an increase in the production of inflammatory cytokines and mediators through the *P2RX7* signaling pathway. Therefore, not only the elevated production of inflammatory cytokines and mediators through the accelerated *P2RX7* signaling pathway due to these polymorphisms, but also a decrease in the suppressed production of inflammatory cytokines and mediators through the TNFR signaling pathway due to the reduction of the IFX actions, may exacerbate inflammation of the intestines in

the patients and eventually lead to secondary loss of response to IFX after 1 year of treatment (Figure 3). Although the decisive factors have not yet been identified, the reduction of the IFX actions after 1 year of treatment may be caused by various clinical risk factors, including a shortened half-life of IFX due to the increased clearance of IFX, the dominant mechanism of inflammation, the production of antibodies to IFX (ATI), and the low serum concentrations of the IFX levels (up to 60%) [29,30]. Indeed, approximately 15-61% of CD patients developed ATI [31-33], although ATI was not examined in this study. Of course, since these CD patients, who showed secondary loss of response after 1 year of treatment, showed response to IFX at the 10-week treatment, at 10 weeks after the start of IFX administration, the condition with the suppressed production of inflammatory cytokines and mediators through the TNFR signaling pathway due to IFX may predominate over that with the slightly elevated production of inflammatory cytokines and mediators through the activation of the *P2RX7* signaling pathway due to the polymorphisms in the genetic background.

With regard to genetic test with the IFX-related polymorphisms, the C/C genotype at rs11670259 in *CARD8* is useful as a biomarker to predict response to IFX for CD patients after 10 weeks of treatment with significant differences (Table 8). As this test showed the sensitivity of 68.1%, we hypothesize that the activation of the *P2RX7* signaling pathway may contribute to inflammation of the intestines in about two-thirds of the CD patients who showed good response to IFX after 10 weeks of treatment. Moreover, the positive predictive value of this test was very higher at 95.2%, thereby indicating the very higher probability that almost all of the CD patients with the C/C genotype of rs11670259 in *CARD8* could show good response to IFX after 10 weeks of treatment.

On the other hand, after 1 year of treatment, genetic test showed the combination marker 6 (T/T or T/G genotype of rs3751143 in *P2RX7*

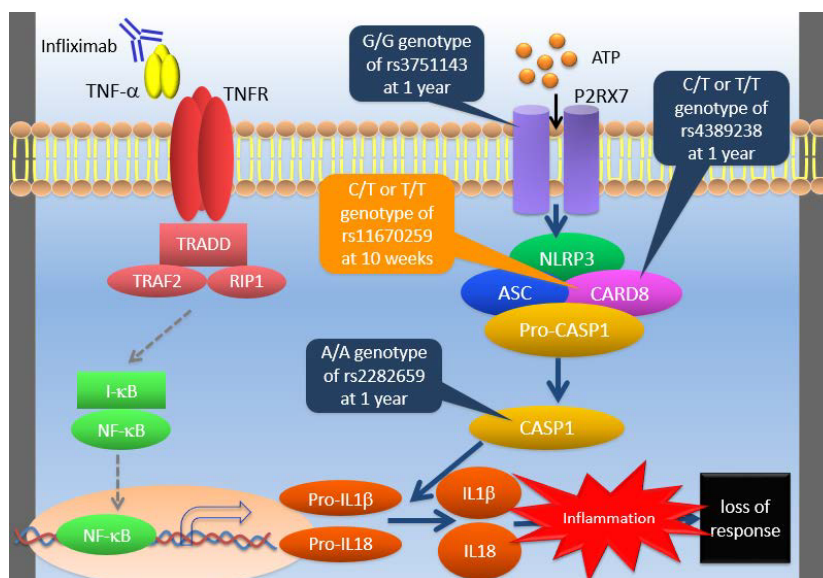


Figure 3. The putative mechanism of loss of response to infliximab for CD patients
 A schematic representation indicates the putative mechanism of primary non-response and secondary loss of response to infliximab after 10 weeks and 1 year of treatment, respectively. In the CD patients with the present polymorphisms of *P2RX7*, *CARD8*, and *CASP1*, the signals in the *P2RX7* pathway may be slightly activated in the genetic background. Whereas, the signals in the TNFR pathway can be suppressed by infliximab.
 Abbreviations: TNF, tumor necrosis factor; TNFR, TNF receptor; TRADD, tumor necrosis factor receptor type 1-associated DEATH domain protein; TRAF2, TNF receptor-associated factor 2; RIP1, receptor-interacting serine/threonine-protein kinase 1; I-κB, inhibitor of kappa B; NF-κB, nuclear factor-kappa B; ATP, adenosine triphosphate; *P2RX7*, purinergic receptor P2X, ligand gated ion channel, 7; NLRP3, NLR family pyrin domain containing 3; *CARD8*, caspase recruitment domain family member 8; ASC, apoptosis-associated speck-like protein containing a carboxy-terminal CARD; CASP1, caspase 1; IL-1β, interleukin 1 beta; IL-18, interleukin 18.

and A/G or G/G genotype of rs2282659 in *CASP1* could be useful as a biomarker to predict response to IFX for CD patients with significant differences (Table 9). As similar to the mechanism observed after 10 weeks of treatment, the sensitivity of 52.6% was seen in the genetic test after 1 year of treatment, indicating that the activation of the P2RX7 signaling pathway may contribute to inflammation of the intestines in about half of all CD patients who showed good response to IFX after 1 year of treatment. Likewise, the positive predictive value of 96.2% shown in the test indicates the very higher probability that almost all of the CD patients with both the T/T or T/G genotype of rs3751143 in *P2RX7* and A/G or G/G genotype of rs2282659 in *CASP1* could show good response to IFX after 1 year of treatment.

In the patients who show secondary loss of response to IFX, remission can be managed by shortening interval between dosing [34], dose intensification [35], and/or switching to other biological agents [36,37]. For example, adalimumab is a fully humanized monoclonal antibody approved for the treatment of CD patients who have failed to respond to conventional agents and anti-TNF- α therapies [30,36]. Certolizumab pegol is a pegylated humanized monoclonal Fab' fragment that binds to TNF- α [30,37,38]. Natalizumab is a humanized monoclonal antibody against $\alpha 4$ integrin [39,40]. When these agents are chosen for the treatment of CD, then useful biomarkers to predict response or loss of response to IFX would be required.

Finally, not only the antagonists of P2RX7 [14,20], but also other molecules involved in the P2RX7 signaling pathway could be targets for newly developed therapeutic agents to combat primary non-response and secondary loss of response to IFX for CD patients.

Conclusion

This is the first report to show that *CARD8* is related to response and primary non-response to IFX after 10 weeks of treatment, and *P2RX7*, *CARD8*, and *CASP1* are related to response and secondary loss of response to IFX after 1 year of treatment for Japanese CD patients. The polymorphism, the C/C genotype at rs11670259 in *CARD8*, and the combination polymorphisms, T/T or T/G genotype of rs3751143 in *P2RX7* and A/G or G/G genotype of rs2282659 in *CASP1*, were found to useful as biomarkers to predict response to IFX after 10 weeks and 1 year of treatment, respectively. These molecules including *P2RX7*, *CARD8*, and *CASP1* in the P2RX7 signaling pathway could therefore become targets for new therapeutic drugs and thereby help patients to overcome primary non-response and secondary loss of response to IFX in CD patients.

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Conflicts of interest

The authors declare that they have no competing interests in association with this study.

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