<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
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<tbody>
<tr>
<td>論文タイトル</td>
<td>ピーアソシエイテッド RX7のシグナルングパスワワロン遺伝的ポリモフィズムの証拠としての予測的バイオマーカーとインフリキマブ治療に対する反応の獲得及び損失</td>
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<tr>
<td>著者</td>
<td>荒木 千鶴</td>
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<tr>
<td>キーワード</td>
<td>新宿大学 博士 薬学 長崎大学 博士</td>
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<tr>
<td>クリック</td>
<td>NAOSITE:長崎大学学術的成績リポジトリ</td>
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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

Abraham C1,2, Yoshimura M1, Fukumitsu Y1, Ma S1, Ishida T3, Urabe S1, Matsushima K4, Honda T1, Uehara S1, Takeshima F1, Higuchi N2, Isomoto H6, Nakao K4 and Tsukamoto K1*

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3Department of Gastroenterology, Oita Red Cross Hospital, Chiyo-machi, Oita, Japan
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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 was 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; AT1: 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-κB: 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 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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Key words: P2RX7 signaling pathway, single nucleotide polymorphism, infliximab, drug-responsibility gene, Crohn’s disease, candidate gene-based association study, DNA-based biomarker

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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 IIaA [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)-1β (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-kB) [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-1β was found to decrease in caspase-1-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 TNF 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 the genetic background and treatment 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.

### 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; ASC1 (OMIM #147678) located at 11q23.1; 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 previously [13,27,28].

#### 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).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (% of CD patients)</th>
</tr>
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<tbody>
<tr>
<td><strong>Responders</strong></td>
<td></td>
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<tr>
<td>10 weeks (n = 127)</td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td>116 (91.3)</td>
</tr>
<tr>
<td>Age, mean ± SD (years)</td>
<td>35.2 ± 11.8</td>
</tr>
<tr>
<td>Male/female (%)</td>
<td>67/59 (49/78.42.2)</td>
</tr>
<tr>
<td>1 year (n = 116)</td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td>97 (83.6)</td>
</tr>
<tr>
<td>Age, mean ± SD (years)</td>
<td>35.2 ± 11.9</td>
</tr>
<tr>
<td>Male/female (%)</td>
<td>56/41 (57.7/42.3)</td>
</tr>
<tr>
<td>2 years (n = 97)</td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td>82 (84.5)</td>
</tr>
<tr>
<td>Age, mean ± SD (years)</td>
<td>35.1 ± 11.9</td>
</tr>
<tr>
<td>Male/female (%)</td>
<td>48/34 (58.5/41.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Non-responders</strong></th>
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<tbody>
<tr>
<td>11 (8.7)</td>
</tr>
<tr>
<td>35.3 ± 11.8</td>
</tr>
<tr>
<td>10/1 (90.9/9.1)</td>
</tr>
<tr>
<td>19 (16.4)</td>
</tr>
<tr>
<td>35.6 ± 11.7</td>
</tr>
<tr>
<td>11/8 (57.9/42.1)</td>
</tr>
<tr>
<td>15 (15.5)</td>
</tr>
<tr>
<td>35.3 ± 11.8</td>
</tr>
<tr>
<td>8/7 (53.3/46.7)</td>
</tr>
</tbody>
</table>
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 T/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 ~3.7-fold loss of response to IFX after 1 year of treatment. Conversely, the possession of a major homozygous T/G 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%, \( P = 0.012, \ OR = 0.231 \); Table 4), indicating that these genotypes are associated with ~3.7-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).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tag SNP</th>
<th>Genotype</th>
<th>Number (% of CD patients)</th>
<th>Inheritance model</th>
<th>P value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<tbody>
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<td>P2RX7</td>
<td>rs884201</td>
<td>MAxF</td>
<td>0.211</td>
<td>0.182</td>
<td>Allele</td>
<td>1.000</td>
<td>1.205</td>
</tr>
<tr>
<td></td>
<td>G &gt; A</td>
<td>G/G</td>
<td>72 (62.1)</td>
<td>7 (63.6)</td>
<td>0.059-21.87</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/A</td>
<td>39 (33.6)</td>
<td>4 (36.4)</td>
<td>0.801-22.61</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>5 (4.3)</td>
<td>0 (0)</td>
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<td>0.306</td>
<td>0.409</td>
<td>Allele</td>
<td>0.320</td>
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<td>52 (48.8)</td>
<td>3 (27.3)</td>
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<tr>
<td></td>
<td></td>
<td>G/C</td>
<td>57 (49.1)</td>
<td>7 (63.6)</td>
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<td></td>
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<td>A/C</td>
<td>4 (3.6)</td>
<td>1 (9.1)</td>
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<tr>
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<td>rs11065450</td>
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<td>0.332</td>
<td>0.409</td>
<td>Allele</td>
<td>0.465</td>
<td>0.718</td>
</tr>
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<tr>
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<td></td>
<td>C/A</td>
<td>53 (45.7)</td>
<td>7 (63.6)</td>
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<td>A/C</td>
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<td>0.951</td>
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<td>5 (45.5)</td>
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<tr>
<td></td>
<td></td>
<td>G/A</td>
<td>45 (38.8)</td>
<td>6 (54.5)</td>
<td>0.059-21.87</td>
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<td>A/G</td>
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<td>G/T</td>
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<td></td>
<td></td>
<td>T/G</td>
<td>17 (14.7)</td>
<td>3 (27.3)</td>
<td>0.059-21.87</td>
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</table>

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.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tag SNP</th>
<th>Genotype</th>
<th>Number (% of CD patients)</th>
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<td>0.138-0.827</td>
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<td>Allele</td>
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<td>Allele</td>
<td>0.029</td>
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<td>4 (36.4)</td>
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<td>Allele</td>
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<td>4 (36.4)</td>
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<td>0.268</td>
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<td>0.172</td>
<td>0.364</td>
<td>Allele</td>
<td>0.029</td>
<td>0.365</td>
<td>0.143-0.927</td>
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<th>G/G</th>
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<td>1.000</td>
<td>1.202</td>
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<td>8 (72.7)</td>
<td>Recessive</td>
<td>0.049 - 0.360</td>
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*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.

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<th>Gene</th>
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<th>Genotype</th>
<th>Responders (n = 97)</th>
<th>Non-responders (n = 19)</th>
<th>Inheritance modela</th>
<th>P value</th>
<th>OR</th>
<th>95% CI</th>
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<td>G/A</td>
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<td>6 (31.6)</td>
<td>Dominant</td>
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<td>11 (57.9)</td>
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**Note:**
- **MAF:** Minor Allele Frequency
- **Allele:** Dominant or Recessive

### 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


**Integr Mol Med, 2016**

**Volume 3(6): 8-15**


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<th>P value</th>
<th>OR</th>
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* 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.
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**PYCARD**

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**CASP1**

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<th>Dominant</th>
<th>Recessive</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs228659</td>
<td>0.317</td>
<td>0.267</td>
<td>0.583</td>
<td>1.277</td>
<td>0.533-3.058</td>
</tr>
<tr>
<td></td>
<td>A &gt; G</td>
<td>35 (42.7)</td>
<td>6 (40.0)</td>
<td>0.445</td>
<td>1.535</td>
<td>0.508-4.632</td>
</tr>
<tr>
<td></td>
<td>G &gt; A</td>
<td>30 (36.6)</td>
<td>5 (33.3)</td>
<td>0.538</td>
<td>1.277</td>
<td>0.533-3.058</td>
</tr>
</tbody>
</table>

**IL18**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>MAF</th>
<th>Allele</th>
<th>Dominant</th>
<th>Recessive</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs5744247</td>
<td>0.457</td>
<td>0.367</td>
<td>0.358</td>
<td>1.456</td>
<td>0.652-3.251</td>
</tr>
<tr>
<td></td>
<td>C &gt; G</td>
<td>24 (29.3)</td>
<td>5 (33.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C &gt; T</td>
<td>41 (50.0)</td>
<td>9 (60.0)</td>
<td>0.765</td>
<td>1.208</td>
<td>0.373-3.909</td>
</tr>
<tr>
<td></td>
<td>G &gt; T</td>
<td>17 (20.7)</td>
<td>1 (6.7)</td>
<td>0.290</td>
<td>3.662</td>
<td>0.449-29.85</td>
</tr>
<tr>
<td></td>
<td>A &gt; A</td>
<td>30 (36.6)</td>
<td>5 (33.3)</td>
<td>0.704</td>
<td>0.859</td>
<td>0.391-1.886</td>
</tr>
<tr>
<td></td>
<td>T &gt; G</td>
<td>37 (45.1)</td>
<td>8 (53.3)</td>
<td>1.000</td>
<td>1.867</td>
<td>0.271-2.775</td>
</tr>
<tr>
<td></td>
<td>A &gt; G</td>
<td>29 (35.4)</td>
<td>5 (33.3)</td>
<td>1.000</td>
<td>1.105</td>
<td>0.122-9.901</td>
</tr>
</tbody>
</table>

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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 G/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, specificity, sensitivity, 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, and lead to non-response to IFX after 10 weeks of treatment (Figure 3).
Table 6. The interaction of genetic and environmental factors for response to IFX after 10 weeks of treatment for CD patients.

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C genotype of rs11670259 in CARD8</td>
<td>5.391 (1.352 - 21.49)</td>
<td>0.017</td>
</tr>
<tr>
<td>G/G genotype of rs1134623 in IL1B</td>
<td>8.293 (0.966 - 71.21)</td>
<td>0.054</td>
</tr>
<tr>
<td>Female</td>
<td>7.364 (0.852 - 63.62)</td>
<td>0.070</td>
</tr>
</tbody>
</table>

*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.

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

*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.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>P2RX7</th>
<th>CARD8</th>
<th>CASP1</th>
<th>Statistical results</th>
<th>Genetic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs3751143</td>
<td>rs4389238</td>
<td>rs2282659</td>
<td>OR (95% CI)</td>
<td>P value*</td>
</tr>
<tr>
<td>C/C genotype of rs11670259 in CARD8</td>
<td>3.736 (1.029 - 13.57)</td>
<td>0.047</td>
<td>68.1</td>
<td>63.6</td>
<td>95.2</td>
</tr>
</tbody>
</table>

*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)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>P2RX7</th>
<th>CARD8</th>
<th>CASP1</th>
<th>Statistical results</th>
<th>Genetic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs3751143</td>
<td>rs4389238</td>
<td>rs2282659</td>
<td>OR (95% CI)</td>
<td>P value*</td>
</tr>
<tr>
<td>marker 1</td>
<td>T/T or T/G</td>
<td>-</td>
<td>-</td>
<td>7.000 (1.961 - 24.99)</td>
<td>0.004</td>
</tr>
<tr>
<td>marker 2</td>
<td>-</td>
<td>C/C</td>
<td>-</td>
<td>4.333 (1.341 - 14.01)</td>
<td>0.012</td>
</tr>
<tr>
<td>marker 3</td>
<td>-</td>
<td>-</td>
<td>A/G or G/G</td>
<td>6.698 (1.831 - 24.50)</td>
<td>0.002</td>
</tr>
<tr>
<td>marker 4</td>
<td>T/T or T/G</td>
<td>C/C</td>
<td>-</td>
<td>5.444 (1.490 - 19.90)</td>
<td>0.006</td>
</tr>
<tr>
<td>marker 5</td>
<td>-</td>
<td>C/C</td>
<td>A/G or G/G</td>
<td>6.592 (0.837 - 51.91)</td>
<td>0.071</td>
</tr>
<tr>
<td>marker 6</td>
<td>T/T or T/G</td>
<td>-</td>
<td>A/G or G/G</td>
<td>9.424 (2.064 - 43.04)</td>
<td>0.001</td>
</tr>
<tr>
<td>marker 7</td>
<td>T/T or T/G</td>
<td>C/C</td>
<td>A/G or G/G</td>
<td>6.250 (0.792 - 49.28)</td>
<td>0.069</td>
</tr>
</tbody>
</table>

*Factors were statistically analyzed by Fisher's exact test. (IFX, infliximab; CD, Crohn's disease; OR, odds ratio; CI, confidence interval).

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-1β 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; IκB, 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.
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 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 high 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...
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 α4 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 be 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.

Acknowledgement

We are grateful to the physicians and CD patients for participating in this study. This work was supported by a Grant-in-Aid for Scientific Research (C) (KAKENHI No. 16K08912) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (K. Tsukamoto) and a research grant from the Non-Profit Organization Aimed to Support Community Medicine Research in Nagasaki, Japan (K. Tsukamoto).

Conflicts of interest

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

References


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