

Expression of ASC splice variant found in Japanese patients with palindromic rheumatism is regulated by rs8056505 single nucleotide polymorphism and interleukin-1 beta

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Abstract

Background: Palindromic rheumatism (PR) is an infrequent form of periodic arthritis. Based on the similarity of the pathogenesis of PR to autoinflammatory syndromes, we previously found that the dominant-active splice variant of the inflammasome adaptor protein, apoptosis-associated speck-like protein containing a CARD (ASC), which lacks exon 2 (Δ exon2), is expressed in Japanese patients with PR.

Objective: Elucidation of the mechanism of Δ exon2 ASC production and the effect of IL-1 β on splicing.

Methods: The genomic DNA of Japanese patients with PR was sequenced. The effect of the observed single nucleotide polymorphisms (SNPs) on ASC splicing was determined via exon trapping using THP-1 cells stimulated with interleukin-1 beta (IL-1 β) or ceramide. To investigate the genes that affect alternative splicing via IL-1 β , we analyzed the transcriptome of IL-1 β -treated THP-1 cells using RNA sequencing.

Results: We found the rs8056505 A>G SNP located in the 5'-untranslated region of the genomic ASC gene in patients and that Δ exon2 expression was induced by this SNP, whereas it was suppressed by IL-1 β or ceramide. We detected 131,426 transcripts and identified 52 differentially expressed genes (DEGs) consisting of 41 downregulated genes and 11 upregulated genes in IL-1 β -stimulated THP-1 cells. The splicing-related gene *MASCRNA* was the most significantly induced gene by IL-1 β .

Conclusion: We propose a cyclic expression model in which ASC alternates between wild-type and Δ exon2 expression regulated by the rs8056505 G allele and splicing factors induced by IL-1 β . This cycle may be correlated with the formation of periodic PR pathologies.

Key words: palindromic rheumatism, inflammasome, ASC, splice variant, IL-1 β

Citation:

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Introduction

Palindromic rheumatism (PR) is an infrequent form of periodic arthritis. The duration of joint swelling persists for several days, but no symptoms or joint swelling are observed after improvement.¹ Although the cause of the pathogenesis of this condition is unknown, the cyclical nature of this phenomenon bears some similarities to familial Mediterranean fever, a prototype autoinflammatory disease.²

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Typical symptoms of autoinflammatory diseases include recurrent periodic fevers, generalized inflammation, skin rashes, abdominal and chest pain, lymphadenopathy, and arthritis. Unlike autoimmune diseases, in which autoantibodies arise in association with dysregulation of the acquired immune system, autoinflammatory diseases are caused by dysregulation of the innate immune system, particularly inflammasome overactivation, rather than the predominance of high-titer autoantibodies or autoreactive T cells.³

The inflammasome is a cytoplasmic protein complex that induces cytokines such as interleukin (IL)-1 β and IL-18 via activation of caspase-1. The main components of the inflammasome are pattern recognition receptors, ASC (apoptosis-associated speck-like protein containing a CARD), and caspase-1.⁴ To date, inflammasome dysfunction has been implicated in the pathogenesis of various autoinflammatory diseases such as cryopyrin-associated periodic syndrome, familial Mediterranean fever, gout, and Alzheimer's disease. The involvement of inflammasome-related genes in the pathogenesis of PR has also been suggested.^{3,5}

Based on findings of prior studies, we previously investigated ASC, which is a common adaptor of inflammasomes, and found that the ASC splicing variant that lacks exon 2 (Δ Exon2) is dominantly expressed in Japanese patients with PR and induces IL-1 β overexpression.⁶ Although this variant lacks exon 2, all three exons of ASC have been found to be intact in genomic DNA.⁶ Since this indicates disruptions in ASC splicing in patients with PR, we analyzed the mechanism of Δ Exon2 ASC production and effect of IL-1 β on splicing.

Methods

Patients

The Japanese patients with PR included in this study were enrolled in a previous study and written informed consent was obtained from them.⁶ All three patients were examined at the Shichikawa Arthritis Center, Osaka Rehabilitation Hospital, as described previously.⁶ The study was approved by the institutional ethical committee of Kobe University Graduate School of Health Sciences and conducted according to the principles of the Declaration of Helsinki (approval no. 140-3).

ASC gene mutation analysis

Genomic DNA was isolated from peripheral blood using the PAXgene blood DNA kit (QIAGEN, Hilden, Germany). Genomic ASC DNA fragments were obtained as five separate fragments. Fragment 1 was amplified via polymerase chain reaction (PCR) for 30 cycles at 94°C for 30 s, 66°C for 30 s, and 72°C for 2 min, followed by 72°C for 5 min using the following primers: hPYCARD3313F forward 5'-TTCAGCAGGGTAGCAGAGC-3', hPYCARD4445R reverse 5'-CCGAAACCTTGCCAGATGCT-3'. Fragment 2 was amplified via PCR for 30 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min using the following primers: hPYCARD4129F forward 5'-ACAGTTAAGTCTCCGATGC-3', hPYCARD4927R reverse 5'-TTACTACACCCTTGGTCCCCT-3'. The amplification conditions

Fragment 3 were as follows: 30 cycles of 94°C for 30 s, 67°C for 30 s, and 72°C for 2 min, followed by 72°C for 5 min. The following primers were used: hPYCARD4770F forward 5'-CAAGCCCAGAGACAAGCAGG-3', hPYCARD5767R reverse 5'-CAGGGTGTGGGTGGTGG-3'. Fragment 4 was amplified using the following reaction conditions: 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 2 min, followed by 72°C for 5 min using the following primers: hPYCARD5634F forward 5'-AGGCCTCCACCCCAG-3', hPYCARD6627R reverse 5'-ATGTGGAAGGTAGGGGAGC-3'. Fragment 5 was amplified using the following reaction conditions: 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 2 min, followed by 72°C for 5 min using the following primers: hPYCARD6339F forward 5'-CTATGCTTCAGAGGCCACCC-3', hPYCARD7854R reverse 5'-GGGAGGACAAGGTAGGAGGT-3'. The number in the primer name indicates the 5' position to which they anneal according to the compared sequence data NCBI accession number NG_029446. The PCR products obtained were purified using the QIAquick PCR Purification Kit (QIAGEN) and subjected to sequence analysis (Eurofins Genomics, Tokyo, Japan).

Exon-trapping assay

We performed an exon-trapping assay to investigate the effect of ASC splicing of the rs8056505 SNP. The wild-type ASC rs8056505 A allele and mutant ASC rs8056505 G allele were amplified from genomic DNA obtained from peripheral blood mononuclear cells of patients with PR containing the heterozygous rs8056505 A \rightarrow G single nucleotide polymorphism (SNP). The reaction mix contained 0.2 mM of each dNTP and 1 \times PrimeSTAR GXL DNA Polymerase (Takara Bio, Shiga, Japan); the amplification conditions included 30 cycles at 98°C for 10 s, 60°C for 15 s, and 68°C for 3 min, followed by a final extension at 68°C for 5 min. The following primers were used for amplification: hPYCARD4129F_Fusion forward, 5'-GGGATCACCAGAATTCACAGTTAAGGTCTCCGATGC-3'; hPYCARD6480R_Fusion reverse, 5'-TCGAGCTCCAGAATTCCTTTAGGGAGCACTGGAGGA-3'. The obtained PCR products were inserted into the EcoRI-digested pSPL3 exon-trapping vector (Thermo Fisher Scientific, Waltham, MA, USA) using the In-Fusion HD Cloning Kit (Takara Bio). THP-1 cells obtained from the JCRB Cell bank were plated at a density of 1.5 \times 10⁶ cells/mL in RPMI 1640 medium (Fujifilm Wako, Tokyo, Japan) supplemented with 100 U/mL penicillin (Thermo Fisher Scientific), 100 μ g/mL streptomycin (Thermo Fisher Scientific), and 10% Equa FETAL (Atlas Biologicals, Fort Collins, CO, USA) and incubated overnight at 37°C. The pSPL3 control vector, pSPL3 vector containing the wild-type ASC rs8056505 A allele, or pSPL3 vector containing the mutant ASC rs8056505 G allele were transfected into THP-1 cells using jetOPTIMUS in vitro DNA transfection reagent (Polyplus-transfection SA, Illkirch, France) according to the manufacturer's instructions and incubated for 48 h. After incubation, the transfected cells were washed and stimulated for 24 h with 50, 100, or 200 pg/mL IL-1 β (R&D Systems, Minneapolis, MN, USA) and 5 or 20 μ M C6-pyridinium-ceramide (Avanti Polar Lipids, Alabaster, AL, USA).

Total RNA fractions from the THP-1 cells were extracted using RNAiso Plus (Takara Bio) and reverse-transcribed to cDNA using the PrimeScript II 1st strand cDNA synthesis kit (Takara Bio). mRNA expression was confirmed via reverse transcription (RT)-PCR using a reaction mixture of 0.2 mM of each dNTP, 1× Gflex PCR Buffer (Takara Bio), 0.2 μM of each primer, and 1.25 U of Tks Gflex DNA Polymerase (Takara Bio). The amplification conditions were as follows: 94°C for 1 min; 30 cycles of 98°C for 10 s and 68°C for 1 min; and 68°C for 2 min 30 s. The primers used for amplification were as follows: pSPL3 SD6 forward, 5'-TCTGAGTCACCTGGACAACC-3'; pSPL3 SA2 reverse, 5'-ATCTCAGTGGTATTTGTGAGC-3'. Second nested PCR was performed at 94°C for 1 min and 30 cycles of 98°C for 10 s, 60°C for 15 s, and 68°C for 30 s, followed by a final extension at 72°C for 5 min. The primers used were as follows: hPYCARD225F forward, 5'-ATGGACGCCTTGGACCTCA-3'; hPYCARD696R reverse, 5'-GGAGTGTGCTGGGAAGGA-3'.

Total RNA sequencing (RNA-seq)

THP-1 cells were plated at a density of 1.0 × 10⁶ cells/mL in RPMI 1640 medium (Fujifilm Wako) supplemented with 100 U/mL penicillin (Thermo Fisher Scientific), 100 μg/mL streptomycin (Thermo Fisher Scientific), and 10% Equa FETAL (Atlas Biologicals), and stimulated with or without 50 pg/mL IL-1β (R&D Systems) for 24 h at 37°C. Total RNA was extracted from the THP-1 cells using RNAiso Plus (Takara Bio), treated with DNase I, and purified using the PureLink RNA Mini Kit (Thermo Fisher Scientific). The purified RNA was subjected to total RNA sequencing (RNA-seq) using a NovaSeq 6000 high-throughput sequencer (Illumina, San Diego, CA, USA). RNA-seq was performed by Eurofin Genomics (Tokyo, Japan). The library insert size was 200 bp, and sequencing was performed using the 150 bp paired-end read method. The assigned indices were

CTTAGGAC-CTTCACTG and ATCTGACC-CTCGACTT. The DDBJ (DNA Databank of Japan) DRA accession number is DRA014002.

RNA-seq data analysis

The RNA-seq data were analyzed using the following pipeline: first, we performed a quality check for each sample using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and low-quality reads were trimmed using Trimmomatic (<http://www.usadellab.org/cms/?page=trimmomatic>; trim primer sequence, read length of 50 bp or less, and Q value base of less than 20 bp from the start or end of the read). After trimming, we performed a quality check again and confirmed that the low-quality reads were removed. The trimmed samples were mapped using HISAT2 (<http://daehwankimlab.github.io/hisat2/>) and the hg38 reference genome, followed by normalization and gene expression level calculations using StringTie (<http://ccb.jhu.edu/software/stringtie/>). We analyzed the data using TCC-GUI; genes with a false discovery rate (FDR) less than 0.05 were deemed to be differentially expressed genes (DEGs).⁷

Results

Presence of rs8056505 A/G heterozygous mutation in the 5'-untranslated region (5'-UTR) of ASC in Japanese patients with PR

Since polymorphisms in genomic DNA affect RNA splicing, we analyzed a total of approximately 4.5 kbp genomic ASC coding regions, including entire exons and introns.⁸ Although no mutations were found in exons and introns (data not shown) in the patients, we found a heterozygous SNP (rs8056505 A/G) in the 5'-UTR (888 bp upstream from 1st ATG) in all three recruited patients (Figure 1a).

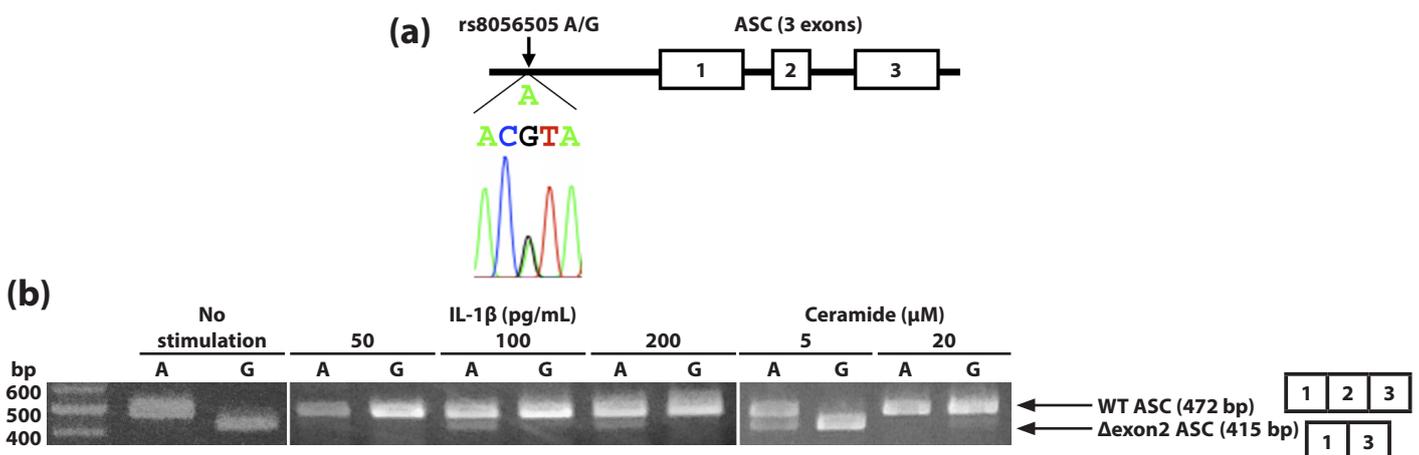


Figure 1. rs8056505 A>G SNP found in Japanese patients with PR affects the expression of ASC variants. (a) rs8056505 A>G heterozygous SNP found in Japanese patients with PR. (b) RT-PCR products of ASC in IL-1β- or ceramide-stimulated THP-1 cells. Cells were transfected with pSPL3 exon-trapping vectors containing whole ASC genomic DNA with the rs8056505 A allele or G allele followed by treatment with 0–200 pg/mL IL-1β or 5–20 μM ceramide.

Δexon2, ASC splicing variant that lacks exon 2; SNP, single nucleotide polymorphism; PR, palindromic rheumatism; IL-1β, interleukin-1β; ASC, apoptosis-associated speck-like protein containing a CARD

ΔExon2 ASC is induced by the rs8056505 G allele compared to the A allele in THP-1 cells and is suppressed by IL-1β or ceramide stimulation

We performed an exon-trapping assay to investigate the effect of ASC splicing of the rs8056505 SNP. ΔExon2 ASC expression was induced by the rs8056505 G allele compared to that observed for the A allele in THP-1 cells. We also evaluated the effect of co-stimulation of cells with IL-1β or ceramide, the latter of which is one of the downstream molecules of IL-1β and functions as a splicing regulator.⁹ We found that ΔExon2 ASC expression was suppressed via stimulation with 50–200 pg/mL IL-1β and 20 μM ceramide (Figure 1b).

Splicing-related genes, such as MASC RNA, are significantly affected in IL-1β-stimulated THP-1 cells

Since ΔExon2 expression was suppressed via IL-1β stimulation, several genes affected by IL-1β may be involved in the splicing regulation of ASC. Hence, we performed total RNA-seq analysis and isolated genes whose expression levels were altered in IL-1β-stimulated THP-1 cells. The number of reads was 44,797,770 and 59,862,218, the number of called bases was 6,720 Mbp and 8,979 Mbp, and the Q30 scores were 92.18% and 92.25% in the control and IL-1β-stimulated cells, respectively.

The obtained data were processed using an analysis pipeline, and gene expression levels between control and IL-1β-treated cells were compared using TCC-GUI. We detected 131,426 transcripts and plotted them on a volcano plot to see trends (Figure 2a). We found 35 genes showing a > 2-fold change in expression and a P-value < 0.05 between the control and IL-1β-stimulated cells, comprising 16 downregulated genes and 19 upregulated genes following IL-1β stimulation (Table 1, Figure 2b). In total, 7/16 (43.8%) of the downregulated genes were non-coding or microRNAs, 6/16 (37.5%) were small nuclear RNAs, such as SNORDs (C/D box small nucleolar RNAs) and scaRNAs (small Cajal body-specific RNAs), and 1/16 (6.3%) was a transcription factor. In contrast, 3/19 (15.8%) of the upregulated genes were typical splicing factors, such as the SR protein modulator MASC RNA (MALAT1-associated small cytoplasmic RNA) and RNVUs (U1 small RNA variants), and 12/19 (63.2%) were non-coding or microRNAs. We also determined genes with an FDR < 0.05 to be DEGs. We found 52 genes following IL-1β stimulation, comprising 41 downregulated and 11 upregulated genes (Table 2, Figure 2c). As splicing factors, SNORD141A was detected as a significantly downregulated gene and MASC RNA was detected as the most significantly upregulated gene.

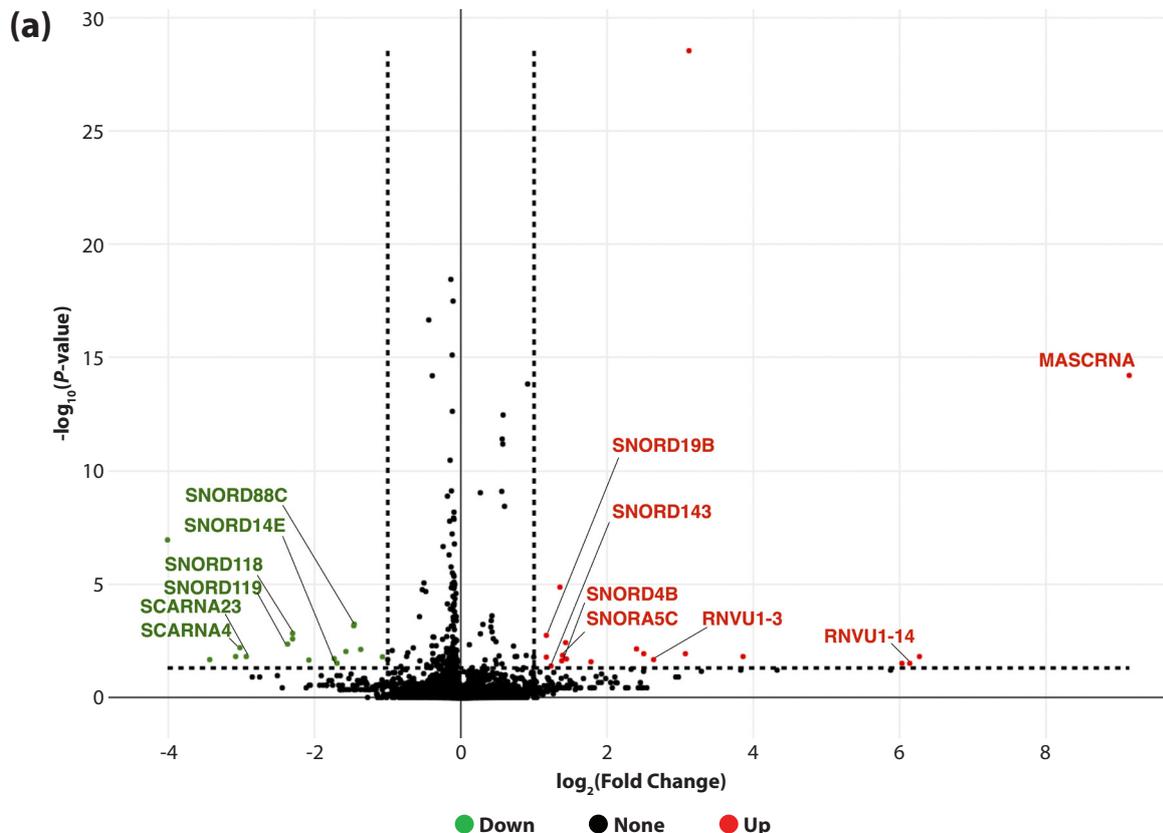


Figure 2. RNA sequencing analysis of IL-1β-stimulated THP-1 cells. (a) Volcano plot of transcripts. Upregulated genes by IL-1β are colored red. Downregulated genes by IL-1β are colored green. Genes whose expression did not vary are colored black. P-value cutoff (0.05) and fold-change cutoff value (2) are indicated by dashed lines. (b) Heatmap of altered genes in the volcano plot. (c) Heatmap of DEGs with FDR values less than 0.05.

DEGs, differentially expressed genes; FDR, false discovery rate; IL-1β, interleukin-1β

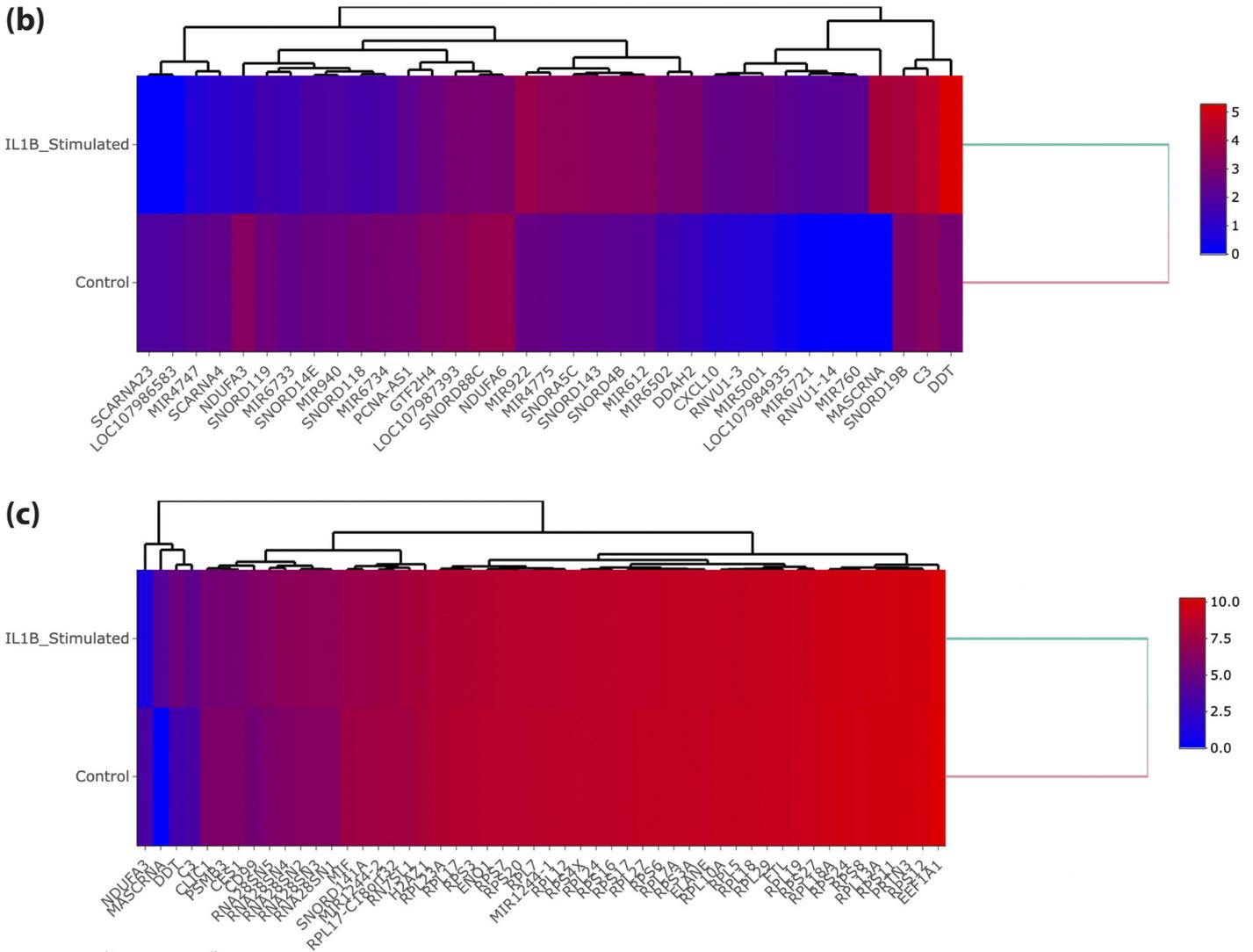


Figure 2. (Continued)

Table 1. Altered genes between control THP-1 cells and IL-1 β -stimulated cells described in the volcano plot.

The genes showing a > 2-fold change in expression and a *P*-value < 0.05 between control and IL-1 β -stimulated cells comprising 16 downregulated genes and 19 upregulated genes following IL-1 β stimulation are shown. (a) Downregulated genes, (b) Upregulated genes.

(a) Downregulated genes in volcano plot

Gene Name	A Value	M Value	P-Value	Q Value (FDR)	TPM Control	TPM IL-1 β Stimulated
SCARNA23	-1.5138	-2.93444	0.015625	1	2.473769	0
LOC107986583	-1.5138	-3.07911	0.015625	1	2.734699	0
MIR4747	1.516202	-3.43325	0.021484	1	3.495543	0.323417
SCARNA4	2.026365	-3.02062	0.006348	0.897492	4.315234	0.531232
NDUFA3	2.843422	-4.01055	1.12E-07	6.25E-05	10.71372	0.664016
SNORD119	2.808638	-2.36926	0.004425	0.667834	5.921486	1.144843
MIR6733	2.515463	-2.07858	0.022461	1	4.369429	1.033339
SNORD14E	2.993346	-1.69362	0.030884	1	5.325241	1.644533
MIR940	3.047133	-1.73312	0.019211	1	5.603714	1.683793
SNORD118	3.056187	-2.30014	0.00149	0.2779	6.863512	1.392097

(a) (Continued)

Gene Name	A Value	M Value	P-Value	Q Value (FDR)	TPM Control	TPM IL-1 β Stimulated
MIR6734	3.188717	-2.30034	0.002599	0.436452	7.524413	1.525928
PCNA-AS1	3.619485	-1.57235	0.009355	1	7.880837	2.647176
GTF2H4	4.111508	-1.36921	0.007632	1	10.33024	3.994588
LOC107987393	4.457921	-1.07262	0.016094	1	11.9526	5.711361
SNORD88C	4.667746	-1.46505	0.000686	0.141669	15.70292	5.681863
NDUFA6	4.747069	-1.45831	0.000584	0.1266	16.55177	6.017056

(b) Upregulated genes in volcano plot

Gene Name	A Value	M Value	P-Value	Q Value (FDR)	TPM Control	TPM IL-1 β Stimulated
MIR922	4.419176	1.431257	0.003802	0.58694	4.844132	13.049709
MIR4775	4.197004	1.167638	0.016589	1	4.550032	10.21038
SNORA5C	4.043417	1.388608	0.013853	1	3.788964	9.909844
SNORD143	3.950346	1.233441	0.04096	1	3.74851	8.8043
SNORD4B	3.882138	1.377682	0.024307	1	3.401069	8.828211
MIR612	3.804063	1.442514	0.020062	1	3.150318	8.553186
MIR6502	3.063624	1.777814	0.026604	1	1.678774	5.750442
DDAH2	2.824525	2.40013	0.007197	0.976517	1.146432	6.044955
CXCL10	1.983587	2.499268	0.011719	1	0.618414	3.492747
RNVU1-3	1.8413	2.63472	0.021484	1	0.534637	3.316823
MIR5001	1.76724	3.06883	0.011719	1	0.436943	3.662419
LOC107984935	0.869244	3.856891	0.015625	1	0.178437	2.582613
MIR6721	-0.43601	6.028913	0.03125	1	0.034019	2.21851
RNVU1-14	-1.5138	6.134473	0.03125	1	0	2.386923
MIR760	-1.5138	6.267965	0.015625	1	0	2.618323
MASCRNA	-1.5138	9.134792	6.11E-15	1.22E-11	0.000187	19.099598
SNORD19B	5.022302	1.167602	0.00182	0.330428	8.0623	18.091555
C3	5.594264	1.35314	1.36E-05	0.004792	11.238561	28.680056
DDT	5.852274	3.117466	2.86E-29	3.85E-25	7.291585	63.212959

FDR, false discovery rate, TPM, transcripts per million

Table 2. Estimated differentially expressed genes (DEGs) between control THP-1 cells and IL-1 β -stimulated cells

Normalized genes with FDR less than 0.05 isolated using TCC-GUI were designated as DEGs between control THP-1 cells and IL-1 β -stimulated cells. (a) Downregulated DEGs, (b) Upregulated DEGs.

(a) Downregulated DEGs

Gene Name	A Value	M Value	P-Value	Q Value (FDR)	TPM Control	TPM IL-1 β Stimulated
NDUFA3	2.843422125	-4.010547812	1.11759E-07	6.25476E-05	10.713716	0.664016
CLIC1	8.132043215	-0.527506405	1.74988E-05	0.005732787	125.234421	86.788475
PSMB3	8.358140478	-0.505354851	8.87393E-06	0.003611959	145.362244	102.295891
CES1	8.379629158	-0.480303786	2.11853E-05	0.006775253	146.268158	104.736389

(a) (Continued)

Gene Name	A Value	M Value	P-Value	Q Value (FDR)	TPM Control	TPM IL-1 β Stimulated
<i>MIF</i>	10.62178682	-0.439716192	2.19304E-17	7.36421E-14	698.490479	521.153076
<i>SNORD141A</i>	10.72203155	-0.391106209	6.33553E-15	1.2157E-11	719.213135	548.185303
<i>MIR1244-2</i>	10.91995502	-0.243282603	2.18316E-07	0.000112785	783.770386	661.434814
<i>RPL17-C18orf32</i>	10.95197345	-0.185033693	7.38783E-05	0.021113466	785.344116	690.069702
<i>H2AZ1</i>	11.58320605	-0.143760781	0.000123859	0.033273467	1199.168945	1084.239014
<i>RPL23A</i>	12.00671952	-0.161879208	5.10944E-07	0.000254185	1618.397339	1445.228027
<i>RPL17</i>	12.17842123	-0.184249712	1.30415E-09	1.03044E-06	1837.128784	1615.133667
<i>RPS3</i>	12.1922498	-0.13141981	1.42694E-05	0.004914538	1821.171021	1660.821899
<i>ENO1</i>	12.34902821	-0.109489102	0.00013531	0.035636849	2014.859985	1865.601562
<i>RPS7</i>	12.42095381	-0.133595682	1.73886E-06	0.000834158	2135.625	1944.653687
<i>RPS20</i>	12.45174619	-0.108828536	8.69818E-05	0.023843654	2163.050049	2003.812378
<i>RPL7</i>	12.50242389	-0.153352992	1.65813E-08	1.01236E-05	2275.22168	2043.588379
<i>MIR1244-1</i>	12.55122503	-0.118296279	9.58395E-06	0.003786225	2325.079102	2139.738037
<i>RPL12</i>	12.61057108	-0.108446657	3.44008E-05	0.010501639	2414.460938	2237.216553
<i>RPS4X</i>	12.74437344	-0.109680182	1.15602E-05	0.004313245	2650.234619	2453.583984
<i>RPL24</i>	12.75005499	-0.116265991	3.19894E-06	0.001481661	2666.834473	2457.649658
<i>RPS16</i>	12.84744467	-0.094771244	8.67848E-05	0.023843654	2831.834473	2648.942139
<i>RPS17</i>	12.96280383	-0.092003115	7.10511E-05	0.020746914	3064.628418	2872.206055
<i>RPL27</i>	13.01534139	-0.085671292	0.000167371	0.043233122	3171.320557	2985.273438
<i>RPS6</i>	13.08355022	-0.147131858	3.40418E-11	3.5173E-08	3396.438721	3063.841309
<i>RPL7A</i>	13.08624211	-0.095932477	1.54887E-05	0.005201098	3342.934326	3124.516846
<i>RPS3A</i>	13.10061078	-0.119671192	6.09248E-08	3.55801E-05	3404.288086	3129.934326
<i>ELANE</i>	13.13209439	-0.10074016	3.96646E-06	0.001775917	3456.641846	3220.046631
<i>RPL10A</i>	13.23878458	-0.094017888	8.14038E-06	0.003416923	3713.295898	3475.288818
<i>RPL5</i>	13.24073211	-0.129364806	7.68298E-10	7.19571E-07	3764.142334	3437.612549
<i>RPL18</i>	13.28758228	-0.090280673	1.3083E-05	0.004749498	3836.070557	3599.506836
<i>RPL29</i>	13.33118727	-0.093584184	4.49801E-06	0.001948945	3958.313721	3705.716309
<i>FTL</i>	13.41037873	-0.081175426	4.35838E-05	0.013009275	4163.720215	3931.736572
<i>RPL19</i>	13.57775038	-0.082729509	1.00912E-05	0.003872701	4678.421387	4412.948242
<i>RPS27</i>	13.7447223	-0.100722088	1.2025E-08	8.07601E-06	5285.317383	4923.617188
<i>RPL18A</i>	13.87815392	-0.088171223	1.68546E-07	9.05562E-05	5772.481445	5424.328613
<i>RPS24</i>	13.94044523	-0.093681876	1.36226E-08	8.71325E-06	6038.510742	5652.78418
<i>RPS8</i>	13.96813105	-0.09476726	6.64796E-09	4.69975E-06	6157.827148	5760.288086
<i>RPL13A</i>	14.04390185	-0.116644614	2.35241E-13	3.51084E-10	6539.275391	6024.897461
<i>RPS11</i>	14.06527489	-0.066578042	2.57853E-05	0.008054621	6522.705566	6221.847656
<i>PRTN3</i>	14.15040106	-0.137197627	3.53529E-19	2.3743E-15	7090.594238	6440.441406
<i>RPS12</i>	14.26133904	-0.118919247	7.71676E-16	2.07303E-12	7608.992188	6999.425781

(b) Upregulated DEGs

Gene Name	A Value	M Value	P-Value	Q Value (FDR)	TPM Control	TPM IL-1 β Stimulated
<i>MASCRNA</i>	-1.5137966	9.13479213	6.10623E-15	1.2157E-11	0.000187	19.099598
<i>DDT</i>	5.85227424	3.11746559	2.86446E-29	3.84754E-25	7.291585	63.212959
<i>C3</i>	5.59426422	1.35313958	1.35572E-05	0.004792115	11.238561	28.680056
<i>CD99</i>	8.29306039	0.912373	1.47016E-14	2.46839E-11	85.010246	159.829346
<i>RNA28SN5</i>	8.72239802	0.59592245	3.67837E-09	2.74488E-06	127.745407	192.872086
<i>RNA28SN4</i>	9.05456044	0.55768402	8.03571E-10	7.19571E-07	162.963989	239.60997
<i>RNA28SN2</i>	9.30195048	0.5718147	6.55212E-12	7.334E-09	192.502594	285.827209
<i>RNA28SN3</i>	9.37453385	0.56431346	3.92339E-12	4.79082E-09	202.962311	299.794922
<i>RNA28SN1</i>	9.43515729	0.57669686	3.4072E-13	4.57655E-10	210.76622	314.005737
<i>RN7SL1</i>	11.1618872	0.26543702	9.20784E-10	7.72998E-07	777.047913	933.309387
<i>EEF1A1</i>	14.7678846	-0.1077724	3.22268E-18	1.4429E-14	10767.99219	9992.490234

FDR, false discovery rate, TPM, transcripts per million

Discussion

Although the Δ Exon2 ASC variant present in Japanese patients with PR lacks exon 2, all three exons of ASC were found to be intact in genomic DNA, which suggests disruptions in ASC splicing.⁶ Therefore, we analyzed the aberrant splicing mechanism of ASC in the present study. We found that the rs8056505 G allele induced Δ Exon2 ASC expression in THP-1 cells without stimulation. According to the NCBI dbSNP, the Japanese allele frequency of rs8056505 is 77.3% for the A allele and 22.7% for the G allele. Our result indicates that the rs8056505 G allele is a major genetic factor that induces Δ Exon2 ASC expression in patients with PR. Although this SNP is located in the 5'-UTR of the ASC gene and is thought to primarily affect transcription, our findings indicate that rs8056505 also affects the splicing of ASC, considering that transcription and alternative splicing are physically and functionally coupled processes.¹⁰ Our findings also suggest that the rs8056505 G allele affects the function of inflammasomes by inducing Δ Exon2 ASC, as we have already addressed.⁶ The SNP position is considered to be in a transcription regulatory region (888 bp upstream of exon 1) and may affect the binding of transcription factors that are linked to splicing. In fact, predictions of transcription factor binding to the rs8056505 region suggested the presence of binding motifs such as Oct1, Xfd3, Hfh2, and Hnf3 (<https://tfbind.hgc.jp>). We also believe that differences in SNPs per cell, i.e., microchimerism, may be involved.

We also found that Δ Exon2 ASC expression was suppressed via IL-1 β stimulation and its downstream molecule ceramide. The involvement of IL-1 β in splicing has also been reported based on the fact that *FOXP3* Δ Exon7 mRNA expression is increased in response to T cell receptor stimulation combined with IL-1 β in human regulatory T cells.¹¹ These findings indicate that IL-1 β affects splicing. We identified genes showing altered expression in IL-1 β -stimulated THP-1 cells using RNA-seq and found that

several genes related to splicing were affected by IL-1 β treatment. Notable DEGs were RNVUs, *MASCRNA*, and non-coding or microRNAs including SNORDs. U1 small nuclear RNA (U1 snRNA) is a major component of the spliceosome and splices pre-mRNA to generate mature RNA.¹² U1 snRNA knockdown has also been reported to cause premature cleavage and polyadenylation (PCPA) in several pre-mRNAs.¹³ U1 snRNA has been suggested to protect entire pre-mRNA molecules from abnormal cleavage and PCPA by binding to various sites on the pre-mRNAs.¹³ In addition, the cathepsin A gene A \rightarrow G SNP results in the absence of the seventh exon in the mRNA, which occurs because U1 snRNA cannot bind to the 5' splice site; moreover, modification of U1 snRNA to complement the 5' splice site improves the splicing abnormality.¹⁴ Based on these results, U1 snRNA induction by IL-1 β may contribute to wild-type ASC expression. *MASCRNA* was a significantly upregulated DEG by IL-1 β stimulation. *MASCRNA*'s parent long non-coding RNA *MALAT1* was reported as a modulator of SR splicing factors, which strongly suggests that upregulated *MASCRNA* by IL-1 β may contribute to wild-type ASC expression.¹⁵ Several non-coding RNAs and microRNAs were also detected in this study. Notably, SNORDs are a highly expressed class of non-coding RNAs, which in addition to their well-established role in rRNA modification, regulate the pre-mRNA splicing of several genes.¹⁶ It has also been reported that *SNORD88C* regulates the alternative splicing of *FGFR3* pre-mRNA.¹⁷ Several recent studies have suggested that alternative RNA splicing may be partly modulated by microRNAs, which are short non-coding RNAs that inhibit the translation of specific mRNA transcripts.¹⁸ As observed in tissues and in diseases, such as cancer and neurological disorders, the dysregulation of microRNA pathways disrupts downstream alternative RNA splicing events by affecting the availability of splicing factors involved in RNA splicing, such as SR proteins

and hnRNP proteins.¹⁸ Future experiments to quantify the expression of candidate genes with qPCR are needed to confirm our RNA-seq results. Overexpression of candidate factors, such as *MASCRNA* and U1snRNAs in THP-1 cells would also be helpful to confirm the effects on ASC splicing.

Based on the results of this study, we propose a splicing cycle in which ASCs alternate between wild-type and Δ exon2 expression regulated by the rs8056505 G allele and splicing regulators, induced by IL-1 β (Figure 3). First, Δ exon2 is induced by the rs8056505G allele. Next, Δ exon2 ASC activates the inflammasome compared to that in the wild-type and increases IL-1 β production as reported previously.⁶ IL-1 β affects the expression of splicing regulators and improves the splicing of ASC, after which wild-type ASC becomes dominant. Owing to ASC splicing repair, the interference of splicing regulators is attenuated due to decreased IL-1 β production via ASC wild-type, and Δ exon2 becomes dominant again. This cycle may lead to the formation of periodic pathologies of PR.

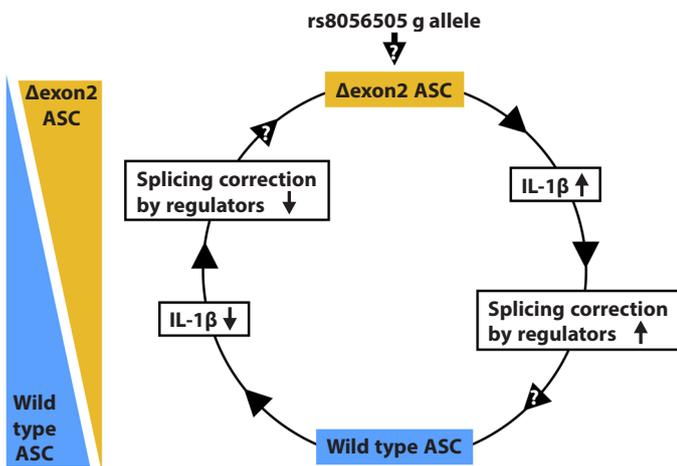


Figure 3. Schema of cyclic expression model of ASC variants. Δ exon2 ASC is induced by the rs8056505 G allele and increases IL-1 β compared to wild-type ASC. Increased IL-1 β affects splicing regulator genes and they induce wild-type ASC expression. Interference of splicing regulators is attenuated due to the decreased IL-1 β by wild-type ASC and Δ exon2 ASC becomes dominant again.

IL-1 β , interleukin-1 β ; ASC, apoptosis-associated speck-like protein containing a CARD

A limitation of this study is the small sample size. To validate the model presented in this study, it will be necessary to use a large number of samples. Although the rs8056505 SNP is reported to be associated with a significantly increased risk of *Helicobacter pylori* infection, whether it affects the splicing of any genes is unknown.¹⁹ The effect of rs8056505 SNP and IL-1 β on the splicing of other genes in other autoinflammatory diseases requires examination. It may also be important to consider in patients receiving IL-1 β suppressive therapy such as canakinumab treatment.

In conclusion, we detected the rs8056505 A \rightarrow G SNP located in the 5'-UTR of genomic ASC in Japanese patients with PR and revealed that Δ exon2 ASC expression is induced by this SNP, whereas it is suppressed by IL-1 β or ceramide treatment. We also found that several genes, such as splicing factors, including *MASCRNA*, are affected in IL-1 β -stimulated THP-1 cells. Based on these results, we propose a cyclic expression model in which ASCs alternate between wild-type and Δ exon2 expression regulated by the rs8056505 G allele and splicing factors induced by IL-1 β . This cycle may be correlated with the formation of periodic PR pathologies.

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Conflicts of Interest

None

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Author contribution

- Masaya Hattori, Atsuko Yabuuchi, and Hongyan Wang performed exon-trapping assays.
- Hayate Tanaka found the Δ Exon2 ASC and rs8056505A \rightarrow G SNPs in patients with PR.
- Masaya Hattori and Taketo Kawara performed RNA-seq analysis.
- Koji Inoue diagnosed the patients with PR.
- Shunichi Shiozawa proposed the basic research concept.
- Koichiro Komai designed all experiments, wrote the grants, and wrote and edited the manuscript.

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