Supplemental materials:

Pathogenic *UBA1* variants associated with VEXAS syndrome in Japanese patients with relapsing polychondritis

Supplemental Methods

DNA extraction

Genomic DNA (gDNA) was extracted from peripheral blood leukocytes or bone marrow aspirate using QuickGene-610 L (Fujifilm, Tokyo, Japan) and from formalin-fixed paraffin-embedded (FFPE) blocks of bone marrow using a QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. DNA concentration was measured using a Qubit dsDNA BR assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

Positive and negative control DNAs

Wild-type and variant DNAs were used to set Tm values and minimize background for the droplet digital PCR (ddPCR) assay. A 349-bp PCR fragment harboring three known *UBA1* p.Met41 variants (c.121A>C, c.121A>G, c.122T>C) [1], and a wild-type allele from patients with relapsing polychondritis (RP) who had the *UBA1* variants was amplified using primers (Table S1). The fragment was cloned into a pCR™ 4-TOPO® vector using a TOPO TA Cloning Kit (Life Technologies, Carlsbad, CA, USA). The inserted fragment in each clone was confirmed by Sanger sequencing.

Droplet digital PCR (ddPCR) targeting pathogenic *UBA1* variants

The ddPCR was performed using a Droplet Digital PCR XQ200 system (Bio-Rad Laboratories, Hercules, CA, USA) as described previously [2]. The region-specific primers and customized locked nucleic acid (LNA) probes for two wild-type (c.121A and c.122T) and three variant alleles (c.121A>C, c.121A>G, c.122T>C) of *UBA1* were purchased from Integrated DNA Technology (Coralville, IA, USA). The primers and probes are listed in Table S1. The PCR mixture contained 66 ng gDNA, 10 µL 2× ddPCR Supermix for probes (no dUTP; Bio-Rad, Hercules, CA, USA), 900 nM target-specific PCR primers, 250 nM variant-specific (FAM) and wild-type-specific (HEX) LNA probes. Twenty µL of PCR mixture and 65 µL Droplet Generation Oil for Probes (Bio-Rad) were mixed and droplets were generated using a QX100 Droplet Generator (Bio-Rad) according to the manufacturer's protocol. The droplet emulsion was thermally cycled as follows: denaturing at 95°C for 10 min, 40 PCR cycles at 94°C for 30 s and 59°C for 2 min, and final extension at 98°C for 10 min. PCR amplification in the droplets was confirmed using a QX200 Droplet Reader (Bio-

Rad). The threshold was determined by comparing wild-type and no-template ddPCR results. All data above the threshold were evaluated. All the experiments were performed in duplicate or triplicate. The data were analyzed using QuantaSoft (version 1.7.4.0917, Bio-Rad). The rare event detection mode setting was used for all ddPCR measurements.

Confirmation of detection limit in ddPCR analysis

Theoretically, 1 ng of gDNA should contain 330 copies. We found that 1 ng of the gDNA from patient RP01 contained 236 copies of the variant allele *UBA1* c.121A>C, according to the fractional abundance obtained by ddPCR. The gDNA of this patient was serially diluted with control wild-type gDNA in variant allele ratios of 5.0%, 1.0%, 0.5%, 0.1%, 0.05%, and 0.01%, and ddPCRs were conducted on the serially diluted samples (using 66 ng of gDNA equivalent to 2.2×10^4 copies per well).

Exome sequencing

Genomic DNA was sheared and captured using a SureSelect Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on a Novaseq 6000 system (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. Exome data processing, variant calling, and annotation were performed as described previously [3]. Briefly, reads were aligned to the human genome reference sequence GRCh37 using Novoalign (http://www.novocraft.com/), and PCR duplicates were removed using Picard (http://broadinstitute.github.io/picard/). Local realignments around indels and base quality-score recalibration were performed using the Genome Analysis Toolkit (GATK). Variants were called using GATK UnifiedGenotyper and filtered according to GATK Best Practices (version 3) (https://software.broadinstitute.org/gatk/). The common variants registered in the single nucleotide polymorphism database dbSNP137 (minor allele frequency ≥ 0.01) without known clinical associations were excluded from further analysis. The included variants were annotated using ANNOVAR (http://annovar.openbioinformatics.org/). The mean coverage depth against the RefSeq coding sequences (CDSs) was 79.9×, and 100% of the CDSs were covered by ≥10 reads. Variants were confirmed by Sanger sequencing.

Peptide nucleic acid (PNA)-clamping PCR

Wild-type-specific PNA corresponding to *UBA1* p.Met41 (c.121A and c.122T) was purchased from Fasmac (Atsugi, Kanagawa, Japan). The PCR mixture was prepared in a 20-μL volume contained 4 μL of 5 × PrimeSTAR GXL Buffer (Takara, Otsu, Shiga, Japan), 1.6 μL of 2.5 mM dNTP Mixture (Takara), 0.2 μL of PrimeSTAR GXL DNA Polymerase (Takara), 10 μM forward and reverse primers, 10 μM PNA, and 50 ng gDNA. PCR

conditions were as follows: denaturing at 94°C for 2 min; 30 cycles at 98°C for 10 s, 60°C for 30 s, and 68°C for 2 min; and extension at 68°C for 5 min. The PCR reaction was analyzed by agarose gel electrophoresis and the product band was visualized on a 2% agarose Tris- borate-EDTA gel after ethidium bromide staining.

Measurement of anti-type II collagen antibody

Human serum anti-type II collagen antibody was measured using an ELISA at Quest Diagnostics (NY, USA).

Supplemental Results

Clinical features of patients with RP who had *UBA1* p.Met41 variants

Patient RP01

Patient RP01 was an 83-year-old man. At 76 years of age, he presented with fever and erythema in the upper body (Figure S2G) and prednisolone (PSL, 5 mg/day) was initiated but with repeated exacerbation. At 78 years of age, he presented with auricular and eyelids swelling (Figure S2A). Biopsy of left auricular cartilage revealed inflammation of the perichondrium to epidermis. Chest computed tomography (CT) showed deformities of the thyroid gland and tracheal cartilage. Positron emission tomography (PET) combined with CT (PET-CT) showed ^{18}F-FDG accumulation at the nasal septum. Skin biopsies of right forearm and left femoral regions showed perivascular neutrophilic infiltration. He was diagnosed with RP and medicated with PSL (30 mg/day, 0.5 mg/kg/day), leading to improved symptoms. Thereafter, he had repeated exacerbations with fever and was treated with increased PSL. Consequently, PSL could not be reduced below 10 mg/day. At 83 years of age, he was hospitalized for high fever and left pleural effusion. He had anemia and thrombocytopenia (Hb 7.0 g/dL, MCV 102.9 fL, MCH 35.5 pg, MCHC 34.5%, RDW 15.9%, Plt 75.0×10^3/μL). A bone marrow examination revealed myelodysplastic syndrome with multilineage dysplasia (MDS-MLD) (Table S2). Bone marrow aspiration showed characteristic vacuolization in myeloid and erythroid precursor cells. MDS was supportively treated with blood transfusion and increased PSL improved his general condition. He was transferred to another hospital but died from an exacerbation of interstitial pneumonia (the details are unknown).

Patient RP03

Patient RP03 was an 83-year-old man. At 81 years of age, he presented with exertional dyspnea, productive cough, and auricular reddening. Chest CT revealed stenosis

of the trachea and bilateral primary bronchus surrounded by thick soft tissues. Mediastinoscope-assisted tracheal biopsy revealed chondritis, leading to the diagnosed of RP. High-dose PSL (65 mg/day) was initiated but with no improvement in tracheal stenosis and stenting of the trachea and bronchial was performed (Figure S3D). He had repeated dyspnea and received intravenous mPSL and medium-dose PSL (30 mg/day). He was treated with azathioprine (AZP) and colchicine, which were both ineffective. At 83 years of age, he developed aspiration pneumonia and was treated in an intensive care unit with antibacterial drugs but died because of respiratory failure. Autopsy revealed that destruction and degeneration of the tracheal cartilage from epiglottis to peripheral bronchus and respiratory tract stenosis may have extended to the entire lung area. He also had diffuse pneumonia, bilateral pleural effusion, and right ventricular enlargement.

Patient RP04

Patient RP04 was a 72-year-old man. At 67 years of age, he presented with fever, ophthalmalgia, swelling of eyelids, and conjunctive hyperemia. He was diagnosed with scleritis at a neighboring clinic. At 68 years of age, he presented with fever, erythema, and auricular swelling (Figure 1A, B). A biopsy of the auricular cartilage revealed chondritis (Figure 1C, D), leading to the diagnosis of RP. Skin biopsy showed severe neutrophilic dermatosis extending from subcutaneous to the perivascular region (Figure 1F). Through the work-up of unknown fever, he was also diagnosed with MDS with isolated del (5q) (46,XY,5q-[16]/46,XY[4]). He was treated with medium to high doses of PSL (17.5–25 mg/day) and methotrexate (MTX), but had repeated exacerbations of RP with fever, auricular chondritis, and infectious events. At 70 years of age, he became transfusion-dependent because of chronic inflammation in addition to MDS. Bone marrow aspiration revealed vacuolization in myeloid and erythroid precursor cells (Figure 1H, I). His status was concluded as progressing MDS (46,XY,5q-[20]/[20]) (Table S2), but he refused to initiate treatment with lenalidomide [4]. At 71 years of age, he presented with progressive dyspnea and a chest CT showed interstitial pneumonia. MTX was discontinued because of suspicion of drug-induced pneumonia. At 72 years of age, cyclosporine A (CyA) was initiated in addition to PSL for exacerbation of fever, general malaise, and polyarthritis. After reinforced immunosuppression, his general condition improved, but he died suddenly. There were no apparent findings including infectious foci or exacerbation of interstitial pneumonia by autopsy, which revealed diffuse endocarditis extending around the heart conducting system, but the cause of the endocarditis was undetermined. This might be a cardiovascular feature of RP.

Patient RP05

Patient RP05 was a 74-year-old man. At 71 years of age, he presented with left auricular swelling, fever, and trunk erythema (Figure S2H), and was treated with PSL 30 mg/day, but the symptoms were recurring. A biopsy of the auricular cartilage showed chondritis, which led to the diagnosis of RP. Regarding erythema, he was diagnosed with Sweet's syndrome. He was treated with PSL (5–30 mg/day) and colchicine but his condition showed little improvement and he had repeated exacerbations of fever, general malaise, rash, and hoarseness. In addition to PSL, AZP, MTX, and mizoribine were used, but were ineffective and discontinued because of thrombocytopenia, liver dysfunction, fatigue, or rash. Several infections including pneumonia and deep neck abscesses occurred. At 73 years of age, he had deep vein thrombosis and chronic pulmonary embolism, and started anticoagulant therapy with warfarin potassium. For repeated exacerbate erythema, skin biopsy was conducted and showed nodular dermatitis with infiltration of inflammatory cells in epidermis and perivascular region, consistent with neutrophilic dermatitis accompanied by RP. Etanercept 50 mg/week was started to control RP exacerbations. Subsequently, he had macrocytic anemia and thrombocytopenia (Hb 10.1 g/dL, MCV 105.5 fL, MCH 34.8 pg, MCHC 33.0%, RDW 19.7%, Plt 67.0×10^3/μL) and MDS was suspected. Bone marrow aspiration revealed several vacuoles and he was diagnosed with MDS with ringed sideroblasts and multilineage dysplasia (MDS-RS-MLD) (Table S2). At 74 years of age, his RP symptoms recurred and the PSL dose was increased, and he was hospitalized for severe pneumonia. He was treated with antibiotics but had sudden cardiopulmonary arrest and underwent cardiopulmonary resuscitation. After resuscitation, he was transferred to the intensive care unit where he had a right temporal lobe cerebral infarction, bilateral occipital lobe cerebral infarction, and gastrointestinal hemorrhage; he died after 26 days of cardiopulmonary arrest. Autopsy revealed sepsis (*Klebsiella pneumoniae*, *Citrobacter freundii*), bacterial and mycotic pneumonia, focal pulmonary embolism and infarction, esophageal ulcer, neutrophilic infiltration at cardiac muscle and liver. He died of assumed sepsis and the cause of cardiopulmonary arrest could not be finally specified.

Patient RP10

Patient RP10, a 43-year-old woman, presented with productive cough and fever. Chest CT suggested peri-tracheal inflammation. Subsequently, she had hoarseness. PET-CT showed ^{18}F-FDG accumulation in the trachea and bilateral primary bronchus (Figure S3C), bilateral costicartilages, and nasal septum cartilage. A biopsy of the nasal septum cartilage revealed inflammation around the perichondrium consistent with the early stage of RP. She was diagnosed with RP and medicated with PSL (70 mg/day, 1.0 mg/kg/day) and MTX, and

her symptoms improved. After gradual reduction of PSL to 12.5 mg/day and increasing the MTX to 16 mg/week over 7 months, she presented with hoarseness and malaise, and a diagnosis of RP exacerbation was made from CT images of peri-tracheal inflammation. She is now taking PSL 10 mg/day, MTX 16 mg/week, tacrolimus 2 mg/day and tocilizumab (TCZ) (162 mg/2-week SC).

Patient RP11

Patient RP11 was an 82-year-old man. At 78 years of age, he presented with macrocytic anemia (Hb 5.6 g/dL, MCV 107.6 fL, MCH 36.2 pg, MCHC 33.7%, RDW 17.5%) and recurrent right auricular swelling and bilateral lower leg edema. He had been anemic during his annual checkup for several years but had not been examined in detail. Biopsy of right auricular cartilage revealed chronic inflammation around the perichondrium consistent with the early stage of RP. He had a skin rash and polyarthritis, which was confirmed by joint ultrasound (Figure S3E) and was considered as a characteristic feature of RP. He was diagnosed with MDS-MLD by bone marrow aspiration (Table S2). Characteristic vacuoles were also confirmed in bone marrow aspiration. He showed chronic heart failure caused by paroxysmal atrial fibrillation. He started taking oral PSL 30 mg/day and AZP 25 mg/day for RP and the auricular swelling and polyarthritis improved. AZP was discontinued due to liver dysfunction and changed to CyA and tapered PSL. He is currently treated with PSL 9 mg/day and CyA 100 mg/day and his symptoms seem to be well controlled except for intermittent erythema.

Patient RP12

Patient RP12 was an 80-year-old man. At 70 years of age, he was diagnosed with RP due to left auricular swelling and medicated with PSL 5 mg/day. At 72 years of age, he presented with macrocytic anemia (Hb 10.5 g/dL, MCV 105.5 fL, MCH 34.9 pg, MCHC 33.1%) and received bone marrow aspiration. He was diagnosed with MDS-MLD and required monthly blood transfusions from 73 years of age. He developed intermittent erythema at head, neck, trunk, and upper extremities, some of which were ulcerated, leading to the suspicion of pyoderma gangrenosum. Elevated C-reactive protein (CRP; approximately 10 mg/dL) persisted under 5 mg/day of PSL. At 80 years of age, his femur and skull were fractured, and he was transferred to another hospital. The subsequent course of this patient is unknown. Bone marrow aspiration at 73 years of age was reviewed and vacuoles were recognized in myeloid and erythroid precursor cells (Figure S4, Table S2). From these clinical findings, he was suspected of having VEXAS, but because his gDNA was unavailable we could not check for pathogenic *UBA1* variants.

Patient RP13

Patient RP13 is a 66-year-old man. He had chronic kidney failure from polycystic kidney and received peritoneal dialysis. At 65 years of age, he presented with erythema on the lower extremities, trunk, and face (Figure S2E, F). He was diagnosed with erythema nodosum by skin biopsy and treated temporarily with PSL (20 mg/day). At 66 years of age, he presented with scleritis and hearing loss and was admitted to the hospital for a short time to investigate fever and abdominal pain. Peritonitis associated with peritoneal dialysis was suspected, but the abdominal pain did not improve with antibiotic treatment. A thorough examination revealed peritonitis, pericarditis, aseptic meningitis (cerebrospinal fluid: 4 cells/μL, IL-6 69.5 pg/mL), erythema nodosum, scleritis, and sensorineural deafness. PET-CT showed ^{18}F-FDG accumulation in the nasal septum and auricles (Figure S3B), but biopsy of the auricular cartilage showed no chondritis. He was treated with PSL 15 mg/day and colchicine and his clinical condition, including audibility, improved. Although no typical pathological findings were identified, he was clinically diagnosed with RP. After a gradual reduction to PSL 9 mg/day over 4 months, the patient presented with anorexia and elevated CRP (8.58 mg/dL) and received TCZ. Currently, he is being treated with TCZ (500 mg/4-week IV) and PSL, and his condition is improving. He presented with macrocytic anemia (Hb 11.7 g/dL, MCV 105.2 fL, MCH 34.7 pg, MCHC 33.0%, RDW 14.6%) and is receiving recombinant erythropoietin on a regular basis for end-stage renal disease.

Patient RP15

Patient RP15 is a 73-year-old man. At 73 years of age, he presented with cough and anorexia with weight loss of 5 kg every 3 months. Chest CT revealed bilateral grand-glass opacity, which improved without antibiotic therapy. Subsequently, he presented with repeated spontaneous remission and exacerbation of fever (37–38°C), erythema at upper truncus (Figure S2C), and subcutaneous nodules at limbs, as well as right auricular swelling (Figure S2B), right testis swelling, and right ankle arthritis. Gallium scintigraphy showed ^{67}Ga accumulation at left submandibular, right forearm, bilateral lower legs, and in bone marrow. A biopsy of the auricular cartilage showed chondritis, which led to the diagnosis of RP. Skin biopsy showed superficial perivascular inflammation. He had macrocytic anemia (Hb 8.7 g/dL, MCV 101.5 fL, MCH 32.7 pg, MCHC 32.2%, RDW 15.1%). Bone marrow aspiration revealed vacuoles in myeloid and erythroid precursor cells and leucocytes. There was normocellular bone marrow with apparent reduction of erythroid lineages (myeloid to erythroid ratio of 9:1), but no apparent morphological dysplasia (myeloid and erythroid dysplasia <10%) (Table S2). He is treated with PSL 20 mg/day and

TCZ (162 mg/2-week SC), leading to improved symptoms.

Patient RP16

Patient RP16 is a 69-year-old man. At 66 years of age, he presented with headache, conjunctive hyperemia, right auricular swelling, rhinitis, hoarseness, erythema multiforme, arthralgia, and high-grade fever. Laboratory tests showed high levels of CRP (24.2 mg/dL) and erythrocyte sedimentation rate (ESR; >100 mm/hr), with no evidence of autoantibodies, including rheumatoid factor, antinuclear antibody, and anti-neutrophil cytoplasmic antibody. Cervical CT revealed wall thickening of the common carotid artery and subclavian artery. Biopsy of the temporal artery revealed infiltration of inflammatory cells. From these findings, he was diagnosed with giant cell arteritis. He was treated with steroid pulse therapy and subsequently maintained with PSL 50 mg/day, which resulted in improvement of symptoms. At 68 years of age, after gradual PSL reduction to 12 mg/day, he showed exacerbation of skin rash and left auricular swelling and started receiving AZP. A biopsy of the left auricular cartilage revealed inflammation around the perichondrium consistent with chondritis, leading to the diagnosis of RP. He was again treated with steroid pulse therapy followed by PSL 60 mg/day (1 mg/kg/day). Thereafter, he presented with progressive anemia and thrombocytopenia (Hb 6.5 g/dL, MCV 107.6 fL, MCH 36.5 pg, MCHC 33.9%, RDW 27.1%, Plt 68.0×10^3/μL). His anemia persisted even after discontinuation of AZP and required regular blood transfusions. Bone marrow aspiration revealed vacuolization in myeloid and erythroid precursor cells, and he was diagnosed with MDS-MLD (Table S2). After gradual reduction of PSL to 30 mg/day, he presented with high-grade fever, skin rash (Figure S2D), and polyarthralgia. Lower extremity venous ultrasonography revealed deep vein thrombosis. Currently, he is being treated with TCZ (162 mg/2-week SC) and PSL, and his condition is improving.

Supplemental Figures

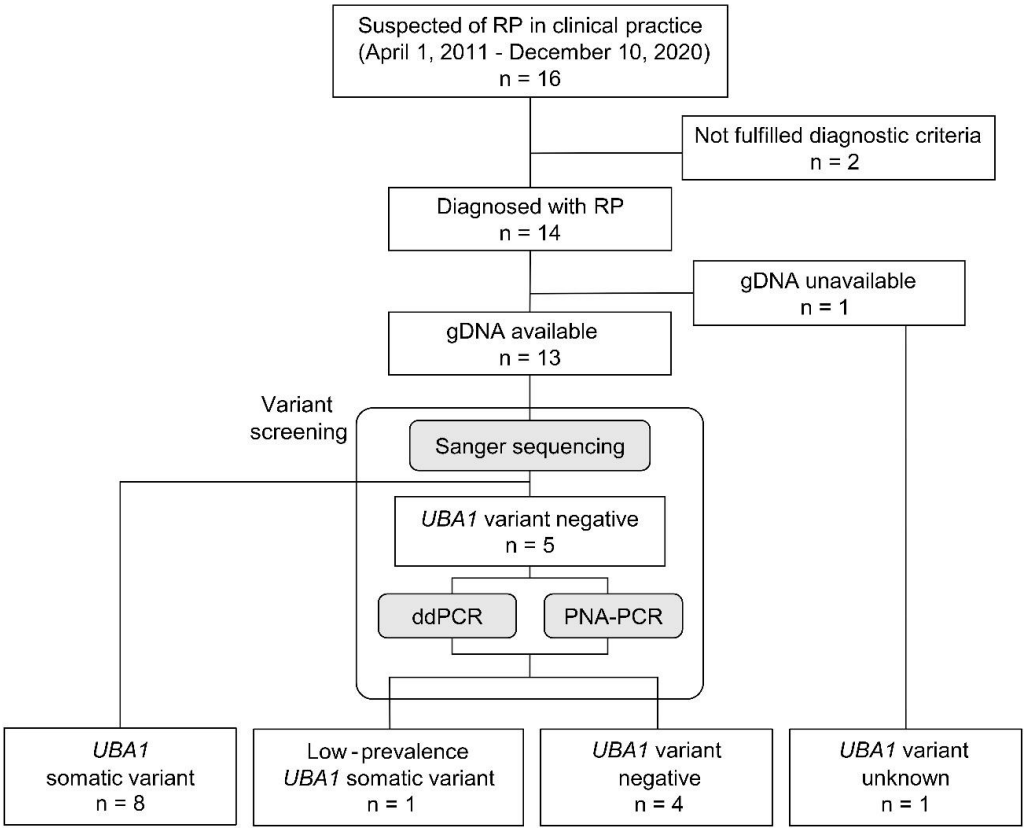

Figure S1. Pathogenic variant screening of patients with relapsing polychondritis recruited in this study.

ddPCR, droplet digital PCR; gDNA, genomic DNA; PNA-PCR, peptide nucleic acid (PNA)-clamping PCR; RP, relapsing polychondritis; *UBA1* variant, variant at *UBA1* p.Met41.

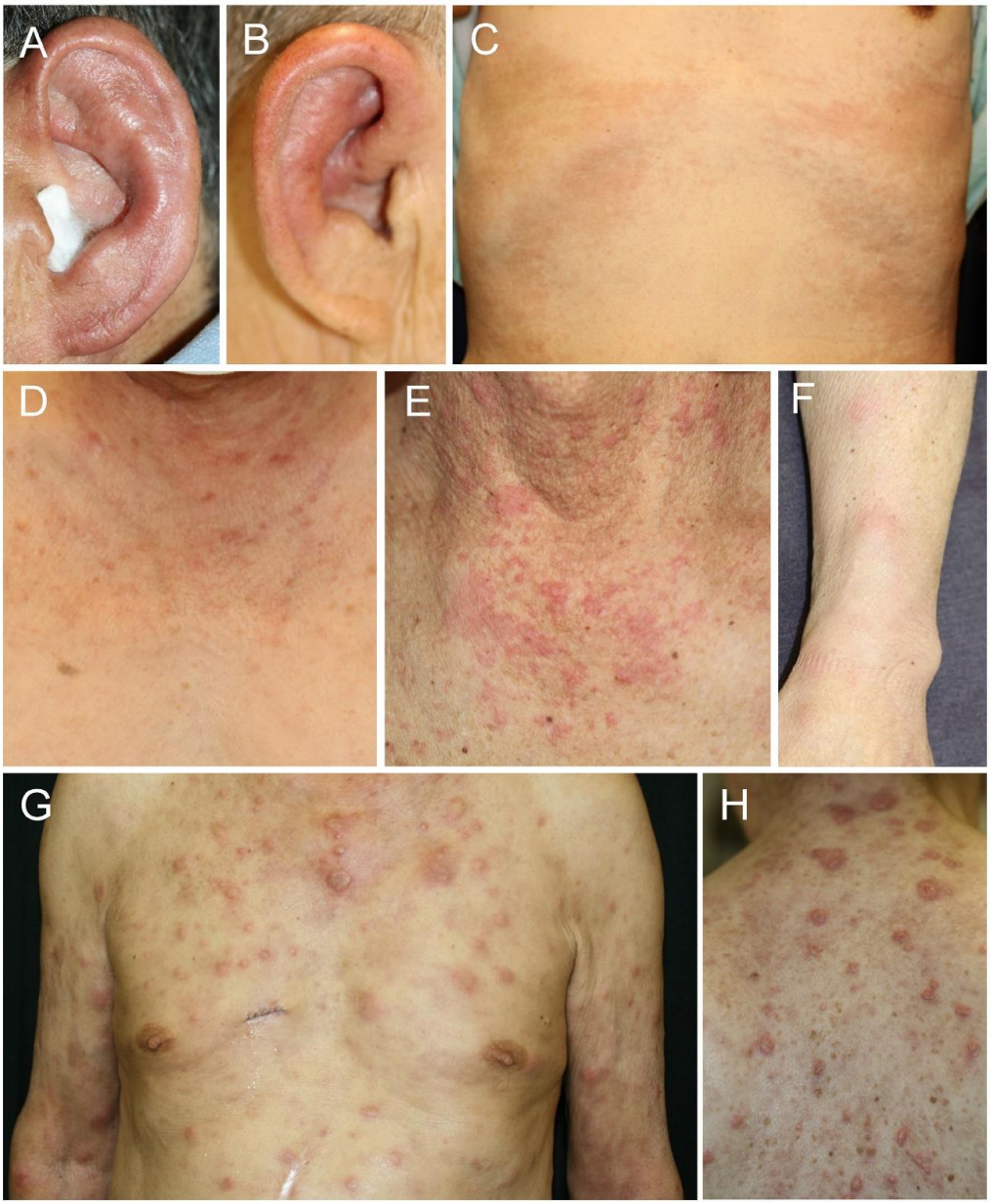

Figure S2. Additional clinical features of patients with relapsing polychondritis.
(A, B) Auricular chondritis of RP01 (A) and RP15 (B). (C, D, E) Erythema of RP15 (C), RP16 (D), and RP (13). (F) Erythema nodosum like lesions of RP13. (G, H) Neutrophilic dermatitis of RP01 (G) and RP05 (H).

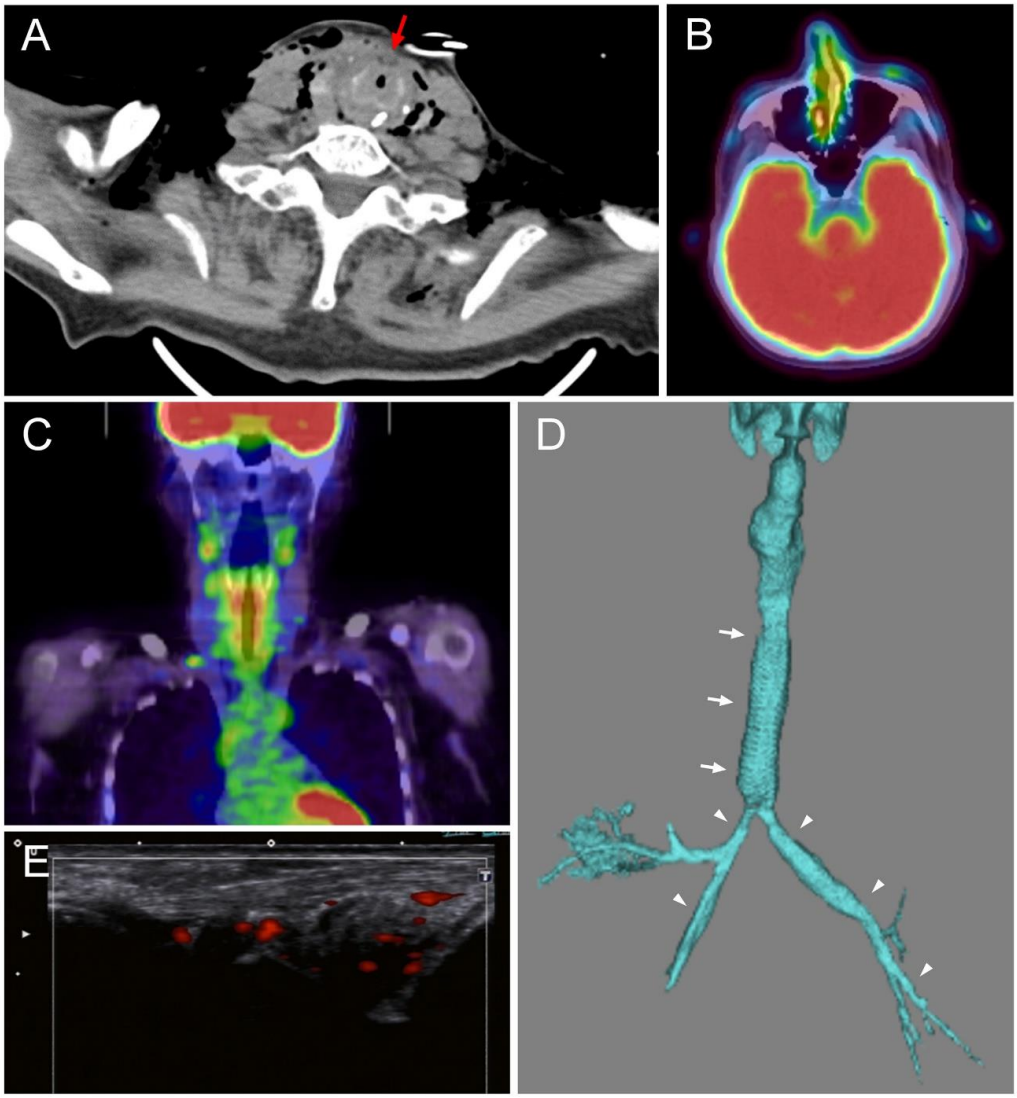

Figure S3. Additional imaging studies of patients with relapsing polychondritis.
(A) Computed tomography (CT) view of tracheobronchomalacia of RP02 (red arrow). (B, C) Positron emission tomography (PET) combined with CT views of nasal chondritis of RP13 (B) and respiratory tract chondritis of RP10 (C). (D) Three-dimensional CT reconstruction image of deformed tracheal cartilage of RP03. Stenting of the trachea (arrows) and stenosis of peripheral lower respiratory tracts (arrowheads). (E) Musculoskeletal ultrasonography views of wrist synovitis of RP11.

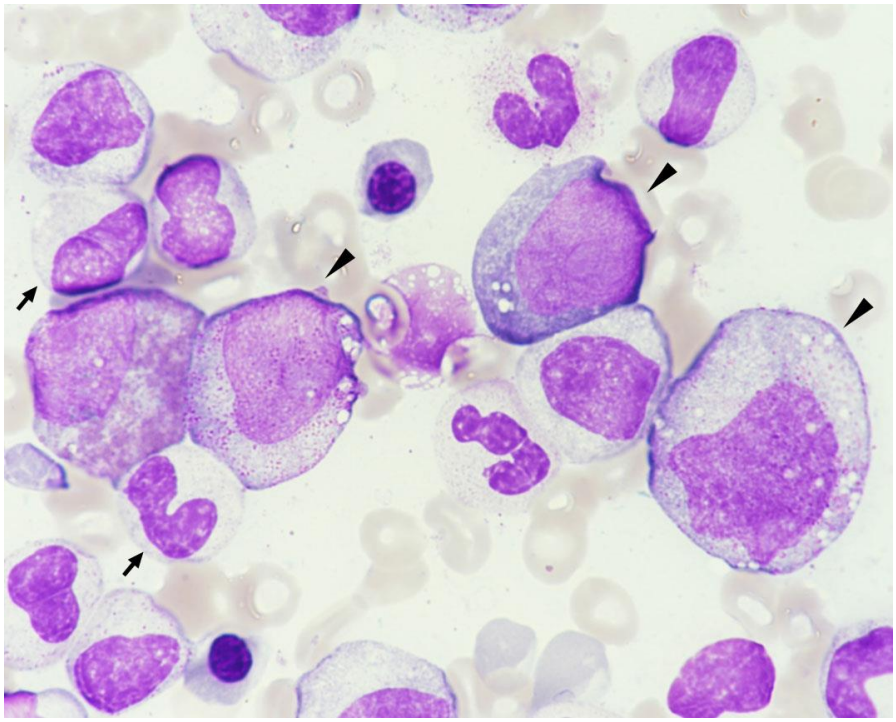

Figure S4. Bone marrow aspiration of patient RP12.

Vacuolization in myeloid and erythroid precursor cells (arrowhead) and degranulation of granulocytes (arrow) are shown (May-Giemsa staining).

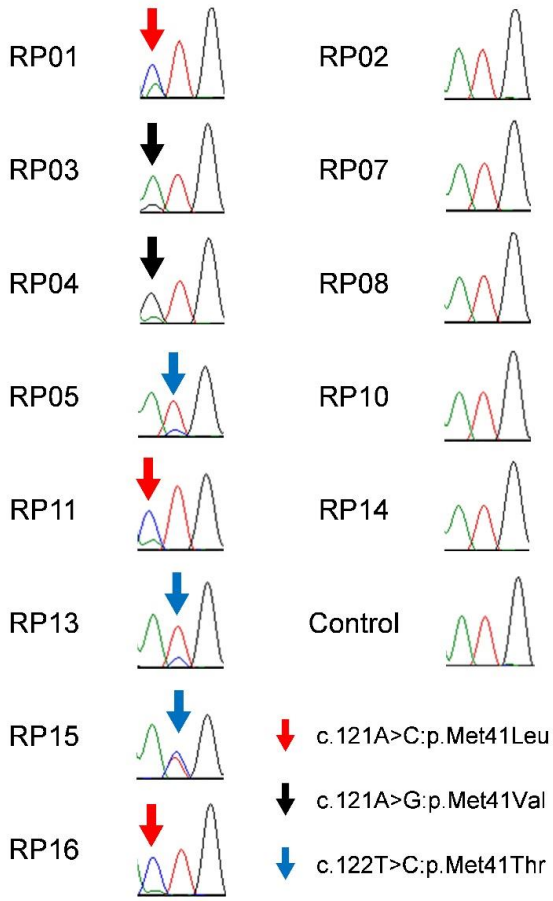

Figure S5. Sanger sequencing of *UBA1* p.Met41.

Chromatograms of the genomic DNA (gDNA) from 13 patients with relapsing polychondritis (RP) obtained by Sanger sequencing. The gDNA was extracted from the peripheral blood of 12 of the patients; for RP05, the gDNA was obtained from formalin-fixed paraffin-embedded [FFPE] blocks of bone marrow. Red, black, and blue arrows indicate the pathogenic variant at c.121A>C:p.Met41Leu, c.121A>G:p.Met41Val, and c.122T>C:p.Met41Thr, respectively. All variants were identified using forward and reverse primers (see Table S1 for details).

Supplemental Tables

Table S1. Sequences of primers and probes used in this study

	Sequence
PCR primers for Sanger sequencing	5′-AAGCCGGGTTCTAACTGCTC-3′ (forward)
	5′-GGTTAGGGGGTACTCTAGGTCA-3′ (reverse)
ddPCR primers	5′-CTCCACTCCTGTGTGTCT-3′ (forward)
	5′-GTAAAGGCCCTCGTCTATGT-3′ (reverse)
LNA probes	5′(HEX)-CTA+G+GG+A+A+TG+GC-3′(IBFQ) (c.121A wild-type)
	5′(FAM)-CT+AG+GG+A+C+TGGC-3′(IBFQ) (c.121A>C variant)
	5′(FAM)-CTA+G+GG+A+G+TGG-3′(IBFQ) (c.121A>G variant)
	5′(HEX)-CTAG+G+GA+A+T+GGC-3′(IBFQ) (c.122T wild-type)
	5′(FAM)-TA+GG+GA+A+C+GGC-3′(IBFQ) (c.122T>C variant)
PNA probe	5′-CTAGGGAATGGCCAA-3′ (wild-type)

ddPCR, droplet digital PCR; LNA, locked nucleic acid; PNA, peptide nucleic acid.

Table S2. Characteristics of patients with relapsing polychondritis and hematological abnormalities

Patient ID	RP01	RP04	RP05	RP07	RP11	RP12	RP15	RP16
Sex (M, male)	M	M	M	M	M	M	M	M
UBA1 variants p.Met41	+	+	+	−	+	NA	+	+
Bone marrow vacuoles	+	+	+	−	+	+	+	+
Macrocytic anemia	+	+	+	+	+	+	+	+
Myelodysplastic syndrome	+	+	+	−	+	+	−	+
WHO categorization[a]	MDS-MLD	MDS with isolated del(5q)	MDS-RS-MLD	−	MDS-MLD	MDS-MLD	−	MDS-MLD
R-IPSS score[b]	3	3	1.5	1	2.5	2	2	3
Hemoglobin (g/dL)	7.0	7.5	10.1	8.5	5.6	8.4	8.7	6.5
Platelets (×10^3/uL)	75.0	72.0	67.0	296.0	182.0	144.0	151.0	68.0
Neutrophils (×10^3/uL)	4.0	7.6	2.9	18.5	2.3	4.1	2.3	2.6
Marrow blasts (%)	< 1%	< 1%	< 1%	< 1%	< 1%	1.2 %	< 1%	< 1%
Ringed sideroblasts (>15%)	NA	NA	26%	NA	3%	NA	NA	4%
Morphological dysplasia lineages[c] (>10%)	E, Mgk	E, M, Mgk	E, Mgk	−	E, Mgk	E, M, Mgk	−	E, Mgk
Cytogenetics	46,XY[20]	46,XY,5q-[20]/[20]	46,XY[20]	46,X,-Y[3] /46,XY[17]	46,XY,del(20)(q1?)[3] /46,XY[17]	46,XY[20]	46,XY[20]	46,XY[20]

Somatic variants were not examined in all patients.

[a]WHO categorization was conducted using the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia [5].

[b]R-IPSS score was according to the Revised International Prognostic Scoring System (R-IPSS) for myelodysplastic syndromes [6]. Risk categories and scores: ≤1.5 very low; >1.5–3 low; >3–4.5 intermediate; >4.5–6 high; >6 very high.

[c]Morphological dysplasia lineages: E, erythrocyte; M, myeloid; Mgk, megakaryocyte.

NA, not available; MDS, myelodysplastic syndrome; MDS-MLD, myelodysplastic syndrome with multilineage dysplasia; MDS-RS-MLD, myelodysplastic syndrome with ringed sideroblasts and multilineage dysplasia.

Table S3. Clinical features of patients with relapsing polychondritis with or without somatic *UBA1* variants by Sanger sequencing

	RP with *UBA1* variants (n=8)	RP without *UBA1* variants (n=5)	*p*
Male, no. (%)	8 (100)	3 (60)	0.05
Age of onset (years), median (IQR)	72.4 (68.2 -78.5)	72.8 (59.9 -84.2)	1.00
Anti-type II collagen antibody, no. (%)	3 (50)	2 (50)	1.00
Pathological findings of chondritis, no. (%)	7 (88)	5 (100)	0.56
Clinical findings			
Fever, no. (%)	6 (75)	2 (40)	0.26
Skin involvement, no. (%)	**7 (88)**	**0 (0)**	**0.0022**
Auricular chondritis, no. (%)	8 (100)	3 (60)	0.05
Nasal chondritis, no. (%)	2 (25)	2 (40)	0.68
Respiratory tract chondritis, no. (%)	4 (50)	3 (60)	0.83
Polyarthritis, no. (%)	3 (38)	1 (20)	0.72
Ocular inflammation, no. (%)	3 (38)	2 (40)	0.84
Audio-vestibular damage, no. (%)	1 (13)	0 (0)	0.56
Macrocytic anemia, no (%)	7 (88)	1 (20)	0.022
Myelodysplastic syndrome, no. (%)	5 (63)	0 (0)	0.036
Bone marrow vacuoles, no. (%)	6 (100)	0 (0)	0.057
Fulfillment of RP diagnostic criteria			
McAdam et al. [7], no. (%)	3 (38)	1 (20)	0.59
Damiani et al. [8], no. (%)	8 (100)	5 (100)	1.00
Michet et al. [9], no. (%)	4 (50)	3 (60)	0.83

Only skin involvement (bold font) was statistically significant between patients with and without *UBA1* variants.

IQR, interquartile range; RP, relapsing polychondritis; *UBA1* variant, variant at *UBA1* p.Met41.

Table S4. Pathogenic variants detected by droplet digital PCR (ddPCR) and peptide nucleic acid (PNA)-clamping PCR

Patient ID	Sex[a]	Sanger	Origin of gDNA[b]	Target variant[c]	Variant copies/well	Wild-type copies/well	Accepted droplet/well	FA%	PNA-PCR
RP01	M	c.121A>C	PB	1	7540.0 (337.4)	146.0 (10.7)	11738.3 (412.4)	72.1 (0.62)	+
RP03	M	c.121A>G	PB	2	1228.0 (1048.6)	3716.7 (3127.9)	12837.3 (1909.7)	24.7 (0.42)	+
RP04	M	c.121A>G	PB	2	9560.0 (65.3)	884.7 (167.6)	13191.0 (481.1)	91.5 (0.29)	+
RP05	M	c.122T>C	BM	NP	NP	NP	NP	NP	NP
RP11	M	c.121A>C	PB	1	8213.3 (57.3)	132.0 (3.3)	11868.7 (1522.9)	75.7 (0.43)	+
RP13	M	c.122T>C	PB	3	2306.7 (131.0)	7960.0 (299.3)	14180.0 (209.9)	22.4 (0.45)	+
RP15	M	c.122T>C	PB	3	6933.3 (893.6)	3173.3 (547.8)	13543.0 (1147.2)	68.8 (1.16)	+
			BM	3	9533.3 (745.5)	3106.7 (261.4)	14195.3 (1267.4)	75.4 (0.14)	+
RP16	M	c.121A>C	PB	1	7066.7 (491.3)	1044.7 (98.0)	15175.0 (883.1)	87.1 (0.30)	+
RP02	F	-	PB	1	0.0 (0.0)	20340.0 (764.4)	13604.7 (156.6)	0.0 (0.0)	−
				2	0.0 (0.0)	12760.0 (7820.0)	10932.0 (1722.0)	0.0 (0.0)	
				3	0.0 (0.0)	14055.3 (2724.1)	14057.0 (725.4)	0.0 (0.0)	
RP07	M	-	PB	1	0.0 (0.0)	11006.7 (264.5)	14822.3 (370.2)	0.0 (0.0)	−
				2	0.0 (0.0)	10580.0 (0.0)	11864.5 (1263.5)	0.0 (0.0)	
				3	0.0 (0.0)	10806.7 (115.9)	15597.3 (112.0)	0.0 (0.0)	
RP08	M	-	PB	1	0.0 (0.0)	456.3 (63.8)	9535.3 (2599.4)	0.0 (0.0)	−
				2	0.0 (0.0)	9940.0 (180.0)	14063.5 (567.5)	0.0 (0.0)	
				3	0.0 (0.0)	10500.0 (520.0)	13285.0 (136.0)	0.0 (0.0)	
RP10	**F**	-	PB	**1**	**30.0 (6.0)**	**21760.0 (1800.0)**	**14674.0 (425.0)**	**0.14 (0.02)**	**+**
				2	0.0 (0.0)	21270.0 (450.0)	13208.0 (738.0)	0.0 (0.0)	
				3	0.0 (0.0)	22580.0 (736.1)	15535.0 (733.2)	0.0 (0.0)	
RP12	M	NA	NA	NA	NA	NA	NA	NA	NA
RP14	M	-	PB	1	0.0 (0.0)	564.3 (15.8)	9421.7 (960.1)	0.0 (0.0)	−
				2	0.0 (0.0)	7716.0 (2188.9)	12486.7 (310.2)	0.0 (0.0)	
				3	0.0 (0.0)	11093.3 (493.8)	15211.0 (514.4)	0.0 (0.0)	

[a]Sex: F, female; M, male. [b]Origin of gDNA (genomic DNA): BM, bone marrow; PB, peripheral blood. [c]Target variants 1, 2, and 3 correspond to c.121A>C, c.121A>G, and c.122T>C, respectively.

SD values are shown in parentheses after the mean values. FA%, fractional abundance; NA, not available; NP, not performed; PNA-PCR, peptide nucleic acid (PNA)-clamping PCR.

Table S5. Variant allele ratios detected by droplet digital PCR (ddPCR).

Variant allele ratio	Variant copies/well	Wild-type copies/well	Accepted droplet/well	FA%	FA-range
5.0%	1078.0	30240	13705	3.44	3.17−3.72
5.0%	1124.0	31940	14029	3.40	3.14−3.66
1.0%	196.0	31700	14734	0.61	0.50−0.72
1.0%	182.0	31260	13308	0.58	0.46−0.69
0.50%	110.0	31020	14285	0.36	0.27−0.44
0.50%	96.0	31580	15176	0.30	0.23−0.38
0.25%	42.0	28660	16861	0.15	0.09−0.20
0.25%	52.0	29140	16982	0.18	0.12−0.24
0.10%	20.0	28780	16011	0.07	0.03−0.11
0.10%	22.0	27180	13134	0.08	0.03−0.13
0.05%	8.4	25800	11276	0.032	0.0−0.066
0.05%	1.8	25780	13275	0.007	0.0−0.023
0.01%	3.6	23660	13094	0.015	0.0−0.038
0.01%	2.0	23680	12051	0.008	0.0−0.028
0.0%	0.0	25520	12284	0.0	0.0−0.0
0.0%	0.0	27360	10820	0.0	0.0−0.0

FA%, fractional abundance.

Supplemental references

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