

Deficiency of Interleukin-1 Receptor Antagonist (DIRA): Report of the First Indian Patient and a Novel Deletion Affecting *IL1RN*

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Abstract

Purpose Deficiency of interleukin-1 receptor antagonist (DIRA) is a rare life-threatening autoinflammatory disease caused by autosomal recessive mutations in *IL1RN*. DIRA presents clinically with early onset generalized pustulosis, multifocal osteomyelitis, and elevation of acute phase reactants. We evaluated and treated an antibiotic-unresponsive patient with presumed DIRA with recombinant IL-1Ra (anakinra). The patient developed anaphylaxis to anakinra and was subsequently desensitized.

Leonardo O. Mendonca and Louise Malle contributed equally to this work.

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Methods Genetic analysis of *IL1RN* was undertaken and treatment with anakinra was initiated.

Results A 5-month-old Indian girl born to healthy non-consanguineous parents presented at the third week of life with irritability, sterile multifocal osteomyelitis including ribs and clavicles, a mild pustular rash, and elevated acute phase reactants. SNP array of the patient's genomic DNA revealed a previously unrecognized homozygous deletion of approximately 22.5 Kb. PCR and Sanger sequencing of the borders of the deleted area allowed identification of the breakpoints of the deletion, thus confirming a homozygous 22,216 bp deletion that spans the first four exons of *IL1RN*. Due to a clinical suspicion of DIRA, anakinra was initiated which resulted in an anaphylactic reaction that triggered desensitization with subsequent marked and sustained clinical and laboratory improvement.

Conclusion We report a novel DIRA-causing homozygous deletion affecting *IL1RN* in an Indian patient. The mutation likely is a founder mutation; the design of breakpoint-specific primers will enable genetic screening in Indian patients suspected of DIRA. The patient developed anaphylaxis to anakinra, was desensitized, and is in clinical remission on continued treatment.

Keywords Deficiency of interleukin-1 receptor antagonist · DIRA · *IL1RN* · SNP array · homozygous deletion

Introduction

Autoinflammatory diseases are a group of disorders caused by dysregulation of the innate immune system, resulting in systemic inflammation [1]. Deficiency of interleukin-1 receptor antagonist (DIRA) is a very rare autosomal recessive autoinflammatory disease first described in 2009 [2, 3]. DIRA presents in early childhood with marked skin and bone

involvement, and systemic inflammation with elevation of acute phase reactants. To date, no untreated patients have lived to adulthood. DIRA is caused by loss-of-function mutations in *IL1RN*, which encodes the endogenous interleukin-1 (IL-1) receptor antagonist (IL-1Ra). IL-1Ra deficiency results in unopposed pro-inflammatory signaling via IL-1 and the IL-1 receptor (IL-1R1) [2] that can escalate to life-threatening systemic inflammation, systemic inflammatory response syndrome (SIRS), and death if untreated [2, 4–8]. The mortality of untreated DIRA based on published reports is over 30% in early infancy (summarized in Table 1). IL-1 blockade results in complete disease remission [2, 3, 5, 7]. The detection of mutations in *IL1RN* is diagnostic and mandatory for a definitive diagnosis. Nineteen patients with DIRA and nine different disease-causing mutations have thus far been reported [8–10] (Table 1). In the present study, we describe the first Indian patient with DIRA, who harbors a novel homozygous 22 Kb deletion spanning four exons (two coding) of *IL1RN*.

Methods

Patient The parents provided consent and the patient was enrolled into an IRB-approved NIH natural history protocol (NCT02974595).

Genetic Analysis

SNP Array

Peripheral blood genomic DNA was obtained from the patient and genotyped for the detection of copy number variations (CNVs) by a high-density single nucleotide polymorphism (SNP) array technique. The DNA sample (~300 ng) was genotyped following the Illumina “infinium assay” protocol [11]. This assay includes whole-genome amplification and fragmentation of DNA, hybridization to the BeadChip with specific oligonucleotide probe array (50-mers), enzymatic extension of the 3' terminal base for incorporation of the allele-specific nucleotide,

Table 1 Summary of published DIRA patients, their disease-causing mutations and outcomes

Gender (n = 18)	Age of first symptoms (days)	Age or disease outcome at time of report	Country of origin	Mutation	Reference
Female	–	Aborted	Turkey	c.355C>T; p.Gln119*	4
Male	7	Deceased	Turkey	c.355C>T; p.Gln119*	4
Male	14	13 months	Canada	c.156_157del; p.Asn52Lysfs*25	2
Male	0	Deceased	Netherlands	c.229G>T; p.Gly77*	2
Female	14	7.2 years	Netherlands	c.229G>T; p.Gly77*	2
Male	0	Deceased	Netherlands	c.229G>T; p.Gly77*	2
Female	2	Deceased	Netherlands	c.229G>T; p.Gly77*	2
Female	17	2 months	Netherlands	c.229G>T; p.Gly77*	2
Male	5	1.8 years	Lebanon	c.160C>T; p.Gln54*	2
Male	2	4 months	Lebanon	c.160C>T; p.Gln54*	2
Male	8	9.5 years	Puerto Rico	c.-64_1696del; p.IL1F9_	2
				IL1RNdel	
Male	10	18 months	Puerto Rico	c.-64_1696del; p.IL1F9_	3
				IL1RNdel	
Male	14	15 years	Puerto Rico	c.-64_1696del; p.IL1F9_	5
				IL1RNdel	
Male	60	5 months	Puerto Rico	c.-64_1696del; p.IL1F9_	6
				IL1RNdel	
Female	0	30 months	Brazil	c.213_227delAGATGTGG	7
				TACCCAT; p.Asp72_Ile76del	
Female	0	27 months	Brazil	c.213_227delAGATGTGG	7
				TACCCAT; p.Asp72_Ile76del	
Male	12	23 days	USA	c.229G>T; p.Gly77* and	8
				c.140delC; p.T47TfsX4 in	
				compound heterozygosity	
Female	12 months	13 years	Turkey	c.76C>T; p.Arg26*	10

An additional DIRA patient has been included in Infefers database (<http://fmf.igh.cnrs.fr/ISSAID/infefers/>) as a personal communication from Berdeli A, Sozeri B, Mir S, Atan, M, and Tigli D. This patient has the following variant in *IL1RN*: c.396delC; p.Thr133Profs*118

detection with fluorescently tagged reagent, and signal amplification. The allele type and intensity were collected using iScan and processed by the GenomeStudio (v2011.1, www.illumina.com) genotyping module. The sample was run on the HumanOmniExpressExome-8v1.2_A Illumina BeadChip, containing 964,193 SNPs. After filtering by GenTrain Score, removing SNPs with a score below 0.7, there were 941,817 SNPs remaining to be used in analysis. cnvPartition v3.2 was used to call copy number aberrations using the following settings: minimum number of contiguous SNPs = 4, GC wave adjust = TRUE, and minimum confidence value = 0. Using the confidence value from the cnvPartition output, copy number variations were filtered based on the following criteria: if CN = 0, then confidence value ≥ 200 ; if CN = 1, then confidence value ≥ 15 ; if CN = 3, then confidence value ≥ 30 . The detected aberrations were visually evaluated using Nexus Copy Number (v7.5, www.biodiscovery.com) to substantiate their validity.

PCR and Sequencing of Breakpoints

Multiple primer sets were designed using Primer3Plus, flanking the breakpoint regions as determined by SNP array. Amplification reactions were carried out with 20–200 ng of genomic DNA using GoTaq Hot Start Master Mix (Promega, Madison, WI) for 35 cycles: denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1.5 min. Amplification products were analyzed by 2% agarose gel electrophoresis, and forward and reverse primers closest to the 5' and 3' end of the deletion, respectively, were used to obtain new amplicons. These PCR products underwent a second PCR reaction with ABI Prism® BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and directly sequenced in both directions using ABI 3100 Genetic Analyzer (Applied Biosystems). Results were analyzed using DNASTar Lasergene software.

Results

Case Report

A 5-month-old north Indian Hindu girl born to healthy non-consanguineous parents, with a birth weight of 3.1 kg and normal Apgars, developed right hand swelling and left hip pain followed by bilateral elbow and shoulder swelling starting at 3 weeks of age. At the age of 5 weeks, she was hospitalized and bone scintigraphy showed multifocal osteomyelitis with involvement of ribs, clavicle and long bones. A bone biopsy performed on her left wrist showed a non-specific inflammatory reaction. At the age of 8 weeks, a mild pustular skin rash limited to the back of the neck and upper forehead developed. Cultures from bone aspirate and

blood were persistently negative for bacteria, fungi, and mycobacteria, and immunodeficiency work-up (including lymphocyte immunophenotyping and HIV testing) was negative. During a 5-week hospitalization, the patient received broad-spectrum antibiotics with no change in clinical manifestations. Oral prednisolone was started (1 mg/kg/day) at the age of 10 weeks and increased to 2 mg/kg/day when the patient clinically improved and became asymptomatic. After 2 weeks, prednisolone was tapered by 0.5 mg every 2 weeks. The patient was clinically suspected to have DIRA and was seen at the National Institutes of Health (NIH) at the age of 5 months when her prednisolone dose was at 0.4 mg/kg/day and could not be tapered further.

On physical exam, her weight was 4.4 kg (<3rd percentile) and she had erythematous and papular skin rashes with healing crusted pustules on posterior neck and forehead extending to hairline and scalp. Her left hip had a significantly decreased range of motion with pain on motion, and the left clavicle was prominent and tender.

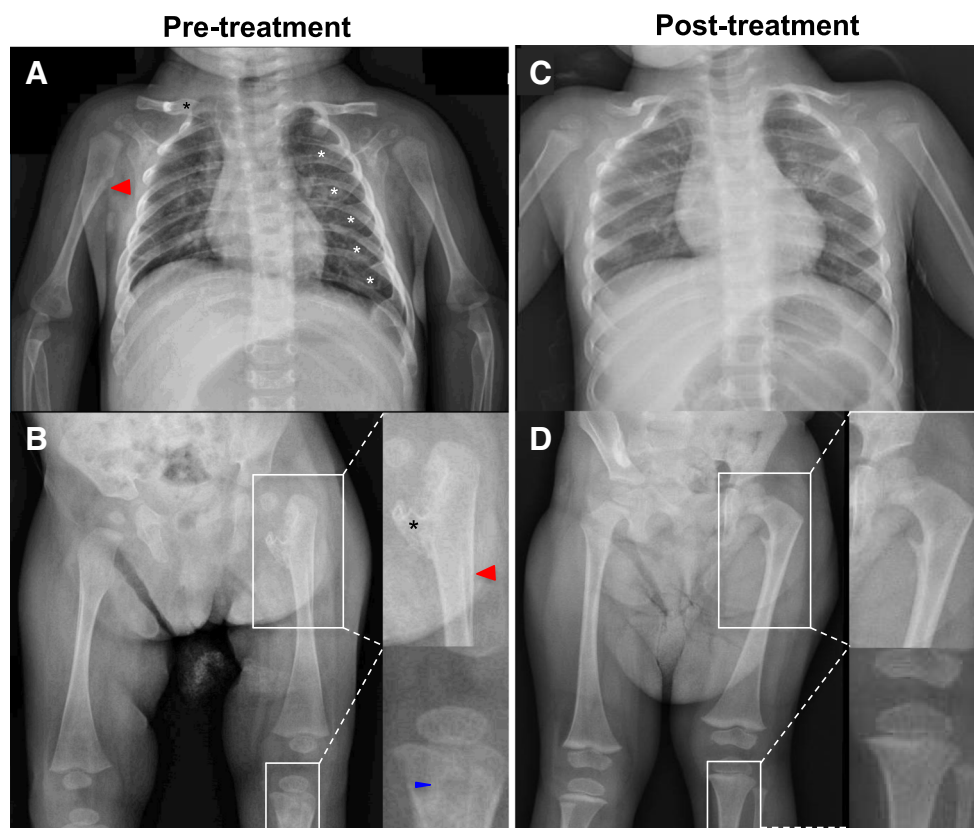
She was anemic with a hemoglobin (Hb) 9.3 g/dL, hematocrit (Ht) 28.8%, white blood cell (WBC) count 24,200 cells/mL (51% of neutrophils), platelet count 729,000 cells/mL, erythrocyte sedimentation rate (ESR) 113 mm/h, and C-reactive protein (CRP) 110.7 mg/L. X-ray studies demonstrated expansion of costochondral transition bilaterally at multiple levels but most prominently at levels 5–7 on the left side and 6 and 8 on the right side (Fig. 1a). She had mixed lytic/sclerotic bone lesions in the proximal portion of the right humerus, on the left proximal femur with adjacent periosteal reaction and mild periosteal reaction on the right proximal femur, and a proximal tibial lesion (Fig. 1b). The distribution and appearance of the bone lesions is pathognomonic for DIRA.

The patient started on treatment with recombinant IL-1Ra anakinra at 0.44 mL daily (1.5 mg/kg/day), and prednisolone was continued. On day 10 of the anakinra treatment, she developed a generalized urticarial rash 15 min after the injection that lasted for 45 min and responded to diphenhydramine (Benadryl®) and corticosteroids. As anakinra is an essential treatment for DIRA, “desensitization” over a period of 25 days was initiated. Anakinra was reduced to 0.025 mL (3.73 mg/day) and gradually increased while pre-medicating with Benadryl. Prednisolone was continued at 0.4 mg/kg/day (Supplemental Table I). On anakinra treatment, the patient achieved complete clinical remission. The bone lesions healed (Fig. 1c, d), she has no clinical symptoms, she is off steroids, and she has normalized inflammatory markers (Table 2).

Genetic Findings

A SNP array analysis of the patient's peripheral blood DNA showed a homozygous deletion of the centromeric end of *IL1RN*, spanning a region of approximately 21.4 kb to

Fig. 1 Radiographic changes before and after treatment with anakinra. **a** Antero-posterior chest X-ray showing expansion of the ribs in the left at 5–7 levels (*white stars*) and widening of the right clavicle (*black star*). There is an osteolytic lesion with periosteal reaction on the right humerus (*red arrow head*). **b** Hip X-ray shows a mixed lytic/sclerotic bone lesion (*black star*) in the left proximal femur with adjacent periosteal reaction (*red arrow head*) and lytic bone lesion (*blue arrow head*) with mild periosteal reaction in the left proximal tibia. **c, d** Chest and hip X-rays show improvement of costal arch widening, resolution of osteolytic lesion on the right humerus, and improvement of the left femur and left tibia bone lesions



23.7 Kb, which included the first four exons of *IL1RN* (NM_173843), two of which are coding (Fig. 2a). After

Table 2 Laboratory parameters before and after anakinra treatment

Laboratory analysis	Pre-treatment with anakinra	1 month after anakinra treatment	1 year after anakinra treatment	Normal range
Hemoglobin (g/dL)	9.3	11.4	10.7	(10.2–12.7)
Platelet (K/uL)	729	426	445	(214–459)
White blood cell (WBC) count (K/uL)	24.2	12.5	12.2	(6.48–13.02)
Neutrophil absolute (K/uL)	12.41	6.75	2.14	(1.27–7.18)
Lymphocyte absolute (K/uL)	9.9	4.87	8.29	(1.52–8.09)
Monocyte absolute (K/uL)	1.89	0.375	0.5	(0.26–1.08)
CRP (C-reactive protein) (mg/L)	110.7	4.2	6.2	<5
ESR (erythrocyte sedimentation rate) (mm/h)	113	17	33	<20

amplifying the boundaries of the predicted deletion using multiple primer pairs 400–500 bp apart, we selected the forward and reverse primers closest to the 5' and 3' end of the deletion (Fig. 2b). PCR using the primers selected generated an amplicon that covered the breakpoint region of the disease-carrying allele (Fig. 2c), and Sanger sequencing of the PCR product indicated the exact breakpoints of the deletion at chr2_hg19_113,865,011 and chr2_hg19_113,887,227 (Fig. 2d). These findings confirm a novel homozygous 22,216 bp deletion affecting *IL1RN*. Further analysis of patient's SNP array demonstrated three regions of homozygosity greater than 2 Mb, suggesting a “distant” relatedness between patient's parents and a founder effect. As the mutation is likely a founder mutation in India, the primers we designed can be used for the detection of suspected DIRA cases in India. The primer sequences are listed in Table 3. Using the primers designed, we performed a multiplex PCR assay, which showed that both parents are heterozygous for the deletion, whereas the patient is homozygous (Fig. 2c).

Discussion

DIRA is caused by autosomal recessive mutations in *IL1RN*, the gene that encodes IL-1 receptor antagonist, a natural antagonist of the IL-1 receptor that, if unopposed, results in

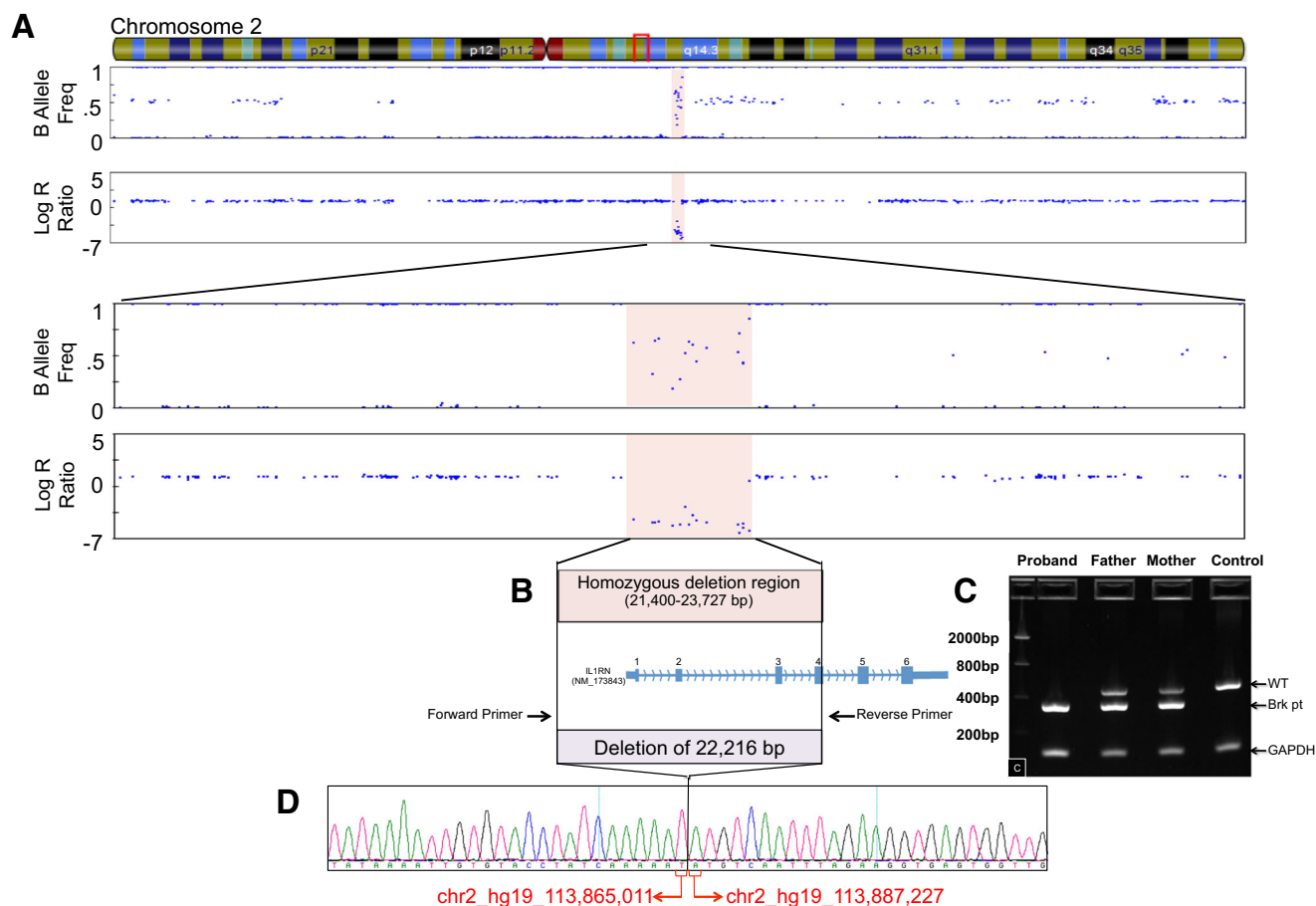


Fig. 2 Genetic analyses for the detection of the *IL1RN* deletion. **a** SNP array data showing a homozygous deletion of *IL1RN*. The region deleted is indicated by a red box on the chromosome diagram. The B allele frequency (BAF) plot conveys the zygosity of a given SNP (blue dot), while the log R ratio (LRR) plot conveys the intensity, or copy number. The homozygous deletion (shaded red) is indicated by the loss of intensity in the LRR plot and the absence of a coherent signal in the BAF plot. The deleted region includes the first four exons (two coding exons) of *IL1RN* (NM_173843). **b** Schematic representation of the deleted region

of *IL1RN* gene predicted by SNP array. **c** Multiplex PCR products generated with three pairs of primers that amplify the wild-type allele the breakpoint junction of the deletion or an internal control (GAPDH) were run on a 2% agarose gel for 20 min. Upper band indicates presence of the wild-type (WT) allele, middle band indicates presence of the deleted allele (Brk pt), and lower band serves as an internal control (GAPDH). **d** Sanger sequencing electropherogram depicting the breakpoint of the *IL1RN* Indian deletion

Table 3 Primer sequences used for the detection of homozygous and heterozygous 22 Kb Indian deletion

Primer name	Oligonucleotide sequence
DIRA22-WT F	TTCCTTCCTCCATCCATTTG
DIRA22-WT R	GAAAAATGGACAGCCTTGGA
DIRA22-BRK F	CAACAACAACGAAAGCAAGG
DIRA22-BRK R	CCTGGGACCACTGTGCCTAT
GAPDH F	CAACGAATTGGCTACAGCA
GAPDH R	CCCAGCAGTGAGGGTCTCT

DIRA22-WT primers amplify the wild-type sequence within the area of the novel deletion (not amplified in deleted allele)

DIRA22-BRK primers amplify the breakpoint sequence (only amplified in carriers of the deleted allele)

GAPDH primers serve as an internal amplification control

increased IL-1 signaling and systemic inflammation. The disease presents with aseptic multifocal osteomyelitis and variable severity of skin pustulosis in the context of elevated inflammatory markers and pathergy. The clinical presentation is similar to that of septic osteomyelitis, which typically prompts treatment with antibiotics that are not beneficial. Failure to start immunosuppressive treatment with steroids results in disease escalation, an inflammatory response syndrome, and potentially death. Treatment with corticosteroids significantly improves the disease but does not result in inflammatory remission and is associated with significant side effects. Daily injections with anakinra, the recombinant IL-1 receptor antagonist is “life-saving” and results in a rapid response and complete inflammatory remission [2, 3, 5, 7]. The disease presented early in life and our reported patient had a clinical course similar to the 18 previously reported patients. Although aseptic multifocal osteomyelitis and pustulosis are the principal

clinical manifestations, additional findings such as CNS vasculitis, hypotonia, respiratory distress, interstitial lung disease, and hemophagocytosis were seen in one patient each. None of these findings were present in our patient.

Most reported cases are homozygotes from areas where *IL1RN* mutations are founder mutations (Table 1). Here we report the first Indian girl diagnosed with DIRA, born to unrelated healthy parents. The diagnosis was confirmed by detection of a deletion of the first two coding exons of *IL1RN* by SNP array, PCR, and Sanger technique. The mutation is novel but the frequency in the ethnic group where both parents come from is unknown. We designed primers to detect the novel deletion that can facilitate screening in Indian patients with a clinical suspicion of DIRA and also in determining the carrier frequency of the deletion. The carrier frequency of a previously described large deletion seen in a geographically isolated Puerto Rican population is 1.3% [2]. In such areas, prenatal screening and counseling may be indicated.

The DIRA-causing mutations in *IL1RN* result in either no protein expression or expression of a truncated non-functional protein [2]. The absence of protein and the treatment with the recombinant IL-1 receptor antagonist, anakinra, have raised concerns about drug reactions to anakinra. Most patients with several monogenic autoinflammatory diseases tolerate anakinra well and have been on treatment with the drug for over 10 years. However, we are aware of drug reactions in two of 12 patients with DIRA who used anakinra but none of over 100 patients with the cryopyrinopathies, familial cold-induced autoinflammatory syndrome (FCAS), Muckle Wells syndrome (MWS), or neonatal-onset multisystem inflammatory disease (NOMID), who have a NLRP3-mediated autoinflammatory syndrome that responds to IL-1 inhibition. Both DIRA patients developed a systemic urticarial drug reaction that was distinct from the common injection site reactions that are observed in up to 30% of patients injected with anakinra. The allergic reaction in our patient occurred within 10 days of anakinra injection, a presentation that is strongly suggestive of a humoral response that developed after supplementation with a protein that had been deficient. The patient harbors a large deletion in chromosome 2 that spans the promoter and the first two coding exons of *IL1RN* that lead to non-expression of IL-1Ra. The other DIRA patient who developed a drug reaction harbors a nonsense *IL1RN* mutation (c.160C>T; p.Gln54*) that leads to a premature stop codon with the presumed expression of a truncated protein. Protein expression has not been tested in vitro; it is possible that the short mRNA transcript is not stable and that the mutation may also result in no protein expression [2]. These findings could indicate that absence or earlier truncations in IL-1Ra would be associated with a higher risk of developing a humoral reaction to the recombinant IL-1Ra, anakinra. Given the critical importance of treatment in these patients, both DIRA patients who have developed a reaction to anakinra have been

desensitized. Our patient was started on lower doses of anakinra and up-titrated (Supplemental Table I), whereas the other patient was rapidly desensitized (Supplemental Table II). Currently, both remain on anakinra treatment at 2.5 and 4 years after desensitization, respectively.

In summary, we report a novel DIRA-causing loss-of-function mutation in *IL1RN* and point to the importance of recognizing and diagnosing DIRA promptly, particularly in clinical circumstances where infectious osteomyelitis is not uncommon. In addition, we provide guidance to IL-1 blocking treatment with anakinra and report two desensitization regimens that were successfully used in two DIRA patients. These patients developed systemic allergic reactions to anakinra and desensitization allowed them to continue their essential treatment.

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Compliance with Ethical Standards The parents provided consent and the patient was enrolled into an IRB-approved NIH natural history protocol (NCT02974595).

Conflicts of Interest Dr. Leonardo O. Mendonca has received speaker fees from Novartis. All other authors declare that they have no conflicts of interest.

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Supplemental Table I. Desensitization with anakinra

Days	Frequency	Divided doses (AM, PM)*	Dose given (ml)	Dose given (mg)	Cumulative dose	Prednisolone	Benadryl
1	Once daily	1/4 th	0.025	3.73	3.73	1.8 mg (0.4 mg/kg) daily	5 mg BID
2	Once daily	1/4 th	0.025	3.73	7.46	1.8 mg (0.4 mg/kg) daily	5 mg BID
3	Once daily	1/4 th	0.025	3.73	11.19	1.8 mg (0.4 mg/kg) daily	5 mg BID
4	Once daily	1/4 th	0.025	3.73	14.92	1.8 mg (0.4 mg/kg) daily	5 mg BID
5	Twice daily	1/4 th , 1/8 th	0.0375	5.59	20.51	1.8 mg (0.4 mg/kg) daily	5 mg BID
6	Twice daily	1/4 th , 1/8 th	0.0375	5.59	26.1	1.8 mg (0.4 mg/kg) daily	5 mg BID
7	Twice daily	1/4 th , 1/8 th	0.0375	5.59	31.69	1.8 mg (0.4 mg/kg) daily	5 mg BID
8	Twice daily	1/4 th , 1/8 th	0.0375	5.59	37.28	1.8 mg (0.4 mg/kg) daily	5 mg BID
9	Twice daily	1/4 th , 1/8 th	0.0375	5.59	42.87	1.8 mg (0.4 mg/kg) daily	5 mg BID
10	Twice daily	1/4 th , 1/8 th	0.0375	5.59	48.46	1.8 mg (0.4 mg/kg) daily	5 mg BID
11	Twice daily	1/4 th , 1/8 th	0.0375	5.59	54.05	1.8 mg (0.4 mg/kg) daily	5 mg BID
12	Twice daily	1/4 th , 1/8 th	0.0375	5.59	59.64	1.8 mg (0.4 mg/kg) daily	5 mg BID
13	Twice daily	1/4 th , 1/8 th	0.0375	5.59	65.23	1.8 mg (0.4 mg/kg) daily	5 mg BID
14	Twice daily	1/4 th , 1/8 th	0.0375	5.59	70.82	1.8 mg (0.4 mg/kg) daily	5 mg BID
15	Twice daily	1/4 th , 1/8 th	0.0375	5.59	76.41	1.8 mg (0.4 mg/kg) daily	5 mg BID
16	Twice daily	1/4 th , 1/4 th	0.05	7.46	83.87	1.8 mg (0.4 mg/kg) daily	5 mg BID
17	Twice daily	1/4 th , 1/4 th	0.05	7.46	91.33	1.8 mg (0.4 mg/kg) daily	5 mg BID
18	Twice daily	1/4 th , 1/4 th	0.05	7.46	98.79	1.8 mg (0.4 mg/kg) daily	5 mg BID
19	Twice daily	1/4 th , 1/4 th	0.05	7.46	106.25	1.8 mg (0.4 mg/kg) daily	5 mg BID
20	Twice daily	1/4 th , 1/4 th	0.05	7.46	113.71	1.8 mg (0.4 mg/kg) daily	5 mg BID *
21	Twice daily	3/8 th , 1/4 th	0.0625	9.32	123.03	1.8 mg (0.4 mg/kg) daily	Benadryl taper
22	Twice daily	3/8 th , 1/4 th	0.0625	9.32	132.35	1.8 mg (0.4 mg/kg) daily	Benadryl taper
							Benadryl taper
23	Twice daily	3/8 th , 1/4 th	0.0625	9.32	141.67	1.8 mg (0.4 mg/kg) daily	Benadryl taper
24	Twice daily	3/8 th , 1/4 th	0.0625	9.32	150.99	1.8 mg (0.4 mg/kg) daily	Benadryl taper
25	Twice daily	3/8 th , 1/4 th	0.0625	9.32	160.31	Start Prednisolone taper (0.3 mg every 2 weeks)	Benadryl taper

*denotes the percentage of the originally administered dose of anakinra

Other comments

- Desensitization pre-medication: Benadryl and prednisolone
- The first anakinra dose was administered in an ICU setting.
- Prednisolone was tapered gradually over a period of 4 months.
- Patient was continued on divided doses of anakinra for 1 year.
- Anakinra dose was increased to 2.5mg/kg/day on the 78th desensitization day (~11 weeks after desensitization started).
- Patient is currently on 3mg/kg/day of anakinra.

Supplemental Table II. Rapid drug desensitization (RDD) with the drug anakinra

Step	Solution	Time (min)	Volume of this solution (ml)	Dose given (mg)	Cumulative dose (mg)
1	1/100 000	0	0.1	0.00015	0.00015
2	1/100 000	30	0.2	0.0003	0.00045
3	1/100 000	60	0.4	0.0006	0.00105
4	1/10 000	90	0.1	0.0015	0.00255
5	1/10 000	120	0.2	0.003	0.00555
6	1/10 000	150	0.4	0.006	0.01155
7	1/1000	180	0.1	0.015	0.02655
8	1/1000	210	0.2	0.03	0.05655
9	1/1000	240	0.4	0.06	0.11655
10	1/100	270	0.1	0.15	0.26655
11	1/100	300	0.2	0.3	0.56655
12	1/100	330	0.4	0.6	1.16655
13	1/10	360	0.1	1.5	2.66655
14	1/10	390	0.2	3	5.66655
15	1/10	420	0.4	6	11.66655

Anakinra desensitization protocol (100mg/0.67 ml or 150 mg/ml): dilutions are done in buffered saline isotonic (as below) and injections are done subcutaneously at 30 min intervals

	Dilution	Amount Anakinra (mg/ml)
Solution 1	1/100 000	0.0015 mg/ml
Solution 2	1/10 000	0.015 mg/ml
Solution 3	1/1000	0.15 mg/ml
Solution 4	1/100	1.5 mg/ml
Solution 5	1/10	15 mg/ml

Premedication with:

Cetirizine 4 mg

Montelukast (Singulair) 4 mg

This treatment is continued for 1 month.

Solu-Cortef was not given prior to RDD out of concern that it can camouflage a more severe reaction.

Other comments:

- RDD is given according to the protocol even if the patient has an allergic reaction, but is stopped if there is a drop in blood pressure or saturation.

- If RDD can be completed according to protocol, the treatment is then continued with at the patient specific dose administered at q 12 hours.
- The patient was cared for at an ICU until 48h after RDD.
- After discharge the recommended Anakinra dose (12 mg for the patient who was treated) is thereafter given once daily for two weeks.
- After 2 weeks the dose as increased to 15 mg Anakinra daily, which was the planned treatment dose.

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