An Autoinflammatory Disease with Deficiency of the Interleukin-1-Receptor Antagonist

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Background

- Autoinflammatory disease: recurrent episodes of inflammatory lesions in association with signs of systemic inflammation
- ex: FMF, TNF receptor-associated periodic syndrome, hyper IgD syndrome
- Cryopyrin-associated syndromes – arise from abnormalities in the control of IL1B, caused by mutations in the *NLRP3* gene which encodes the NALP3 protein
- NALP3 forms a complex that activates caspase 1, which cleaves inactive IL1B into its active form
- Anakinra (recombinant human IL-1-receptor antagonist) – blocks the proinflammatory effects of IL1B and blocks systemic inflammation in patients with cryopyrin-associated periodic syndrome
- 9 patients with an autoinflammatory syndrome of skin and bone caused by recessive mutations in *IL1RN* (gene encoding the IL1-receptor antagonist)
- Propose the term deficiency of the interleukin-1 receptor antagonist (DIRA) to denote this illness

IL1 and the IL1 receptor antagonist

- IL1 is a highly active proinflammatory cytokine that causes fever, anorexia, and tissue damage/remodeling, elevated markers of systemic inflammation
- IL1RN is structurally similar to IL1B, the antagonist binds to the IL1 receptor thereby blocking access of IL1 to the receptor
- both are produced in patients with infection, trauma, or inflammatory conditions and compete for the IL1 Receptor
- animal studies indicate that severity of inflammation is influenced by the IL1/IL1RN balance

Methods

- all protocols approved by IRBs, informed consent obtained
- empiric treatment with anakinra was initiated in all patients at a dose of 1mg/kg subQ
- dose was increased to titrate to a CRP < 0.5mg/dL and ESR<15mm/hr
- functional assays were conducted on blood from patients, siblings, parents, and controls

Characteristics of Study Patients and Their Clinical Disease

Table 1. Characteristics of Study Patients and Their Clinical Disease.*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Age at diagnosis	13 Mo	Deceased	7.2 Yr	Deceased	Deceased	2 Mo	1.8 Yr	4 Mo	9.5 Yr
Sex	Male	Male	Female	Male	Female	Female	Male	Male	Male
Country or region of origin†	Newfoundland, Canada	Netherlands	Netherlands	Netherlands (residing in Canada)	Netherlands	Netherlands	Lebanon (residing in Sweden)	Lebanon (residing in Sweden)	Puerto Rico (residing on U.S. mainland)
Relation to other patients		Brother of Patient 3	Sister of Patient 2		Sister of Patient 6	Sister of Patient 5	Brother of Patient 8	Brother of Patient 7	
Gestational age (wk)	37.5	35, with fetal distress	38, with fetal distress	36, with fetal distress	36, with fetal distress	37	38	38	34, with fetal distress
Birth weight (g)	4640	Not known	2880	2880	3000	3780	3220	2815	1930
Clinical outcome	Alive and well	Deceased at 2 mo, from SIRS	Alive and well	Deceased at 9.5 yr	Deceased at 21 mo, from SIRS	Alive	Alive but failure to thrive	Alive with vertebral collapse	Alive with skeletal deformities
Age or time of clinical presentation	2 Wk	Birth	Birth	Birth	2 Days	2.5 Wk	5 Days	2 Days	8 Days
Symptoms at initial presentation	Finger swelling, vesicular stomatitis	Respiratory distress	Aspiration pneumonia, rash at 2 weeks	Rash on forehead, joint swelling (proximal interphalangeal joint and wrist), stomatitis	Fever, multifocal osteomyelitis, pathergy	Pustular dermatitis on cheeks, oral candidiasis	Pustular dermatitis, mouth ulcers	Respiratory distress, mouth ulcers	Chorioamnionitis, swelling of right foot and ankle
Presentation of rash	Mild-to-severe pustulosis, pathergy	Mild pustulosis	Mild pustulosis	Severe pustulosis	Not known	Severe pustulosis	Severe pustulosis, pathergy	Mild pustulosis	Mild pustulosis, pyoderma gangrenosum
Nail changes	Yes (pits)	No	No	Yes	Not known	Yes	Not known	Yes	No
Skeletal abnormalities on radiography‡	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Widening of ribs	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Periosteal cloaking	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
Periosteal elevation	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Multifocal osteolytic lesions	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Cervical vertebral fusion	No	No	Yes	Yes	No	No	Not known	Yes	Yes
Findings of bone-tissue culture	Negative	Negative	Negative	Negative	Negative	None (tissue culture not performed; blood cultures negative)	None (tissue culture not performed; blood cultures negative)	Negative	Negative
Hepatosplenomegaly	No	Yes	Yes	Yes	Yes	Yes	No	No	Not known
Other manifestations	Vasculitis (on biopsy), central nervous system vasculitis or vasculopathy			Interstitial lung disease, hypotonia, developmental delay			Conjunctival injection	Conjunctival injection	
Treatments before anakinra§	Antibiotics, indomethacin, prednisolone, IV immune globulin	Antibiotics	Antibiotics, indomethacin, prednisolone, methotrexate, cyclosporine, azathioprine, etanercept, thalidomide	Antibiotics, prednisone, methotrexate, cyclosporine	Antibiotics, indomethacin, prednisolone	None	Antibiotics, antiviral and antifungal agents, ibuprofen, methylprednisolone sodium succinate, prednisolone	Antibiotics, ibuprofen, prednisolone	Antibiotics, prednisone, interferon-γ
Maximal prednisone dose	2 mg/kg/day	Not known	2 mg/kg/day	2 mg/kg/day	2 mg/kg/day		2 mg/kg/day	0.25 mg/kg/day	2 mg/kg/day

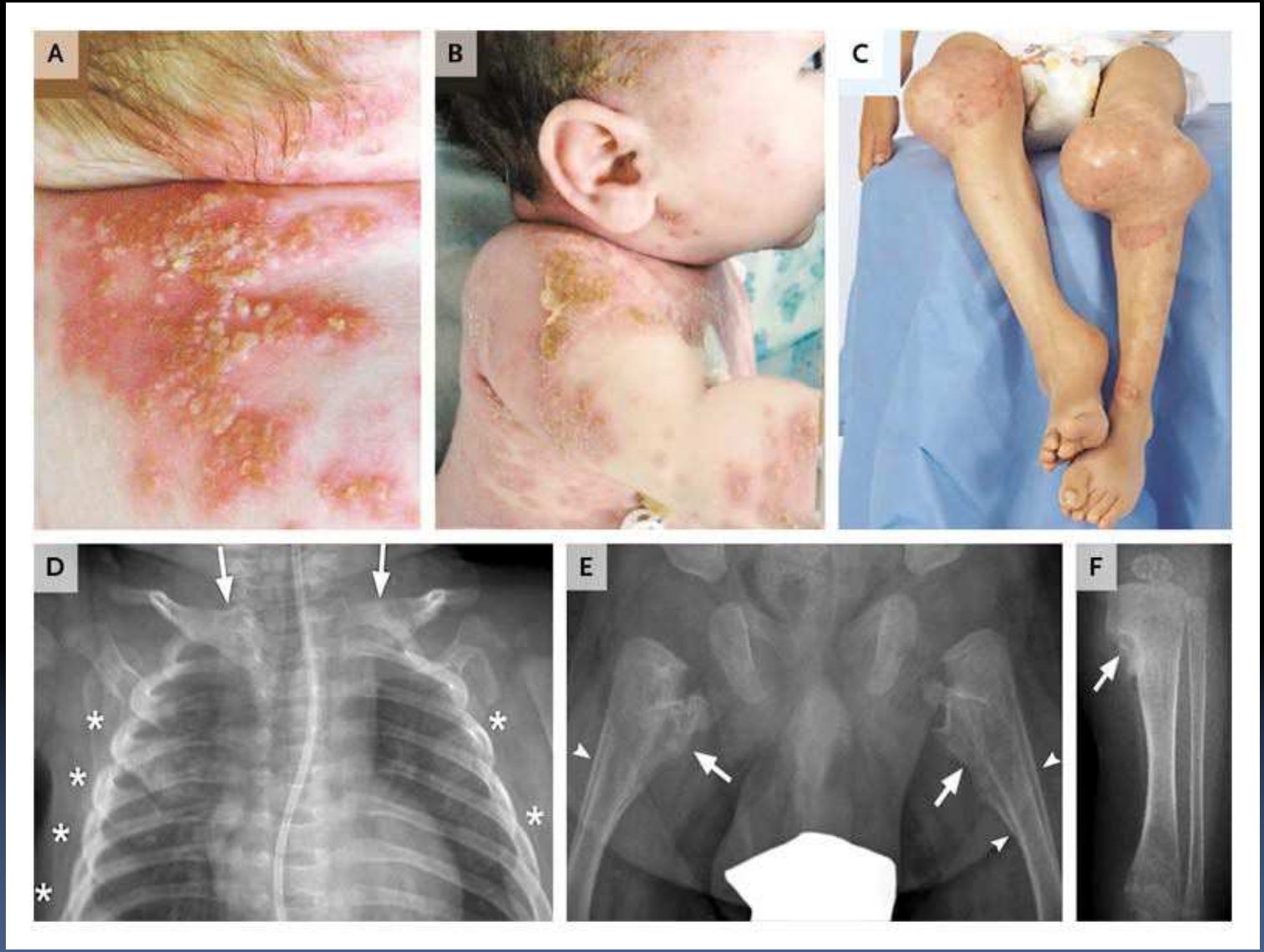
* IV denotes intravenous, and SIRS the severe inflammatory response syndrome.
† Country of origin was reported by the parents of the patients.
‡ Examples of radiographic features are depicted in Figure 1D, 1E, and 1F, and in Figure 1E, 1F, and 1G in the Supplementary Appendix.
§ Patient 9 had an incomplete response to anakinra at a dose of 4 mg per kilogram per day. His symptoms have improved but he continues to have elevated acute-phase reactant levels 6 months after treatment with anakinra.

Table 1: Demographics

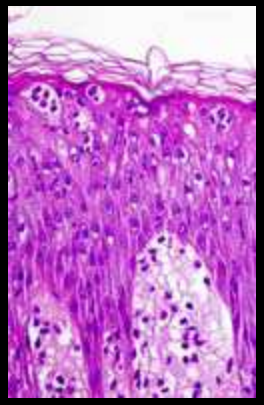
- demographic characteristics and clinical presentation of the affected children
- all patients presented at birth or by 2.5 weeks of age
- 9 patients total
  - 1 from Newfoundland, Canada
  - 5 from the Netherlands (two sets of sibs)
  - 2 sibs from Lebanon
  - 1 from Puerto Rico
- No pts had fever, ESR and CRP markedly elevated
- Therapy with DMARDS and high dose corticosteroids only partially controlled symptoms
- Two infants died of multiorgan failure at 2 months and 21 months, third child died at 9,5 yrs secondary to complications of pulmonary hemosiderosis with progressive interstitial fibrosis

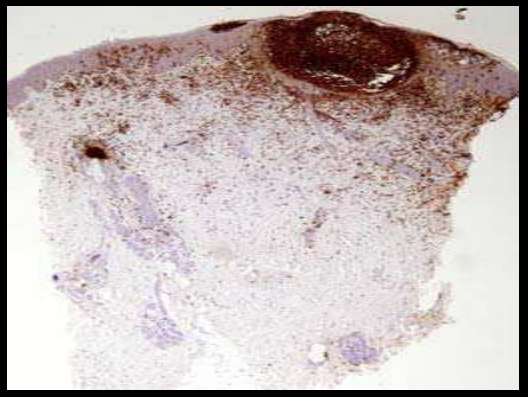
Inflammatory Skin and Bone Manifestations in Patients with DIRA

A. Pustular rash
B. Generalized pustulosis
C. Epiphyseal ballooning
D. Widening of ribs and clavicles
E. Heterotopic ossification
F. Osteolytic lesion with sclerotic rim

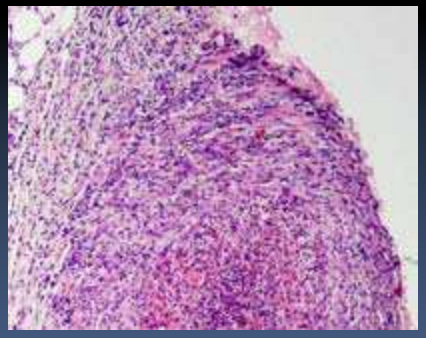


Histopathologic Changes in Patients with DIRA

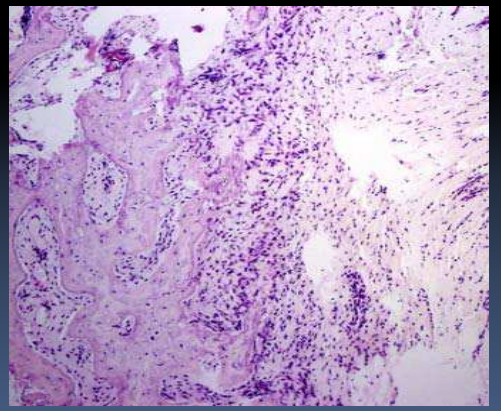




A. Extensive infiltration of epidermis/dermis by neutrophils, pustule formation along hair follicles, acanthosis, hyperkeratosis.



B. Vasculitis in connective and fat tissue



C. Bone-biopsy specimens were sterile, histologic analysis revealed purulent osteomyelitis, fibrosis, and sclerosis

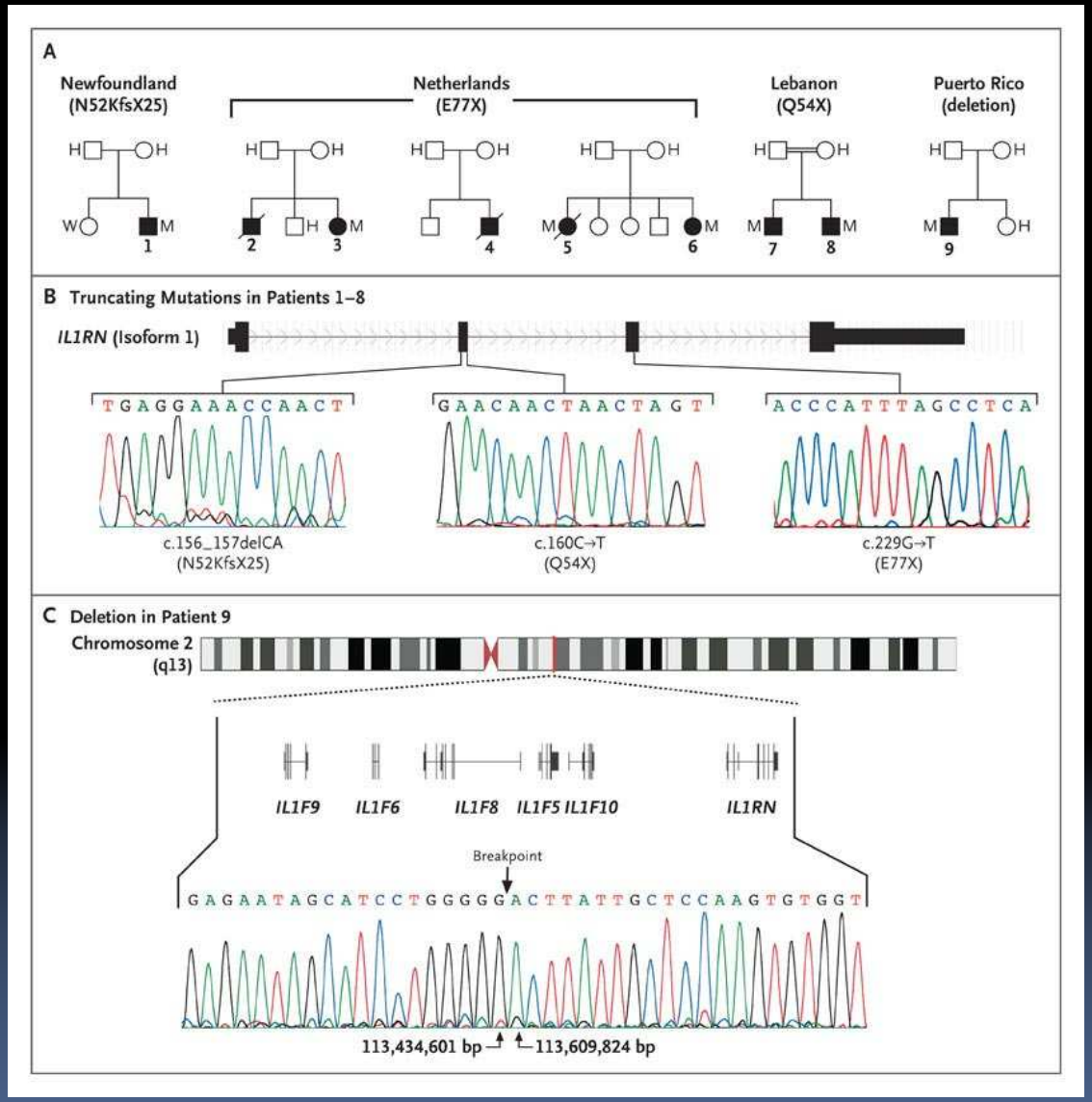
Mutations in the IL1RN Gene Encoding Interleukin-1-Receptor Antagonist and a Genomic Deletion in the Study Patients

Figure 2.

A. Mutations in the IL1RN Gene, pedigrees of the six families. H denotes heterozygous mutant, W wild type, and M homozygous mutant.

B. Structure of IL1RN (isoform 1) exons (black boxes) and the sequences of the homozygous mutations

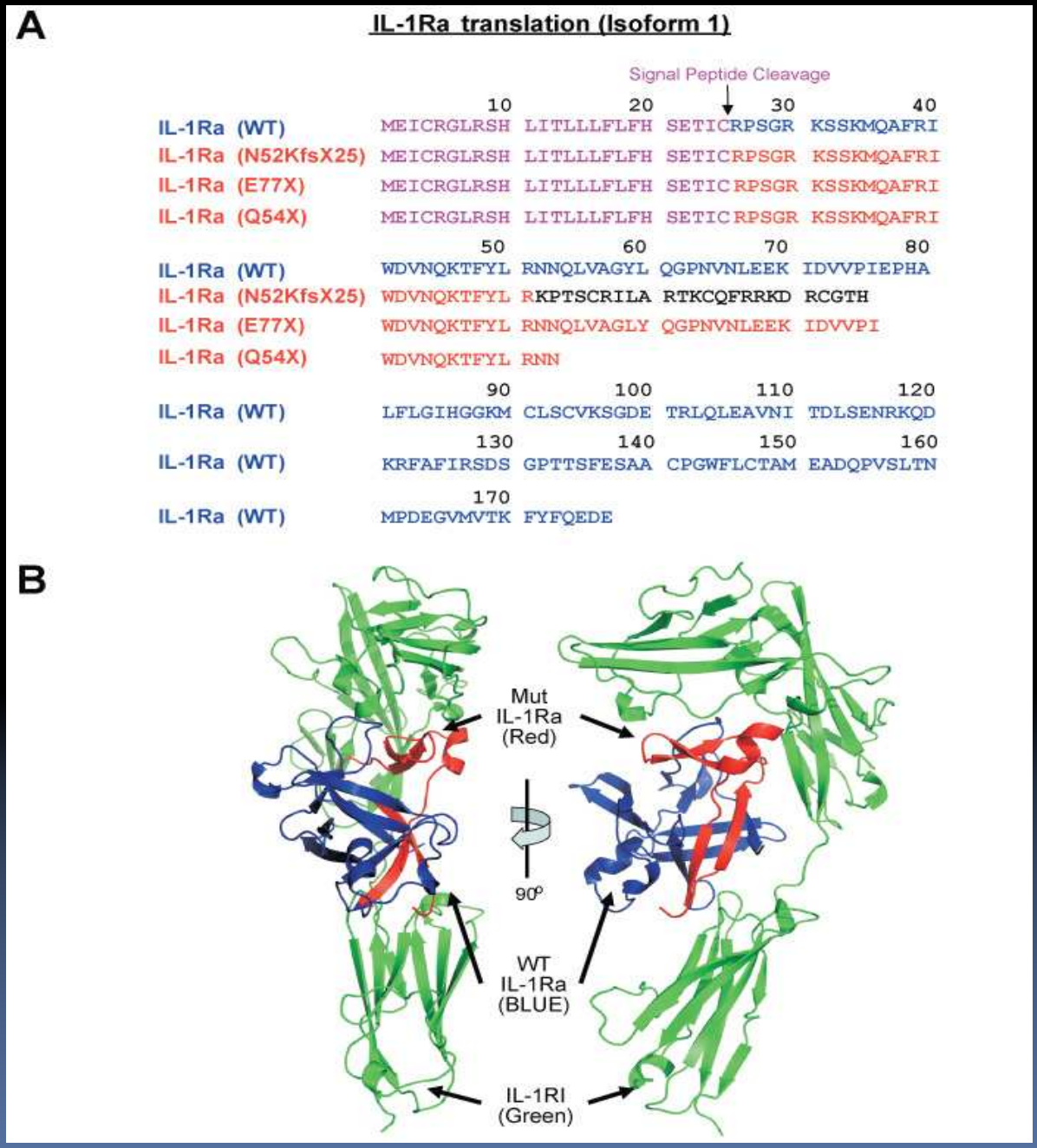
C. A genetic isolate in northwestern Puerto Rico is a founder population for a 175-kb genomic deletion on chromosome 2 that deletes genes encoding six interleukin-1-related genes: IL1RN and the genes encoding interleukin-1 family, members 9 (IL1F9), 6 (IL1F6), 8 (IL1F8), 5 (IL1F5), and 10 (IL1F10).



Structural Consequences of the IL1RN Mutations

A. DIRA results in premature truncation of the IL1R amino acid sequence, residues 1-25 represent a segnal peptide that is cleaved when secreted.

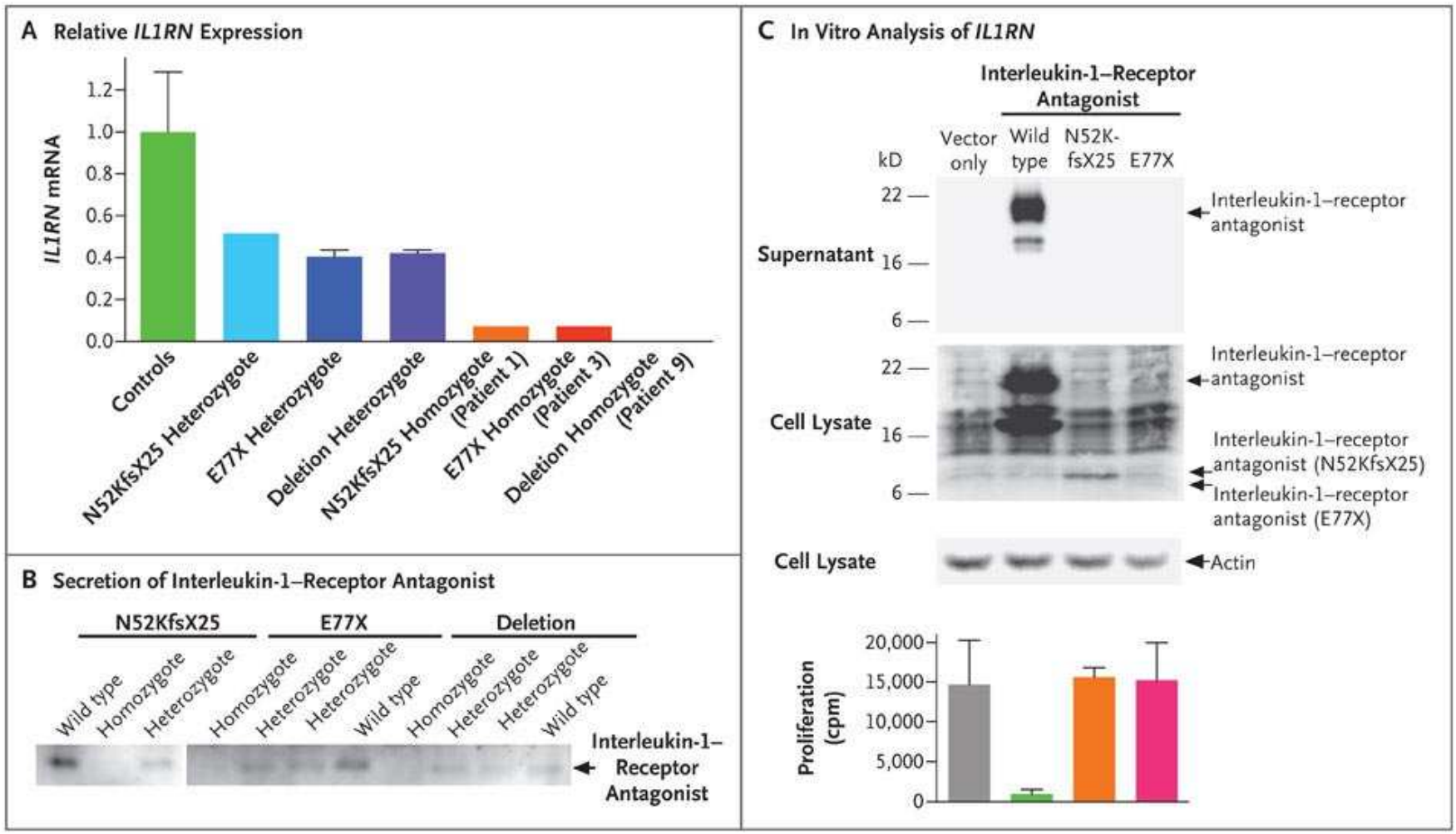
B. Crystal structure of IL1R1 (green) in complex with IL1Ra.



IL1RN Mutations

- all nine patients were homozygous for IL1RN mutation (7 pts) or parents were heterozygous carriers (2 pts)
- mutations included deletions, frame shift mutations, incorporation of aberrant amino acids and termination codons, and nonsense mutations
- none of these mutations were found in DNA specimens obtained from a panel of 364 white control from the New York Cancer Project
- frequency of each mutation was tested in DNA samples from controls from the patient's country of origin (except the Lebanese family)
- 2/555 controls from Newfoundland carried the mutation from patient 1
- 0/351 Dutch controls carried the same mutation as the Dutch patients, all three unrelated Dutch families were geographically matched and carried the same mutation strongly suggesting a founder effect
- 3/119 Puerto Rican controls carried the same mutation as the Puerto Rican patient who came from a genetically isolated population

Mechanism of Disease Caused by DIRA



A. IL1RN messenger RNA diminished in DIRA pts. B. IL1RN protein diminished in DIRA pts and heterozygotes. C. Embryonic kidney cells transfected with mutant or wt IL1RN; mean growth of an IL1 responsive cell line in the presence of IL1RN (wt efficiently inhibits proliferation)

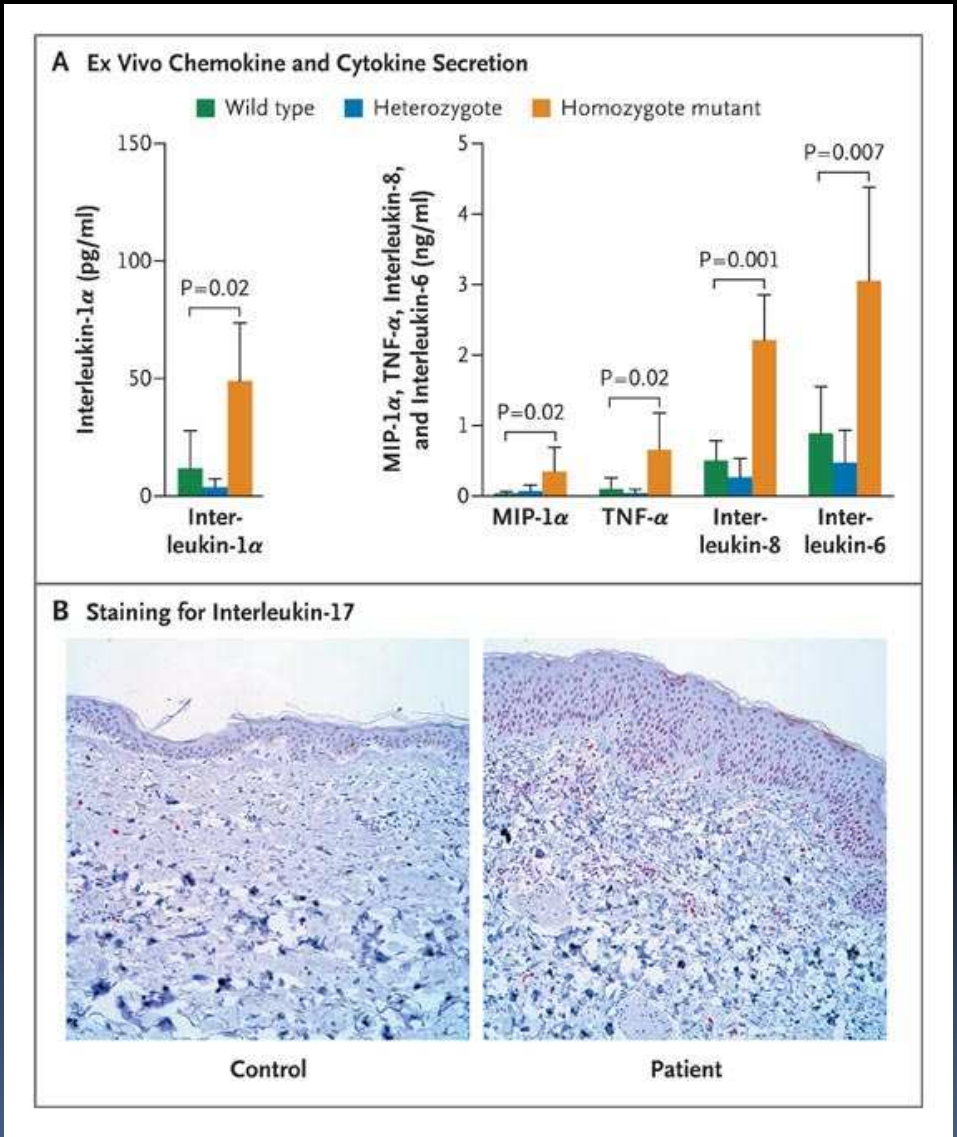
Supplementary Table 1. Cytokines assayed post stimulation with human recombinant IL-1 β

Group I Cytokines		Group II Cytokines	
IL-1b	Eotaxin	IFN-a2	MIF
IL-1ra	FGF basic	IL-1a	MIG
IL-2	G-CSF	IL-2ra	B-NGF
IL-4	GM-CSF	IL-3	SCF
IL-5	IFN-g	IL-12p40	SCGF-B
IL-6	IP-10	IL-16	SDF-1a
IL-7	MCP-1(MCAF)	IL-18	TNF-B
IL-8	MIP-1a	CTACK	TRAIL
IL-9	MIP-1b	GRO-a	VCAM-1
IL-10	PDGF bb	HGF	
IL-12(p70)	RANTES	ICAM-1	
IL-13	TNF-a	LIF	
IL-15	VEGF	MCP-3	
IL-17	M-CSF		

Functional Consequences of DIRA

A. Monocytes from pts, carriers, and controls stimulated with IL1B, 50 cytokines/chemokines measured, 5 significantly overexpressed (IL1a, MIP1a, TNFa, IL8, IL6).

B. Cytohisochemical analysis of IL-17 expression from biopsy samples of inflamed skin from patients with DIRA versus controls.

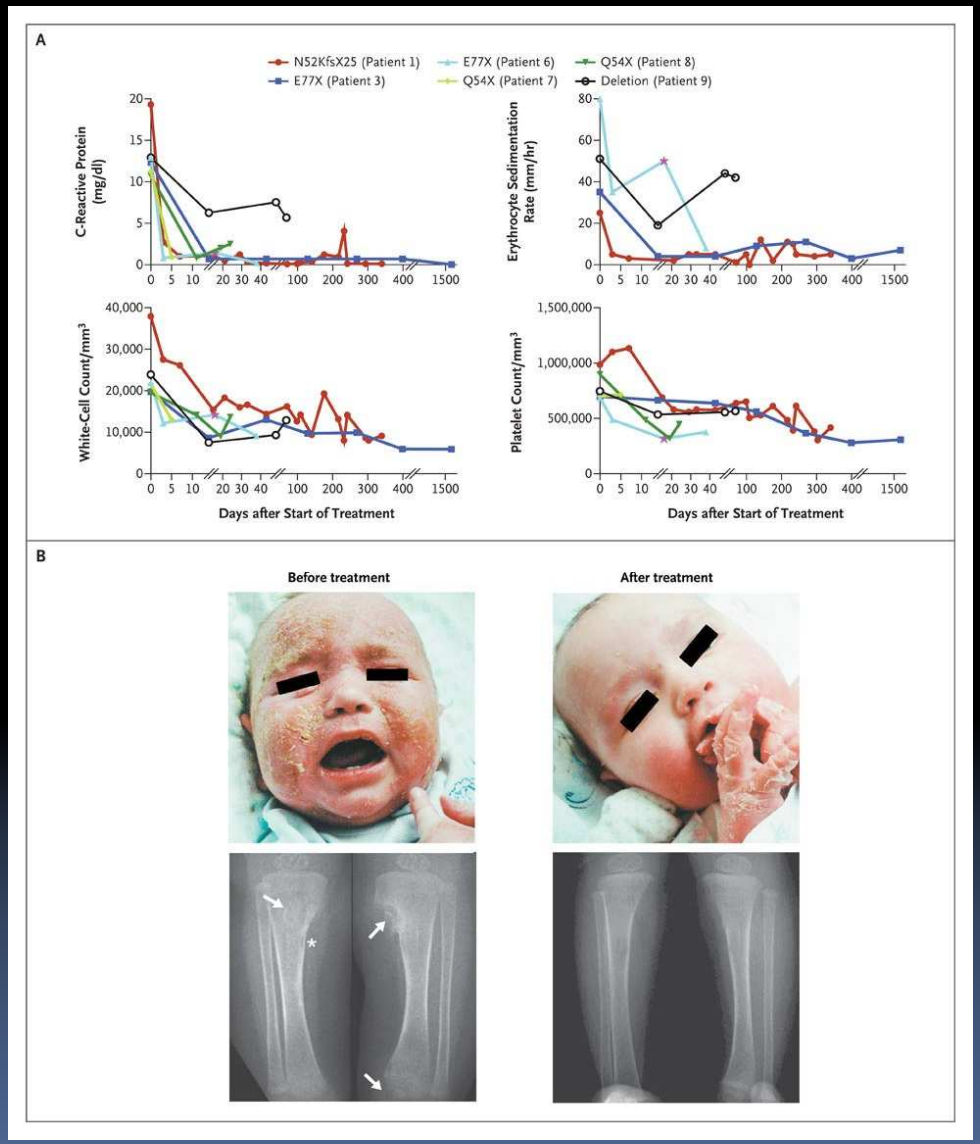


Clinical and Laboratory Response of Patients with DIRA to Treatment with Anakinra

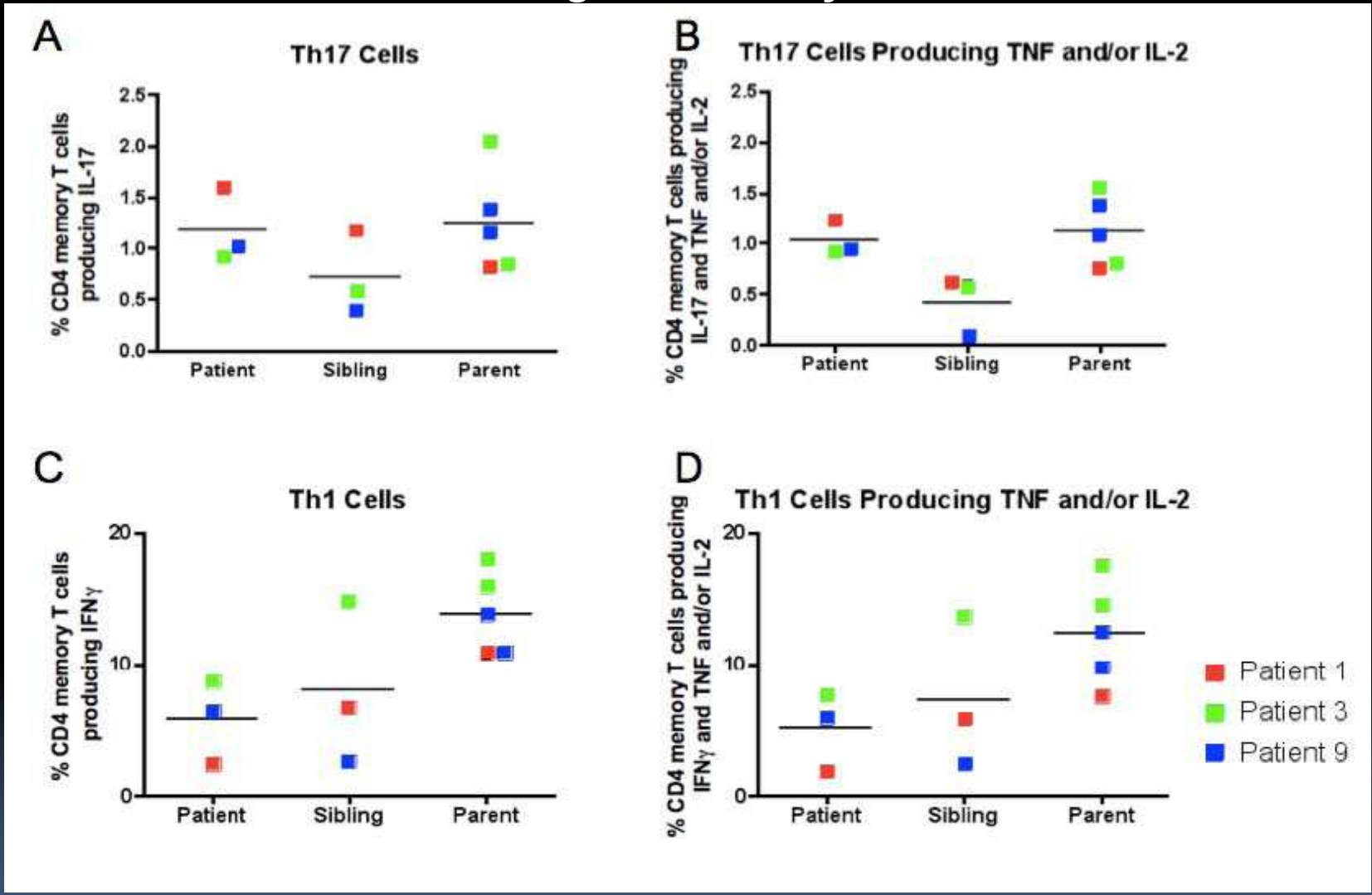
- Empiric therapy with anakinra started in 6 pts, all had rapid response to treatment.
- Discontinuation of anakinra lead to a relapse of disease in the Dutch patient.
- Three patients had transient injection site reactions of anakinra, one had anaphylaxis and subsequent discontinuation caused disease flare.

A. All but the Puerto Rican patient had clinical remission and normalization of acute-phase reactant levels and CBC.

B. Skin and Bone manifestations resolved within weeks to months.



Supplementary Figure 3. TH17 and IFNγ producing cells in DIRA patients, their siblings and family controls



Cytokine production by memory CD4+ T cells after stimulation with Staph toxin.

A. Frequency of CD4+ memory T cells that produce IL-17.

B.Frequency of CD4+ memory cells that produce IL-17 (Th17 cells) but also TNF and/or IL-2.

C.Frequency of CD4+ memory T cells that produce IFNγ.

D.Frequency of CD4+memory cells that produce IFNγ (Th1 cells) but also TNF and/or IL-2.

Summary

- Describe 9 patients with an autosomal recessive autoinflammatory syndrome, deficiency of the interleukin-1-receptor antagonist, which begins near birth with multifocal osteomyelitis, periostitis, and pustulosis
- Identified homozygous truncating mutations in the IL1RN gene in six patients and, by inference, in two additional patients in families in which both parents were carriers of the mutation.
- A ninth patient had a deletion in chromosome 2 that includes IL1RN and five other members of the IL1 gene family.
- The mutation causes a decrease in IL1RN which normally inhibits the proinflammatory cytokines IL1a and IL1B.

Allele Frequencies

- Prevalence of Newfoundland founder mutation = 0.2%
- Prevalence of Puerto Rican founder mutation = 1.3%
- Did not find the Dutch mutation in the controls, but occurrence of this mutation in three independent families (one residing in Canada) strongly suggests a founder effect.
- Screening of newborns may be warranted in these high risk populations?
- No controls for the Lebanese mutation but could be the result of consanguinity.
- Other case descriptions fit this clinical picture (Ivker et al, Pediatric Dermatology 1993; Leung et al, Journal Pediatric Orthopedics 1985)
- Another patient with IL1RN mutation was also recently identified (Reddy et al, NEJM 2009)

Clinical Correlations

- Syndrome of infantile cortical hyperostosis – self-limited disease caused by an autosomal dominant mutation on COL1A1 (encodes major component of type 1 collagen)
- Neonatal-onset multisystem inflammatory disease (NOMID) – caused by gain-of-function mutations in $NLRP_3$ which causes constitutive activation and hypersecretion of IL1B
- In contrast, DIRA permits over-activity of IL1B because of the lack of IL1BN, and also allows over-activity of IL1a which acts and a proinflammatory cytokine, potent osteoclast activator, and autocrine growth factor

Other Points of Interest

- Homozygous genomic deletion on chromosome 2q in the Puerto Rican patient includes IL1RN and five other members of the IL1 gene family (IL1F6, IL1F8, IL1F9, IL1F5, IL1F10), this phenotype appears to be more refractory to anakinra.
- Mouse models with knocked out IL1RN have arthritis, psoriasis, and arteritis (Horai et al, J Exp Med 2000; Nicklin et al, J Exp Med 2000).
- Unopposed IL1 signaling could drive differentiation of type 17 helper T cells that contribute to inflammation in the animal model.
- Although deficiency of IL1RN is rare, it may point to clues about the mechanisms of more common illnesses that affect the balance between IL1 and IL1RN, including type 2 DM, osteoarthritis, and Alzheimer's.