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A FAMILY AFFECTED BY ATYPICAL IPEX SYNDROME

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Immunedysregulation Polyendocrinopathy Enteropathy X-linked
IPEX

A rare genetic disorder of **immune regulation** characterized by overwhelming systemic autoimmunity

It is caused by mutations in the ***FOXP3 gene*** , located on X-chromosome, key molecular factor driving T cell tolerance and crucially important for **$CD4^+CD25^+$ regulatory T cell** development and function

What does *FOXP3* do?

Plays an essential role in the development and function of $CD4^+CD25^+$ Regulatory T cells

Hori S, et al., Science 2003
Khattri R, et al., Nat. Immunol. 2003
Fontenot JD, et al., Nat. Immunol. 2003

FOXP3 may function as a transcriptional repressor of cytokine promoters

Schubert L, et al., J. Biol. Chem. 2001

Rheostat of the immune response

Khattri R, et al., J. Immunol. 2001

Foxp3

MPNPRPAKPMAPSLALGPSPGVLPSWKTAPKGSELLGTRGPGGPFQGRDLRSGAHTS

Δ2

SSLNPLPPSQLQLPTVPLVMVAPSGARLGPSPHLQALLQDRPHFMHQLSTVDAHAQT

PVLQVRPLDNPAMISLPPPSAATGVFSLKARPGLPPGINVASLEWVSREPALLCTFP

RSGTPRKDSNLLAAPQGSYPLLANGVCKWPGCEKVFEEPEEFLKHCQADHLLDEKGK

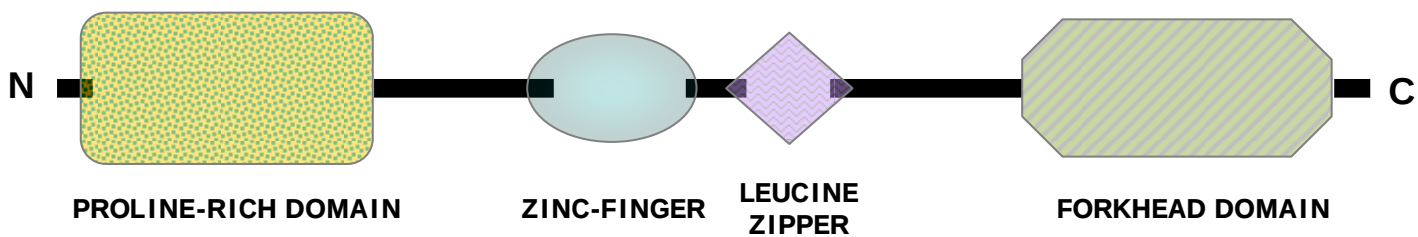
AQCLLQREVVQSLEQQLELEKEKLGAMQAHLAGKMALAKAPSVASMDKSSCCIVATS

*

TQGSVLPAWSAPREAPDGGLFAVRRHLWGSHGNSTFPEFFHNMDYFKYHNMRPPFTY

ATLIRWAILEAPERQRTLNEIYHWFTRMFAYFRNHPATWKNAIRHNLSLHKCFVRVE

SEKGAVWTVDEFEFRKKRSQRPNKCSNPCP



$CD4^+CD25^+$ Regulatory T Cells

Make up 5-10% of the normal CD4+ T cell population.

Characterized by expression of CD4 and $CD25^{bright}$ at baseline and of intracytoplasmatic expression of FOXP3. They are positive for Cytotoxic T Lymphocyte associated Antigen-4 (CTLA-4) e Glucocorticoid-Induced Tumor-necrosis factor receptor-Related protein (GITR); recently, it has been reported that they have low expression of CD127.

Require activation and cell contact to repress proliferation of other T cells but do not appear to proliferate themselves after activation.

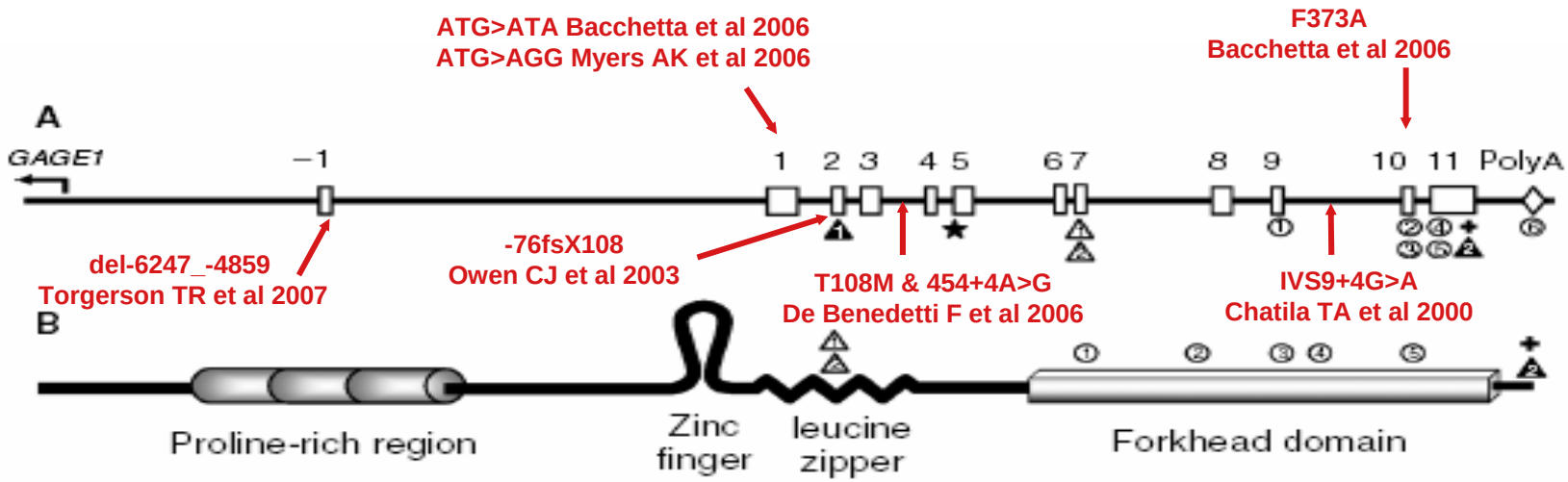
BASIC Clinical & Laboratory Features:

Enteropathy	✓ Watery diarrhea (rarely bloody) with villous atrophy at the biopsy ✓ Inflammatory bowel disease
Dermatitis	✓ Eczema ✓ Erithroderma ✓ Psoriasiform dermatitis ✓ Alopecia
Endocrinophaties	✓ Type I diabetes ✓ Thyroid abnormalities
Elevated IgE	Mainly present

Other Clinical & Laboratory Features:

Hematologic	✓ Coombs (+) hemolytic anemia, ✓ Autoimmune thrombocytopenia ✓ Autoimmune neutropenia
Renal	Nephrosis or Nephritis
Hepatic	Autoimmune hepatitis
Lymphadenopathy	Rare
Arthritis / Vasculitis	Rare
Autoantibodies	May be present : ✓ AIE-75 (Ab against gut and kidney specific antigen) ✓ ANA ✓ Ab against different organs (thyroid, pancreatic islets, erythrocytes, platelets, smooth muscle)

Locations of *FOXP3* mutations reported



C

	cDNA	Protein
▲1	227 delT	L76fsX128 (29)
★	543C>T	Splicing (22)
△2	747_749delAAG	ΔK250 (22)
△3	750_752delGGA	ΔE251 (1)
①4	1040G>A	R347H (22)
②5	1087A>G	1363V (29)
③6	1113T>G	F371L (3)
④7	1150G>A	A384T (2,3)
⑤8	1189C>T	R397W (3)
✚1	1290_1309del/ins TGG	G430fsX452(3)
▲2	1293_1294delCT	P431fsX457(2)
⑥9	AAUAAA>AAUGAA	Polyadenylation(30)

Legend	
○	Point mutation
△	Deletion, inframe
▲	Deletion, frameshift
★	Splice site mutation
✚	Complex mutation

From *Ochs HD et al.* Immunol Rev. 2005 Feb;203:156-64. Review.

MILD PHENOTYPES

	CASE #	ONSET	FAILURE TO THRIVE	ECZEMA	ENDOCRINOPATHY	ENTEROPATHY	AUTOIMMUNE DISORDERS	INFECTIONS	OTHERS	TREATMENT	OUTCOME	FOXP3 MUTATIONS
Powell J Pediatr 1982	2	9 mo	yes	yes	IDDM at 16 years of age	Episodic watery diarrhea at 9 mo			Non-hemolytic anemia Sarcoidosis at 29 yrs		Died at 30 yrs	Poly A
	3	early	?	yes	?	episodic diarrhea, early infancy		pneumonia	Non-hemolytic anemia at 10 yrs athralgias at 5 yrs glomerulonephritis at 7 yrs Ulcerative Colitis		Alive 10 yrs	
Sakaki Eur J Ped 1993	1	neonatal	yes	?	IDDM Hypotyroidism	yes	hemolytic aanemia			CsA	Alive 18 mo	NA
Wildin J Med Gen 2001	2	1 mo		yes	IDDM at 6 yrs Hypotyroidisms at 2 yrs	Intractable diarrhea started at 1 mo of age. Episodic diarrea after 1 yr	emolitic anemia at 2 yrs neutropenia	upper and lower respiratory infections	linphoadenopathy hepatomegaly	steroids rituximab	Alive 5 yrs	intron 9
Walker Smith Lancet 1982	1	1 yr	yes	?	?	yes						NA
Ferguson Am J Med Genet 2000	1	1 mo	yes	yes		yes					Alive 8 yrs	Locus maps to Xp11.23 to Xq21.1
Di Rocco Arch Dis Child 1996						diarrhea responsive to gluten-free diet					Alive 7 yrs	NA
De Benedetti Clin Gastr Hepatol 2006	1	neonatal		Yes 18 yrs		Early-onset diarrhea responsive to gluten-free diet			Arthritis at 15 yrs		Alive 22 yrs	454+4A>G (intron 3)
	2	14 mo				Diarrhea started at 14 mo			Arthritis at 5 yrs		Alive 7 yrs	T108M

Male born to healthy unrelated parents after 37 wks of pregnancy, complicated with diabetes and IUGR.

At birth, weight was 2580g. The neonatal period was unremarkable.

At age of 1 month he was admitted to our hospital for vomiting and **severe bloody diarrhea**

Lab evaluation resulted negative for common GI infections, total protein were low (3.6 g/dl) and anemia was present (Hb 10g/dl)

Severe and intractable diarrhea persisted and because of his progressive clinical deterioration **total parenteral nutrition** as well as **treatments of support** were started.

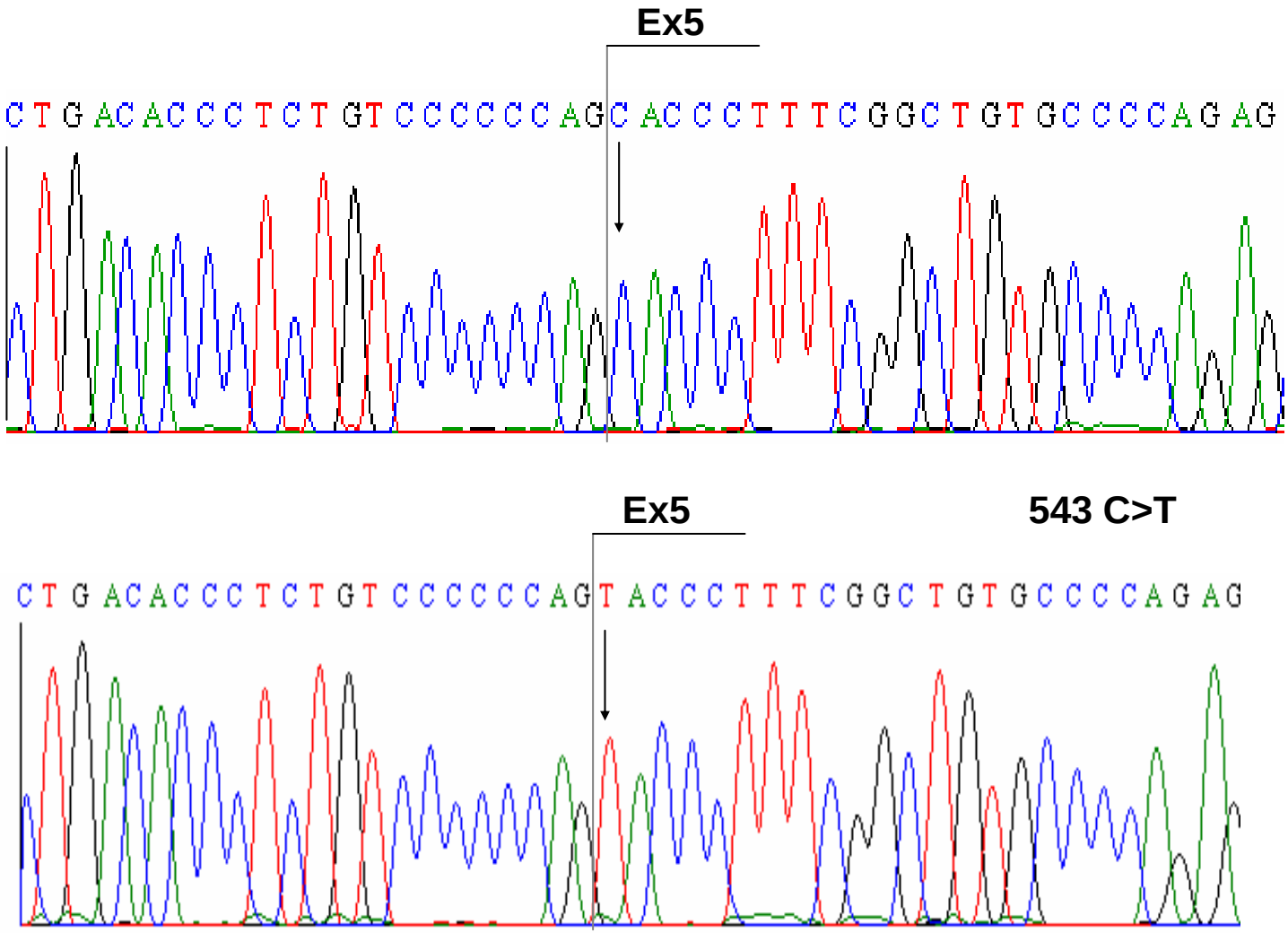
Intestinal biopsies showed histological findings compatible with **autoimmune enteropathy**

Clinical improvement was slowly obtained when **immunosuppressive therapy** was started (high-dose intravenous corticosteroids combined to oral cyclosporin)

Although absence of any endocrine dysfunction and skin lesions, due to life threatening enteropathy, a diagnostic procedure for **IPEX** syndrome was considered.

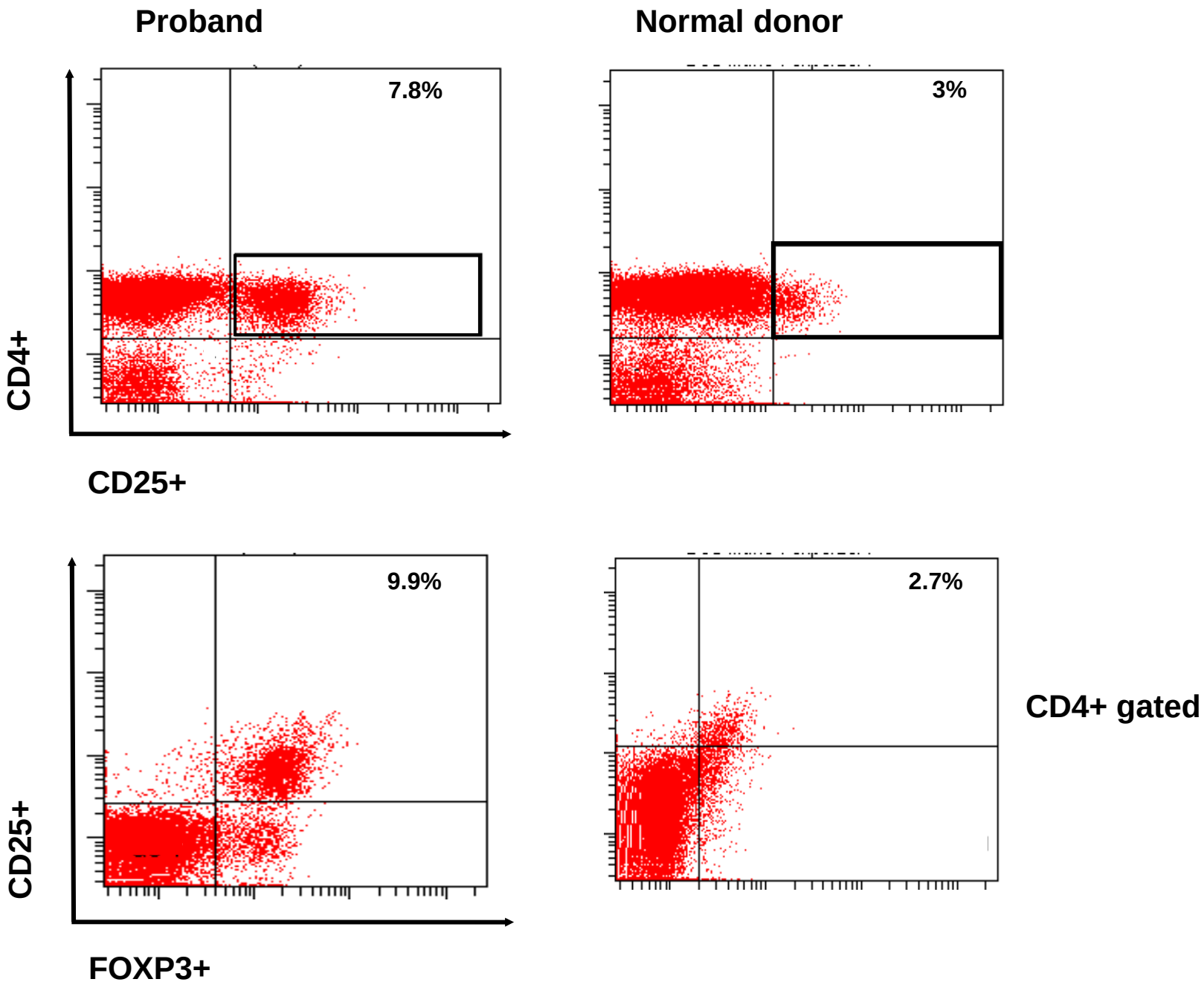
Unfortunately the patient **died prematurely at age of 5 month** while he was on immunosuppression

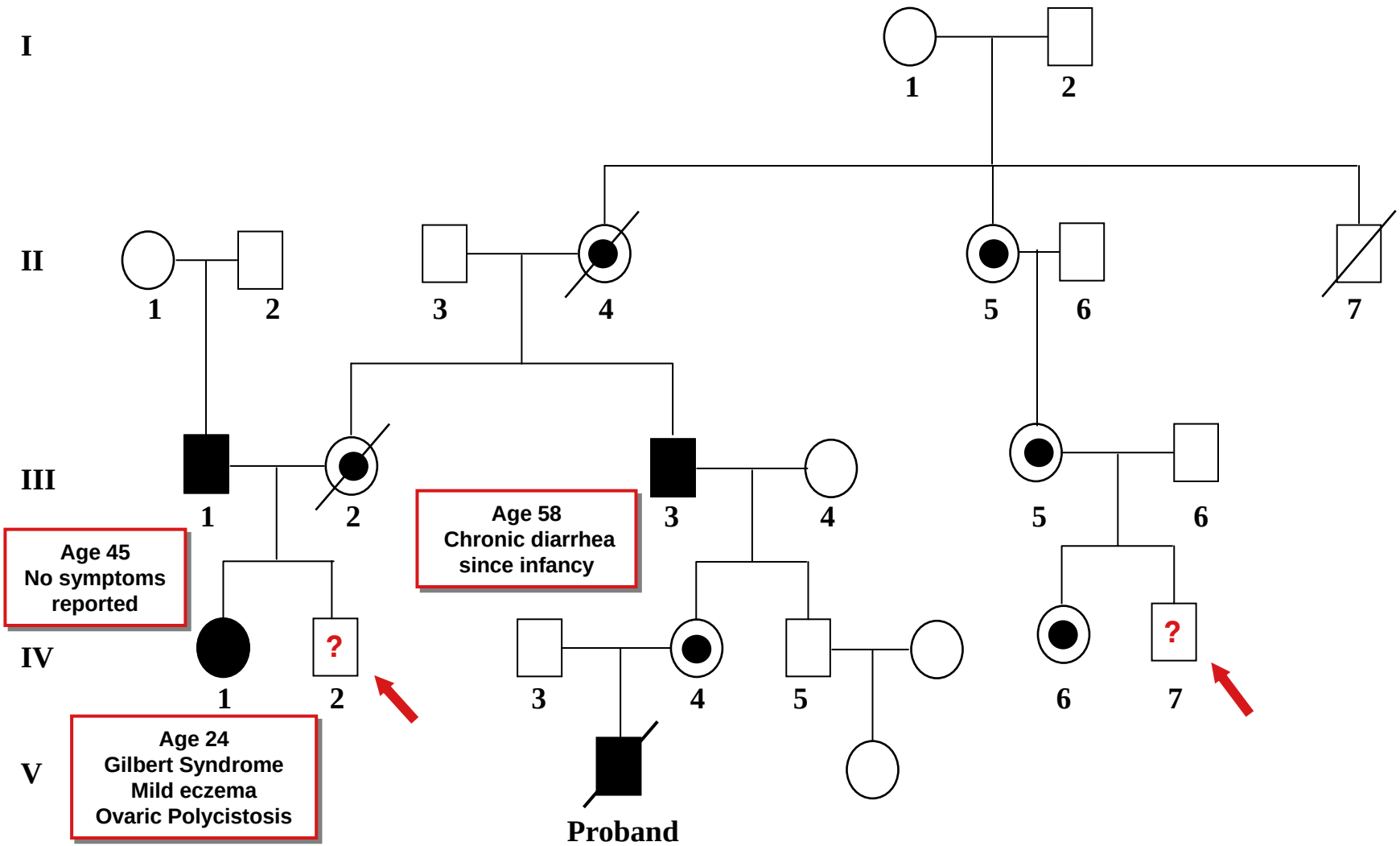
Molecular analysis of *FOXP3*



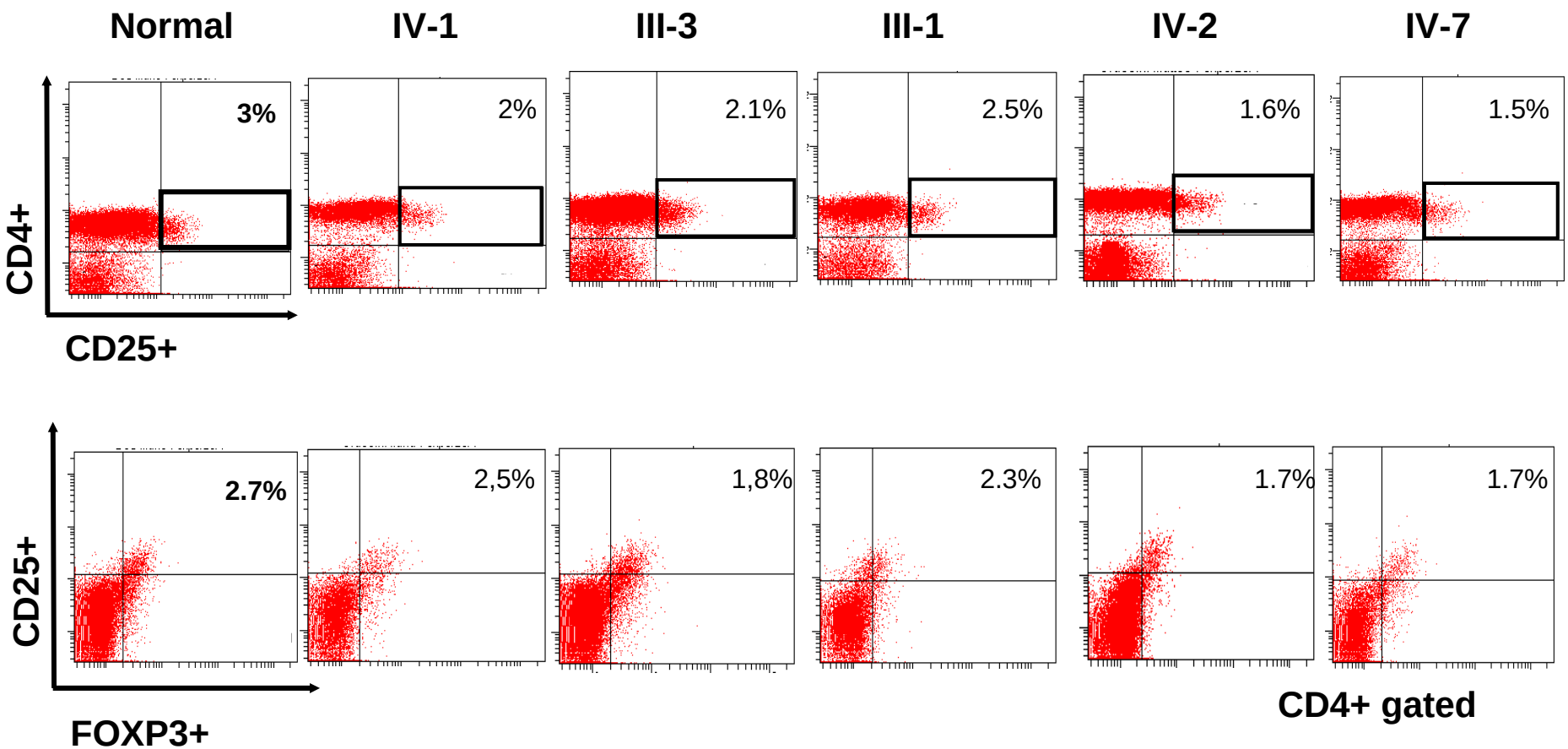
Bennet C. et al. Curr Opin Pediatr, 2001
Gambineri E. et al. Curr Opin Rheumatol, 2003

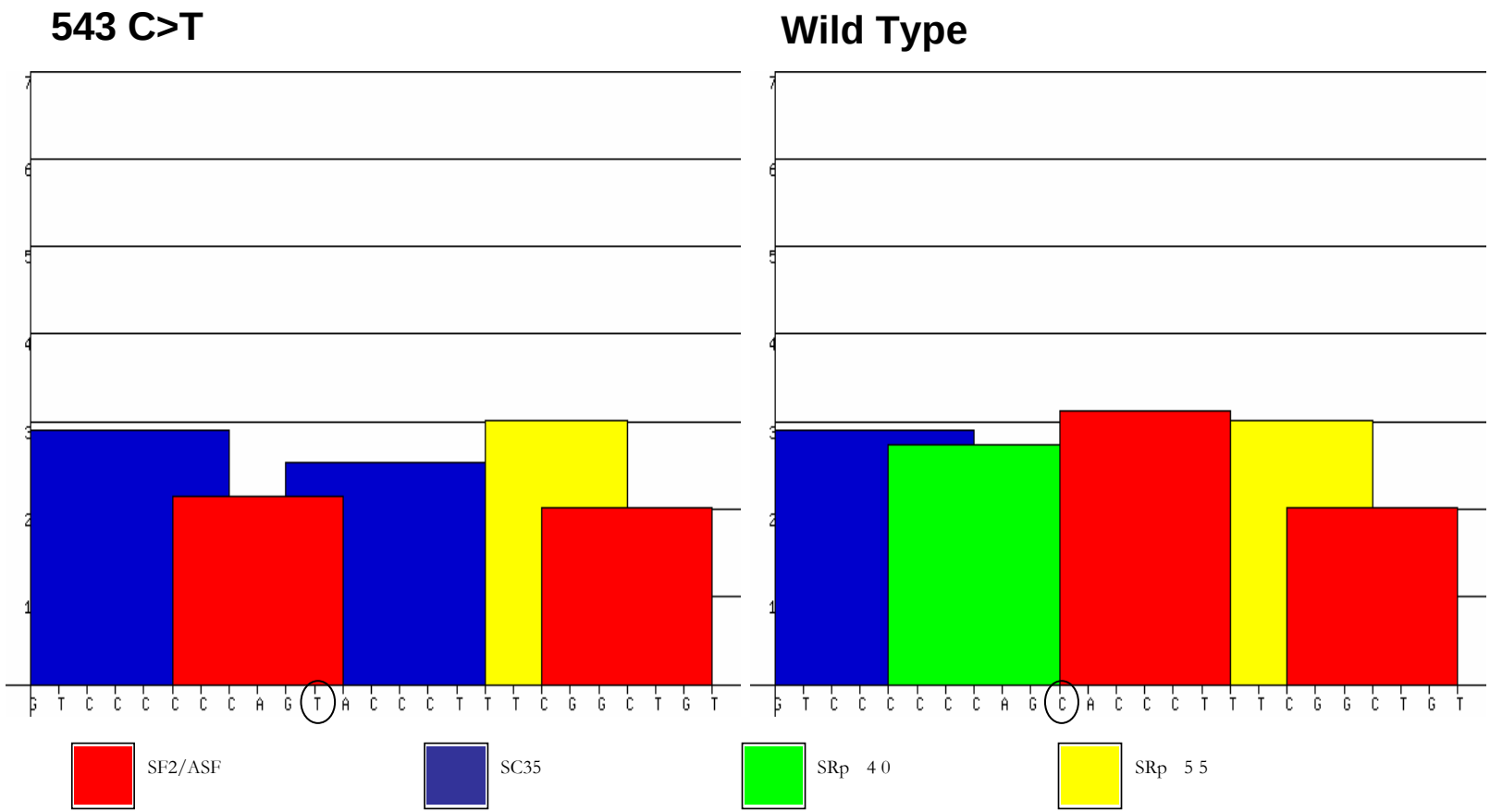
FOXP3 PROTEIN EXPRESSION by Flow Cytometry




I
1
2
II
1
2
3
4
5
6
7
III
1
2
3
4
5
6
Age 45
No symptoms
reported
Age 58
Chronic diarrhea
since infancy
IV
1
?
2
3
4
5
6
?
7
V
Age 24
Gilbert Syndrome
Mild eczema
Ovaric Polycistosis
Proband

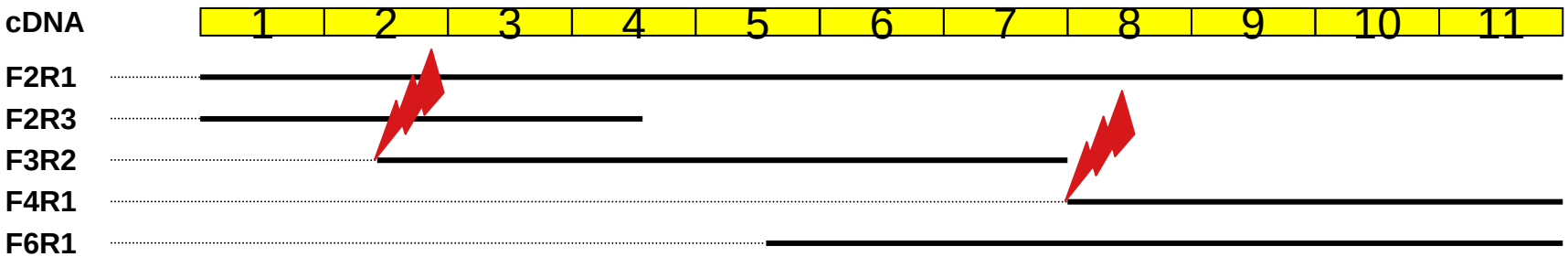
FOXP3 PROTEIN EXPRESSION by Flow Cytometry

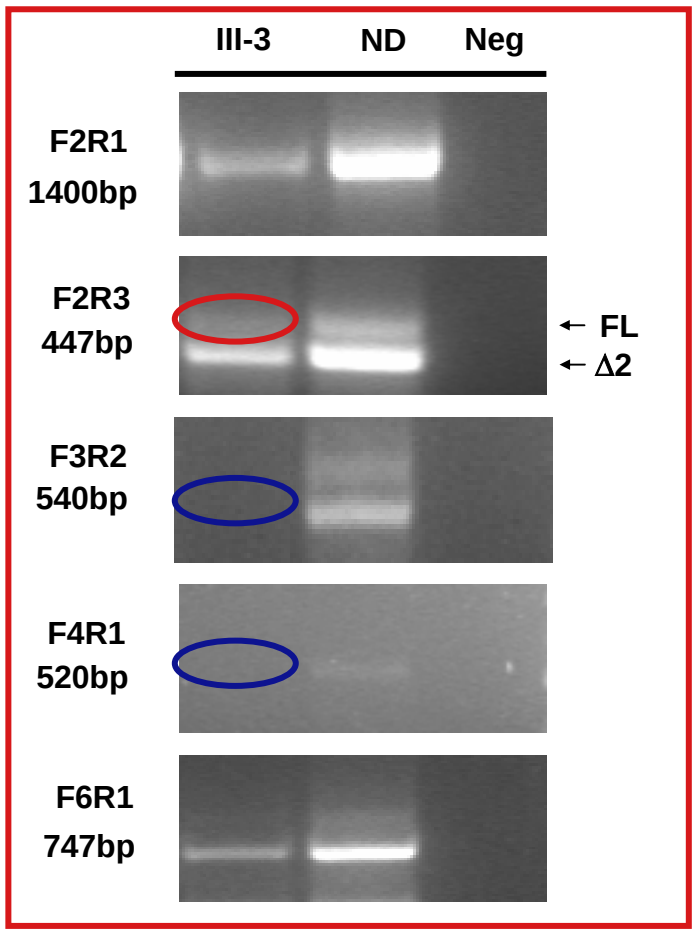




Disruption of exonic splicing enhancer (ESE) motifs and modification of SR protein binding sites in mutated of *FOXP3*

FOXP3 cDNA fragments @ PCR amplification





- ✓ **Lower cDNA expression**
- ✓ **Lacks full-length isoform**
- ✓ **No amplification of fragments F3R2 and F4R1**

- ✓ In the majority of cases IPEX is a severe disease. Patients develop symptoms early in infancy and most die within the first 2 years of life. Milder and late-onset forms of the disease have been described.
- ✓ We found a family harboring a silent mutation within a splice site that do not alter protein expression but see… only 2

IS IPEX UNDERESTIMATED OR MISDIAGNOSED?

IS REALLY FOXP3 RESPONSIBLE FOR THE DISEASE?

WHAT IS MODULATING THE PHENOTYPE VARIABILITY?

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