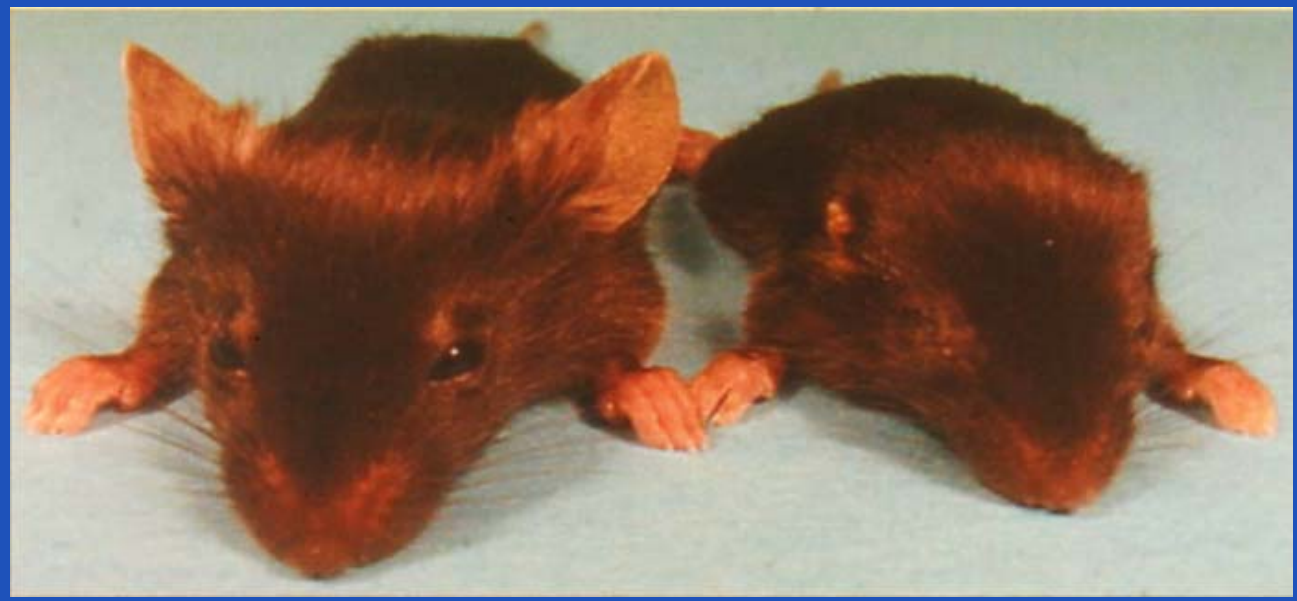
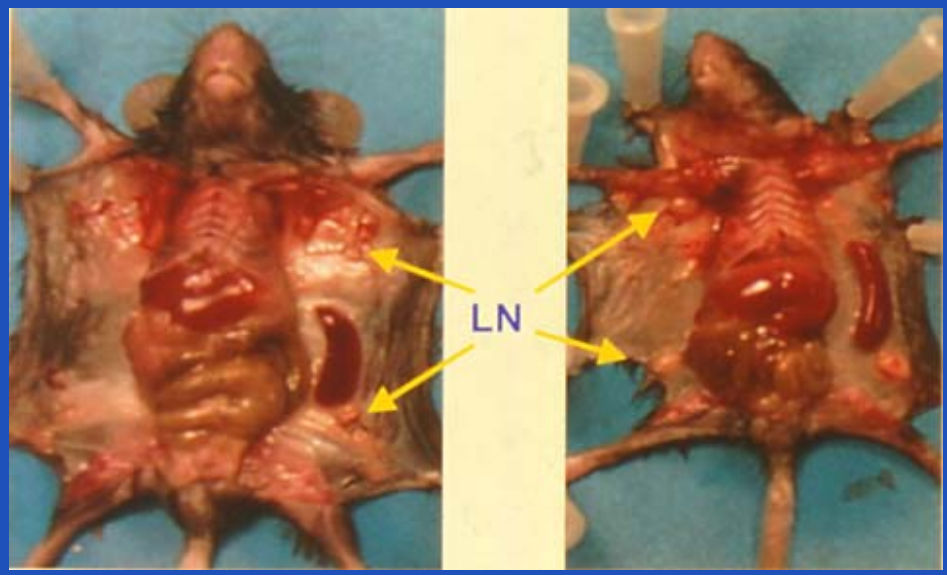
Scurfy Mouse (outside)



Wild type *Scurfy*

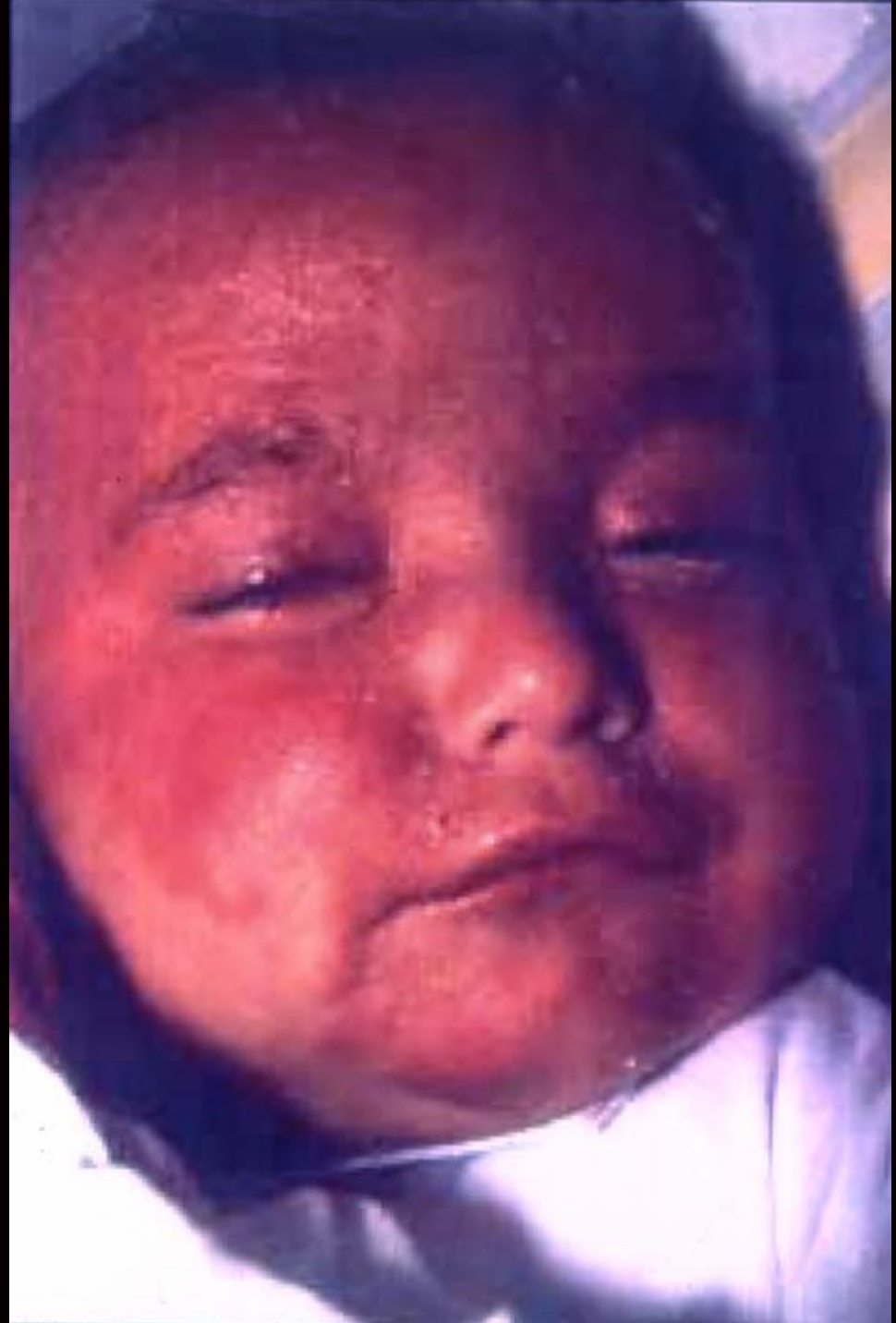
- X-linked recessive inheritance
- Lethality at 21-25 days
- Wasting syndrome
- Exfoliative dermatitis
- Small, thickened ears

Scurfy Mouse (inside)

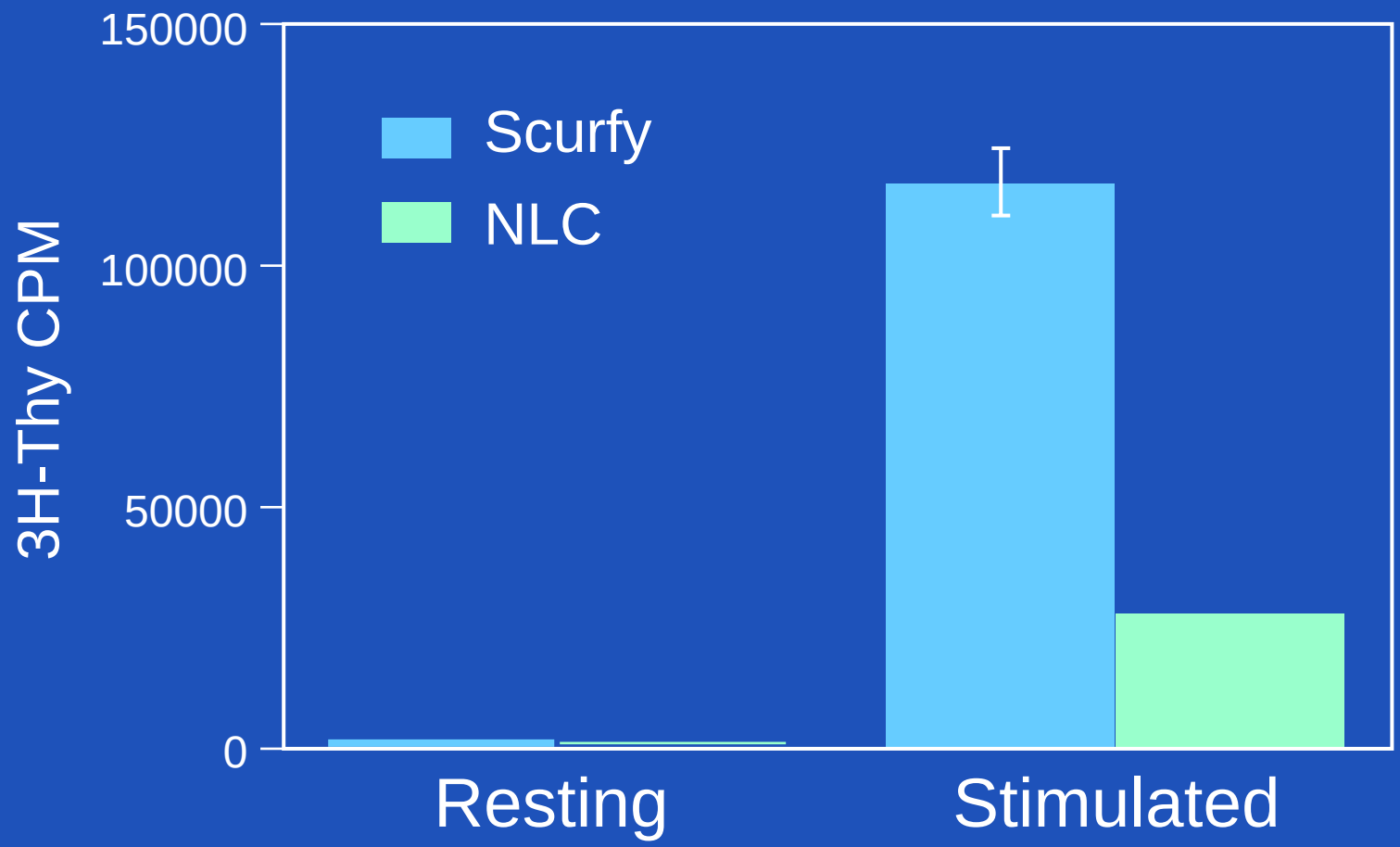


Wild type *Scurfy*

- Severe autoimmune disorder
- Hepatosplenomegaly
- Enlarged lymph nodes
- Multi-organ lymphocytic infiltrates
- Elevated cytokines (GM-CSF, IL-2, -4, -5,-6,-7, -10, IFN-γ, TNFα)



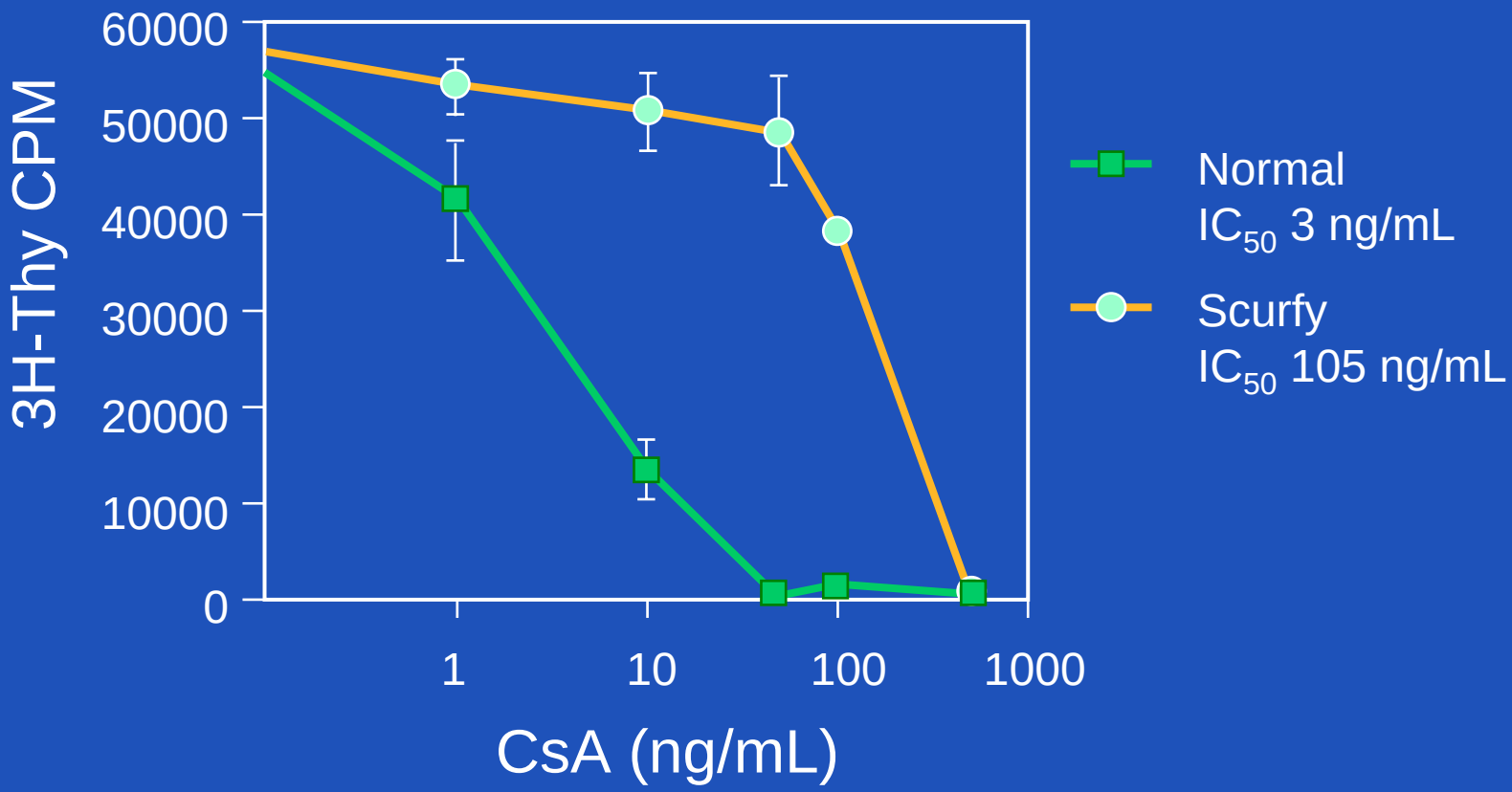
CD4+ T cells are hyper-active in Scurfy mice



Clark LB, et al., J. Immunol. 1999

Scurfy T cells are insensitive to inhibitors

Cyclosporin A dose vs. IL2 response



Clark LB, et al., J. Immunol. 1999

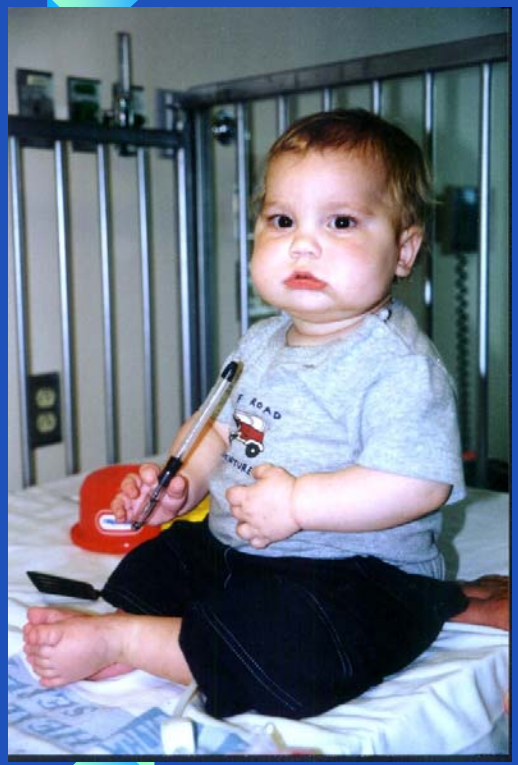
I **I**mmune deficiency/dysregulation

P **P**olyendocrinopathy

E **E**nteropathy (Often have Ab against gut epithelium)

X **X**-linked inheritance

IPEX – Outside (Clinical Findings)



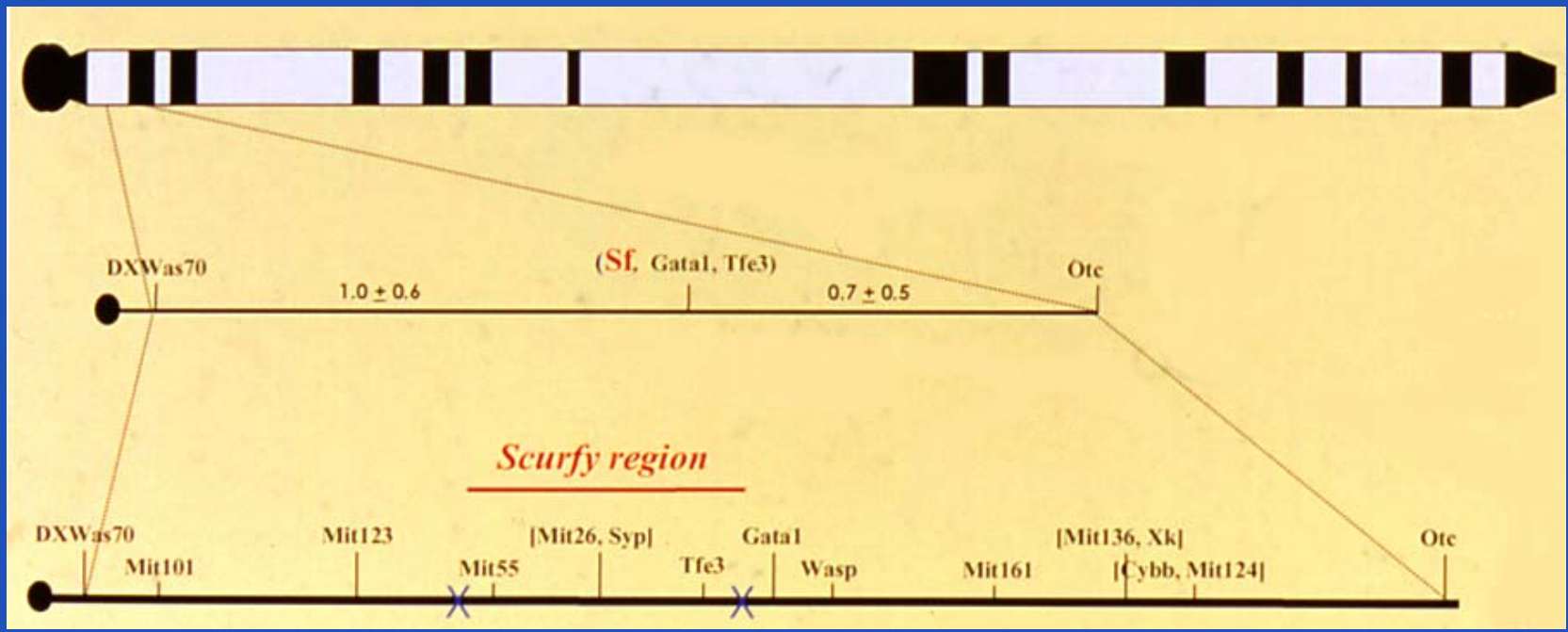
- First described in 1982 by Powell et al. as a syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy.
- Neonatal onset diabetes mellitus
- Hypothyroidism
- Enteritis (diarrhea/villous atrophy)
- Hemolytic anemia & thrombocytopenia.
- Dermatitis
- Dermatitis (eczema)
- Death by 1-2 years of age

IPEX – Inside (Autopsy Findings)

- Pancreas: islets of Langerhans absent with lymphocytic infiltrates
- Intestine: villous atrophy with lyphocytic infiltrates
- Liver: Cholangitis
- Spleen: enlarged
- Lymph nodes: Follicular hyperplasia
- Thyroid: Lymphocytic infiltrates
- Lungs: Consolidation/Inflammation
- Thymus: Atrophy

Mapping of the Scurfy genetic region

X Chromosome ~500 genes



FOXP3 Encodes a Novel Forkhead/ Winged-Helix Protein

Foxp3

MPNPRPAKPMAPSLALGPSPGVLPSWKTAPKGSELLGTRGPGGPFQGRDLRSGAHTS

SSLNPLPPSQLQLPTVPLVMVAPSGARLGPSPHLQALLQDRPHFMHQLSTVDAHAQT

PVLQVRPLDNPAMISLPPPSAATGVFSLKARPGLPPGINVASLEWVSREPALLCTFP

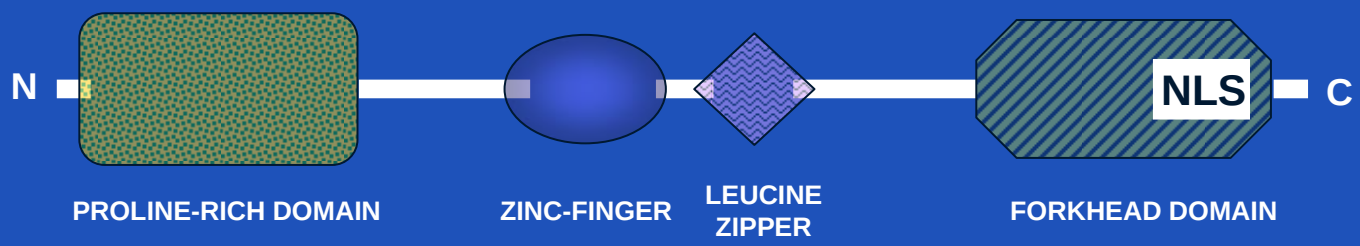
RSGTPRKDSNLLAAPQGSYPLLANGVCKWPGCEKVFEEPEEFLKHCQADHLLDEKGK

AQCLLQREVVQSLEQQLELEKEKLGAMQAHLAGKMALAKAPSVASMDKSSCCIVATS

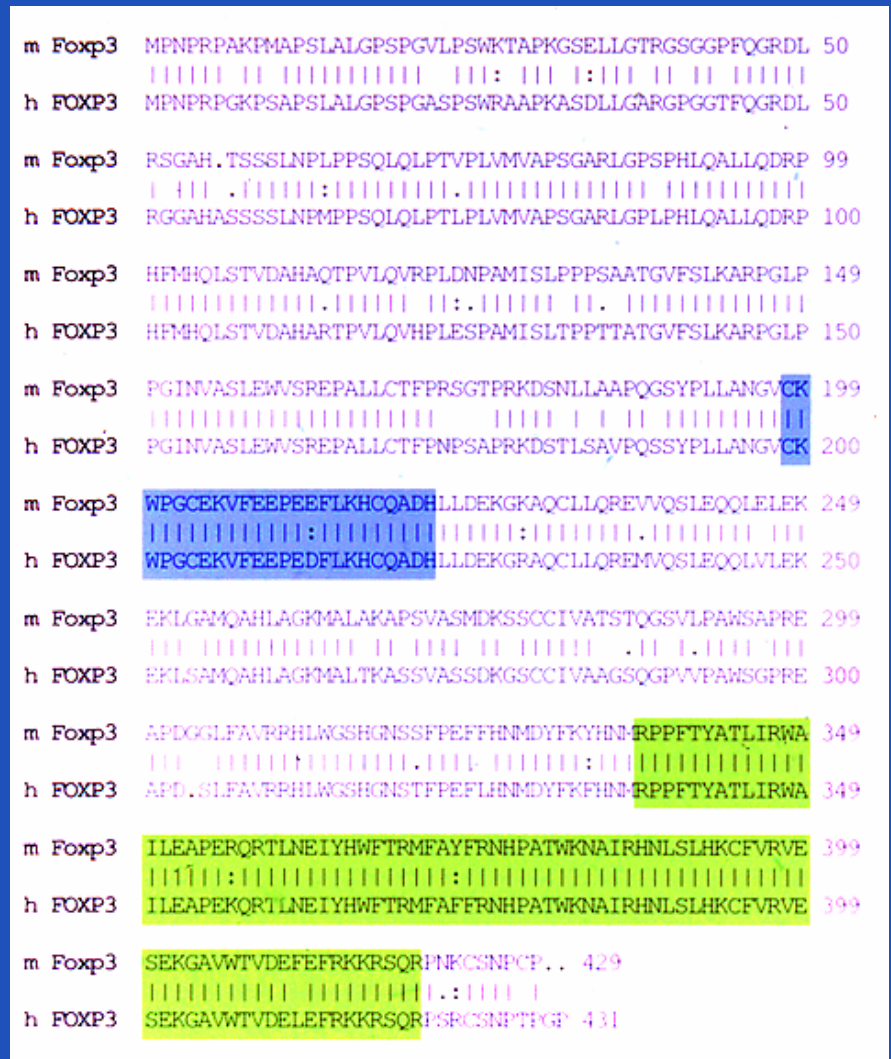
TQGSVLPAWSAPREAPDGGLFAVRRHLWGSHGNSTFPEFFHNMDYFKYHNMRPPFTY

ATLIRWAILEAPERQRTLNEIYHWFTRMFAYFRNHPATWKNAIRHNLSLHKCFVRVE

SEKGAVWTVDEFEFRKKRSQRPNKCSNPCP



Mouse and human scurfy gene products are highly conserved (86% identity)



What does FOXP3 do?

1 – Rheostat of the immune response

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

2 – 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

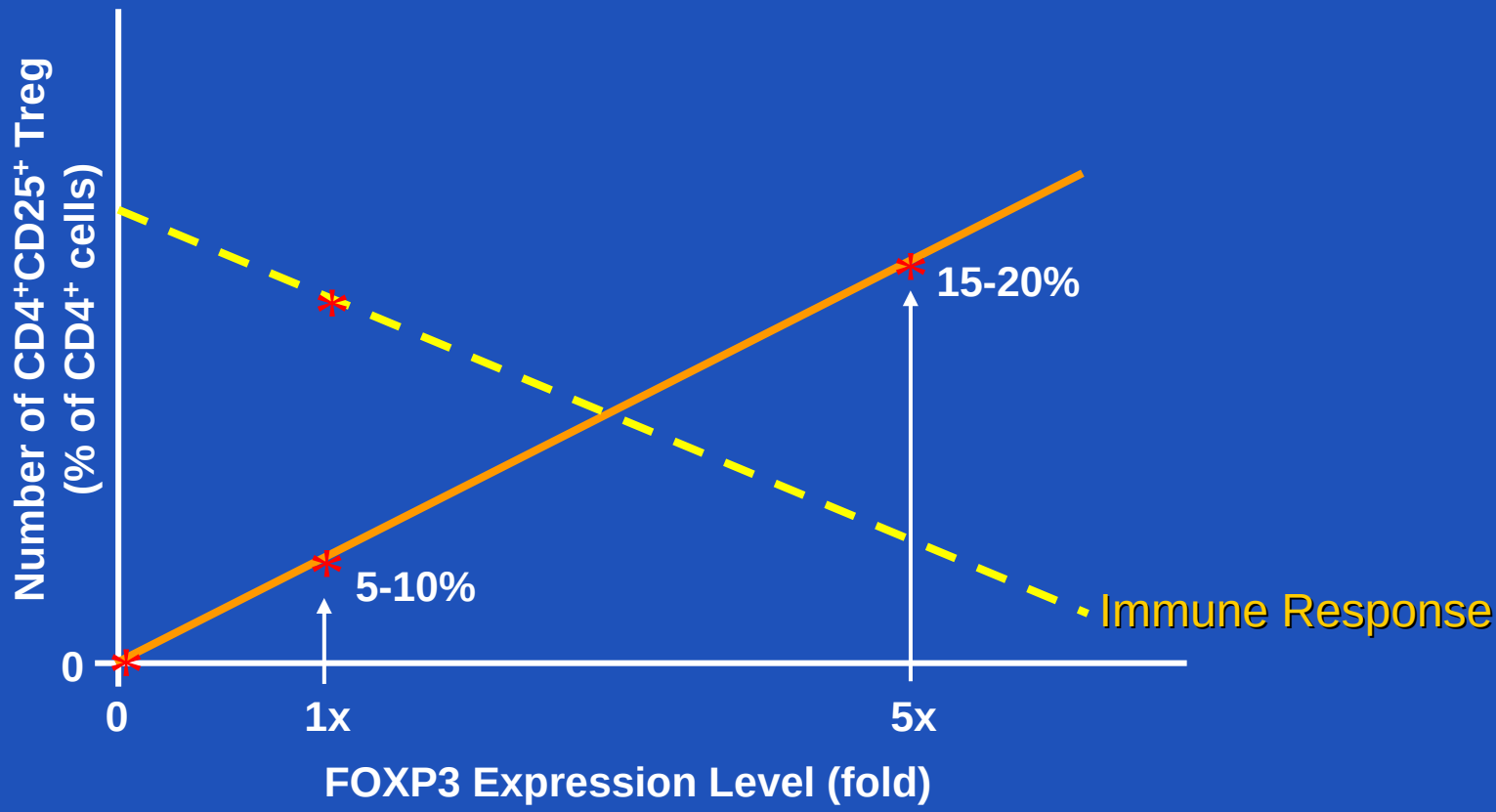
3 – FOXP3 may function as a transcriptional repressor of cytokine promotors

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

5 Habits of Highly Effective Transcription Factors

1. Nuclear import – may be regulated
2. Interaction with partners (homo- or heterodimerization)
3. DNA binding
4. Transcriptional enhancement or suppression at specific gene promotors
5. Down regulation (nuclear export / degradation)

FOXP3 is a rheostat of the immune response



What is known about FOXP3 mediated gene transcription

1 -- FOXP3 may function as a transcriptional repressor of cytokine promotors

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

2 – PBMC from patients with IPEX show poor up-regulation of IFN-γ production in response to activation

Chatilla TA, et al., JCI 2000
Neives D, et al., Arch. Dermatol. 2003

$CD4^+CD25^+$ Regulatory T Cells

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

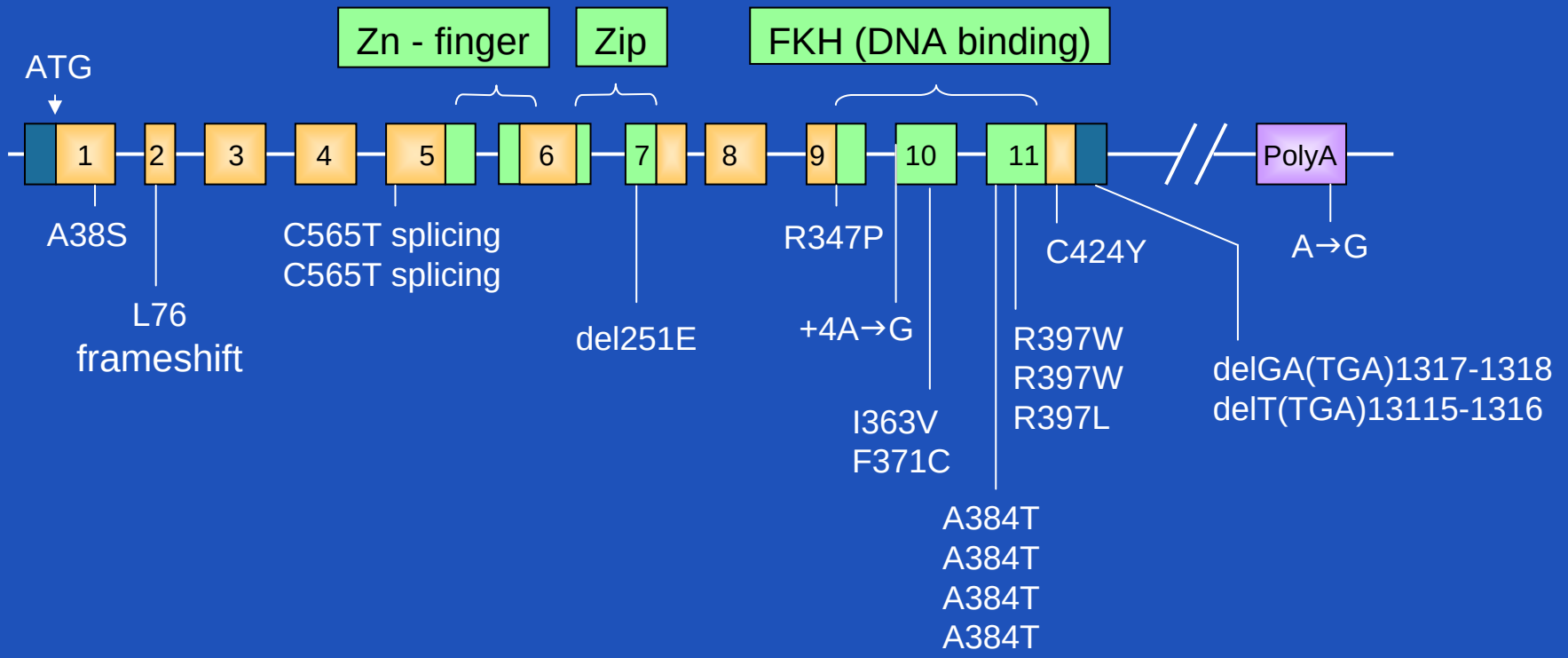
- Characterized by expression of CD4 and CD25 on the cell surface at baseline. Cytotoxic T-Lymphocyte associated Antigen-4 (CTLA-4), Glucocorticoid-Induced Tumor-necrosis factor receptor-Related protein (GITR), Transforming Growth Factor β (TGFβ), and Interleukin-10 (IL-10) have all been reported to play a role in T_{reg} function but are not specific to T_{reg} cells

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

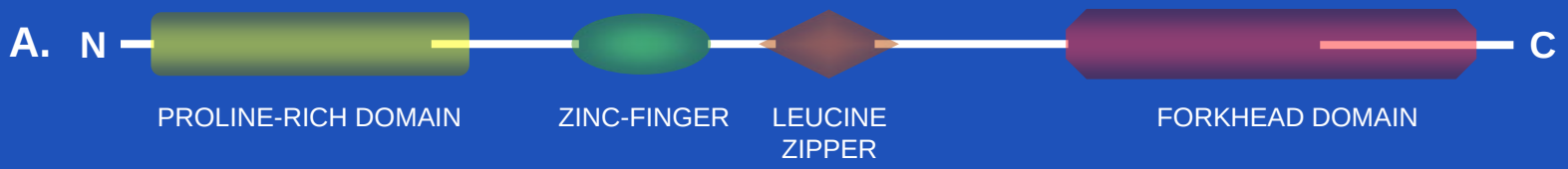
Goals

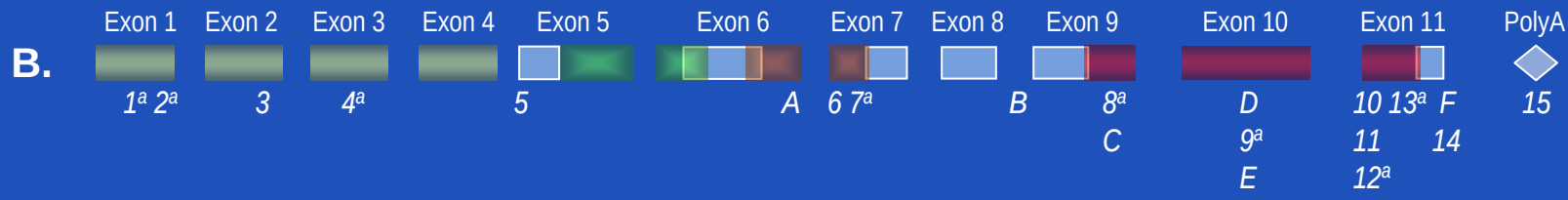
- **To better define the clinical phenotype of IPEX**
- **To understand the molecular structure and function of FOXP3:**
  - **What does it do – transcriptional regulation?**
  - **How is it regulated?**
  - **What does it regulate?**
- **To use the Scurfy mouse and naturally occurring human mutations as models to study the development and function of Regulatory T cells**

FOXP3 Mutations



FOXP3 Mutations in IPEX





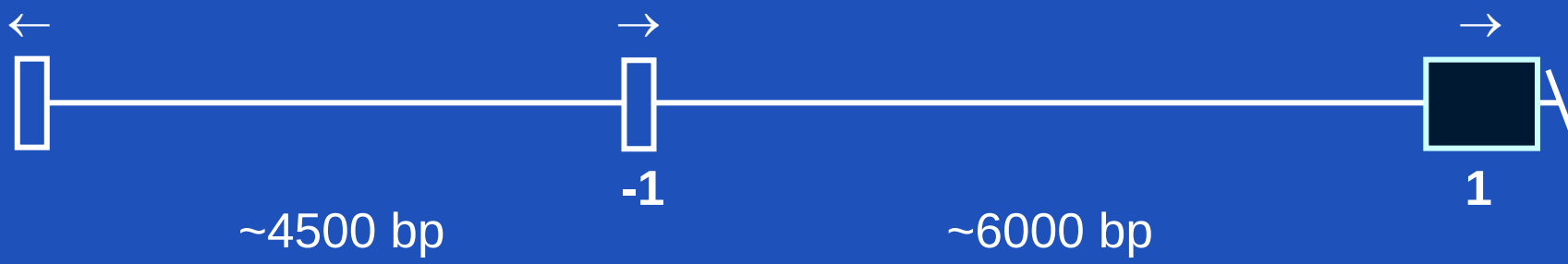
	Nucleotide	Amino Acid	Ref
1.	112G>T[a]	A38S	
2.	210G>T[a]	Q70H	
3.	227delT	L76fsX128	8
4.	303_304delTT[a]	F102fsX101	
5.	543C>T	Splicing	20
6.	750_752delGGA	E251del	5
7.	776A>C[a]	H259P	
8.	1010G>A[a]	R337P	
9.	1099T>C[a]	F367L	
10.	1150G>A	A384T	6,7
11.	1189C>T	R397W	7
12.	1190G>T[a]	R397L	
13.	1271G>A[a]	C424Y	
14	1293_1294delCT	P431fsX457	6
15.	AAUAAA>AAUGAA	Polyadenylation	16

	Nucleotide	Amino Acid	Ref
A.	747_749delAAG	K250del	21
B.	IVS9+459A>G		5
C.	1040G>A	R347H	21
D.	1087A>G	I363V	8
E.	1113T>G	F371L[b]	7
F.	1290_1309del/insTGG	G430fsX452[b]	7

[a] Newly identified mutation

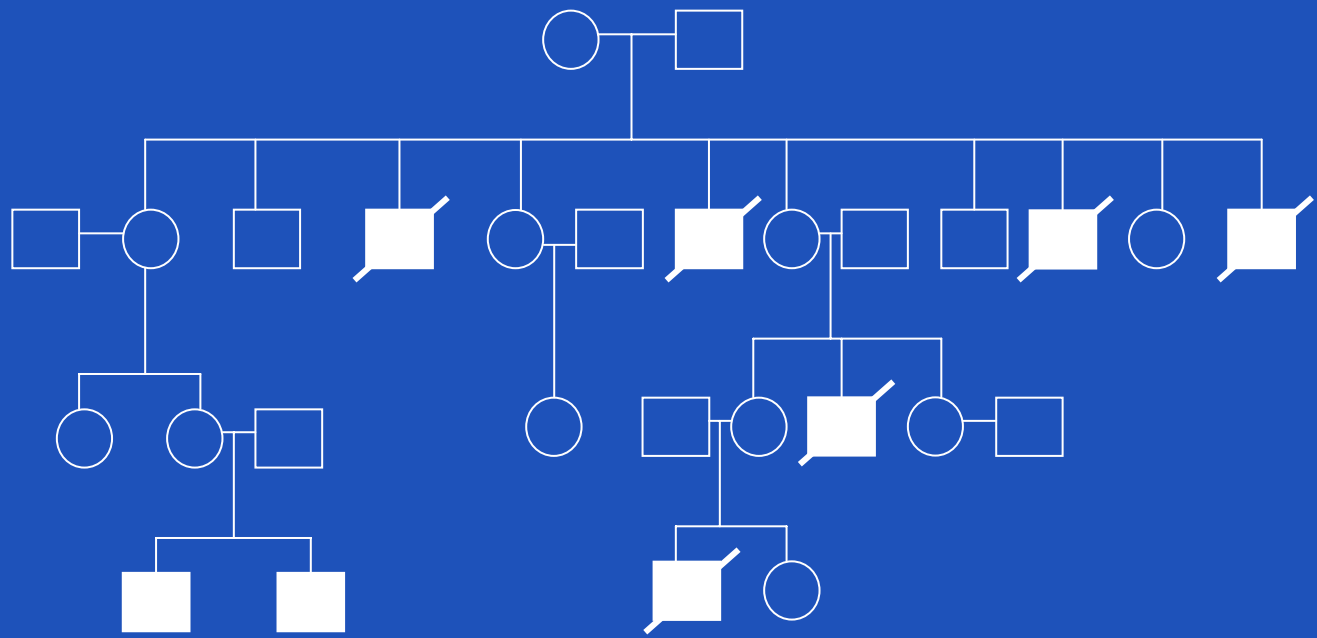
[b] The indicated amino acid change differs from that originally reported but is the sequence that results from the reported nucleotide change.

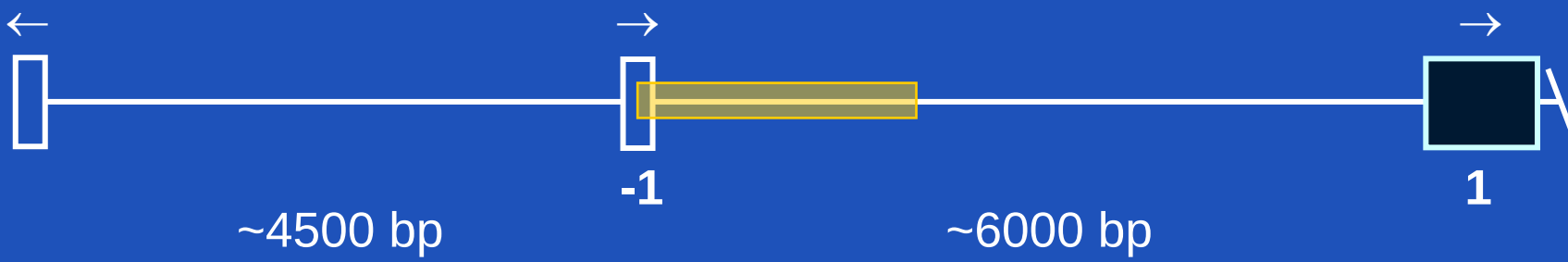
Human *FOXP3* Promotor Structure



- Is there a tissue-specific repressor that limits FOXP3 expression to the $CD4^{+}CD25+$ population (similar to the CD4 or Btk promoters) or are there tissue-specific enhancers that lead to expression only in this cell type?

Human *FOXP3* Promoter Structure





Summary

1. We have identified 16 different mutations in patients with IPEX syndrome.

- Patients with FOXP3 mutations have a more severe phenotype than those without mutations.

- We have identified domains of the FOXP3 protein important for its function.

- We have identified a deletion in the FOXP3 promoter that leads to low FOXP3 mRNA expression levels.

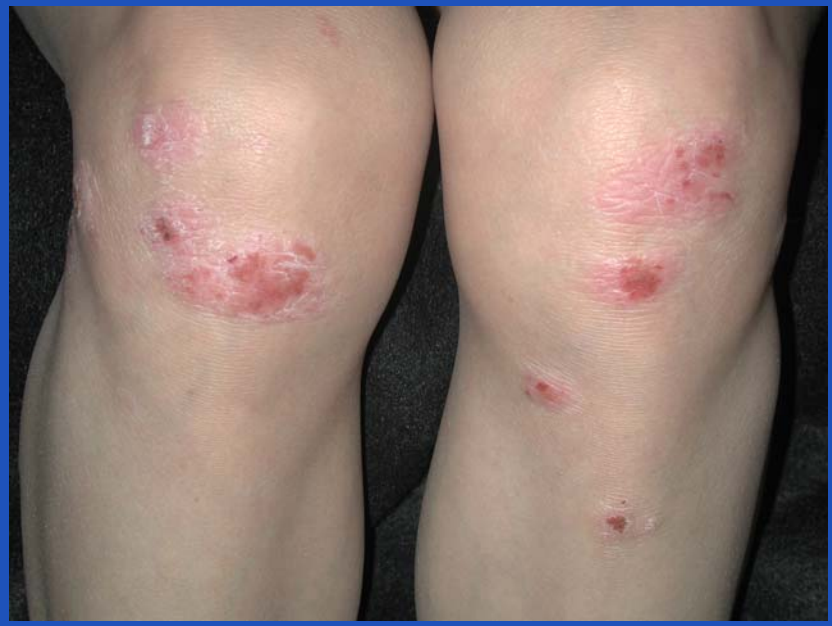
A Better Picture of IPEX

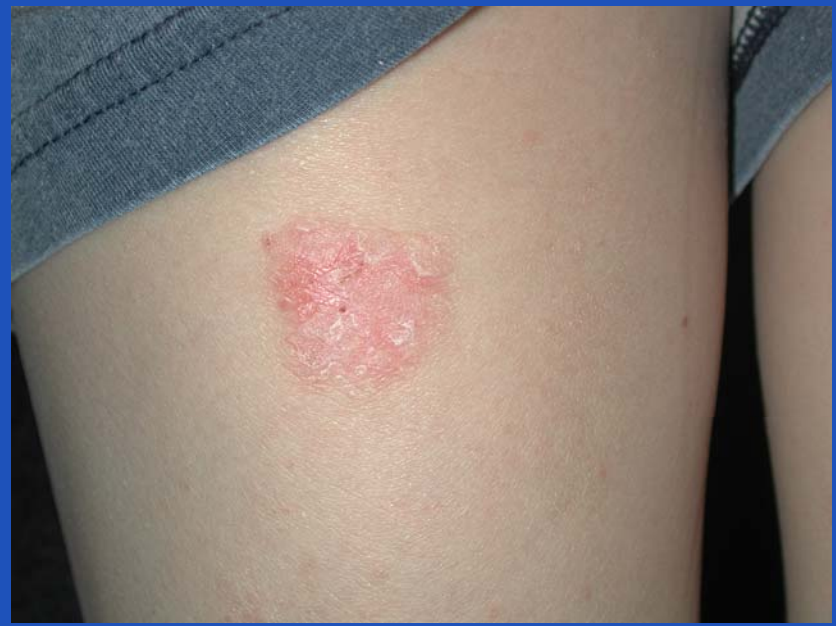
25 kindreds (31 patients) with the IPEX phenotype:
Clinical data obtained from 24 kindreds by questionnaire to PMD:
-*FOXP3* mutations found in 14 kindreds (17 patients) – "IPEX"
-No *FOXP3* mutation in 10 kindreds (10 patients) – "IPEX-like"

IPEX: 100% have enteropathy diarrhea, often bloody
100% have skin disease – eczema (most), erythroderma
80-90% have one endocrinopathy – usually IDDM or thyroid
60% have glumerolonephropathy – often mild
50% have autoimmune hematologic problems: hemolytic anemia, thrombocytopenia, or neutropenia.
90+% have failure to thrive and ~40% have developmental delay or neurologic abnormality (seizures, etc).
50-60% have one or more major infections (sepsis, osteo, etc.)

Treatment Options: CsA, FK506, Steroids, BMT

Skin Disease in IPEX





The Expanding IPEX Phenotype

Kindred I-9:

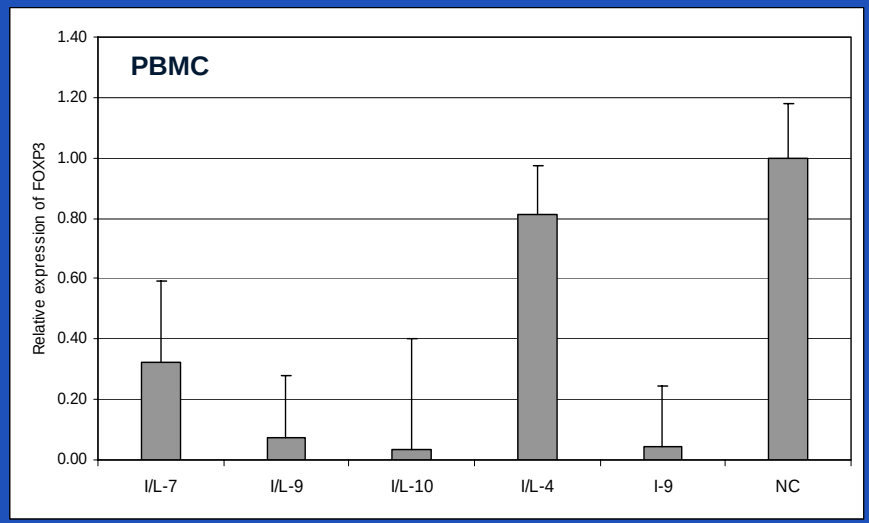
- **The original IPEX kindred. A large family with several affected males, some who lived into adulthood.**
- **Mutation in the first Polyadenylation site – leads to decreased mRNA levels. Variations in mRNA processing may account for variable phenotype.**
- **Immune activation (severe illness/vaccinations) caused rapid worsening of disease in some patients.**

Kindred I-11:

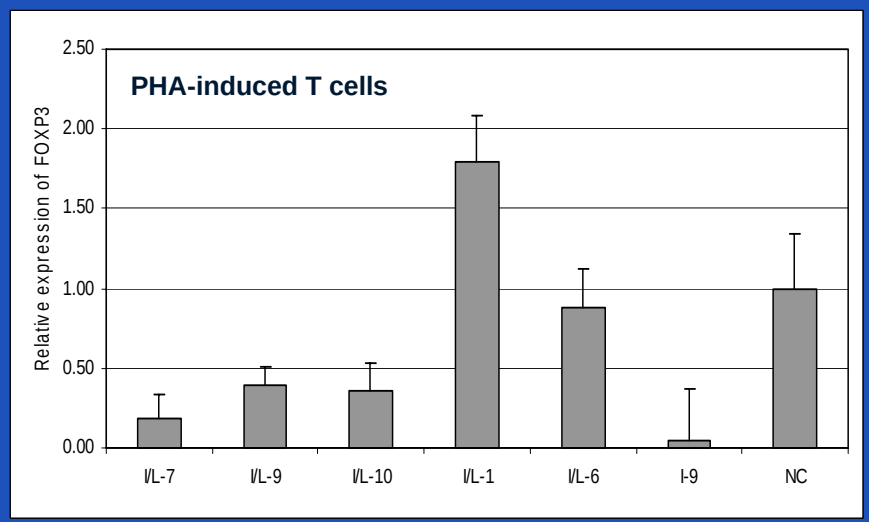
- **12 year old male with Diarrhea starting at 6 m/o, bloody stools ~ 1 year ago – Dx'd with Crohn's disease by biopsy**
- **Autoimmune Hyperthyroidism diagnosed at 5 y/o and ablated with I^{125} therapy**
- **Severe eczema over last 2 years with numerous superinfections**
- **Neutropenia over last 1-2 years with anti-neutrophil antibodies**
- **Elevated IgE (1080 gm/dl)**
- **Mutation in the first base of Exon 5 (Splicing?)**

Quantitative RT-PCR of *FOXP3* Expression in IPEX-like Patients

A



B



Scurfy Mouse - Immunology

- Mediated by $CD4^+$ T cells – adoptive transfer
- Overproduction of cytokines IL-2, 4,5,6,10; IFNγ, TNFα, GM-CSF
- $\uparrow$ $Mac\text{-}1^+$, $\downarrow$ $B220^+$
- $\uparrow$ Activation antigens CD69, CD25, CD80, CD86
- Scurfy T cells $\downarrow$ sensitivity to tyrosine kinase Inhibitors (genistein, herbimycinA) and to cyclosporin A

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Expression of Foxp3

- Spleen — strong
- Thymus — strong
- CD4+ CD8- — strong
- Cd4- cd8+
- B220

> Barely detectable

All Forkhead/HNF3 Proteins Possess "Winged-Helix" Domain

- ~110 amino acid domain
- Involved in DNA binding
- Forkhead/hnf3 proteins act as transcriptional regulators
  - (Examples of activators and repressors)
- Large gene family:
  - Found in all species; Involved in many different processes (eg., Development, organogenesis, tumorigenesis)

Scurfy gene/mutation

- Proximal X-chromosome
- Tightly linked (but not synonymous) to Wasp
- Novel forkhead gene
- 2 base pair deletion, frameshift
- Exon 8 (proximal to forkhead domain)
- Early termination – truncated protein
- Loss of function

Scurfin
(transcriptional regulator)

- 47 KD protein
- 429 a.a. (mouse)
- 431 a.a. (human)
- 3 a helices
- 2 loop regions (“wings”)
- Zinkfinger domain
- Forkhead domain (116 a.a. downstream of ZF mediates protein – DNA contact

Scurfin - mutated

- Failure to control T cell function
- Mutations of : Scurfin
- CD95, CD95 ligand
- CTLA-4
- TGF-β
- Scurfy mouse resembles
- Knockout of CTLA-4 and TGF-β

Syndrome	WAS	XLT/IXLT	XNP	XLT/ thalassemia trait	IPEX
gene	WASP prot. –	WASP prot.+	WASP L270P	GATA-1	FOXP3
Thrombo-cytopenia	yes	yes / (yes)	no	yes	yes
anemia	(yes)	(yes)	no	yes (Hgb synthesis)	yes
neutropenia	no	no	yes	no	(yes)
endocrine	no	no	no	no	yes
GI-problems	(yes)	no	no	no	yes

FOXP3 - function (1)

- Forkhead (FKH) proteins regulate lineage commitment, developmental differentiation
- Scurfin localizes to the nucleus
  FKH domain required
- FKH binding cites adjacent to NFAT sites were identified in cytokine Promotors

FOXP3 - function (2)

- FOXP3 transgenic mice overexpress scurfin
  decrease of Tcell number (ly nodes, spleen)
  poor proliferation
  decreased IL-2. IFN gamma
  decreased expression of CD40L
  reduced Ab responses to T dependent Ag

APECED

- Autoimmune
- Polyendocrinopathy
- Candidiasis
- Ectodermal Dysplasia

APECED - Clinical phenotype

- Chronic mucocutaneous candidiasis
- Hypoparathyroidism
- Addison Disease
- Diabetes, type 1, gonadal atrophy, pernicious anemia, hypothyroidism
- Autoimmune hepatitis
- Ectodermal dysplasia: enamel, nails, alopecia, vitiligo, calcification of TM

APECED -Founder Effect

	Finnish	Sardinia	Iran Jews	N-America
Incidence	1:25K	1:14K	1:9K	16
N	67	11	16	75
MCC	100%	83	18	100
Hypopara	79%	93	96	93
Adrenal	72%	73	22	not rep.
Nails	52%	not rep.	most	C322
Predomin. AIRE	R257X	R139X	Y85C	de113
mutation	Exon 6	Exon 3	Exon 2	bp>stop

APECED - genetics (1)

- Mapped to 21 822.3
- Gene: Autolimmune REgulator (AIRE)
- Function: transcriptional regulator, has characteristic domains for Induction and maintenance of immune function
- 14 exons, encoding a 545 aa protein
- Expressed in Thymus epithelium, lymphnodes, spleen, fetal liver

APECED genetics (3)

- 45 known mutations of AIRE
- Distributed throughout the AIRE gene
- Variable clinical phenotype

  type of mutation

  environmental

  HLA-DR (B 103 - Addison disease)

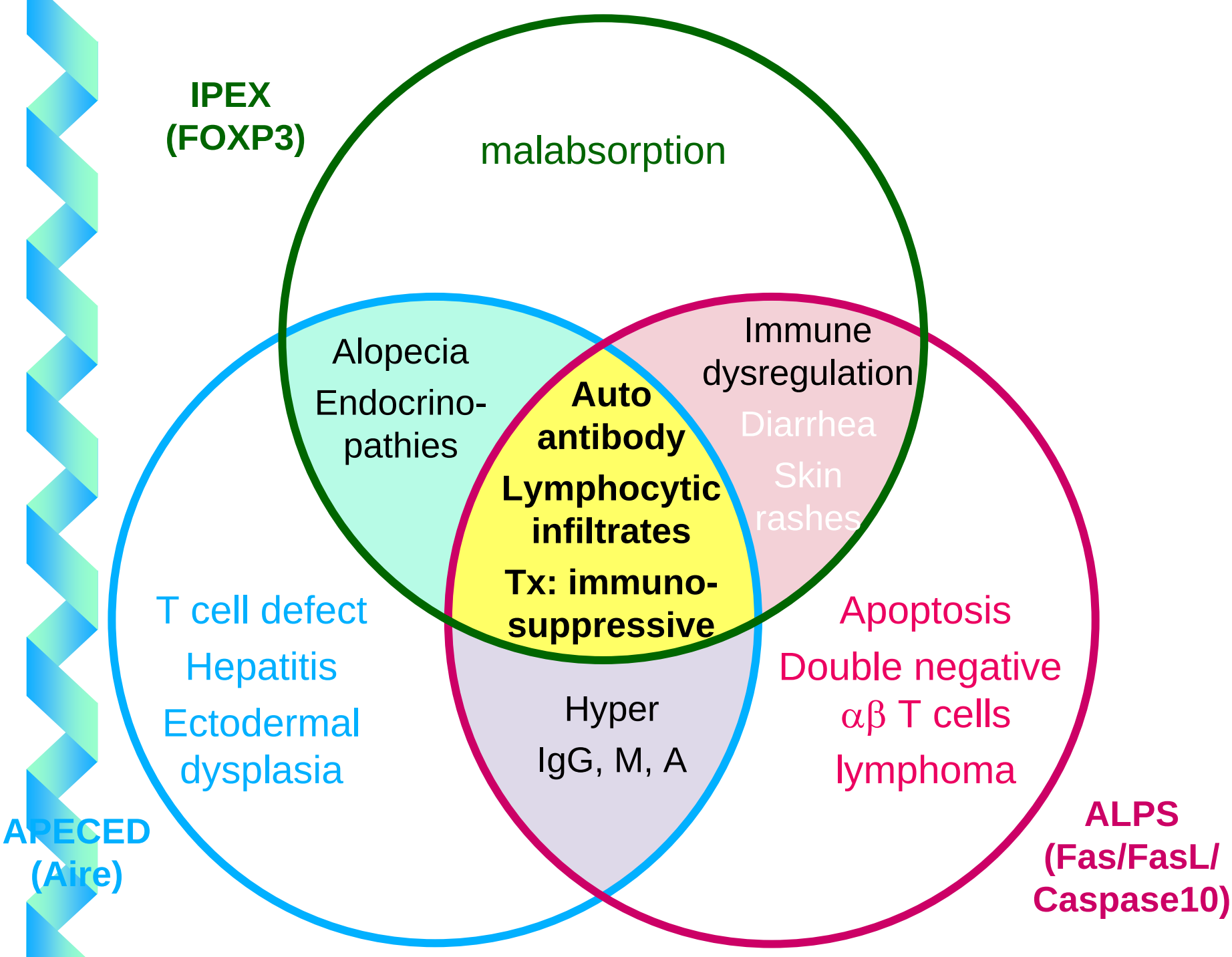
  (B 104 - alopecia)

APECED Aire-knockout mice

- APECED features
- Lymphocytic infiltrates (ovary, liver)
- Atrophy of thymus, adrenals, ovaries (42%)
- Autoimmune hepatitis (50%)
- Autoantibodies (73%), testes (8/15), liver (3/15), pancreas (7/15), adrenal gland (3/15)
- In vitro hyperproliferation of sensitized Ly

ALPS - genetics

- Autosomal dominant (mostly), or recessive
- Mutations of *APT1* encoding Fas/APO-1/CD95 is present in most ALPS
- Mutation of FasL or Caspase 10 is rare
- Animal models: *lpr* mutation (Fas)
  gld mutation (FasL)
  phenotype is strain dependent


IPEX
(FOXP3)
malabsorption
Alopecia
Endocrino-
pathies
Immune
dysregulation
Diarrhea
Skin
rashes
Auto
antibody
Lymphocytic
infiltrates
Tx: immuno-
suppressive
T cell defect
Hepatitis
Ectodermal
dysplasia
Hyper
IgG, M, A
Apoptosis
Double negative
αβ T cells
lymphoma
APECED
(Aire)
ALPS
(Fas/FasL/
Caspase10)