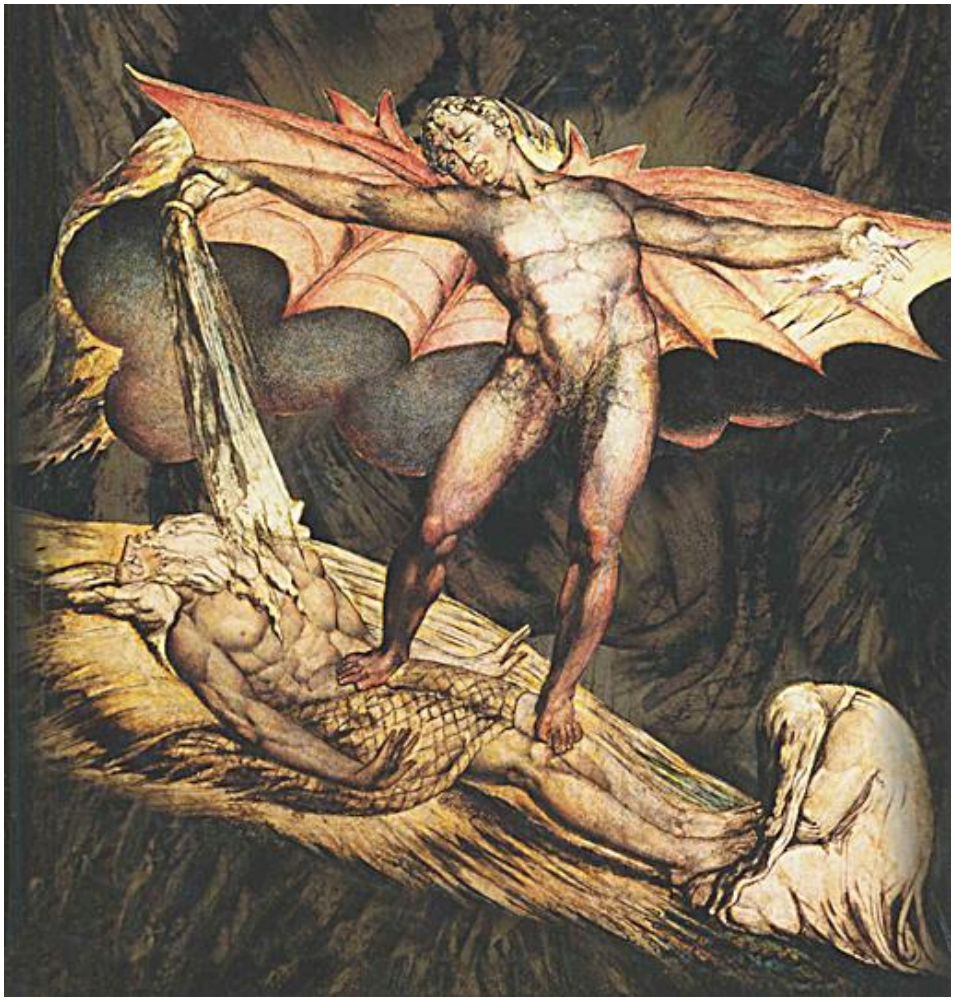
STAT MUTATIONS

Objectives

- To introduce the audience to the previous phenotype of Job's Syndrome/ Hyper IgE Syndrome
- To introduce the audience to the translational genotype of of Job's Syndrome
- To introduce the audience to the expanded phenotype using the genotype of STAT-3

Historical Significance



Historical Significance

- First identified in1966 in two red haired girl
- Characterized by recurrent staphylococcal abscesses, sinopulmonary infections and severe eczema

Signal Transducers and Activator of Transcription

- **Aka: signal transduction and transcription proteins**

Original Phenotype

Translational genotype

Expanded phenotype

Regulates

- cell growth
- survival
- differentiation

STAT Family

- The first two STAT proteins were identified in the interferon system
- The seven mammalian STAT family members identified are: STAT1, STAT2, STAT3, STAT4, STAT5 (STAT5A and STAT5B), and STAT6.

STAT STRUCTURE

Pathogenesis

- STAT 3 is critical in signaling pathways of
  - IL-6 (pyrogen and acute phase response)
  - IL-10 (anti-inflammatory)
  - Differentiation pathways of IL-17 producing CD4+ T cells (helps defend against extracellular pathogens)
  - IL-22 (stimulates β defensin in skin and lungs)
  - Down-regulation of osteoclasts
- STAT 3 deletions have shown higher levels of TNFα and INFγ

STAT FUNCTION

- STAT proteins were originally described as latent cytoplasmic transcription factors that require phosphorylation for nuclear retention.
- The unphosphorylated STAT proteins shuttles between cytosol and the nucleus waiting for its activation signal.
- Once the activated transcription factors reaches the nucleus it binds to consensus DNA-recognition motif called gamma activated sites (GAS) in the promoter region of cytokine inducible genes and activates transcription of these genes.

Activation

- Extracellular binding of cytokines induces activation of the intracellular Janus kinase that phosphorylates a specific tyrosine residue in the STAT protein which promotes the dimerization of STAT monomers via their SH2 domain.
- The phosphorylated dimer is then actively transported in the nucleus via importin a/b and RanGDP complex.
- Once inside the nucleus the active STAT dimer binds to cytokine inducible promoter regions of genes containing gamma activated site (GAS) motif and activate transcription of these genes.
- The STAT protein can be dephosphorylated by nuclear phosphatases which leads to inactivation of STAT and the transcription factor becomes transported out of the nucleus by exportin crm1/RanGTP.

JAK

- Just another Kinase
- The name is taken from the two-faced Roman god of doorways, Janus, because the JAKs possess two near-identical phosphate-transferring domains.
- One domain exhibits the kinase activity while the other negatively regulates the kinase activity of the first.

There are four JAK family members:

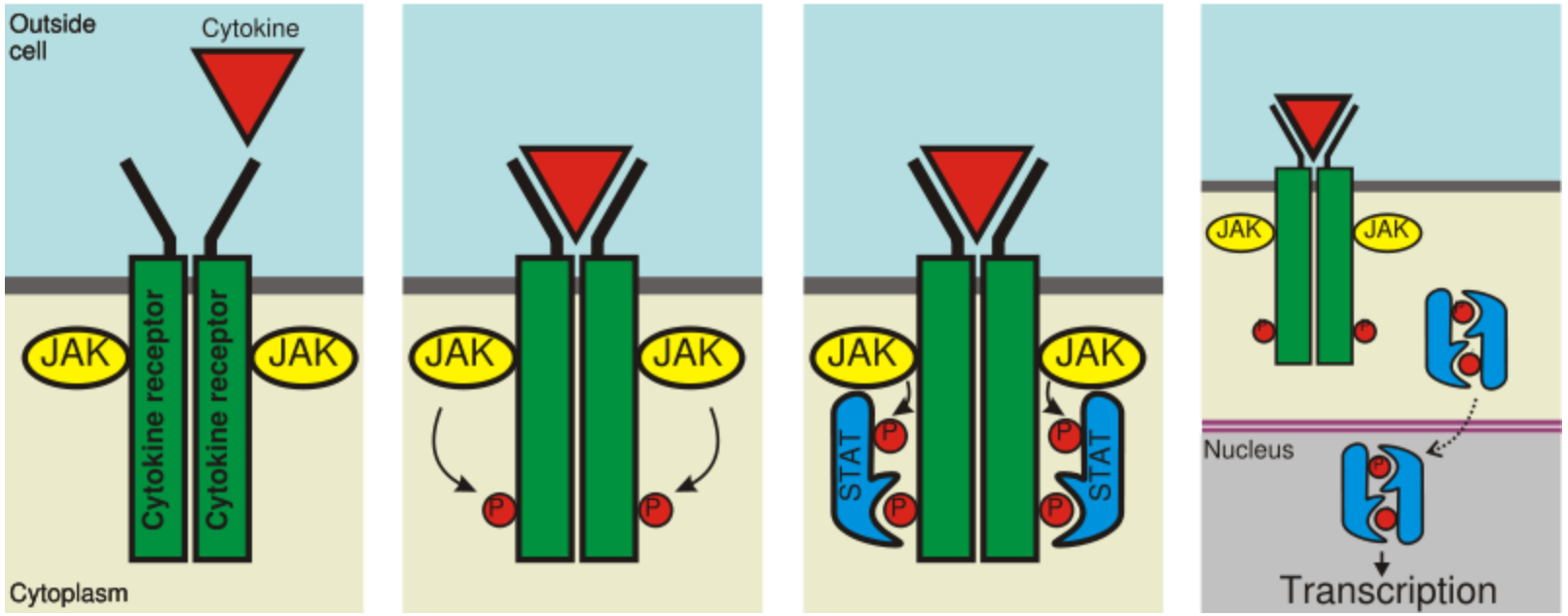
- Janus kinase 1 (JAK1)
- Janus kinase 2 (JAK2)
- Janus kinase 3 (JAK3)
- Tyrosine kinase 2 (TYK2)

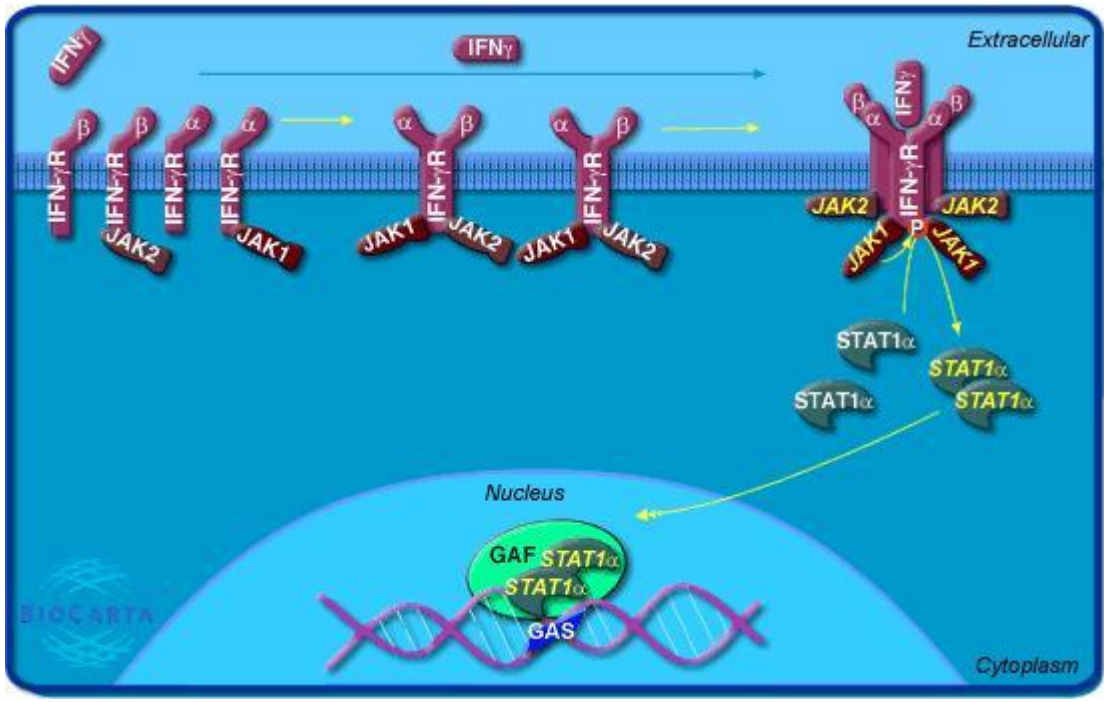
JAK structure

- JAKs range from 120-140 kDa in size and have seven defined regions of homology called Janus homology domain 1–7 (JH1-7).
- JH1 is the kinase domain important for the enzymatic activity of the JAK and contains typical features of a tyrosine kinase such as conserved tyrosines necessary for JAK activation (e.g. Y1038/Y1039 in JAK1, Y1007/Y1008 in JAK2, Y980/Y981 in JAK3, and Y1054/Y1055 in Tyk2).
- Phosphorylation of these dual tyrosines leads to the conformational changes in the JAK protein to facilitate binding of substrate.
- JH2 is a *pseudokinase domain*, a domain structurally similar to a tyrosine kinase and is essential for a normal kinase activity yet lacks enzymatic activity.
- This domain may be involved in regulating the activity of JH1. The JH3-JH4 domain of JAKs shares homology with Src-homology-2 (SH2) domains.
- The amino terminal (NH_2) end (JH4-JH7) of Jaks is called a FERM domain (short for band 4.1 ezrin, radixin and moesin); this domain is also found in the focal adhesion kinase (FAK) family and is involved in association of JAKs with cytokine receptors and/or other kinases [4].


2HN
JH7
JH6
JH5
JH4
JH3
JH2
(pseudokinase)
JH1
(kinase)
COOH

JAK STAT Pathway




IFNγ
IFNγ
Extracellular
IFN-γR
JAK2
JAK1
JAK1
JAK2
JAK1
JAK2
IFNγ
IFN-γR
JAK2
JAK2
JAK1
P
JAK1
STAT1α
STAT1α
STAT1α
STAT1α
Nucleus
GAF
STAT1α
STAT1α
GAS
BIOCARTA
Cytoplasm

STAT 3 Mutation (Hyper IgE)

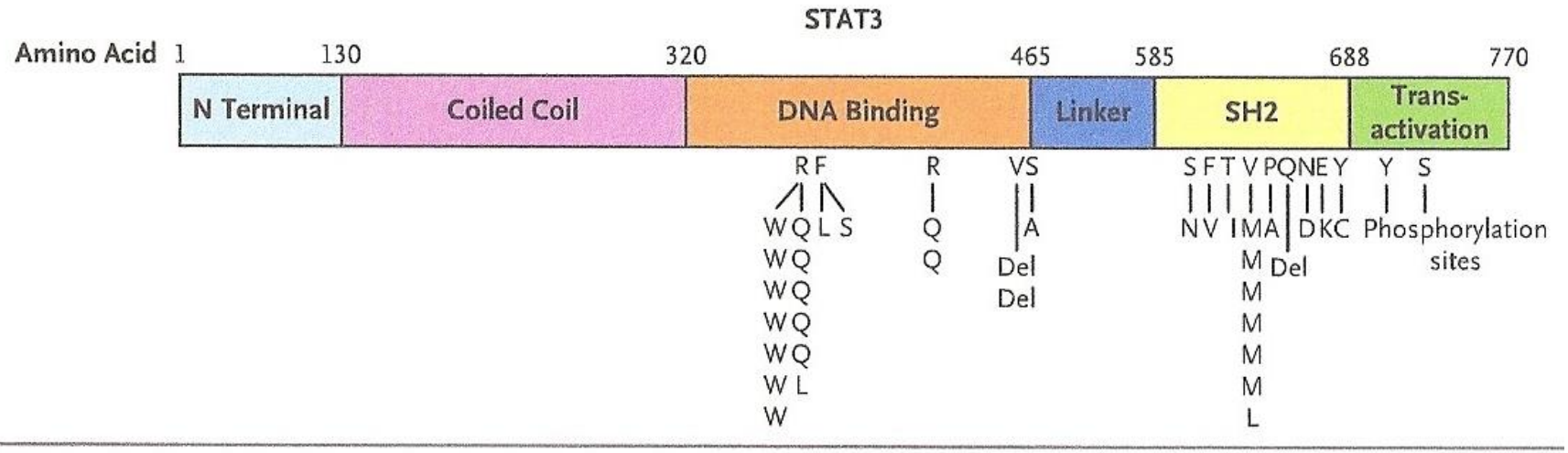


Figure 1. *STAT3* Mutations.

Wild-type amino acids at the loci where mutations were found are listed immediately below the STAT3 domains, with all the mutant amino acids identified in our study listed below that. The five hot-spot sites, those with multiple mutations, were found in both the DNA-binding and SRC homology 2 (SH2) domains. The amino acid mutations shown (and the underlying nucleotide mutations) in the DNA-binding domain are as follows: R382W (1144C→T), R382Q (1145G→A), R382L (1145G→T), F384L (1150T→C), F384S (1151T→C), R423Q (1268G→A), V463del (1387delGTG), and S465A (1393T→G). The amino acid mutations shown (and the underlying nucleotide mutations) in the SH2 domain are as follows: S611N (1832G→A), F621V (1861T→G), T622I (1865C→T), V637M (1909G→A), V637L (1909G→T), P639A (1915C→G), Q644del (1930delCAG), N647D (1939A→G), E652K (1954G→A), and Y657C (1970A→G).

Stat3 mutation pedigree

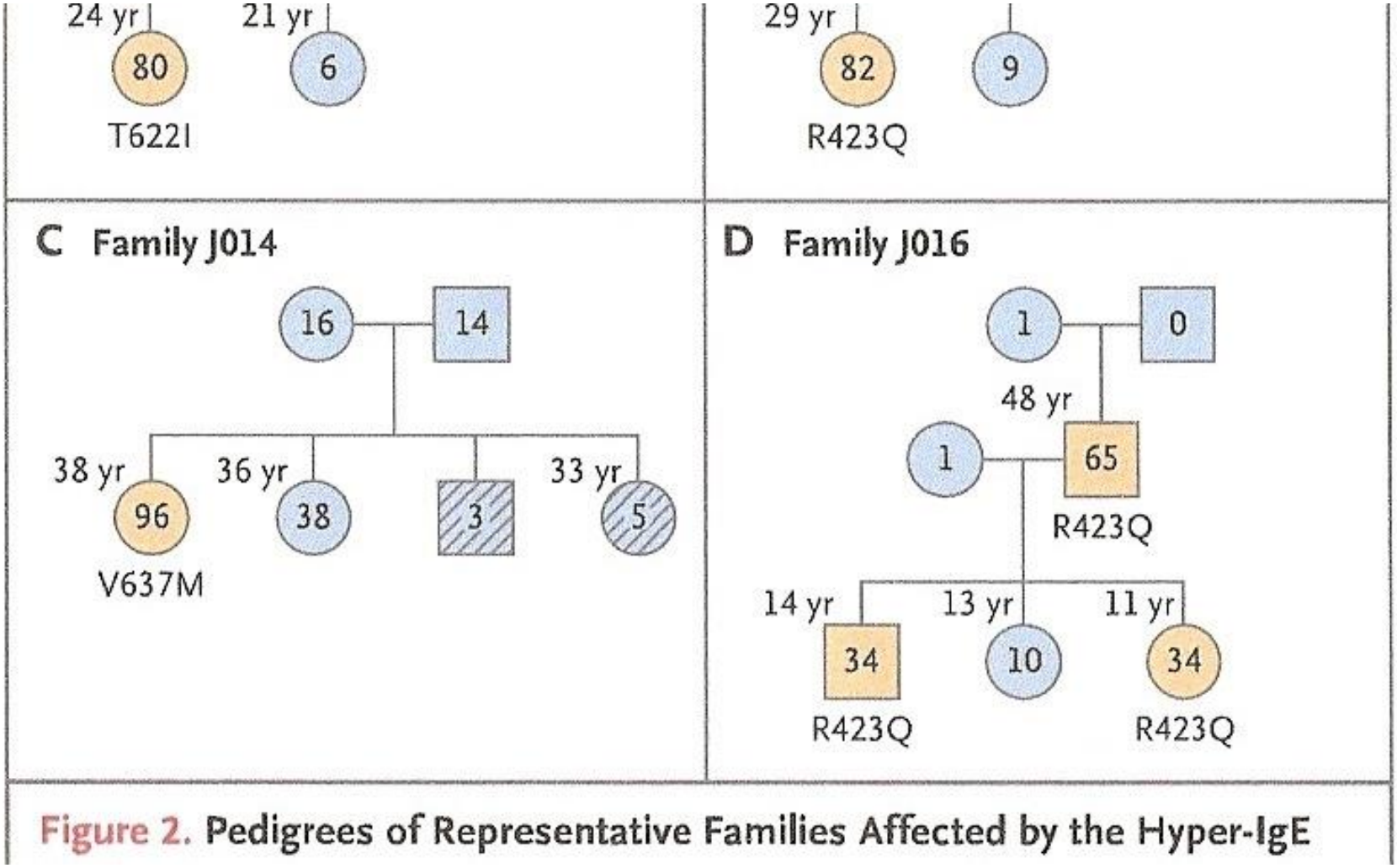


Figure 2. Pedigrees of Representative Families Affected by the Hyper-IgE