STAT1 Mutations in Autosomal Dominant Chronic Mucocutaneous Candidiasis

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Background

Chronic Mucocutaneous Candidiasis (CMC)

- PID characterized by susceptibility to candida and dermatophytes infection
- Infection most often of skin, nails, mucous membranes
- Three known forms:
  - Isolated autosomal recessive
  - Autosomal dominant CMC with or without thyroid disease
  - Autosomal recessive autoimmune polyendocrinopathy candidiasis with ectodermal dystrophy (APECED) – autoimmune regulator (AIRE) gene mutation

Background

APECED

- AIRE gene mutation
- Autoantibodies to IL-17 and IL-22
- Th17 cells are crucial for mucosal antifungal immunity

Autosomal dominant CMC

- AD CMC found to have defective Th1-IFNg and Th17 responses
- Dectin-1-CARD9 pathway mutations can lead to increased susceptibility to fungus but less severe clinical picture than AD CMC

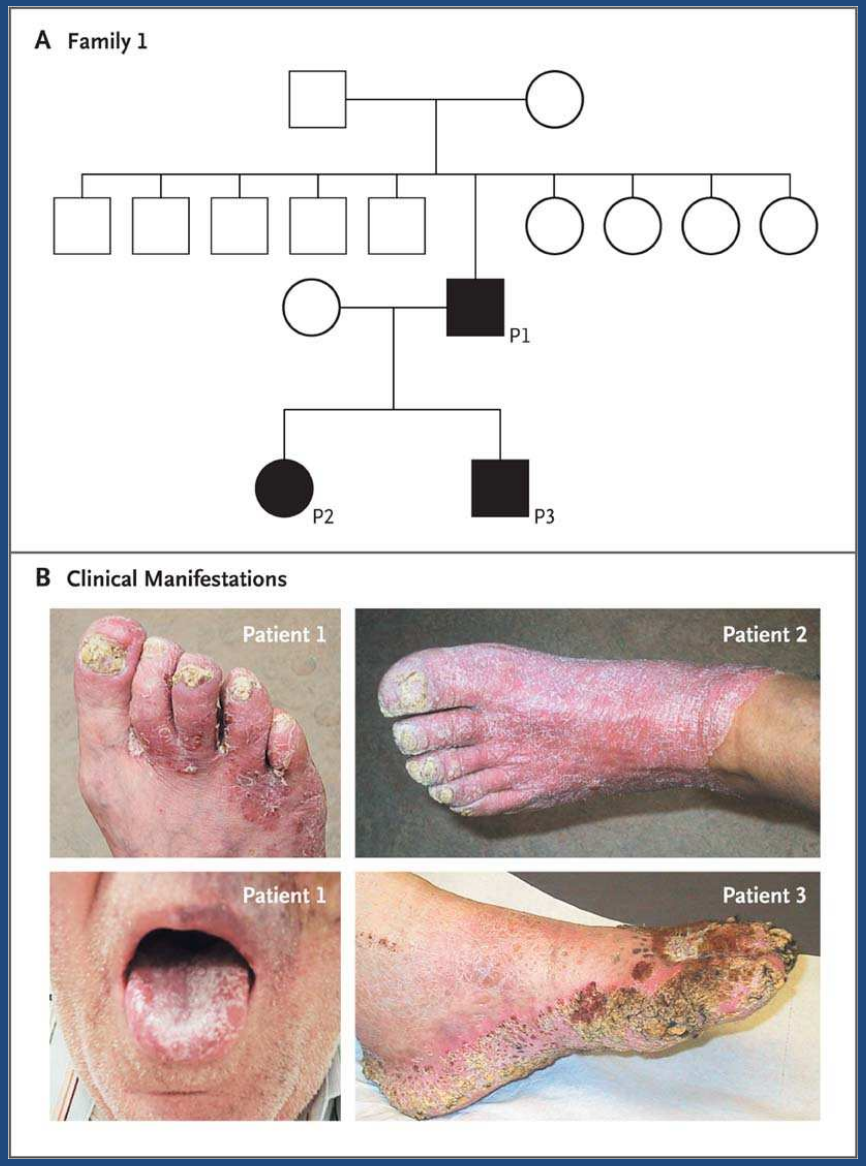
Objective:

Investigation of the genetic cause of susceptibility to mucocutaneous fungal infection in families with AD CMC

Methods

- Family 1
  - Non-consanguineous family of Dutch descent
  - Father, daughter, and son have had severe CMC since early childhood
  - Father (67M) – autoimmune hepatitis
  - Daughter (38F) – autoimmume hemolysis, pernicious anemia, APL antibodies, pulmonary embolism, PCP and CMV infections
  - Son (37M) – extensive *T. rubrum* (dermatophytosis) infection of feet, no autoimmunity
  - Mother – unaffected
  - Father had 9 unaffected siblings

Pedigree of Family 1 with Autosomal Dominant Chronic Mucocutaneous Candidiasis (CMC) and Clinical Signs in Affected Family Members.



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Methods

- Family 2 – nonconsanguanous UK family, all with fungal infections of mouth, skin, nails
  - Female (59) –hypothyroidism, thyroid abs, eczema, enamel dysplasia, oral cancer, blepharitis, iron-deficient anemia
  - Male (36) – eczema, enamel dysplasia, chest infections, blepharitis
  - Male (29) – thyroid antibodies, eczema, enamel dysplasia, blepharitis, reflux disease
  - Female (27) – eczema, enamel dysplasia, blepharitis, deratopathy, iron-deficiency, reflux disease
  - Female (6) – oral and skin fungal infections

Methods

- Family 3 – nonconsanguanous UK family, all with fungal infections of mouth, skin, nails
  - Female (37) – eczmea, iron deficient anemia
  - Male (32) – hypothyroid, eczema, blepharitis
  - Male (5) – eczema

Methods

- Family 4 – female (40, UK) with oral, nail, skin, vaginal candidiasis, hypothyroid, thyroid abs, chest infections, iron-deficient anemia

- Family 5 – nonconsanguenous Dutch family
  - Female (48) – oral, nail, skin fungal infections, enamel dysplasia, esophageal cancer
  - Male (15) – oral and skin fungal infections, enamel dysplasia

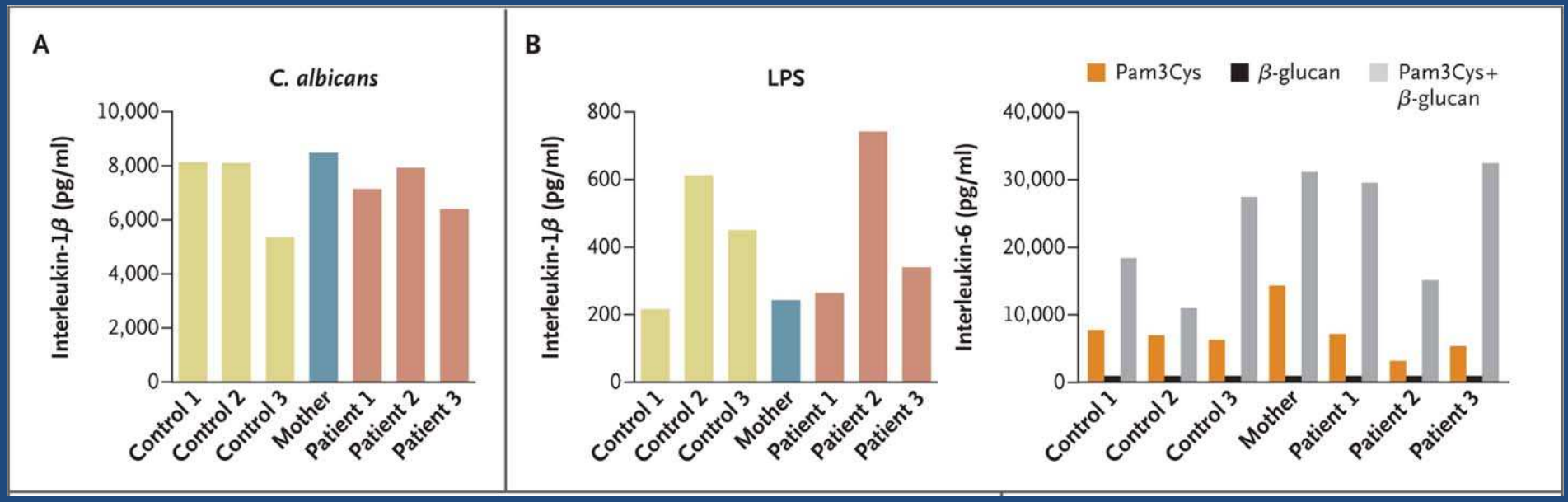
Methods

- Controls
  - 301 unrelated healthy Dutch controls and 56 healthy British controls of European ancestry
  - Analyzed in-house Nijmegen database of 100 exome data sets derived from healthy European subjects
  - Questionnaire concerning ethnic origin of parents and grandparents of healthy subjects confirmed ancestry in Netherlands and UK

Methods

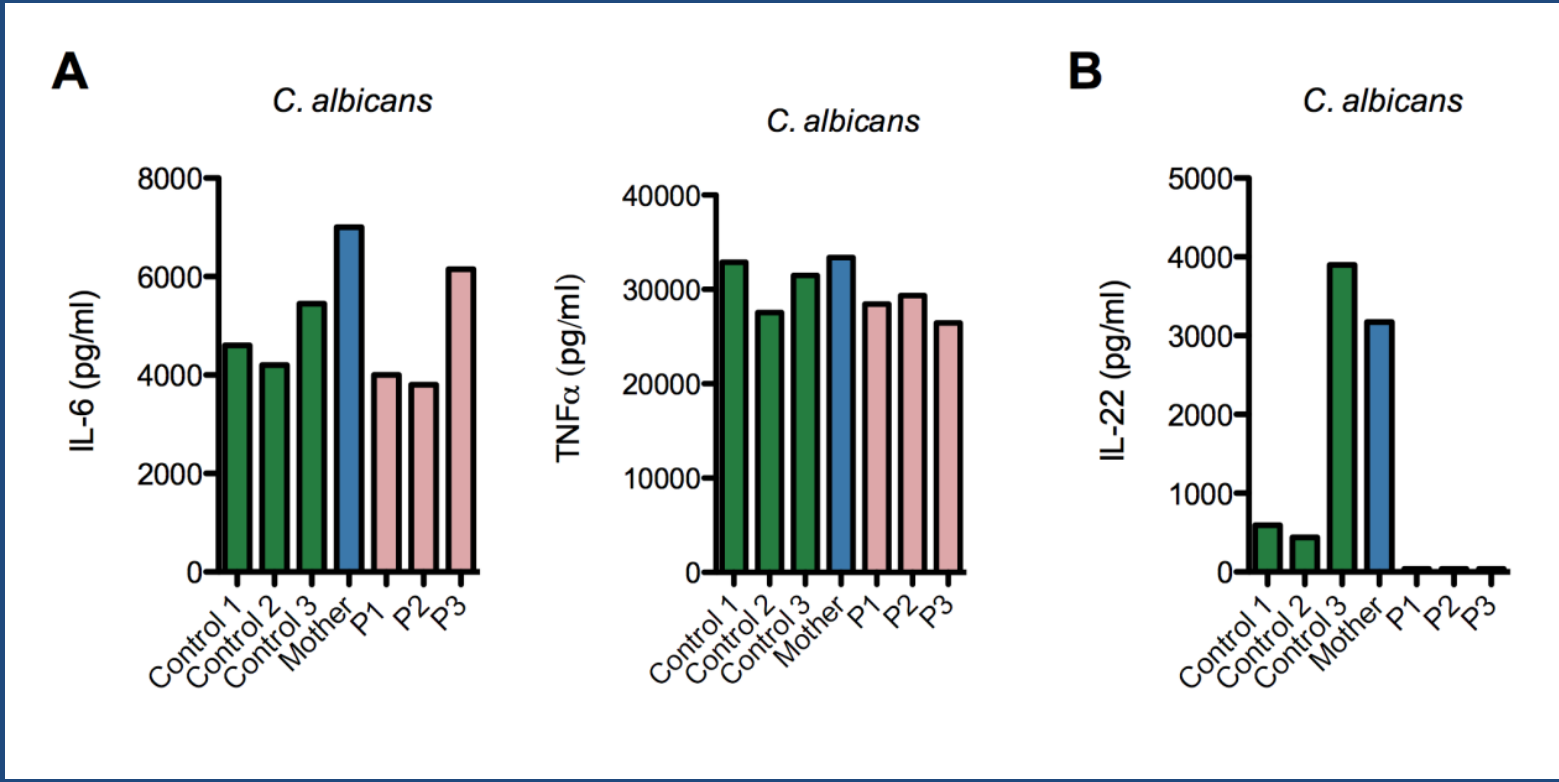
- Immunologic studies, sequencing
  - Incubation with candida or E. coli
  - ELISA for IL-1B, TNFa, IL-17, IL-22, IFNg, IL-6
  - Array-based sequence capture followed by next-generation sequencing to analyze 100 genes from known immunologic pathways
  - PCR used to analyze identified mutations
  - AIRE mutations were excluded

Immunologic Defects in the Affected Members of Family 1.



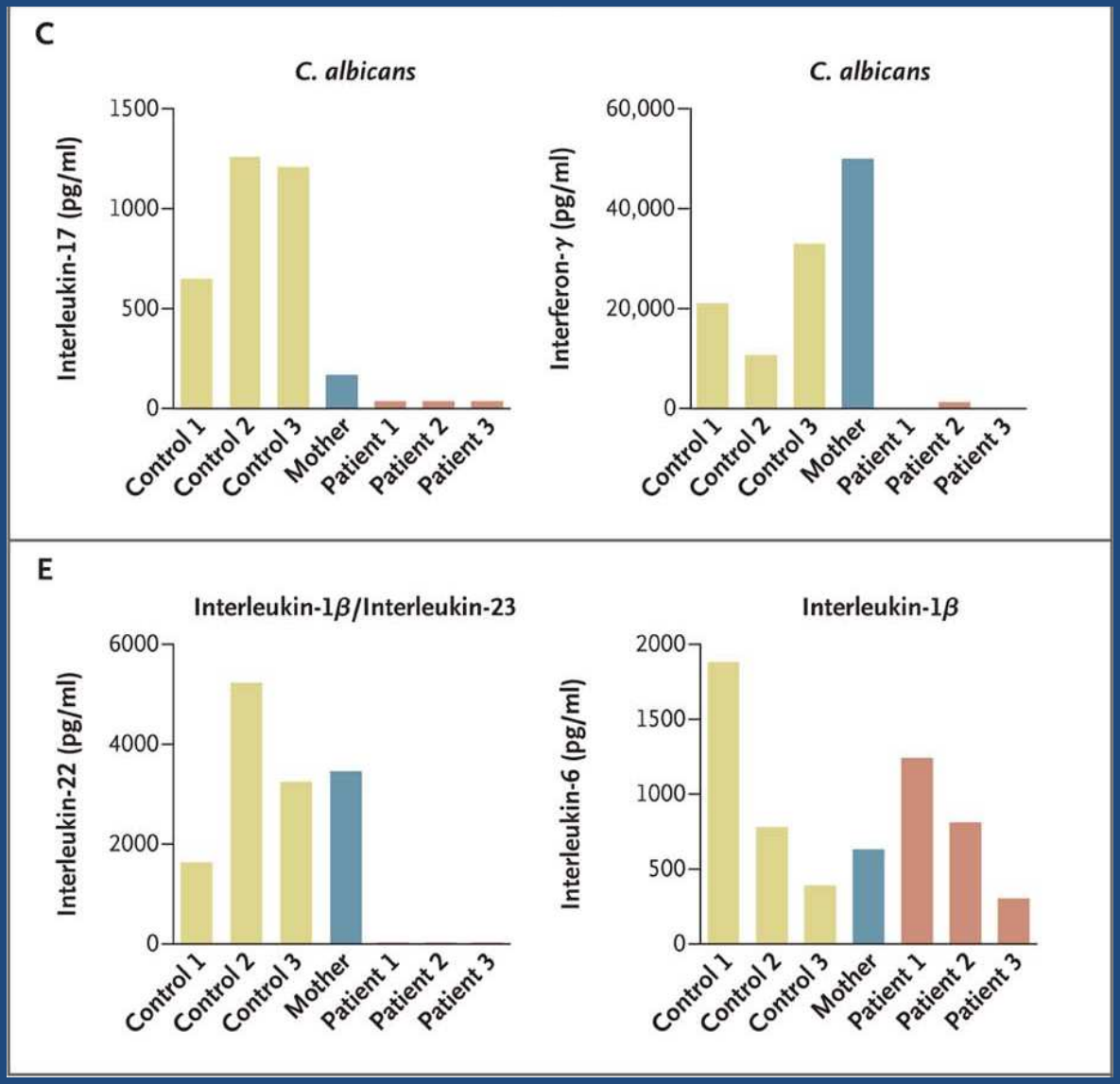
A. PBMCs stimulated with candida - controls and subjects produced normal amounts of IL-1B
B. Activation of TLR4 (LPS), TLR2 (Pam3Cys), and dectin-1-receptor (B-glucan) signaling pathways – controls and subjects had similar levels

Immunologic Defects in the Affected Members of Family 1.



A. PBMCs stimulated with candida - controls and subjects produced normal amounts of IL-6 and TNFa
B. Subjects produced lower levels of IL-22 in response to candida stimulation

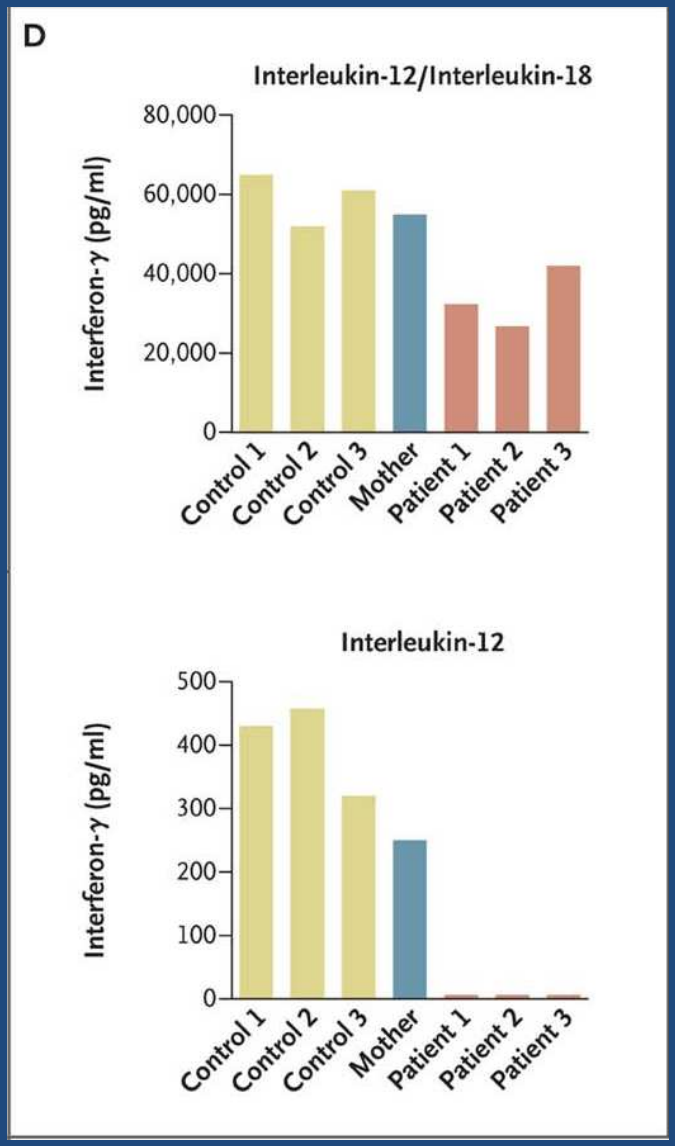
Immunologic Defects in the Affected Members of Family 1.



- Subjects had lower IL-17 and IFNg production in response to candida stimulation

- Subjects had lower IL-22 production in response to IL-1B/IL-23 stimulation
- Subjects produced normal levels of IL-6 in response to IL-1B

Immunologic Defects in the Affected Members of Family 1.

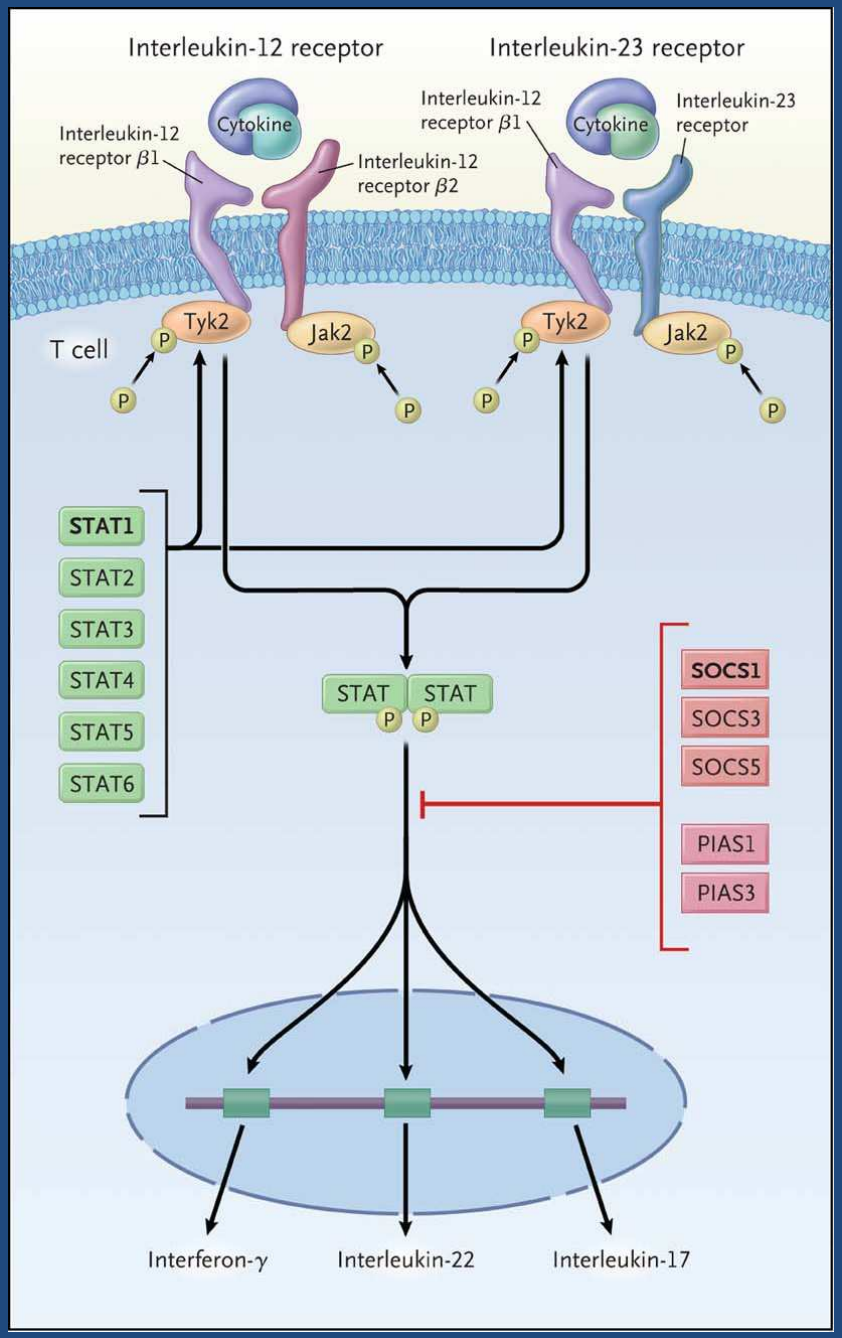


- Subjects had normal IFNg production in response to IL-12/IL-18 stimulation

- Subjects had lower IFNg production in response to IL-12 stimulation

- mitogen stimulation produced normal IFNg (not shown)

Conclusion: IL-12 and IL-23 pathways are affected



- shared adaptor proteins: Tyk2, Jak2

- shared downstream proteins: STAT, SOCS, PIAS

- unresponsiveness to IL-12 and IL-23 suggests defect in a shared protein

- no mutations found in STAT4

- selected 100 genes encoding proteins relevant in IL-12, IL-23, Th1, and Th17 responses

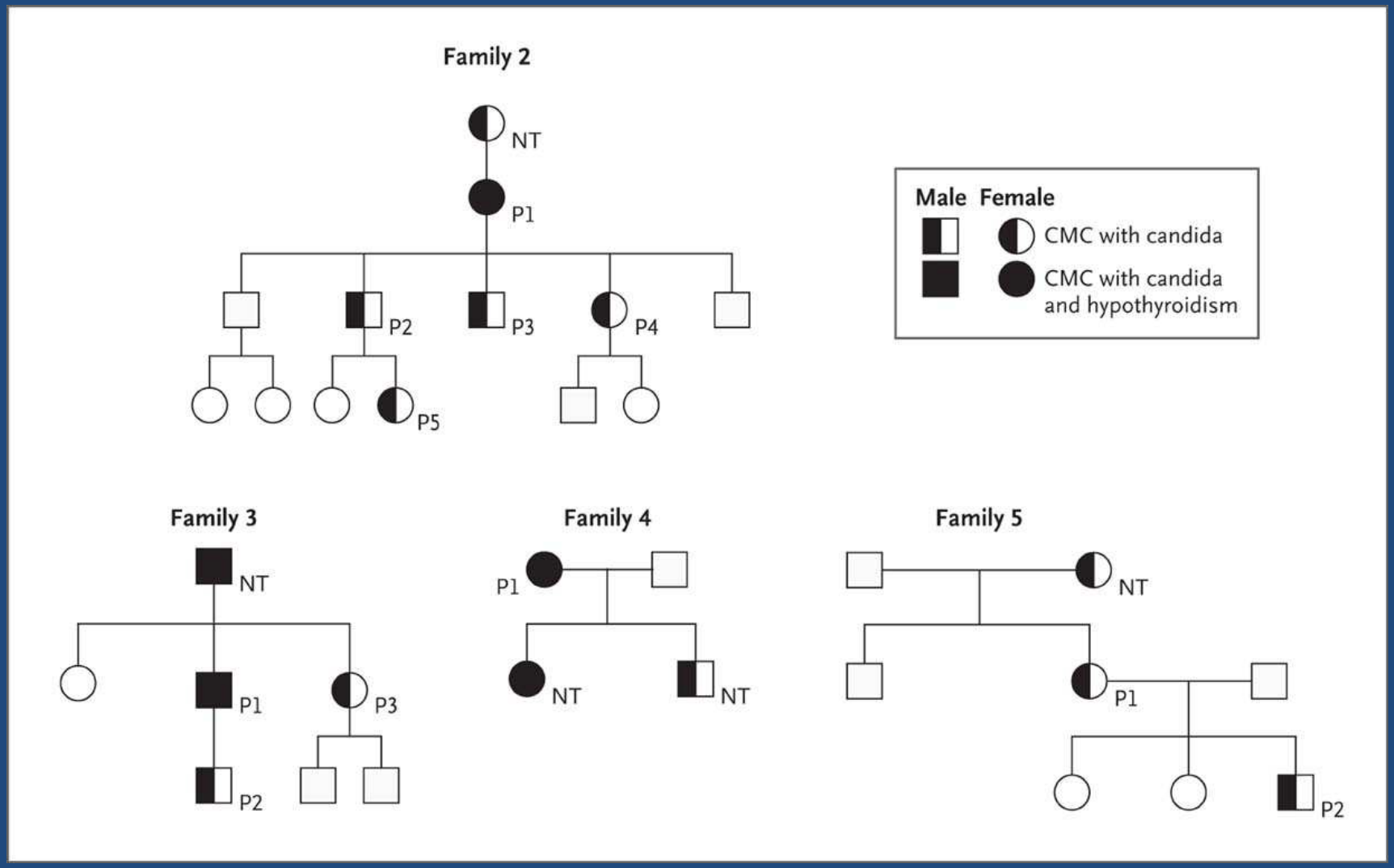
Analysis of Candidate Genes – Family 1

- Observed average of 723 variants per sample, 651 corresponded with known SNPs or were located in polymorphic region
- Identified 11 candidate variants, 3 co-segregated in the 3 affected members of family 1
- Each carried a heterozygous variant of STAT1 gene in exon 10 mapping to chromo 2
- Mutation predicts amino acid change of arginine to tryptophan in the CC domain of STAT1
- Unrelated healthy subjects did not carry this mutation

Confirmation of STAT1 mutations

- Investigated the 3 UK families with AD CMC
- Observed a different STAT1 variant also affecting exon 10
- Presence of the mutation confirmed with PCR amplification of DNA sequence coding the CC domain of STAT1 followed by Sanger sequencing
- Screened controls and banked DNA for these mutations and they were not found

Confirmation of *STAT1* Mutations in Patients with Chronic Mucocutaneous Candidiasis (CMC).

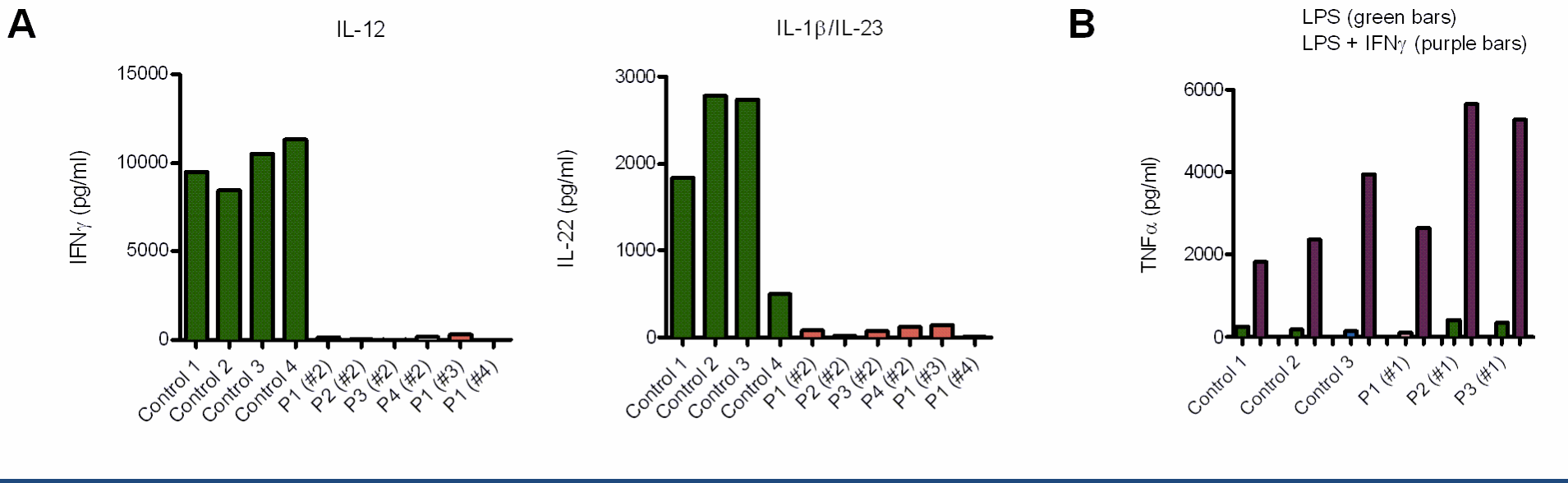


Three families from the United Kingdom (Families 2, 3, and 4) and one Dutch family (Family 5). 11 patients were tested for *STAT1* mutations. Patients in Families 2, 3, and 5 were found to have mutation Ala267Val, and those in Family 4 were found to have mutation Arg274Trp.

STAT1 mutation analysis

- Haplotype analysis performed to determine if STAT1 mutations were founder effects
- High-density SNP arrays and Sanger sequencing of genomic DNA from affected and unaffected members of all families in the study
- Arg274Trp and Ala267Val mutations on different haplotypes common to all families bearing the mutation
- Suggests founder effect for each mutation
- In family 1, only one patient of nine sibs was affected – suggests de novo mutation

Immunologic defects in other families



A. PBMCs stimulated with IL-12 – subjects produced lower levels of IFNg
   PBMCs stimulated with IL-1B/IL-23 – subjects produced lower levels of IL-22

B. Subjects and controls produced similar levels of TNFa in response to LPS/IFNg

Conclusions

- Mutations in the CC domain of *STAT1* underlie autosomal dominant CMC
- Lead to defective Th1 and Th17 responses (decreased production of IFNg, IL-17, IL-22)
- May explain the increased susceptibility to fungal infection
- Patients with HIES also have defective responses in Th1 and Th17 pathways and are susceptible to fungal infections

Discussion

- STAT1 mutations also described in patients with susceptibility to viruses and mycobacteria
- These mutations located in Src homology or DNA-binding domains and result in defective IFNg and type-1 INF receptor pathways
- Other mutation in CC domain of STAT1 resulted in decreased expression of STAT1 protein or blocked dimerization

Discussion

- Mutations found in this cohort exclusively affect Th1 and Th17 responses, may modify interaction of STAT1 with STAT 3 and 4
- STAT1/STAT1 homodimers mediate signaling by IFNg receptor, induces resistance to intracellular microorganisms
- IFNg signaling preserved in AD CMC - normal susceptibility to mycobacteria and viruses

Discussion

- Affected members in two families with Arg274Trp mutation had autoimmune disorders
- Some patients had hypothyroidism
- Thyrotropin may act as cytokine inhibitor in thyroid tissue, mutated STAT1 may hamper the rescue of thyroid cells by thyrotropin and contribute to hypothyroidism
- STAT1-deficient mice have decreased iodine accumulation which may contribute to hypothyroidism
- Three patients who had Ala267Val mutation had esophageal or oral carcinoma
- Loss of function of STAT1 linked to esophageal carcinoma in other reports

Case

- KB (25F) diagnosed with CMC at age 1
- Recurrent candidal infections of skin and mucus membranes, responsive to treatment with diflucan
- Diagnosed with SLE on the basis of rashes, joint pains, Raynaud's, treated with plaquenil
- Experienced shortness of breath and occasional wheezing that improved with bronchodilators
- Frequent episodes of bronchitis were responsive to antibiotics
- Found to have hypertension and was treated with HCTZ

Case

- Age 20 found to have a thyroid nodule which was biopsied and reported as benign
- TSH 1.05 IU/mL, thyroid abs negative
- She was started on daily voriconazole, 200mg
- Continued plaquinel and was started on daily low dose prednisone 5mg for SLE

Case

- Over 1 year the mass doubled in size to 6x4x2 cm with right thyroid lobe hypervascularity
- Repeat needle biospy showed papillary carcinoma of the thyroid
- Thyroidectomy was performed follow by treatment with radioactive iodine (I-131)
- Synthroid treatment was started

Case

- Developed increasingly common and persistent oral ulcers
- Treatment with nystatin was not effective
- Initiated high dose corticosteroid therapy (40-60mg prednisone daily) during flares which occurred every few weeks
- Oral ulcers limited her ability to take PO
- Age 23 admitted to NYU for severe ulcerations and esophageal candidiasis, treated with high dose antifungals and steroids

Case

- Vaginal ulcerations developed and the diagnosis of Bechet's disease was considered although her rheumatologist did not favor this diagnosis
- Facial erythema worsened, thought to be possibly due to prolonged voriconazole therapy so this was changed to daily fluconazole
- Developed recurrent episodes of MRSA hydradenitis suppertiva treated surgically and with bactrim

Case

- Genetic analysis did not show any evidence of AIRE mutation although her clinical picture is consistent with APECED

Nijmegen Breakage Syndrome

- Autosomal recessive chromosomal instability disorder
- Mutations in the NBS1 gene, encodes the protein nibrin
- Nibrin forms a complex with MRE11 and RAD50, rapidly localizes to the site of double-strand DNA breaks
- DNA breaks not efficiently repaired in the absence of nibrin
- Protein complex involved in meiotic recombination and telomere maintenance