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) - autoimmune 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.

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) - eczea, 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 TNFα
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 \textit{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 \textit{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-1β/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 IFNγ 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 IFNγ receptor, induces resistance to intracellular microorganisms

- IFNγ 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 biopsy showed papillary carcinoma of the thyroid
- Thyroidectomy was performed followed 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 hydadenitis 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