Immunology - the first steps
Variation of FCN3 (FCN3+1637delC - FCN3 L117fs)

Ficolin-3 multimer

- Mutation
- Signal peptide
- Cysteine rich region
- Collagen-like domain
- Linker region
- FBG domain
- Non-sense sequence

Allele frequency 0.01, homozygosity expected in 1:10,000

Hummelshøj, Munthe-Fog et al. 2005
Search for FCN3 deletion variant in patients with suspected immunodeficiencies

- A total of 1282 patients referred to Department of Clinical Immunology over a period of 12 years for routine immunologic investigation of various immunodeficiencies (not HIV related)
- Sequencing of exon 5 of FCN3 in all 1282 patients

*Munthe-Fog, Hummelshøj, Honoré et al., 2009, NEJM*
Serum Ficolin-3 of index patient and the family

Polyclonal anti-Ficolin-3 Ab

Lanes
1: Heterozygous sister
2: Wild-type sister
3: Heterozygous mother
4: Heterozygous father
5: Index patient
6: Wild-type control
7: Wild-type rFicolin-3

Munthe-Fog, Hummelshøj, Honoré et al., NEJM, 2009
Immunological characteristics of index patient

- Normal blood count
- Normal concentration of B, T, and NK lymphocytes
- Responded normally in vitro to mitogens
- Responded normally in vitro to microbial antigens
- Serum levels of IgG and IgA were normal, IgM was slightly decreased
- Low response to pneumococcal polysaccharide vaccine but normal response to *H. influenzae*, diphtheria and tetanus vaccines
- Normal activity of the classical, MBL, and alternative complement pathways
- Normal level of MBL (2.0 µg/ml)
Medical history of index patient

• 32-years old man (unrelated parents of Macedonian/Albanian origin)

• Since early childhood
  – Repeated lower respiratory tract infections

• Since age 17
  – Recurring warts on his fingers

• Age 20
  – Spleen removed because of unexplained thrombocytopenia

• Age 26
  – Treated for bilateral frontal cerebral abscesses with non-hemolytic streptococci

• Since age 26
  – Several episodes of bacterial pneumonia requiring hospital admission
  – Severe bronchiectasis and pulmonary fibrosis
  – Progressively decreased lung capacity and obstructive lung disease

Munthe-Fog, Hummelshøj, Honoré et al NEJM., 2009
Effect of frame shift mutation in FCN3 normal and mutated recombinant Ficolin-3 protein

ELISA

1. Wild type rFicolin-3
2. Leu117fs supernatant
3. Non-transfected CHO-cell supernatant
4. Negative control
5. Assay control

Western blot

1. Wild type rFicolin-3
2. Leu117fs supernatant
3. Non-transfected CHO-cell supernatant
4. Leu117fs CHO-cell lysate
5. Non-transfected CHO-cell lysate

Munthe-Fog and Ma et al. 2008
Ficolin-3 specific complement activation ELISA

Ficolin-3 binding to acetylated compounds

Hein et al. 2010, PLoS ONE
Ficolin-3 specific complement activation ELISA

Hein et al. 2010, PLoS ONE
Complement pathway ELISA

Classical pathway

Lectin pathway

Ficolin-3 pathway

Alternative pathway
Suggestion for Persimune primary immunodeficiency project

Utilize biological material from patients admitted to routine investigation
For primary immunodeficiencies from the last 15 years, n=1500

Utilize the routine immunological data present in the Department databases
matched with Persimune datawarehouse

Utilize DNA kept, for NGS targeted sequencing of large immunodeficiency panels to reach coverage acceptable for clinical diagnostics or exom sequencing

Investigate for chromosomal imbalances using comparative genomic hybridization arrays (CGH)

Genetic imprinting on mRNA from cells

Validation by functional studies using mutated recombinant proteins and functional assays
Perfect vs. imperfect match

Graft Survival after 1. Deceased Donor Renal Transplantation
Scandiatransplant 2004-2013
Effect of HLA AB and DR Match

Sørensen and Leivestad for ”The Nordic Kidney Group”, Scandiatransplant
Kidney allograft-survival, donor specific antibodies (DSA) and complement C1q binding status after transplantation.
Suggestion for Persimune primary kidney allograft transplantation project

Utilize stored serum from patients monitored for kidney transplantation in the last 10 years, n=1000 to monitor for effect of DSA and complement

Utilize these data matched with Persimune data warehouse data and MATCH platform to investigate influence on graft survival and infection rate in these patients

Utilize recipient DNA kept, to perform NGS sequencing in order to find genetic variations that may predict outcome

Validation by functional studies using mutated recombinant proteins and functional assays
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