Diagnosis and Phenotypic Characterization of ROSAH Syndrome through Whole Exome Sequence and Segregation Analysis

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ROSAH Syndrome

ROSAH syndrome named for the consistent clinical features: **R**etinal dystrophy, **O**ptic nerve edema, **S**plenomegaly, **A**nhidrosis, and migraine **H**eadache

Epidemiology
- Characterized in 2019
- ~8 families and 18 patients worldwide

Inheritance
- De novo in 1st affected family member, then inherited in autosomal dominant fashion
- 100% penetrance

Clinical Findings

Ocular: Nearly all patients develop vision impairment
- Earliest feature decreased vision and optic nerve edema, onset 4 - 12 yoa
- Low-grade ocular inflammation resistant to steroid or immunosuppression
- Initially abnormal cone function followed by loss of rod function
- Severe visual dysfunction by third decade
- 4/5 families had cataract development

Spleen/Immune System: Splenomegaly, onset childhood to 2nd decade
- Episodic fevers and polyarthralgia
- Increased susceptibility to viral illnesses especially URIs

Hematologic: Chronic pancytopenia
- Exacerbated by infections

Neurological: Episodic headaches and migraines

Renal: Mild renal dysfunction

Skin/Musculoskeletal: Anhidrosis and nail dystrophy

Dental: Peg shaped teeth, enamel defects and predisposition to dental caries

Pathophysiology

- Same de novo variant in all families → mutational hotspot
- Locus evolutionarily conserved in most species
- ALPK1 encodes an alpha kinase protein expressed in all cells, preferentially expressed in:
  - Retinal pigment epithelium
  - Optic nerve tissue
  - Myoepithelial cells of sweat glands

ALPK1 involved in:
- Establishing cell polarity→ mechanism of anhidrosis
- Centrosome and ciliary function→ mechanism in other retinal dystrophies
- NF-kB inflammatory pathway→ other ALPK1 variants linked to inflammatory bowel disease
- RBC cytoskeleton formation→ elliptocyte splenic sequestration

Case Report

We report a case of a 58 year old man with disparate findings in multiple organ systems notably: anhidrosis, pancytopenia, splenomegaly, renal impairment, juvenile onset blindness, periodic episodes of fever/malaise, numerous dental caries and diabetes mellitus type II (DMII). This patient had substantial workup without a definitive diagnosis over the course of his life including repeated bone marrow biopsies and CT scans.

PMH: First presented at 10 yoa with painless, bilateral vision loss that started in central visual fields. Initially attributed to uveitis 2/2 scarlet fever. S/P bilateral cataract removal.

Fhx: Thin brother with DMII

Physical exam: Thin non dysmorphic blind man BMI:21.7 Height:183cm
Abdominal: Splenomegaly
Ocular: Neovascular fibrotic disease in macula and chronic swelling/compression of optic nerve

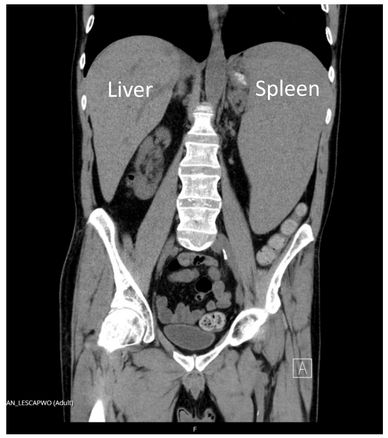
Workup:

Pancytopenia Testing :
WBCs: 1.7-2.8 (x10E9/L)
ANC: 900s to 2000s (cells/dL)
Hemoglobin: 10s (g/dL)
Platelets: 60-80s (x10E9/L)
Hemolytic anemia labs/peripheral smear: Normal

Metabolic Testing:
Urine organic acids-Normal
Homocysteine level-Normal
Lactic acid-Normal

Bone Marrow Biopsy:
Hypercellular with maturing trilineage hematopoiesis, no evidence of dysplasia

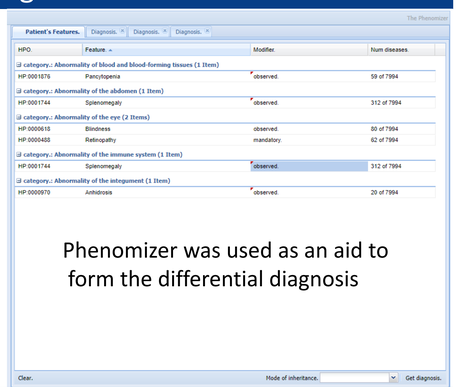
Previous Genetic Testing:
From Bone Marrow:
JAK2(V617F): negative
FISH negative for deletion 5q, monosomy 7, deletion 7q, trisomy 8, deletion 17p and deletion 20q
Karyotype: Normal 46 XY
MDS Gene Panel: Normal
From Blood:
Hereditary Hemolytic Anemia Panel: Normal



Diabetes mellitus type I testing:
Insulin antibodies- Negative
Islet cell antibodies- Negative
Glutamic acid decarboxylase antibodies- Negative

Differential Diagnosis

- Unifying diagnoses
  - Mitochondrial disease
  - Inborn Error of Metabolism:
    - Isolated methylmalonic acidemia
    - Fabry Disease
    - Gaucher Disease Type I
  - Inflammatory process ex: Hemophagocytic lymphohistiocytosis
- Organ System Based Diagnosis
  - Ocular: Retinitis pigmentosa/Sorsby fundus dystrophy
  - Pancytopenia: Splenic sequestration, dyskeratosis congenita , MDS, hemolytic anemia
  - Endocrine: Mature onset diabetes mellitus of the young
  - Renal: Secondary to DMII



Phenomizer was used as an aid to form the differential diagnosis

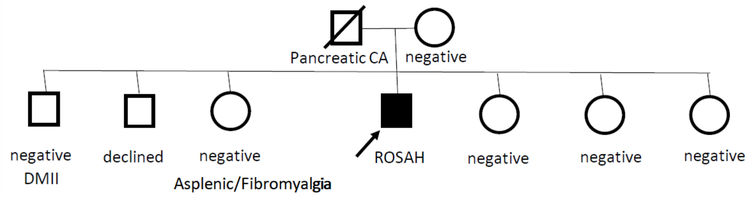
Whole Exome Sequence

- Due to broad differential a whole exome sequence duo (with patient's mother) was sent
- Identified one pathogenic variant in ALPK1, which is associated with autosomal dominant ROSAH syndrome

GENE	VARIANT	ZYGOSITY	MOTHER	VARIANT CLASSIFICATION
ALPK1	c.710C>T (p.Thr237Met)	heterozygous	absent	PATHOGENIC

- Variant not present in population databases
- Patient's clinical findings an excellent match for ROSAH syndrome

Segregation Analysis and DMII



Why does this thin man have DMII?
- MODY and DMI ruled out by WES and antibody testing
- Brother with DMII negative for ROSAH variant
- DMII not found in other patients with ROSAH syndrome, DM could be an uncommon manifestation but not enough data to make this determination at this time
- Possible mechanisms of DM in ROSAH syndrome include chronic inflammation of pancreas or problems with insulin secretion
- Most likely patient has DMII for unrelated reasons though he lacks typical profile
- Segregation analysis shows de novo inheritance, as seen in other families with ROSAH

Treatment

- Adalimumab, a biologic TNF-inhibitor, used as experimental treatment in 2 Chinese children with ROSAH syndrome
- In 1 patient there was improved ocular inflammation, but adalimumab did not halt loss of visual acuity or improve optic disk edema
- Splenectomy can improve abdominal pain, pancytopenia and episodic fevers/malaise

Future Research

This patient recently underwent splenectomy after learning of the improved quality of life other ROSAH syndrome patients had experienced. The spleen was sent for research analysis the results of which are pending. This is the first time a ROSAH spleen has been investigated in detail as previous ROSAH patients had splenectomy years before diagnosis. Analyses include:
- Spleen was 1599 g ~10x the weight of a normal spleen
- Immunohistochemistry to identify where ALPK1 localizes in the ROSAH spleen
- RNA extraction to determine level of ALPK1 expression compared to normal spleen
- Staining and flow cytometry to determine if/what type of cells, for instance elliptocytes, are sequestering in the spleen

Conclusions

- Repeating studies will generally not yield an explanation for a rare phenotype
- Genetic workup in adults with impressive unexplained phenotypes can be high yield
- When differential is large WES may be a superior approach to multiple gene panels
- When possible apply "parsimony of diagnosis" to look for a unifying diagnosis rather than multiple diagnoses to explain disparate findings in multiple organ systems
- A patient can have an (extremely) rare disease and common diseases of adulthood

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