Next-Generation Sequencing for Craniofacial Gene Discovery

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Study Design

• Study a group of patients with syndromic forms of craniofacial anomalies who have eluded a specific diagnosis through traditional expert genetic evaluation

• Perform exome or genome sequencing on about 150 unrelated families with undiagnosed syndromic craniofacial anomalies
  – Sample collect so successful increased to 500 families
Clinical Whole Exome Sequencing Reanalysis

• 50 families “negative” from reference labs
• Referred by Samatha Vergano
• At four years presented with hypotonia, developmental delay, and macrocephaly
• Sister also demonstrated hypotonia and delay without macrocephaly
TBCK discovery

• CAG by Dong Li, PhD finds both sisters to be compound heterozygote for the TBCK (TBC1 domain containing kinase) variants:
  • c.2060-2A>G (splice site variant)
  • c.803_806delTGAA:p.M268fsX26 (frameshift variant)
Cohort of 13 similar patients

- Hypotonia
- Variable Developmental Delay
- May present like a leukodystrophy or storage disorder
- May include seizures
- No common facial gestalt
Just how common is it?

• We’re now aware of at least 25 affected families worldwide.
Decreased TBCK and mTOR signalling

- Western blot for specific protein levels in patient lymphoblastic cell lines:
  - Absent TBCK protein
  - Equal total mTOR and S6
  - Decreased phosphorylated S6

In collaboration with Peter Crino, Temple University
Quantifiable defect in mTOR

• >70% decrease in mTOR activation in two different patient fibroblast cells lines
Towards targeted therapy

• One of the many activators of the mTOR pathway has been shown to be leucine, one of the essential amino acids, through mTORC1.

• Leucine supplementation via mTOR activation has been studied in the role of adipogenesis, increased muscle mass, and diabetes control.

• Current pediatric trials using leucine as treatment for Diamond-Blackfan anemia
Leucine activates mTOR in TBCK-/- cells

- Leucine (600µg/ml) added to patient TBCK-/- fibroblasts recovers PS6 phosphorylation
- This suggests a potential therapeutic target for these patients

In collaboration with Peter Crino, Temple University
Mutations in *TBCK*, Encoding TBC1-Domain-Containing Kinase, Lead to a Recognizable Syndrome of Intellectual Disability and Hypotonia

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TBCK Future Directions

• Currently awaiting IRB approval for a TBCK-patient registry, designing leucine trial
• RNAseq in progress on patient cells
• Breeding *tbck*-/- mice and plan to perform neurobehavioral testing
  – Autopsies (including careful brain examination)
  – Leucine to pregnant mothers and pups
• Autophagy and proteosomal degradation studies on patient cell lines
Additional Findings

• Currently 24 novel genes from this cohort undergoing additional study

• Focusing on treatable conditions
  – New projects on novel genes working through epigenetic mechanisms that may be new targets for precision therapies
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• All our participating families
Questions?