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Next-Generation Sequencing for Craniofacial Gene Discovery

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

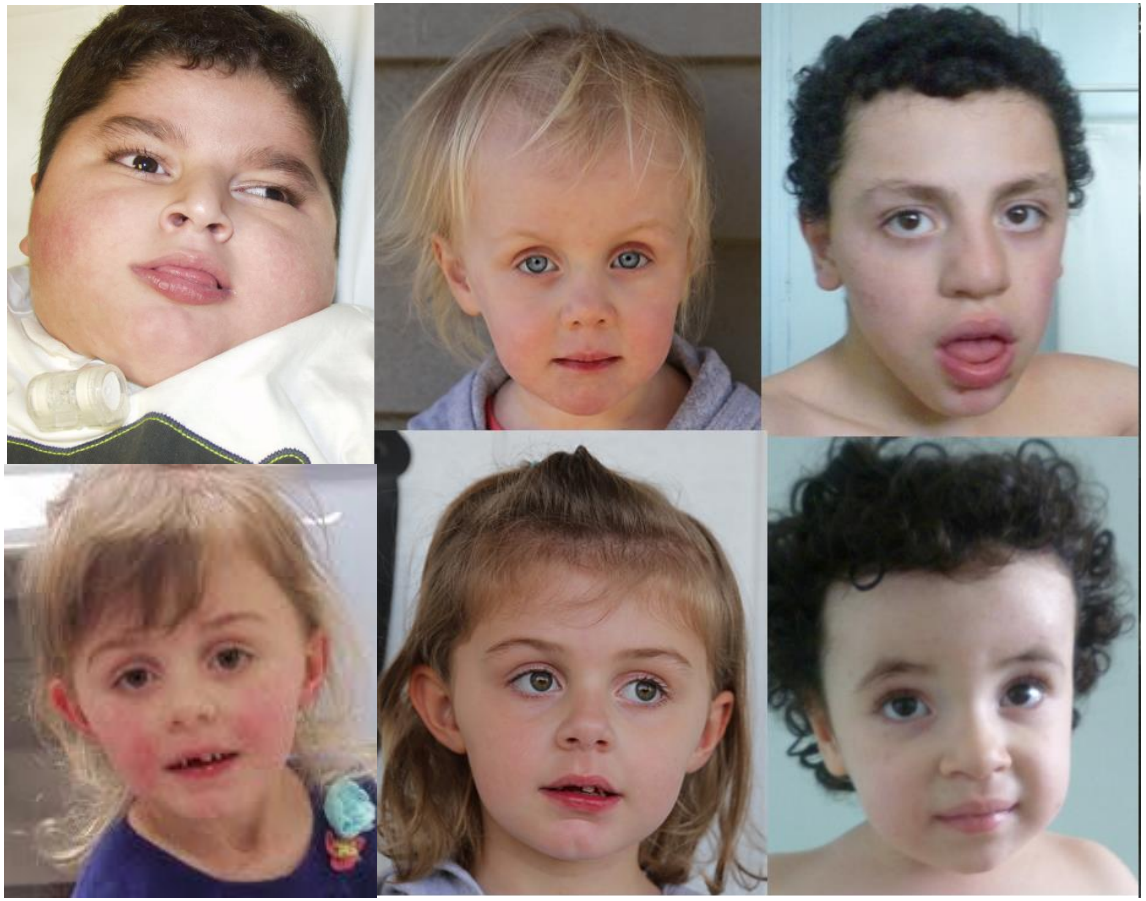
- 50 families “negative” from reference labs
- Referred by Samatha Vergano
- At four years presented with hypotonia, developmental delay, and macrocephaly
- Sister also demonstrated hypotonia and delay without macrocephlay

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

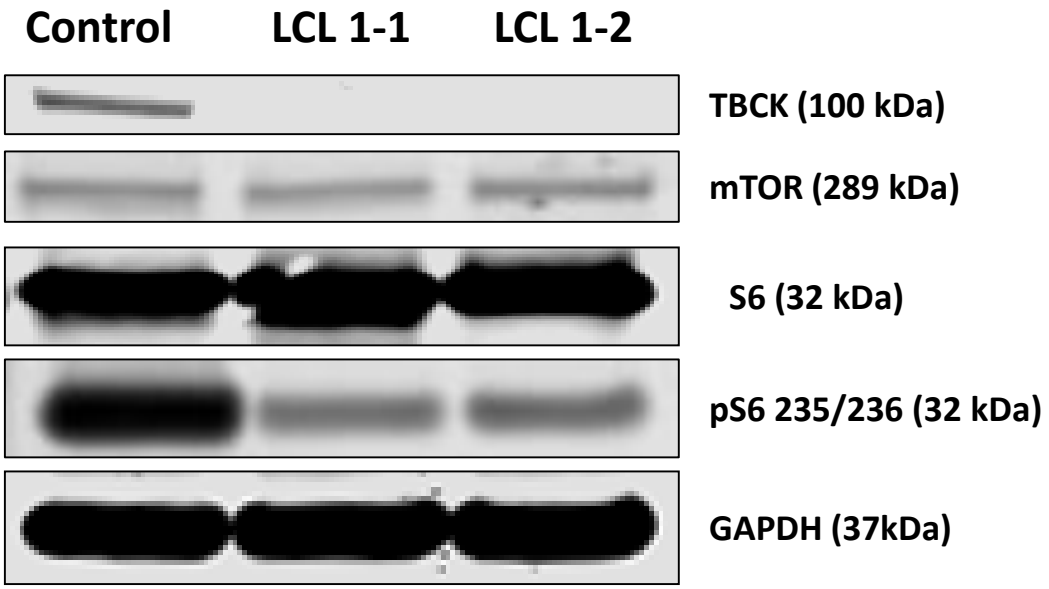


Just how common is it?

- 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



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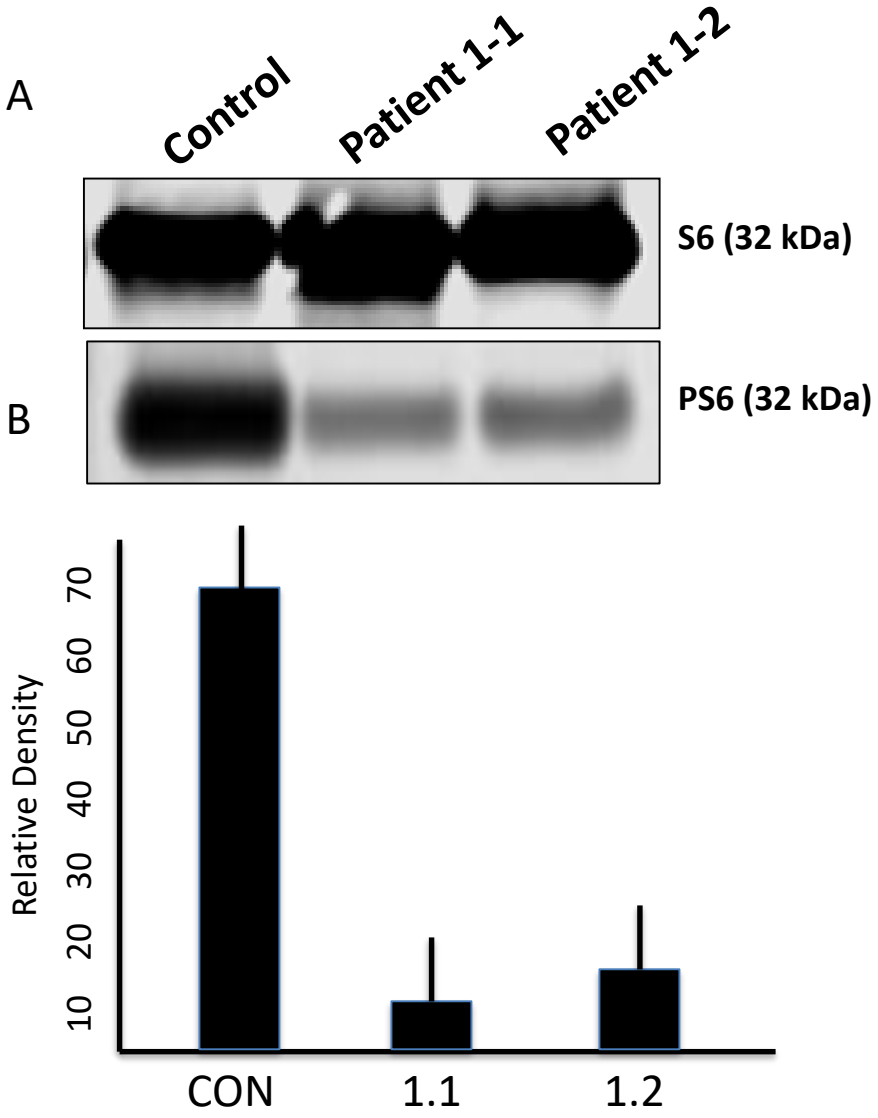
Quantifiable defect in mTOR

- >70% decrease in mTOR activation in two different patient fibroblast cells lines

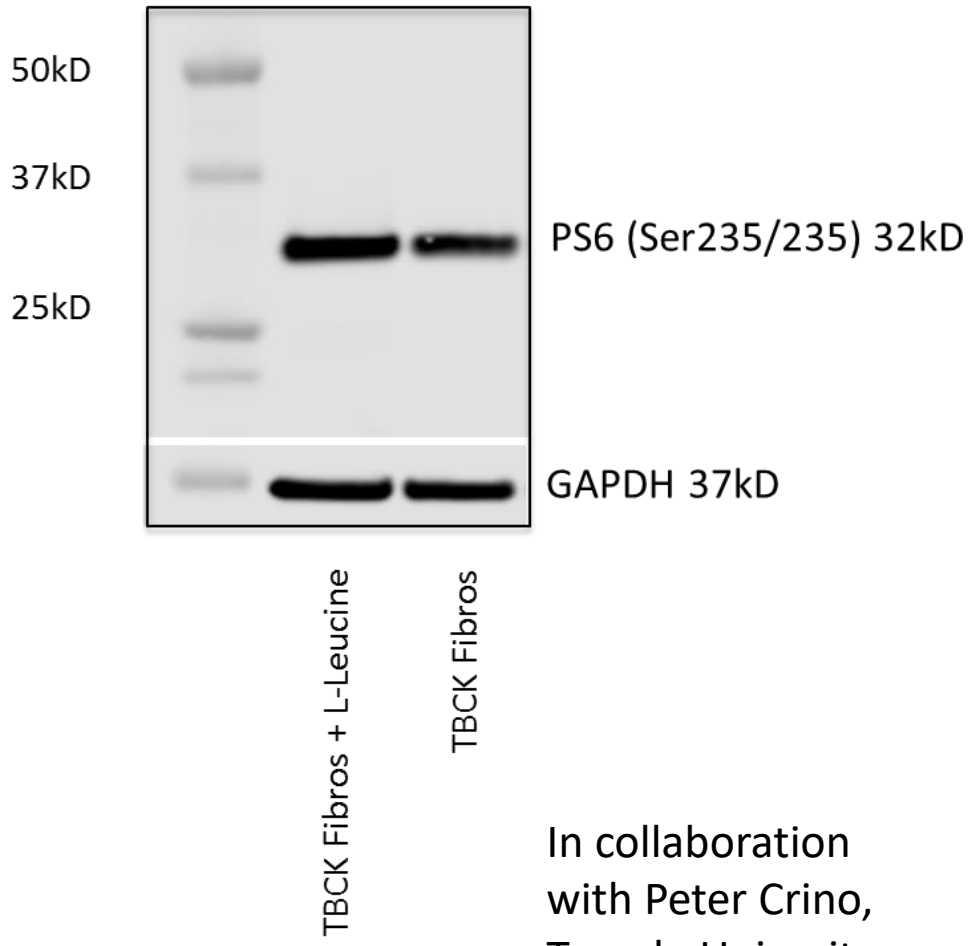


Towards targeted therapy

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



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REPORT

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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- Peter Crino for helping with the functional work, and Laurence Colleux for sharing his TBCK antibody
- All our participating families

Questions?