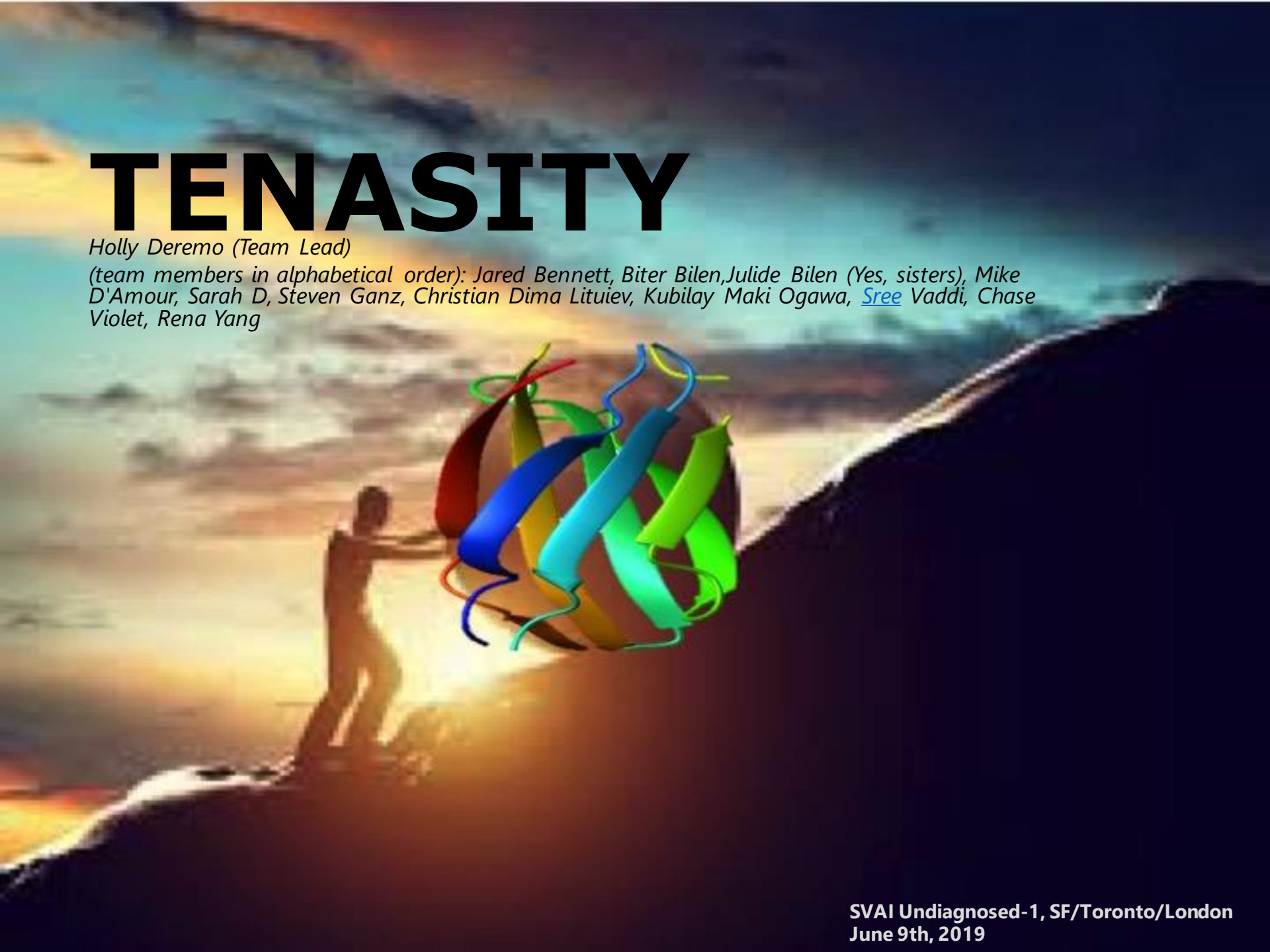
TENASITY

Holly Deremo (Team Lead)

(team members in alphabetical order): Jared Bennett, Biter Bilen,Julide Bilen (Yes, sisters), Mike D'Amour, Sarah D, Steven Ganz, Christian Dima Lituiev, Kubilay Maki Ogawa, Sree Vaddi, Chase Violet, Rena Yang

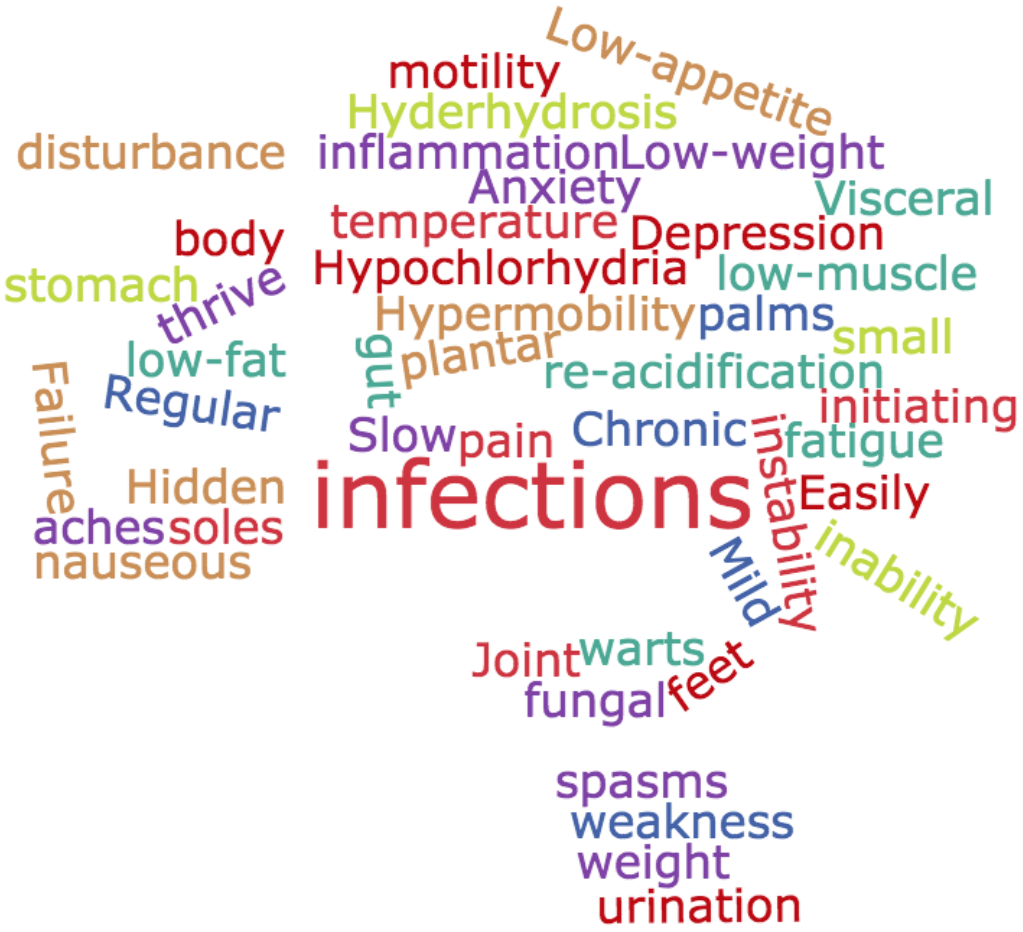


SVAI Undiagnosed-1, SF/Toronto/London
June 9th, 2019

Outline

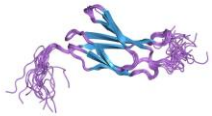
1. Research Summary
2. Project Outline
3. Deep Dive into Recommended Targets
4. Conclusions and Strategic Recommendations

John's Symptoms



Tenacity Specific Targets for Future Research

TNXB Tenascin is the top priority to pursue in the near term according to our analysis and primary research



TNXB

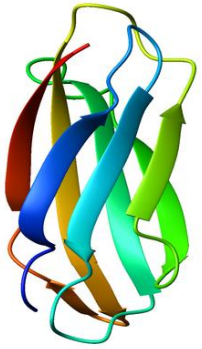
John should next consider **a variety of high value targets** due to high potential and reasonable inherent risks

Alg11 ATP7B PLCD4

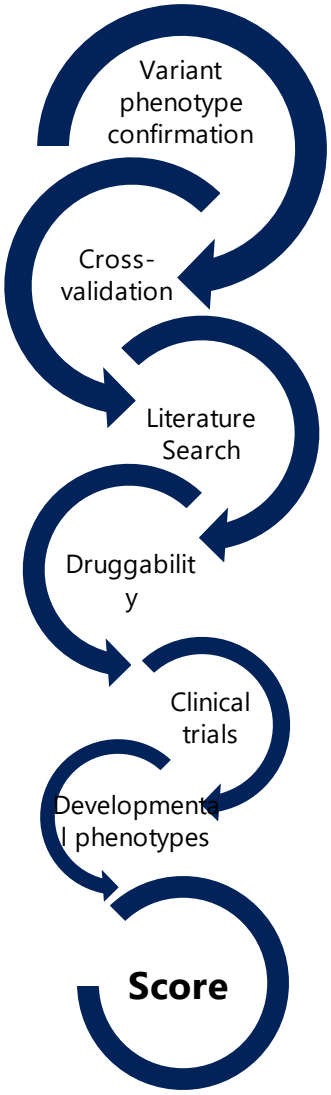
John should avoid these targets due to false-positiveness and further check genome-wide Q/C

CCK, CCK_RA, CCK_RB

Project Outline



Tenacity Workflow for Gene-Variant Analysis



Is the variant passes certain confidence criteria

Can it be confirmed with deep-exon sequencing

Is this target closely related to a disease state?

Is there clinical evidence exist on this target?

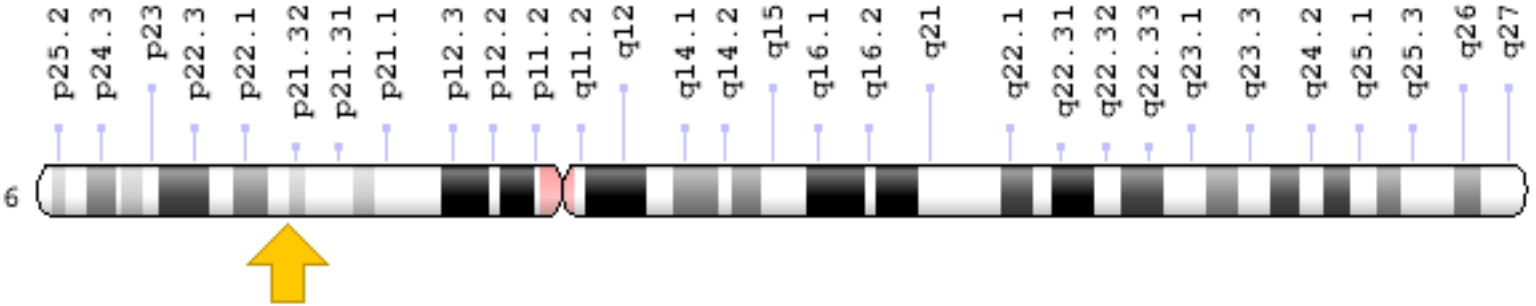
Is it of clinical interest

Is gene's function required early on development

Weighted final scoring

Deep Dive into Recommended Targets

Tenascin-X(TNXB) Partially Lost in John's Case



- An extracellular-matrix protein
- Required for organismal architectural
- Evolutionary conserved suggesting architectural protein, as it displays features of a matricellular matrix by modulating cell adhesion
- TNXB-deficiency leads to greater symptoms of reflux, indigestion and abdominal pain.
- Partial relief from GABA-B agonists, like benzodiazepines (which John currently takes and have worked)
- Known to be mutated in Ehlers-Danlos type III disease patients

TNXB Variant is Heterozygous

Ehlers Danlos III Syndrome

- Group of connective tissue disorders
- Reduces the amount of functional tenascin-X produced

- Weakens connective tissues in many parts of the body, which leads to signs and symptoms of hypermobility

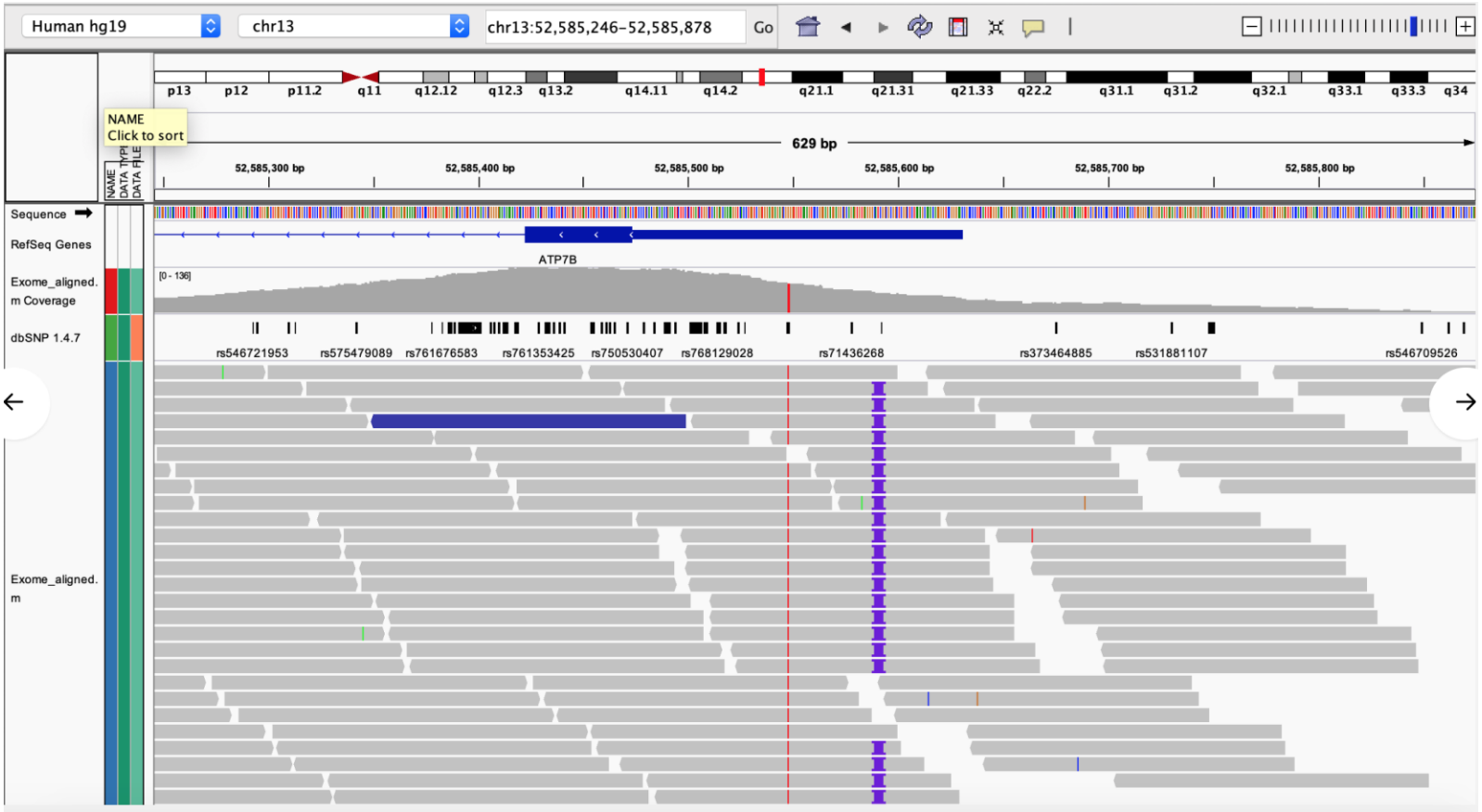
- Additional symptoms include numerous ties to GI dysmotility disorders, as well as other functional disorders like Gastric Reflux, Constipation, Nausea, Vomiting, Heartburn, abnormal gastric emptying, and abnormal colonic transit time

Secondary Recommendations 1

- **ATP7B**: implicated in osteo-muscular type, growth failure
  Potential connection with ATPase (small intestine) / Lysyl oxidase (connective tissue, LOX)
  3 homozygous variants:
    2 affecting transcription factor binding site
    1 of the 2 involving promoter loss

- **ALG11**: implicated in failure to thrive, developmental delay, persistent vomiting (via glycosylation)
  The 2 above variants affecting TFBS

ATP7B/ALG11 is Homozygous



Secondary Recommendations 2

- **TTN**: implicated in implicated in limb girdle muscular dystrophy, muscle weakness, growth failure
  - 5 heterozygous variants:
    - 1 affecting stop gain on translation
    - 2 classified as possibly damaging (PolyPhen-2)

- **SCN5A**: implicated in diabetic neuropathy, depression, acute pain, renal impairment, urinary issues, constipation, pelvic pain, vomiting, pharyngitis
  - 3 heterogynous variants

Next Steps

Short-term

- Focus on high value target TNXB, and verify the loss-of-function by conventional methods with contract lab.
- Complement gene variant analysis with incoming proteomics data

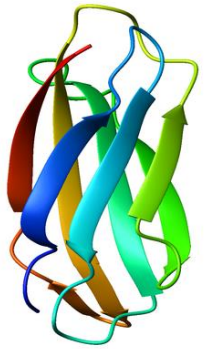
Mid-term

- Determine up- or down-regulation of RNA expression using existing small intestine biopsy tissue
- Continue investigating secondary targets

Long-term

- Combine tissue-specific protein data, as well as single-cell sequencing in gut and connective tissue
- Work with academic labs to create an organoid model for ex vivo drug testing

Conclusion



BONUS SLIDES!

- The remainder of this presentation is for understanding our process later.

Ehlers Danlos III

- EDS is a group of connective tissue disorders that affect the skin, bones, blood vessels, and many other organs and tissues.
- Mutations that cause this form of the disorder occur in one copy of the TNXB gene in each cell. These mutations reduce the amount of functional tenascin-X that cells produce, which decreases the ability of tenascin-X to interact with collagens and elastic fibers. These changes weaken connective tissues in many parts of the body, which results in the signs and symptoms of the hypermobile type of Ehlers-Danlos syndrome.7
- EDS III has been linked numerous times to GI dysmotility disorders, as well as other functional disorders like Gastric Reflux, Constipation, Nausea, Vomiting, Heartburn, abnormal gastric emptying, and abnormal colonic transit time 3,4.

TNXB and Tenascin-X contd.

- In adults, TN-X mRNA is observed at varying levels in a number of organs and tissues with a higher level in the digestive tract (pancreas, stomach, jejunum, ileum, and colon).[2]

- Previously, it has been shown that TNX is required for neural control of the bowel by a specific subtype of mainly cholinergic enteric neurons and regulates sprouting and sensitivity of nociceptive sensory endings in mouse colon.

- These findings correlate with symptoms shown by TNX-deficient patients and mice[5].

Tenascin-X, ECM, and the GI System

- TNX was found to be present in calretinin-immunoreactive extrinsic nerve endings in mouse and human stomach.

- TNX deficient mice had increased vagal afferent responses to gastric distension that could be rescued with GABA-B receptor agonist.

- In TNXB-deficient patients, significantly greater symptoms of reflux, indigestion and abdominal pain were reported. A study published in only march of this year is the first to outline the role for TNX in gastric function.[7]

- **Further studies are required in TNX-deficient patients to classify groupings of symptoms and to determine whether symptoms can be relieved even partially using GABA-B agonists, like benzodiazepines, which the patient currently takes, and has found some relief using them.**

Tenasity recommends for next steps

Short-term

- Focus on high value target TNXB, and verify the loss-of-function by conventional methods with contract lab.
- Complement gene variant analysis with incoming proteomics data
- Explore **DNA Pols** as a potential high-risk, high-reward option

Mid-term

- Determine up- or down-regulation of RNA expression using existing small intestine biopsy tissue
- Continue investigating secondary targets

Long-term

- Start pursuing currently unvalidated targets like **FEN1, CDC20, CAD, and Raptor** as more validation and in-vivo models emerge

Secondary Recommendations

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  3 homozygous variants:
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  2 of the 3 above variants, including those mentioned

More Secondary Recommendations

- **TTN**: implicated in implicated in limb girdle muscular dystrophy, muscle weakness, growth failure
  - 5 heterozygous variants:
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