The Elephant in the Room: TTN

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COI disclosure

Fulltime employee at Invitae
Owns 23andMe stock options

Spouse (Sarah Garcia, PhD, MS, CGC)
Fulltime employee at 10X Genomics
Owns Personalis stock options
“Let’s try some role playing. I’ll be the elephant in the room and you address me.”
Outline

• Titin’s function and structure
• Associated clinical phenotypes
• What we know, and what we don’t
• Practical variant interpretation suggestions
Sarcomere and cardiac disease
Titin’s function and structure
<table>
<thead>
<tr>
<th>Condition</th>
<th>Overt symptoms</th>
<th>Inheritance</th>
<th>Types of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titinopathies (multiple forms of muscular dystrophies and myopathies)</td>
<td>Skeletal</td>
<td>Autosomal recessive</td>
<td>LoF</td>
</tr>
<tr>
<td>Late-onset tibial muscular dystrophy</td>
<td>Skeletal</td>
<td>Autosomal dominant</td>
<td>Founder variant (11-bp indel)</td>
</tr>
<tr>
<td>Hereditary myopathy with early respiratory failure</td>
<td>Skeletal</td>
<td>Autosomal dominant</td>
<td>Specific missense variants</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>Cardiac</td>
<td>Autosomal dominant</td>
<td>LoF</td>
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LoF = nonsense, frameshift, and canonical splice variants
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<th>Evidence?</th>
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<td>Biallelic (TTN) LoF variants</td>
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<td>One (TTN) LoF variant</td>
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Biallelic $TTN$ LoF variants cause titinopathies

- Dozens of families observed
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Heterozygous TTN LoF variants cause dilated cardiomyopathy

- Hundreds of families observed
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<td>+++</td>
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TTN LoF variants are common in the population

- 1-2% of the general population has a *TTN* LoF variant
  - Most people with a *TTN* LoF variant have no signs of disease (PMID: 27869827)
# Recap

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<td>+++</td>
</tr>
<tr>
<td>(no phenotype)</td>
<td>N/A</td>
<td>One $TTN$ LoF has no effect</td>
<td>+</td>
</tr>
</tbody>
</table>
What we know:

Most recessive titinopathies involve $TTN$ LoF within the M-band

Most DCM-associated $TTN$ LoF are within the A-band

Most $TTN$ LoF in the general population are found in the I-band or Z-disk
Many I-band exons are not consistently transcribed

*TTN* cardiac isoforms
TTN LoF variants in the A-band are significantly enriched in individuals with dilated cardiomyopathy.
What we don’t know:

• Why does location matter?
  – Nonsense mediated decay (NMD) should make most *TTN* LoF variants equivalent
    • No evidence for NMD in humans
What we don’t know:

• Why does location matter?
  – Nonsense mediated decay (NMD) should make most \textit{TTN} LoF variants equivalent
    • No evidence for NMD in humans
  – If truncated protein is expressed, phenotypes may be driven by function of truncated proteins
    • No evidence for significant presence of truncated titin
What about missense variants in \textit{TTN}?

- Filed under “What we don’t know”
  - No enrichment of \textit{TTN} missense variants in DCM cases vs controls (PMID: 22335739, 24980681)
  - No missense variants have conclusively segregated with DCM (PMID: 26567375)
    - Small \# segregate with myopathy and early respiratory failure (PMID: 23606733)
  - 25\% of people in this room have novel \textit{TTN} missense variants (PMID: 26516846)

Rare/novel \textit{TTN} missense variants are of limited clinical significance without additional information
Practical variant interpretation suggestions

• Novel missense variant anywhere in \textit{TTN}
  – Treat as ‘synonymous’
    • For most labs, this means moving the variant to a supplemental report
  – Use clinical judgement before de-emphasizing
    • Check for literature, splicing predictors, other pathogenic \textit{TTN} variants at the same codon, etc.
Practical variant interpretation suggestions

• The Z/A/I/M boundaries depend on the transcript you’re using
  – See http://cardiodb.org/titin/titin_transcripts.php for a full list
  – For the “meta” transcript (NM_001267550.2):

<table>
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<th>Exon number</th>
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<tr>
<td>1-28</td>
<td>Z</td>
</tr>
<tr>
<td>29-251</td>
<td>I</td>
</tr>
<tr>
<td>252-357</td>
<td>A</td>
</tr>
<tr>
<td>358-363</td>
<td>M</td>
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</table>
Practical variant interpretation suggestions

• M-band *TTN* LoF
  • Suggested interpretation: (Likely) Pathogenic
  • Clinical impact: Carrier
    – Heterozygous family members are not expected to be at risk for dilated cardiomyopathy

• A-band *TTN* LoF
  • Suggested interpretation (Likely) Pathogenic
  • Clinical impact: At risk
    – Heterozygous family members at risk for dilated cardiomyopathy
Practical variant interpretation suggestions

• Z- or I-band *TTN* LoF:
  • Suggested interpretation: Variant of Uncertain Significance
  • Clinical impact: Uncertain
    – No conclusive associations with dominant disease
    – No conclusive associations with recessive disease
  • *TTN* LoF variants found in skipped exons (e.g., some exons in the I-band) have not been associated with any disease (yet)
Conclusions

• The *TTN* gene should be part of routine cardiovascular and neuromuscular genetic testing, and we shouldn’t be dissuaded by its size and complexity
• We can give clinically relevant answers to patients and families right now
• The more we learn, the better we can serve our patients in the future
Acknowledgements

Matteo Vatta, PhD, FACMG
Amy Daly, MS, LCGC
Tom Winder, PhD, FACMG
Jody Westbrook, PhD
Jackie Tahiliani, MS, LCGC
Chris Tan, MS, LCGC
Emily James, MS, LCGC
Ariadna Martinez, MS, LCGC
Sienna Aguilar, MS, LCGC
Emily Decker, MS, LCGC
Rachel Harte, PhD
Daniel Beltran, PhD
Laura Murillo, PhD
Blanca Herrera, DPhil
Tom Callis, PhD