SUPPLEMENTAL MATERIAL

Role of Titin Missense Variants in Dilated Cardiomyopathy

Running title: *Begay et al. TTN missense variants in DCM*

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Supplemental Figure

Figure S1. Venn Diagram of SIFT/PolyPhen2/GERP Relationships

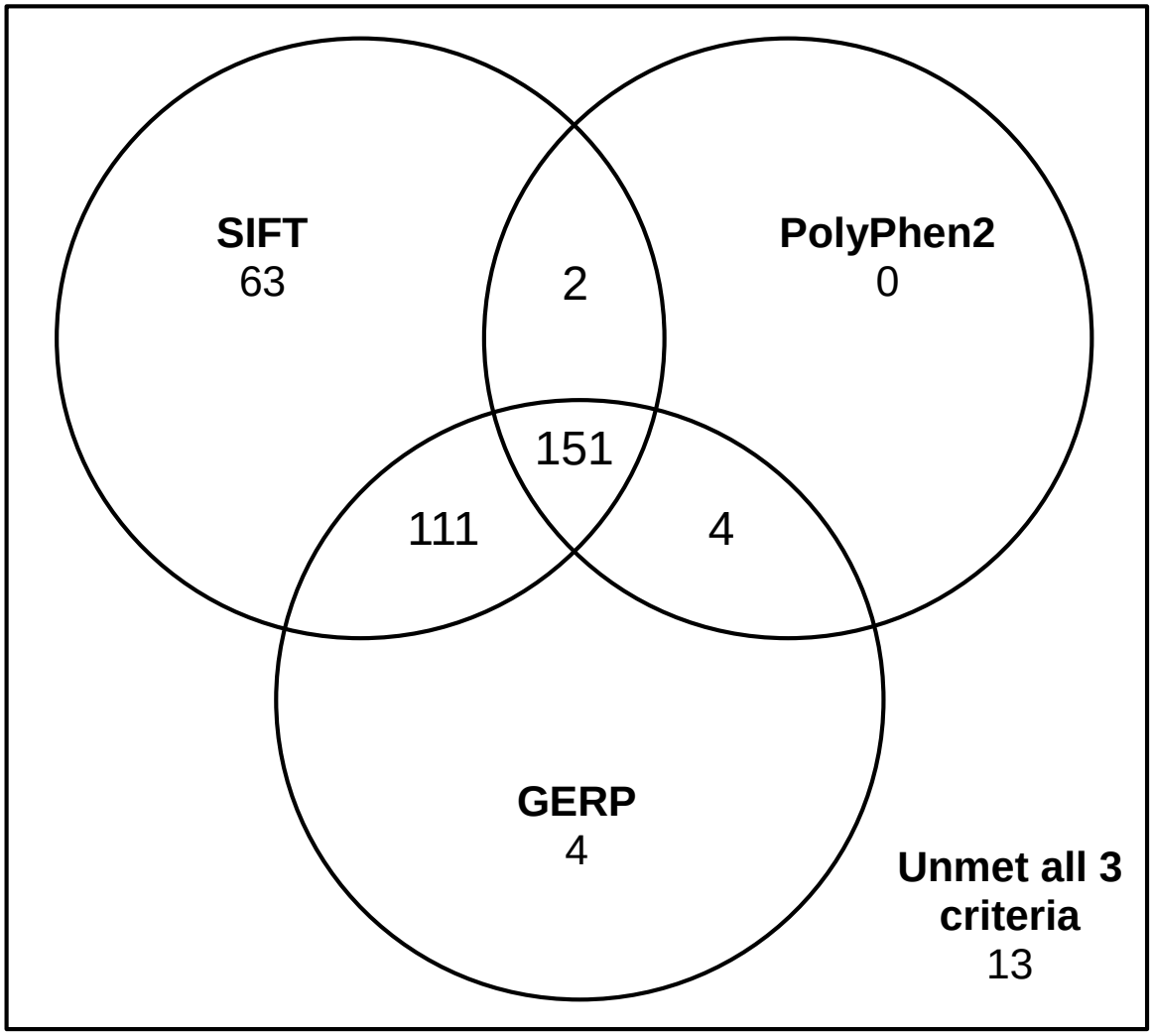

Figure S1. Displayed are GERP, PolyPhen2 prediction, and SIFT scores for all 348 *TTN* missense variants. This figure shows the distribution of the variants that met all cutoff criteria, none, or met only two or one of the three bioinformatic filtering criteria.

Supplemental Tables

Table S1. Sequential Bioinformatic Filter Criteria of *TTN* Variants.

Variant Filter Inclusion Criteria	Number of Variants
1) Total sequence data of 147 families (non-synonymous, synonymous, intronic, frameshift, exonic, and intergenic)	814
2) Non-synonymous variants only - Excluded 13 families harboring truncation mutations*	348
3) Variants with SIFT score = 0	327
4) PolyPhen2 HVAR 'possibly damaging' and 'damaging' predictions	170
5) Number of Mutations ≤ 2	124
6) Exclude, if present in 1000 Genome database	83
7) Exclude if allele frequency ≥ 0.04% in Exome Sequencing Project (n=5,400 subjects)	65
8) GERP RS score ≥ 4.2	44

* Previously reported by Herman, et al.[1]

Table S2. Bioinformatic Data for the 44 'severe' *TTN* Rare Variants.

ID	Mutation	Polyphen2 Prediction	RS Number	GERP+RS	ESP5400 AA; EA Frequency
TTN-238839	Pro23170Arg	P;P;P;P	72646900	4.39	0; 0
TTN-088762	Ser7409Pro	P	17452588	4.71	0; 0
TTN-178217	Arg12999Cys	D;D;D;D	72650088	4.87	0; 0
TTN-245906	Arg25922Cys	P;P;P;P	72648214	4.95	0; 0
TTN-245973	Ser25944Tyr	P;P;P;P	72648215	4.96	0; 0
TTN-095345	Gly8569Arg	P	72648991	4.98	0; 0
TTN-236246	Ala22702Thr	P;P;P;B	72646895	5.21	0.000316; 0
TTN-172724	Ser12061Pro	D;D;D;D	72650078	5.38	0; 0
TTN-275613	Lys32652Glu	P;P;P;P	72629783	5.41	0; 0.0003
TTN-259628	Trp24693Arg	D;D;D;D	72648246	5.48	0; 0
TTN-189481	Arg13843Met	D;D;D;D	72677229	5.49	0; 0
TTN-026760	Glu1039Gly	B;B;B;B;P	72647868	5.54	0; 0
TTN-233699	Tyr21853His	P;P;P;P	72646888	5.6	0; 0
TTN-034409	Ala2258Val	D;D;D;D;D	72647881	5.62	0; 0
TTN-199079	Arg30897His	D;D;D;D	72632860	5.63	0.000319; 0.00015
TTN-272197	Gly31678Arg	D;D;D;D	72648279	5.65	0; 0
TTN-254388	Leu27858Arg	P;P;P;P	72648234	5.66	0; 0.000301
TTN-254689	Gly27921Asp	D;D;D;D	72648235	5.66	0; 0.000296

TTN-230280	Phe21012Leu	D;D;D;D	72646877	5.68	0; 0
TTN-232757	Glu21576Gly	D;D;D;D	72646884	5.72	0; 0
TTN-254772	Glu27949Gln	P;P;P;P	72648236	5.76	0; 0
TTN-257524	Gly28717Glu	D;D;D;D	72648243	5.76	0; 0
TTN-251996	Ser27662Gly	B;P;P;P	72648231	5.77	0; 0
TTN-263807	Arg30215Gly	D;D;D;D	72648261	5.81	0; 0
TTN-263385	Ile30116Thr	D;D;D;D	72648259	5.82	0; 0.000295
TTN-188497	Ile13613Phe	P;P;P;P	72677226	5.83	0; 0
TTN-275968	Ile32770Asn	D;D;D;D	72629784	5.83	0; 0
TTN-226981	Arg20388His	D;D;D;D	72646868	5.86	0; 0.000151
TTN-270677	Arg31411His	D;D;D;D	72648276	5.87	0.000313; 0
TTN-244241	Tyr30201Asp	D;D;D;D	72648211	5.88	0; 0
TTN-009517	Glu222Lys	P;P;P;P;P	72647844	5.92	0; 0
TTN-197931	Glu15297Gln	P;P;P;P	72677250	5.95	0; 0
TTN-274444	Arg32262Leu	D;D;D;D	72629782	5.95	0; 0
TTN-276694	Arg33012Leu	P;P;P;P	72629786	5.95	0; 0
TTN-221566	Leu19535Ser	D;D;D;D	72646854	6.01	0; 0
TTN-175395	Thr12542Ala	P;P;P;P	72650081	6.06	0; 0
TTN-195470	Leu21219His	D;D;D;D	72677245	6.07	0; 0
TTN-236765	Val22875Ile	D;D;D;D	72646897	6.07	0; 0

TTN-247572	Arg26477His	D;D;D;D	72648220	6.1	0; 0
TTN-218441	Ser18632Tyr	D;D;D;D	72646844	6.11	0; 0
TTN-216342	Arg18064Cys	D;D;D;D	72646839	6.16	0.00032; 0
TTN-224394	Pro19922Ala	D;D;D;D	72646860	6.17	0; 0.000152

Table S2. Displayed are Polyphen2 HDVAR predictions as described,[3,4] and the multiple predictions in PolyPhen2 refer to their multiple isoforms. D: Damaging by Polyphen2, P: probably damaging by Polyphen2 criteria. The variants listed are from families with ≤ 2 mutations predicted, per our bioinformatic filtering strategy. The table also includes GERP scores for 'severe' *TTN* missense variants that had SIFT scores of zero and were absent in the 1,000 Genomes dataset. The data show ESP scores below the cutoff of 0.04%.[2]

Table S3. Primer Sequences and PCR Conditions.

Mutation	Primer Name (RS number)	Primer Sequence
Ser>Gly	rs72648231 F	TCGAGAAAGCATCACTGTGG
	R	GCCAAGACTGGTTCAGAAGG
Arg>His	rs72648220 F	ATATGATGGTGGCTGCCAAA
	R	GGATACTCTGCAACAACAGCT
Arg>Cys	rs72650088 F	TTAGCAAAGCAGATGCACCA
	R	GCACAAATAACCCCAATGCT
Glu>Gln	rs72648236 F	GGCCTCCAGCTGATGATG
	R	CAAATGCGTTCTTGGCTACA

References

1. Herman DS, Lam L, Taylor, MR, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, Teodorescu DL, Cirino AL, Banner NR, Pennell DJ, Graw S, Merlo M, Di Lenarda A, Sinagra G, Bos JM, Ackerman MJ, Mitchell RN, Murry CE, Lakdawala NK, Ho CY, Barton PJ, Cook SA, Mestroni L, Seidman JG, Seidman CE. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med* 2012;366:619-628.

2. Norton N, Robertson PD, Rieder MJ, Zuchner S, Rampersaud E, Martin E, Li D, Nickerson DA, Hershberger RE, National Heart, Lung and Blood Institute GO Exome Sequencing Project. Evaluating pathogenicity of rare variants from dilated cardiomyopathy in the exome era. *Circ Cardiovasc Genet* 2012;5:167-174.

3. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248-9.

4. Maathuis MH, Colombo D, Kalisch M, Bühlmann P. Predicting causal effects in large-scale systems from observational data. *Nat Methods* 2010;7:247-8.