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Supplemental Data

Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium

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Supplemental Note

The following section describes in more detail specific points of discussion that arose in evaluating different types of evidence and their associated codes.

Population data (PS4, PM2, BA1, BS1 and BS2)
Whether or not to apply PM2 (variant is absent in population databases or at an extremely low frequency), was a point of discussion for several variants with discordant interpretations. The group struggled with quantifying an 'extremely low frequency' with some sites only invoking this rule when the variant was not present in population databases while others created an arbitrary cut off (for example <0.001%). The development of disease and/or gene-specific allele frequency thresholds would help to standardize the use of this rule. Some sites incorrectly invoked PM2 for a 20 bp indel variant not seen in ExAC (http://exac.broadinstitute.org), ESP (http://evs.gs.washington.edu/EVS/) or 1000 Genomes (http://www.1000genomes.org/). As stated in the ACMG/AMP rules, indel detection by next generation sequencing technologies can be challenging and therefore one should not assume accurate detection, particularly of longer indels. The guidelines also highlight consideration of whether a variant call in a population database passed quality metrics. In this exercise PM2 was mis-applied when a variant appeared to be missing from ExAC, but instead had been filtered out based on a low quality call. Similarly, some regions are not sequenced by exome-capture methods, so variants maybe missed and therefore not be found in exome-based databases. One must check coverage data and whether mapping was reliable before assuming an allele is absent. It should be noted that many of these points of caution relate to the other codes using population databases. For example, BS1 and BS2 also cannot be applied based on frequency data from low quality calls.

A point of confusion arose related to which disease frequency to use when analyzing whether to invoke rule BS1 (the allele frequency of the variant is too common to cause disease) for genes associated with more than one disease. In this case, it was concluded by discussion that if calling a variant benign for all diseases, one must use the most common disease prevalence. On the other hand, one can call a variant benign with respect to a single condition (relevant to the diagnostic test being performed for example), but make no claim on another condition. For example, in the *PTPN11* [MIM:176876] gene, primarily associated with Noonan syndrome [MIM: 163950], a variant may be under consideration for either Noonan syndrome, or, if observed in a patient with cardiomyopathy, could be evaluated with respect to this alternate condition. Because cardiomyopathy is more common, one can either evaluate the variant for all conditions by using the more common condition (cardiomyopathy), or consider its role only in one condition and use that prevalence.

It was common that laboratories used their own criteria to call a variant B or LB when the ACMG/AMP rules were less able to achieve these interpretations and labeled more VUS. For example, the ACMG/AMP rules contain, BA1, a stand-alone rule that if the allele frequency is >5% the variant can be called B. These discrepancies were not unexpected given that the ACMG/AMP rules had to be designed in a generic manner to address all genes and diseases. The 5% criterion is very conservative for highly penetrant alleles, particularly for rare autosomal dominant disorders. However, for very rare diseases, many laboratories have implemented lower cutoffs as stand-alone classification criteria for B and LB classifications, particularly if no other evidence is present to implicate the variant in disease. It was concluded that a widespread effort to define appropriate allele frequency cutoffs for each disease would be a useful resource for the community and aid in improved consistency of variant interpretation. These disease-specific efforts would require expertise to address possible underestimation of disease prevalence and reduced or age-dependent penetrance and take into account the genetic

heterogeneity of each disease to refine a more realistic highest possible allele frequency for a novel pathogenic variant. Special consideration would also be necessary for autosomal recessive conditions where the incidence of disease is not clearly known.

For certain variants laboratories struggled with defining 'fully penetrant' regarding the BS2 line of evidence (the variant is observed in a healthy adult for a disorder with full penetrance at an early age), leading to discordance. For instance some sites applied this rule for gene-condition pairs such as *DSP* [MIM:125647] and cardiomyopathy and *BRCA1* [MIM:113705] and breast cancer; however, pathogenic variants in these genes are not associated with full penetrance or presence at an early age. Consensus concluded that the BS2 rule should not be applied based on individuals in population databases, as these individuals are not well-characterized and could have cardiomyopathy or breast cancer.

Computational and predictive data (PVS1, PS1, PM5, PM4, PP3, BP7 BP4, BP3 BP1)
PVS1, a null variant where LOF is a known mechanism of disease, was incorrectly invoked by laboratories for a variant near the 3' end of the gene that would likely escape nonsense mediated decay[20; 21]. The ACMG/AMP guidelines warn against invoking PVS1 in this context, reinforcing the importance of considering the caveats already published in the guideline for each line of evidence. For some variants, laboratories disagreed on whether or not to apply PVS1 because not all agreed LOF was a known mechanism for the condition. Development of a resource to define which genes LOF is an established mechanism for disease would be useful and improve consistent application of this rule.

One source of discrepancy occurred in one site's usage of PS1, the variant results in the same amino acid as a previously described pathogenic variant regardless of the nucleotide change. One site erroneously used this to apply prior publication of the same exact variant while this rule, as described in more depth on the ACMG/AMP guideline, only applies when the established pathogenic variant has a different nucleotide change than the variant being interpreted. Discussion clarified that analysis of prior cases was more appropriately applied to rule PS4, where multiple occurrences of the same variant in cases can be considered evidence if there is a statistically significant increased occurrence in cases compared to controls. In addition to clarifying the intended usage of PS1, another point of discordance in applying both PS1 and PM5 was in circumstances where the other published variants were not universally agreed to be "well-established" as pathogenic, a requirement for usage of both rules.

For criteria PP3 and BP4 (multiple lines of computational evidence do, or do not, support a deleterious effect on the protein), it was clarified that these lines of evidence can only be invoked when ALL lines of evidence evaluated are consistent (for either missense or splicing evaluation, both is not necessary). This is clear in the more detailed text of the ACMG/AMP guidelines, but is not listed in the brief rule description. Clarifying this point resolved some discordant use of these rules; however, discordance remained when laboratories used different computational programs. Consistent use of these computational lines of evidence would be aided by the development of recommendations for which computational evidence programs are best to use and what thresholds are appropriate. Use of such algorithms and standards may also be gene specific or only applicable to a type of variation. There were several variants that were discordant by ACMG/AMP rule usage simply because laboratories allowed a computational line of evidence that conflicted with strong evidence supporting pathogenicity, to be used to call the variant a VUS. While the ACMG/AMP guidelines do state that conflicting lines of evidence (e.g. segregation supports pathogenicity but functional evidence does not) generally result in a VUS classification, it was noted that professional judgement must be used. All sites agreed that computational predictions of missense or splice variants are well known to

have reduced accuracy and therefore should not be used to override other strong evidence. For example, if a variant has ample criteria to be called pathogenic, it should not be reduced to VUS just because computational algorithms did not predict an impact.

For several variants the definition of “primarily” was a source of discordance with respect to whether or not to invoke BP1 (a missense variant in a gene where primarily truncations cause disease); “primarily” may range from a simple majority (>50%) to nearly all (e.g., >90%). If the former, some laboratories felt that this was not the level of truncations that would lead them to determine a SNV was less likely to cause disease. A member of the ACMG/AMP guideline committee noted that the rule was intended to focus on disorders where all, or nearly all, (e.g. > 90%) were due to truncations. All sites agreed that more quantitative guidance to establish a threshold to invoke this rule would increase pathogenicity classification concordance.

Functional data (PS3, PM1, PP2, BS3)
Whether or not laboratories invoked PS3, well-established functional studies support a damaging effect, was dependent on whether the lab trusted the clinical relevance of the results of such studies. Defining ‘well-established’ was the critical component and familiarity with the assay seemed to be a factor when deciding to invoke this rule. There was considerable variability in the functional study thresholds among groups. After discussion, sites agreed that, at a minimum, the assay must be validated with known pathogenic and benign variants and the output of the assay must have an established mechanistic relevance to the associated phenotype. Developing a resource curated by disease experts which lists functional assays that meet the ‘well-established’ threshold would increase consistency in applying the PS3 rule. Of note, the strength of PS3 can be reduced to moderate or supporting if a lab does not feel confident enough in the functional study to invoke it as a strong line of evidence.

One source of discrepancy identified that the PM1 rule (variant is located in a mutational hot spot and/or a critical and well-established functional domain), should only be invoked for missense variants, not truncations, and it should only be applied if the variant occurs in domains that are devoid of benign variation as described in more detail in the ACMG/AMP guideline. Defining ‘well-established’ also led to discordance in PM1 rule usage. The group defined a mutational hot spot as a location where there are multiple changes in the same domain that are known to be pathogenic; however, there was still disagreement regarding how many pathogenic variants constitute ‘multiple’, how well-defined the domain must be and how close other benign variants can be to the domain and variant in question.

Laboratories used various techniques to determine whether or not to apply PP2, a missense variant in a gene with a low rate of benign missense variation and in which missense variants are a common mechanism of disease. Establishing a quantitative metric for “a low rate” would clarify when to correctly invoke PP2.

Segregation data (PP1, BS4)
PP1, cosegregation in affected family members, and BS4, lack of cosegregation, were inconsistently invoked. This is likely due to the absence of a quantitative metric to establish whether or not these rules apply. For example, one lab invoked PP1 based on a single family with two affected individuals shown to carry a variant, but other sites did not deem this evidence sufficient. PP1 was the most commonly modified line of evidence illustrating that laboratories did consider how many affected individuals in a family tested positive for a variant and/or how many families with the variant showed segregation; however, whether PP1 was made a moderate or strong line of evidence, or modified at all, was based solely on the opinion of the laboratories. As stated above, the ACMG/AMP guidelines support the use of expert judgment when

classifying variants; however, quantitatively analyzing segregation data would increase concordance in using these rules. For one variant (NM_017636.3 (TRPM4) [MIM:606936]: c.2531G>A (p.Gly844Asp)) the segregation data in the literature was from a family with a different phenotype (right bundle branch block, RBBB) than the phenotype of the individual in whom the variant was found (long QT syndrome, LQTS). The group concluded that in this context PP1 could not be invoked since the variant was being interpreted for the LQTS phenotype; however, this data was used to invoke BS2, observed in a healthy adult, since the affected individuals in the literature did not have LQTS, but downgraded it from strong to supporting based on incomplete penetrance of the LQTS phenotype.

De novo data (PS2, PM6)
De novo data was not commonly found for variants reviewed in this project; however, the group did discuss how and when to downgrade PS2, the variant is *de novo* in a patient with a disease and no family history with both maternity and paternity confirmed, for one analyzed variant. The guidelines support invoking PM6 when maternity and/or paternity is not confirmed, and the discussions supported use of PS2 downgraded to moderate if the individual is mosaic for a variant that is thus presumed *de novo* and high enough frequency to be associated with the phenotype. PS2 could also be invoked even if maternity and paternity were not confirmed if there were multiple *de novo* occurrences published or observed (i.e. parental testing was performed but maternity and paternity assessment was either not performed or not documented in the literature.)

Allelic data (PM3, BP2)
Laboratories discussed when to modify the strength of PM3, the variant is seen in *trans* with a pathogenic variant for recessive disorders. Published literature may not always explicitly state the phase of variants found in affected individuals which raises a challenge for invoking PM3. When phase has not been established, some felt that PM3 could be invoked as supporting evidence. Also, if the variant is seen in *trans* with a pathogenic variant in more than one individual it was felt that PM3 can be upgraded to strong. However, sites did not agree on how many additional observations were necessary to call the evidence strong (2 vs. 3) but concluded that such guidance would be useful.

Other databases (PP5, BP6)
Both PP5 and BP6 (a reputable source reports the variant as pathogenic or benign respectively), was commonly invoked incorrectly when any P or B interpretation was present in a database, for example ClinVar. As stated in the ACMG/AMP guideline, these rules should only be invoked when the supporting evidence for the assertions in the database is not available for review, for example interpretations from the Sharing Clinical Reports Project (https://www.clinicalgenome.org/data-sharing/sharing-clinical-reports-project-scrp/) could correctly invoke these rules. Invoking PP5 when the evidence to support the P classification made by the reputable source is the same as the evidence the lab is using to evaluate the variant's pathogenicity would be counting the same evidence twice. Furthermore, it was clarified that ClinVar is not a reputable source itself and this judgement must be placed on the individual ClinVar submitters.

Other data (PP4, BP5)
As noted above, for rules invoked more than 10 times overall, PP4 (the patient's phenotype or family history is highly specific for a disease with a single genetic etiology), was used the most inconsistently. Laboratories discussed several contexts in which PP4 should not be applied. As stated in the ACMG/AMP guidelines, PP4 should not be invoked for cases where the phenotype has locus heterogeneity such as intellectual disability, breast cancer, or hearing loss. This rule

was misapplied by laboratories that interpreted it to mean the gene is known to be associated with the disorder, rather than the gene was the sole gene known to cause the disorder. PP4 will also typically not be invoked when interpreting a variant that has been identified as an incidental finding since it is unlikely the individual has the phenotype specific to the gene of interest.

For two variants, sites discussed and clarified the use of BP5 vs BP2. Invoking BP5 (the variant was found in a case with an alternate molecular basis for disease), requires that a pathogenic variant in a *different* gene has been found in an individual who also has the variant being evaluated. This differs from BP2 (the variant was observed in *trans* with a dominant variant or in *cis* with a recessive variant), which should be invoked if that pathogenic variant is seen in the *same* gene as the variant being evaluated.

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Table S1. Final ACMG classifications for consensus variants and range of classifications for discordant variants

	Variant	Number of sites	Classification consensus or range	How consensus achieved (if applicable)
1	NM_005228.3(*EGFR*)[MIM:131550]:c.2369C>T(p.Thr790Met)	9	Pathogenic	Conference Call
2	NM_000465.3(*BARD1*)[MIM:601593]:c.1075_1095delTTGCCTGAATGTTCTTCACCA(p.Leu359_Pro365del)	3	Benign	Originally agreed
3	NM_000122.1(*ERCC3*)[MIM:133510]:c.325C>T(p.Arg109*)	3	Likely Pathogenic	Originally agreed
4	NM_000546.5(*TP53)* [MIM:191170]:c.743G>A(p.Arg248Gln)	3	Pathogenic	Originally agreed
5	NM_001127510.2(*APC*)[MIM:611731]:c.3920T>A(p.Ile1307Lys)	3	Benign/VUS	NA
6	NM_000038.5(*APC*)[MIM:611731]:c.3386T>C(p.Leu1129Ser)	3	Likely Benign/Benign	NA
7	NM_004360.3(*CDH1*)[MIM:192090]:c.1568A>G(p.Tyr523Cys)	3	VUS	Originally agreed
8	NM_033084.3(*FANCD2*)[MIM:613984]:c.1278+1G>T	3	Likely Pathogenic/VUS	NA
9	NM_000257.3(*MYH7*)[MIM:160760]:c.2717A>G(p.Asp906Gly)	3	Pathogenic/Likely Pathogenic	NA
10	NM_000535.5(*PMS2*)[MIM:600259]:c.1532C>T(p.Thr511Met)	3	Benign	Conference Call
11	NM_003242.5(*TGFBR2*)[MIM:190182]:c.383delA(p.Lys128Serfs*35)	3	Benign	Conference Call
12	NM_001163817.1(*DHCR7*)[MIM:602858]: c.964-1G>C	3	Pathogenic	Originally agreed
13	NM_001042351.2(*G6PD*)[MIM:305900]:c.202G>A (p.Val68Met)	3	Pathogenic	Email
14	NM_031844.2(*HNRNPU*)[MIM:602869]:c.2304_2305del (p.Gly769Glufs*83)	3	Likely Pathogenic	Originally agreed
15	NM_000363.4(*TNNI3*)[MIM:191044]:c.485G>A (p.Arg162Gln)	3	Pathogenic/Likely Pathogenic	NA
16	NM_078485.3(*COL9A1*)[MIM:120210]: c.70C>A (p.Gln24Lys)	3	VUS	Originally agreed
17	NM_024022.2(*TMPRSS3*)[MIM:605511]:c.1152G>T (p.Met384Ile)	3	VUS	Email
18	NM_001089.2(*ABCA3*)[MIM:601615]:c.2614A>G(p.Ser872Gly)	3	VUS	Email
19	NM_000238.3(*KCNH2*)[MIM:152427]:c.442C>T (p.Arg148Trp)	3	VUS	Originally agreed
20	NM_017636.3(*TRPM4*)[MIM:606936]:c.2531G>A (p.Gly844Asp)	9	Likely Benign	Conference Call
21	NM_007294.3(*BRCA1*)[MIM:113705]:c.3119G>A (p.Ser1040Asn)	3	Pathogenic/Likely Pathogenic	NA
22	NM_018848.3(*MKKS*)[MIM:604896]: c.724G>T (p.Ala242Ser)	3	VUS/Likely Benign	NA
23	NM_000546.5(*TP53*)[MIM:191170]:c.455C>T (p.Pro152Leu)	3	Pathogenic	Originally agreed
24	NM_007294.3(*BRCA2*)[MIM:600185]:c.7762_7764delinsTT (p.I2588Ffs*60)	3	Pathogenic	Email
25	NM_000535.5(*PMS2*)[MIM:600259]:c.1096G>C (p.Asp366His)	3	VUS	Originally agreed
26	NM_024642.3(*GALNT12*)[MIM:610290]: c.1278_1293delGTGGTTCTTGGAGACT (p.Trp427Cysfs*23)	3	VUS	Email
27	NM_006231.2(*POLE*)[MIM:174762]:c.2214G>C (p.Lys738Asn)	3	VUS	Originally agreed
28	NM_001943.3(*DSG2*)[MIM:125671]:c.2568A>C (p.Lys856Asn)	3	Likely Benign	Conference Call
29	NM_000059.3(*BRCA2*)[MIM:600185]:c.4779A>C (p.Glu1593Asp)	9	VUS/Likely Benign	NA
30	NM_000138.4(*FBN1*)[MIM:134797]:c.2956G>A (p.Ala986Thr)	3	Likely Benign	Email
31	NM_000540.2(*RYR1*)[MIM:180901]:c.4178A>G (p.Lys1393Arg)	3	Likely Benign	Email
32	NM_000256.3(*MYBPC3*)[MIM:600958]:c.977G>A (p.Arg326Gln)	3	Benign	Originally agreed
33	NM_000540.2(*RYR1*)[MIM:180901]:c.13513G>C (p.Asp4505His)	3	VUS/Likely Benign	NA
34	NM_1048171.1 (*MUTYH*)[MIM:604933]:c.536A>G (p.Tyr179Cys)	3	Pathogenic	Originally agreed
35	NM_003119.23 (*SPG7*)[MIM:602783]:c.1529C>T (p.Ala510Val)	9	Pathogenic/VUS	NA
36	NM_000262.2 (*NAGA*)[MIM:104170]:c.606C>A (p.Tyr202*)	3	Likely Pathogenic	Email
37	NM_003060.3 (*SLC22A5*):c.1463G>A (p.Arg488His)	3	VUS	Email
38	NM_133259.3 (LRPPRC):c.3286delC (p.His1096Thrfs7*)	3	Likely Pathogenic	Email
39	NM_000124.3 (ERCC6):c.3289A>G (p.Met1097Val)	3	Benign	Originally agreed
40	NM_000059.3 (*BRCA2*)[MIM:600185]:c.4061C>T (p.Thr1354Met)	3	Likely Benign/Benign	NA
41	NM_000506.3(*F2*)[MIM:176930]:c.598G>A (p.Glu200Lys)	3	VUS	Originally agreed
42	NM_000060.2 (*BTD*)[MIM:609019]:c.1330G>C (p.Asp444His)	3	Pathogenic/Benign	NA
43	NM_000445.3 (*PLEC*)[MIM:601282]:c.4732C>T (p.Arg1578Cys)	3	VUS	Originally agreed
44	NM_015506.2 (*MMACHC*)[MIM:609831]:c.271dupA, (p.Arg91Lysfs14*)	3	Pathogenic	Originally agreed
45	NM_153717(*BBS2*)[MIM:606151]:c.1864C>T(p.Arg622*)	3	Pathogenic	Email
46	NM_005859(*PURA*)[MIM:600473]:c.698T>C(p.Phe233Ser)	3	VUS	Email
47	NM_078480(*PUF60*)[MIM:604819]:c.436C>T(p.Arg146Cys)	3	VUS	Originally agreed
48	NM_015560(*OPA1*)[MIM:605290]:c.113_130del18(p.R38_S43del)	3	VUS/Likely Benign	NA
49	NM_001197104(*KMT2A*)[MIM:159555]:c.6572G>A(p.Arg2191Gln)	4	Likely Benign	Conference Call
50	NM_004541(*NDUFA1*)[MIM:300078]:c.G94C(p.G32R)	3	Likely Benign/Benign	NA
51	NM_000531(*OTC*)[MIM:300461]:c.118C>T(p.Arg40Cys)	3	Pathogenic/Likely Pathogenic	NA
52	NM_000138(*FBN1*)[MIM:134797]:c.1328-2A>G	3	Pathogenic	Originally agreed
53	NM_004006(*DMD*)[MIM:300377]:c.4233+2C>T	9	Likely Benign	Conference Call
54	NM_000179(*MSH6*)[MIM:600678]:c.4068_4071dupGATT(p.Lys1358fs)	3	Benign	Conference Call
55	NM_000492(*CFTR*)[MIM:602421]:c.3705T>G(p.Ser1235Arg)	3	Benign	Email
56	NM_004992.3(*MECP2*)[MIM:300005]:c.27-6C>G	3	Pathogenic/Likely Pathogenic	NA
57	NM_013334 (*GMPPB*)[MIM:615320]: c.860G>A(Arg287Gln)	3	Pathogenic/Likely Pathogenic	NA
58	NM_000059 (*BRCA2*)[MIM:600185]:delTG (p.V220IfsX3)	3	Pathogenic	Originally agreed
59	NM_000833 (*GRIN2A*)[MIM:138253]:c.4375T>C (p.Ser1459Gly)	9	Pathogenic/Likely Pathogenic	NA
60	NM_005445 (*SMC3*)[MIM:606062]:c.283G>A (p.Glu95Lys)	3	Likely Pathogenic	Originally agreed
61	NM_007325 (*GRIA3*)[MIM:305915]:c.466T>C (Tyr156His)	3	VUS	Originally agreed
62	NM_000138 (*FBN1*)[MIM:134797]:c.8176C>T (Arg2726Trp)	3	VUS/Likely Benign	NA
63	NM_001005463.2 (*EBF3*)[MIM:607407] c.1101+1G>T	3	VUS	Email
64	NM_014801 (*PCNXL2*):c.3526C>T (His1176Tyr)	3	VUS	Email
65	NM_000249 (*MLH1*)[MIM:120436]:c.394G>C (p.Asp132His)	3	Likely Benign	Email
66	NM_000492 (*CFTR*) [MIM:602421]:c.2991G>C (p.Lys997Phe)	3	Benign	Originally agreed
67	NM_144612.6(*LOXHD1*)[MIM:613072]: c.1028G>A (p.Arg343His)	3	Likely Benign	Email
68	NM_000257.2(*MYH7*)[MIM:160760]:c.327C>T (p.Tyr109Tyr)	3	Likely Benign/Benign	NA
69	NM_001114753.1(ENG)[MIM:131195]:c.818C>T (p.Thr273Ile)	3	VUS	Originally agreed
70	NM_024422.3(*DSC2*)[MIM:125645]: c.631-2A>G	3	Pathogenic	Email
71	NM_000169.2(*GLA*)[MIM:300644]:c.639+919G>A	3	Pathogenic	Conference Call
72	NM_001369.2(*DNAH5*)[MIM:603335]:c.7468_7488del (p.Trp2490_Leu2496del)	9	Likely Pathogenic/VUS	NA
73	NM_000484.3(*APP*)[MIM:104760]:c.2137G>A (p.Ala713Thr)	3	Likely Pathogenic/VUS	NA
74	NM_174916.2(*UBR1*)[MIM:605981]:c.4107T>A (p.Cys1369*)	3	Likely Pathogenic	Email
75	NM_000391.3(*TPP1*)[MIM:607998]:c.1678_1679delCT (p.Leu560ThrfsX47)	3	Likely Pathogenic	Email
76	NM_000053.3(*ATP7B*)[MIM:606882]:c.2972C>T (p.Thr991Met)	3	VUS	Originally agreed
77	NM_005633.3(*SOS1*)[MIM:182530]:c.1010A>G (p.Tyr337Cys)	3	VUS	Originally agreed
78	NM_003476.4(*CSRP3*)[MIM:600824]:c.286_287delCC (p.Pro96fs)	3	VUS	Conference Call

79	NM_000142.4(*FGFR3*)[MIM:134934]:c.2310C>G (p.Tyr770*)	3	VUS	Originally agreed
80	NM_001001431(*TNNT2*)[MIM:191045]:c.391C>T (p.Arg131Trp)	3	Pathogenic/Likely Pathogenic	NA
81	NM_001142605.1(*EFTUD2*)[MIM:603892]:c.1258G>A (p.Gly420Ser)	3	Benign/VUS	NA
82	NM_ 032335.3(*PHF6*)[MIM:300414]:c.865A>G (p.Thr289Ala)	3	VUS	Originally agreed
83	NM_022068.2(*PIEZO2*)[MIM:613629]: c.8057G>A (p.Arg2686His)	3	Pathogenic	Originally agreed
84	NM_000552.3(*VWF*):[MIM:613160]c.6937C>T (p.Arg2313Cys)	3	VUS	Conference Call
85	NM_001008844.1(*DSP*)[MIM:125647]:c.3701A>T (p.Glu1234Val)	9	Likely Benign/Benign	NA
86	NM_000321.2(*RB1*)[MIM:614041]:c.920C>T (p.Thr307Ile)	3	Likely Benign/Benign	NA
87	NM_198056.2(*SCN5A*)[MIM:600163]:c.3956G>T (p.Gly1319Val)	3	Likely Pathogenic	Conference Call
88	NM_003620.3(*PPM1D*)[MIM:605100]:c.1437dupT(p.Lys480fs)	3	Pathogenic	Conference Call
89	NM_000069.2 (*CACNA1S*)[MIM:114208]:c.4060A>T (p.Thr1354Ser)	3	VUS	Originally agreed
90	NM_000540.2 (*RYR1*)[MIM:180901]:c.1840C>T (p.Arg614Cys)	3	Pathogenic	Originally agreed
91	NM_144997.5 (*FLCN*)[MIM:607273]:c.1285dupC (p.His429Profs*27)	3	Pathogenic	Email
92	NM_000257.2 (*MYH7*)[MIM:160760]:c.2359C>T (p.Arg787Cys)	3	VUS	Conference Call
93	NM_001103.2 (*ACTN2*)[MIM:102573]:c.26A>G (p.Gln9Arg)	3	VUS	Originally agreed
94	NM_133378.4 (*TTN*)[MIM:188840]:c.94398G>A (p.Asp31467Asn)	3	VUS/Likely Benign	NA
95	NM_015311.1 (*OBSL1*)[MIM:610991]:c.4951G>T (p.Glu1651*)	3	VUS	Originally agreed
96	NM_015166.3 (*MLC1*)[MIM:605908]:c.353C>T (p.Thr118Met)	3	Pathogenic/Likely Pathogenic	NA
97	NM_000018.2 (*ACADVL*)[MIM:609575]:c.1844G>A (p.Arg615Gln)	3	VUS/Likely Benign	NA
98	NM_007103.3 (*NDUFV1*)[MIM:161015]:c.753_756del (p.Pro252Glnfs*44)	9	Likely Pathogenic	Conference Call
99	NM_000057.2 (*BLM*)[MIM:210900]:c.2603C>T (p.Pro868Leu)	3	Benign	Conference Call

Table S2. Mean and standard deviation of the coefficient of variation for all lines of evidence

Line of Evidence	Used	Mean CV	SD CV
BP3	1	3.00	.
PP2	9	2.07	0.54
BP1	5	1.81	0.76
PM6	6	1.80	0.68
PP4	30	1.74	0.58
BS3	8	1.73	0.00
BS4	9	1.71	0.08
BP5	11	1.71	0.19
PS1	23	1.68	0.54
BS2	19	1.62	0.52
PM1	25	1.58	0.65
PM5	5	1.56	0.39
PM4	11	1.53	0.75
PS4	21	1.52	0.36
BP2	10	1.51	0.79
PM3	15	1.44	0.40
PP5	31	1.43	0.61
BP6	22	1.36	0.45
BA1	10	1.30	0.74
BP4	28	1.27	0.68
PM2	71	1.19	0.78
PP3	65	1.08	0.80
PS1	11	1.08	0.80
BS2	41	1.08	0.67
PP1	21	1.06	0.56
PS3	29	1.06	0.67
PVS1	25	0.55	0.65