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A commentary on Actionable secondary findings in the 73 ACMG-recommended genes in 1,559 Thai exomes

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We read the article by Chetruengchai and Shotelersuk [1] with interest as it provided an estimate of ACMG v3.0 secondary findings [2] in an unselected Thai population. In a cohort of 1,559 unrelated individuals a finding of 22 pathogenic/likely pathogenic variants was asserted in 15 genes associated with 13 diseases in 85 individuals for a secondary variant rate of 5.5%. We question the validity of this claim, based on our understanding of current variant classification standards [3]. The 22 variants detailed in Chetruengchai and Shotelersuk as secondary findings were classified as pathogenic (P) or likely pathogenic (LP) by VarSome [4] and at least one submitter in ClinVar [5], without further assessment by the authors. It is widely recognized that a substantial number of individual classifications in ClinVar are incorrect and that they must be reviewed to confirm evidence is sufficient to support the final classification and applicable to the phenotype in question. While tools such as VarSome are useful in providing evidence that can inform the ACMG/AMP/ClinGen criteria, VarSome is not a replacement for expert opinion and does not strictly adhere to ACMG/AMP/ClinGen guidance. Final classification as to whether a variant is P/LP should be determined after a review of all available data using the relevant ACMG/AMP/ClinGen standards [3,6]. In addition to using tools such as VarSome and ClinVar, mining primary data that support or refute criteria relevant to ACMG/AMP pathogenicity classification is critical to providing the best possible variant classification, based on the current state of knowledge. The primary literature and variant databases can inform criteria including PS2/PS4/PP4/BS2/BP2/BP5 (case information), PP1/BS4 (segregation) and PS3/BS3 (functional data). The individual classifying the variant must confirm that sufficient data have been identified and are correctly applied to the ACMG criteria.

It is also important to use the current ACMG/AMP/ClinGen guidance on application of evidence and the final combining rules when classifying variants. Chetruengchai and Shotelersuk assigned PVS1 and PP3 in combination to several variants, however, PVS1 assumes loss of function and thus no additional weight should be awarded for the prediction

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Conflict of Interest Notification Page

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Supplementary information is available at Journal of Human Genetics website.

of such (PP3, bioinformatic prediction) [7]. Several variants that are relatively common in gnomAD (popmax maf>0.1%) were assigned criterion PM2, which gives weight for rarity in the population. For variants in *KCNQ1*, *SCN5A,* and *RYR1* it appears that PM2 was assigned based on autosomal recessive inheritance (allowing a higher minor allele frequency) even though the associated disorders on the ACMG SF v3.0 gene list demonstrate autosomal dominant inheritance. For genes that are associated with multiple phenotypes the variant scientist must consider the phenotype under consideration when assigning criteria [2,8]. As well, variants were not strictly classified using either the combining rules as presented in Richards et al. or the Bayes combining metric [3,9]. Variant classifiers that conform to current ACMG/AMP/ClinGen standards will be instrumental in allowing larger data sets to be analyzed although providers will still hold responsibility for final classifications.

We have reviewed the 22 variants classified as P/LP and mined the primary literature to identify case and functional data. When available, we have used gene-specific criteria as presented by ClinGen variant curation expert panels (*MYH7* [10], *LDLR*, *RYR1* [11]). This approach mirrors current variant classification standards. Overall, by correctly applying Richards et al and relevant updates, 15 of the 22 variants were reclassified as VUS/LB. Seven variants (24 individuals) remained classified as P/LP (Table 1) for a rate of secondary variant return of 24/1,559 or 1.5%. Using the Bayes combining metric, three additional variants were classified as LP for a rate of secondary variant return of 35/1,559 or 2.2%. Of course, we cannot re-interpret variants classified as VUS in their data set because those were not individually listed. It is entirely possible that additional variants would be reclassified as P/LP with additional analysis increasing the secondary variant return rate above 2.2%.

Analyses of exome and genome data for secondary findings provide the opportunity to identify variants that, when returned to patients, can improve health outcomes by making them aware of undiagnosed disorders and/or increased risk allowing them to pursue medical treatment and/or increase screening [8]. It is essential in this process to classify variant pathogenicity as accurately as possible given current knowledge. Currently it is suggested that variants with a pathogenicity likelihood of >90% (likely pathogenic) be returned as secondary variants [3]. The American College of Medical Genetics and Genomics (ACMG) has provided general guidance on which genes and associated disorders should be considered for secondary variant return and ACMG/AMP have specified criteria that should be considered for variant classification. ClinGen is working toward refining and clarifying guidance (PVS1 [7], PP1 [10], PM2) and specifying gene-specific criteria where necessary. Variant scientists must review the relevant primary literature and understand the limitations of the tools they use to provide the most accurate variant classification possible. This also applies to scientific publications that evaluate the returnable yield of variants from the ACMG secondary findings recommendations. Furthermore, when publishing variant classifications authors should consider providing the raw data that support their conclusions to allow others to critically assess the evidence. We appreciate that Chetruengchai and Shotelersuk provided these data for their P/LP variants (their Table 1), as it allowed us to critically evaluate their classifications. As variant interpretation is an evolving science, and new data are continually being discovered, variant classifications may change over time.

However, it is essential that current best practices and all readily available data are used when classifying variant pathogenicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Variants presented as pathogenic/likely pathogenic by Chetruengchai and Shotelersuk. ACMG pathogenicity classifications are presented from the original manuscript along with adjusted ACMG criteria and resulting classifications using both Richards et al. and the Bayes combining method.

Gene	Genomic Position	cDNA	Protein	# of Individuals	ACMG Criteria Presented[a]	Classification Presented	Richards et al.	ClinVar Classification[b]	GnomAD PopMax	ACMG Criteria[c] Post Literature Review	Reclassified Richards	Reclassified Tavtigian Bayes
BRCA1	chr17:41244913	NM_007300.4: c.2635G>T	p.(Glu879*)	1	PVS1, PM2, PP3, PP5	P	P	P	not in gnomAD	PVS1, PS4, PM2_Su	P	P
PALB2	chr16:23641062	NM_024675.4: c.2411_2412del	p.(Ser804Cysfs*10)	1	PVS1, PM2, PP5	P	P	P/LP	SAS, maf=0.00011291	PVS1, PS4	P	P
PTEN	chr10:89720649	NM_000314.8: c.802–2A>G	p.(?)	6	PVS1, PM2, PP3, PP5	P	P	P	not in gnomAD	PVS1, PS4_Su, PM2_Su[e]	P[e]	P[e]
TGFBR2	chr3:30713619	NM_003242.6: c.944C>T	p.(Thr315Met)[d]	19	PM1, PP2, PP3, PP5, BS2	LP	VUS	CIP; B(3), LB (5), LP (1), VUS(2)	EAS, maf=0.014436	PM1, BS1	VUS	LB
DSP	chr6:7579930	NM_004415.4: c.3507C>A	p.(Tyr1169*)	9	PVS1, PM2, PP3, PP5	P	P	LP	not in gnomAD	PVS1, PM2_Su[e]	VUS	LP[e]
PKP2	chr12:32994140	NM_004572.4: c.1511–1G>C	p.(?)	1	PVS1, PM2, PP3, PP5	P	P	P	not in gnomAD	PVS1, PM2_Su	VUS	LP
RYR2	chr1:237540658	NM_001035.3: c.499A>G	p.(Lys167Glu)	1	PM1, PM2, PP3, PP5	LP	LP	LP	not in gnomAD	PM1, PM2_Su, PP3	VUS	VUS
TNNT2	chr1:201328372	NM_001276345.2: c.863G>C	p.(Arg288Pro)	6	PM2, PM5, PP2, PP3	LP	LP	CIP; LP(4), P (1), VUS(1)	not in gnomAD	PS4_M, PM2_Su[e], PP3	VUS	VUS
TTN	chr2:179418821	NM_001256850.1: c.84094C>T	p.(Arg28032*)	1	PVS1, PM2, PP3, PP5	P	P	P/LP	not in gnomAD	PVS1, PM2_Su	VUS	LP
TTN	chr2:179415988	NM_001256850.1: c.86348–1G>A	p.(?)	1	PVS1, PM2, PP3, PP5	P	P	LP	not in gnomAD	PVS1_M, PM2_Su	VUS	VUS
LDLR	chr19:11213463	NM_000527.5: c.313+1G>A	p.(?)	1	PVS1, PM2, PP3	P	P	CIP; LB(1), LP (2), P(17)	NFE, maf=0.00006156	PVS1_St, PS4, PP1_St, PM2_Su, PS3_M, PP4	P	P

Gene	Genomic Position	cDNA	Protein	# of Individuals	ACMG Criteria Presented[a]	Classification Presented	Richards et al.	ClinVar Classification[b]	GnomAD PopMax	ACMG Criteria[c] Post Literature Review	Reclassified Richards	Reclassified Tavtigian Bayes
LDLR	chr19:11215926	NM_000527.5: c.344G>A	p.(Arg115His)	1	PM1, PM2, PP2	LP	VUS	CIP; LB(1), LP(2), P(1), VUS (3)	EAS, maf=0.0022597	PS3	VUS	VUS
LDLR	chr19:11227576	NM_000527.5: c.1747C>T	p.(His583Tyr)	3	PM1, PM2, PM5, PP2, PP3	P	LP	CIP; LB(1), LP(5), P(7), VUS (1)	EAS, maf=0.0012029	PS3_Moderate, PS4 (founder), PM3, PP1_M, PP3	P	P
LDLR	chr19:11221443	NM_000527.5: c.1056C>A	p.(Cys352*)	10	PVS1, PM2, PP3, PP5	P	P	P/LP	not in gnomAD	PVS1, PS4_Su, PM2_Su	P	P
MYBPC3	chr11:47367848	NM_000256.3: c.1000G>A	p.(Glu334Lys)	16	PM2, PP2, PP3, PP5	LP	VUS	CIP; B(2), LP(1), P(2), VUS(6)	EAS, maf=0.0033385	PS4, PP3, BS1	VUS	VUS
MYH7	chr14:23894566	NM_000257.4: c.2348G>A	p.(Arg783His)	1	PM1, PM2, PM5, PP2, BP4	LP	LP	CIP; LP(3), VUS (3)	AFR, maf=0.000040054	PM1, PM2_Su, BP4	VUS	VUS
KCNQ1	chr11:2608860	NM_000218.3: c.1189C>T	p.(Arg397Trp)	1	PM2, PM5, PP2, PP3	LP	LP	CIP; B(1), LP (3), VUS(9)	NFE, maf=0.0003021; AJ, maf=0.00086839	PS4_M, PS3_Su, PP3, BS1	VUS	VUS
KCNQ1	chr11:2594172	NM_000218.3: c.877C>T	p.(Arg293Cys)	1	PM2, PP2, PP3, PP5	LP	VUS	CIP; LP(1), VUS (5)	SAS, maf=0.0001307	PP3	VUS	VUS
SCN5A	chr3:38655260	NM_001160161.2: c.677C>T	p.(Ala226Val)	1	PM1, PM2, PP2, PP3, PP5	LP	LP	CIP; B(1), LB(4), LP (1), VUS(5)	EAS, maf=0.0013628	PS4, PM1, PP3, BS1	VUS	VUS
SCN5A	chr3:38603958	NM_001160161.2: c.3749C>T	p.(Thr1250Met)	1	PM1, PM2, PP2, PP3, PP5	LP	LP	CIP; B(1), LP(2), VUS(11)	NFE, maf=0.00031312	PM1, PP1, PP3, BS1	VUS	VUS
RYR1	chr19: 38951020	NM_000540.3: c.2366G>A	p.(Arg789Gln)	1	PM1, PM2, PP2, PP3	LP	LP	CIP; LB(10), LP (1), VUS(2)	AMR, maf=0.0033578	BS1	LB	LB
RYR1	chr19: 38931469	NM_000540.3: c.130C>T	p.(Arg44Cys)	2	PM2, PP2, PP3, PP5	LP	VUS	CIP; LP (3), P(1), VUS(8)	AMR, maf=0.000029433	PS4_M, PS3_M, PM1, PP3_M	LP	LP

[a] Criteria not applicable are shown in grey.

[b] CIP, Conflicting interpretations of pathogenicity; Pathogenic, P; Likely Pathogenic, LP; Variant of Uncertain Significance, VUS; Likely Benign, LB.

[c] For modified strength levels: St, Strong; M, moderate; Su, supporting.

[d] Alternate nomenclature NM_001024847.2:c.1019C>T; NP_001020018.1:p.Thr340Met.

[e] Variant allele frequency higher than expected in the sample set, correct application of PM2/BS1 requires more information regarding cohort.