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The Forefront of Genomics

Specification of frequency criteria for secondary findings genes to improve classification concordance

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Abstract

ACMG policy recommends that secondary findings (SF) from clinical sequencing be offered to individuals. Toward standardizing this process, the ACMG Secondary Findings Working Group curates a list of genes for variant return, v3.2 contains 81 genes. In 2015, the ACMG/AMP put forward criteria for classifying the pathogenicity of variants applicable to mendelian disease. Prior work has shown discordance in variant classification amongst laboratories using these classification criteria. To increase concordance, ClinGen is providing additional guidance through the Sequence Variant Interpretation group and Variant Curation Expert Panels (VCEPs). However, 50 genes suggested for SF return do not currently have published specified criteria. Having specified criteria for SF genes is important as laboratories are expected to classify variants in these genes for SF return whether or not they have specialized expertise in the gene. Classification concordance could be increased by providing basic specified criteria. Our goal is to provide thresholds for BA1 (stand-alone benign) and BS1 (allele frequency greater than expected for disorder) along with guidance for application of PVS1 (loss of function) prior to VCEP criteria publication. Disease frequency, gene contribution and penetrance values identified from the literature were used to define a maximum allele frequency for pathogenic variants (maxAF). It is reasonable, for genes without a VCEP, to define a more stringent BA1 at 10X the maxAF value. We have compared calculated maxAF and BA1 values with popmax filtering allele frequencies (FAF) of the most common P/LP variants in gnomAD v4.1.0. P/LP variants were defined as variants with a ClinVar classification of P/LP or novel loss of function variants in the MANE transcript for genes with evidence supporting haploinsufficiency. For eight genes without published VCEP BA1 values, the popmax FAF of the most common P/LP variant was > the calculated maxAF, none were > the calculated BA1. For BS1, we suggest using a value 3X the maxAF when published VCEP criteria are not available. Four genes, *BTD*, *CACNA1S*, *RET*, and *TTR*, had a known P/LP variant with a popmax FAF > the calculated BS1. For PVS1, we have used the ClinGen dosage sensitivity data and variant information from HGMD and ClinVar to determine if loss of function variants contribute to disease. Specification of basic criteria should allow for improved classification concordance for the return of secondary findings.

Introduction

- Defined criteria for variant classification allow for increased concordance
- General criteria defined by ACMG/AMP/ClinGen
- ClinGen VCEPs specifying criteria for individual monogenic disorders
- Fifty genes (beyond HFE) on the SF v3.2 list do not have VCEP specified guidance

- Setting BA1, BS1, and PVS1 should increase classification concordance for monogenic disorders included on the SF v3.2 list

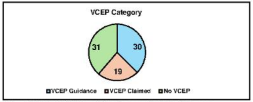

Figure 1. State of VCEP specified criteria availability for SF genes.

Methods

- Identify published VCEP guidance for monogenic disorders recommended for return as SF
- Work with VCEPs to identify unpublished guidance
- Identify disease frequency, gene contribution and penetrance values from the literature to calculate the maximum pathogenic allele frequency (maxAF) for SF monogenic disorder
- When multiple monogenic disorders are listed for a gene on the ACMG SF list, the provided maxAF for the gene will be the maximum as determined for all associated disorders

$$\text{maxAF (AD)} = \frac{\text{Disease frequency x Gene Contribution}}{\text{Penetrance}} \times 0.5$$

- Calculate BA1 (10x maxAF) and BS1 (3x maxAF)

- Use ClinGen dosage sensitivity data and variant information from HGMD to inform use of PVS1

References

1. Green, *et al. Genet Med*, 2013
2. Miller, *et al. Genet Med*, 2023
3. Richards, *et al. Genet Med*, 2015
4. Amendola, *et al. Am J Hum Genet*, 2020
5. Rehm, *et al. N Engl J Med*, 2015

Results

- Forty-seven SF genes had calculated BA1 < 0.05
- PopMax AF for common P/LP variants identified in gnomAD were < calculated gene specific BA1
- PopMax AF for common P/LP variants identified in gnomAD were < gene specific maxAF for 42/50 genes
- Four P/LP variants had PopMax AF > BS1 (3x maxAF)
  - NM_001370658.1(*BTD*):c.1270G>C; p.Asp424His
  - NM_000069.2(*CACNA1S*):c.520C>T; p.Arg174Trp
  - NM_020975.4(*RET*):c.2410G>A; p.Val804Met
  - NM_000371.4(*TTR*):c.424G>A; p.Val142Ile
- PVS1 is recommended for 32 genes without restrictions and for five additional genes with caution

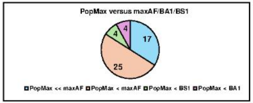

Figure 2. Comparison of calculated maxAF/BA1/BS1 for 50 SF genes without VCEP guidance to popmax values in gnomAD for P/LP variants in these genes. (<< maxAF defined as < 0.1x maxAF).

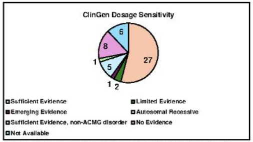

Figure 3. ClinGen dosage sensitivity categories for 50 SF genes without VCEP guidance.

Future Directions

- Provide BA1/BS1/PVS1 values to the field
- Update values as VCEPs contribute expert opinion
- Provide BA1/BS1/PVS1 for new SF genes as the ACMG list expands

Table 1. Data for BA1/BS1/PVS1 for SF monogenic disorders included on the ACMG v3.2 list.

Monogenic Disease	Calculated maxAF	BA1 (10x maxAF)	BS1(3x maxAF)	gnomAD P/LP Variants with Highest PopMax MAF	gnomAD PopMax MAF	Comparison of BA1/BS1/maxAF to PopMax	ClinGen Dosage Sensitivity HGMD pLoF^	PVS1-Applicable
ACTA2- related Loeys-Dietz syndrome ACTA2- related familial thoracic aortic aneurysm and dissection	0.00034	0.0034	0.001	p.Arg118Gln (P/LP)	0.00000028	PopMax<<maxAF	Limited Evidence, 9 pLoF reported for TAAD	Yes, With Caution, consider reducing strength
ATP7B- related Wilson disease	0.0056	0.056	0.017	p.Met645Arg (P/LP)	0.0021	PopMax<maxAF	Autosomal Recessive, many pLoF	Yes
BAG3- related dilated cardiomyopathy	0.00075	0.0075	0.0023	c.72del (P/LP)	0.0000076	PopMax<<maxAF	Sufficient Evidence	Yes
BMPR1A- related juvenile polyposis syndrome	0.0000097	0.000097	0.000029	p.Arg361X (P/LP)	0.00000028	PopMax<<maxAF	Sufficient Evidence	Yes
BTD- related biotinodase deficiency	0.0056	0.056	0.017	p.Gln436His (P/LP) p.Asp424His (P/LP&)	0.00084 0.042	PopMax<BA1	Autosomal Recessive, many pLoF	Yes
CACNA1S- related malignant hyperthermia susceptibility	0.00005	0.0005	0.00015	p.Arg1086Ser (P/LP) p.Arg174Trp (Drug Response&)	0.00023 0.00033	PopMax<BA1	No Evidence, pLoF reported for non-ACMG disorder	No
CALM1- related long QT syndrome	0.000005	0.00005	0.000015	NA	NA	PopMax<maxAF	No Evidence	No
CALM2- related long QT syndrome	0.000005	0.00005	0.000015	NA	NA	PopMax<maxAF	No Evidence	No
CALM3- related long QT syndrome	0.000005	0.00005	0.000015	NA	NA	PopMax<maxAF	No Evidence	No
CASQ2- related catecholaminergic polymorphic ventricular tachycardia	0.0022	0.022	0.0067	p.Pro308Leu (P/LP)	0.000066	PopMax<<maxAF	Autosomal Recessive, many pLoF	Yes
COL3A1- related Ehlers-Danlos syndrome, vascular type	0.00001	0.0001	0.00003	p.Gly666Ser (P/LP)	0.000005	PopMax<maxAF	Sufficient Evidence	Yes
DES- related dilated cardiomyopathy	0.00025	0.0025	0.00075	p.Lys449Thr (P/LP)	0.00000028	PopMax<<maxAF	NA, pLoF reported for non-ACMG disorder	No
DSC2- related arrhythmogenic right ventricular cardiomyopathy	0.000033	0.00033	0.0001	p.Trp69X (P/LP)	0.000036	PopMAx<BS1	Limited Evidence, many pLoF	With Caution, consider reducing strength
DSG2- related arrhythmogenic right ventricular cardiomyopathy	0.00043	0.0043	0.0013	c.523+1G>A (P/LP)	0.000061	PopMax<maxAF	Emerging Evidence, many pLoF	With Caution, consider reducing strength
DSP- related arrhythmogenic right ventricular cardiomyopathy DSP- related dilated cardiomyopathy	0.00065	0.0065	0.00195	p.Leu1619TrpfsX (P/LP)	0.000043	PopMax<<maxAF	Sufficient Evidence	Yes
FLNC- related dilated cardiomyopathy	0.001	0.01	0.003	p.Gln1522X (P/LP)	0.0000037	PopMax<<maxAF	Sufficient Evidence	Yes
GLA- related hypertrophic cardiomyopathy GLA- related dilated cardiomyopathy	0.0008	0.008	0.0024	p.Asn215Ser (P/LP)	0.000031	PopMax<<maxAF	Sufficient Evidence	Yes
KCNH2- related long QT syndrome	0.00015	0.0015	0.00046	c.78+2T>G (P/LP)	0.0000091	PopMax<<maxAF	Sufficient Evidence	Yes
KCNQ1- related long QT syndrome	0.0002	0.002	0.0006	p.Cys122LeufsX (P/LP)	0.00017	PopMAx<maxAF	Sufficient Evidence	Yes
LMNA- related dilated cardiomyopathy	0.0015	0.015	0.0045	p.Arg48Gly (P/LP)	0.000044	PopMax<<maxAF	Sufficient Evidence	Yes
MAX- related hereditary paraganglioma- pheochromocytoma syndrome	0.000032	0.00032	0.000096	p.Tyr71X (P/LP)	0.0000062	PopMax<maxAF	Sufficient Evidence	Yes
MEN1- related multiple endocrine neoplasia	0.000095	0.00095	0.00028	p.Arg516ProfsX (P/LP)	0.0000012	PopMax<<maxAF	Sufficient Evidence	Yes
MUTYH- related familial adenomatous polyposis	0.0071	0.071	0.021	p.Gly368Asp (P/LP)	0.0058	PopMax<maxAF	Autosomal Recessive, many pLoF	Yes
MYH11- related Loeys-Dietz syndrome MYH11- related familial thoracic aortic aneurysm and dissection	0.000016	0.00016	0.000048	NA	NA	PopMax<maxAF	No Evidence, pLoF reported for non-ACMG disorder	No
NF2- related neurofibromatosis	0.00001	0.0001	0.00003	p.Arg588X (P/LP)	0.0000029	PopMax<maxAF	Sufficient Evidence	Yes
PKP2- related arrhythmogenic right ventricular cardiomyopathy	0.0012	0.012	0.0037	c.2014-1G>C (P/LP)	0.000087	PopMax<<maxAF	Sufficient Evidence	Yes
PRKAG2- related hypertrophic cardiomyopathy	0.00001	0.0001	0.00003	p.Arg302Gln (P/LP)	0.00000028	PopMax<<maxAF	No Evidence, no pLoF reported	No
RB1- related retinoblastoma	0.000013	0.00013	0.00004	p.Arg7GlufsX (P/LP)	0.0000041	PopMax<maxAF	Sufficient Evidence	Yes
RBM20- related dilated cardiomyopathy	0.0005	0.005	0.0015	p.Arg636His (P/LP)	0.00000029	PopMax<<maxAF	NA, many pLoF reported for DCM	With Caution, consider reducing strength
RET- related multiple endocrine neoplasia RET- related familial medullary thyroid cancer	0.000029	0.00029	0.000086	p.Ser891Ala (P/LP) p.Val804Met (P/LP&)	0.000013 0.00011	PopMax<BA1	NA, pLoF reported for non-ACMG disorder	No
RYR2- related catecholaminergic polymorphic ventricular tachycardia	0.000044	0.00044	0.00013	p.Gly357Asp (P/LP)	0.000032	PopMax<maxAF	No Evidence, Few pLoF	No
SCN5A- related long QT syndrome SCN5A- related Brugada syndrome	0.00025	0.0025	0.00075	p.Arg225Trp (P/LP)	0.00011	PopMax<maxAF	Sufficient Evidence	Yes
SDHAF2- related hereditary paraganglioma- pheochromocytoma syndrome	0.000008	0.00008	0.000024	p.Arg77GlyfsX (P/LP)	0.0000029	PopMax<maxAF	Sufficient Evidence	Yes
SDHB- related hereditary paraganglioma- pheochromocytoma syndrome	0.0002	0.002	0.0006	p.Asp74Ala (P/LP)	0.000023 0.00018	PopMax<maxAF	Sufficient Evidence	Yes
SDHC- related hereditary paraganglioma- pheochromocytoma syndrome	0.00008	0.0008	0.00024	p.Tyr126Cys (P/LP)	0.000052	PopMax<maxAF	Sufficient Evidence	Yes
SDHD- related hereditary paraganglioma- pheochromocytoma syndrome	0.000047	0.00047	0.00014	p.Pro81Leu (P/LP)	0.000029	PopMax<maxAF	Sufficient Evidence	Yes
SMAD3- related Loeys-Dietz syndrome SMAD3- related familial thoracic aortic aneurysm and dissection	0.000032	0.00032	0.000096	p.Pro182LeufsX (P/LP)	0.00000029	PopMax<<maxAF	Sufficient Evidence	Yes
SMAD4- related juvenile polyposis syndrome	0.0000097	0.000097	0.000029	p.Arg496Cys (P/LP)	0.0000012	PopMax<maxAF	Sufficient Evidence	Yes
STK11- related Peutz-Jeghers syndrome	0.00002	0.0002	0.00006	NA	NA	PopMax<maxAF	Sufficient Evidence	Yes
TGFBR1- related Loeys-Dietz syndrome TGFBR1- related familial thoracic aortic aneurysm and dissection	0.00008	0.0008	0.00024	p.Gly174Val (P/LP)	0.000013	PopMax<maxAF	Sufficient Evidence, LoF cause non-ACMG disorder	No
TGFBR2- related Loeys-Dietz syndrome TGFBR2- related familial thoracic aortic aneurysm and dissection	0.00008	0.0008	0.00024	p.Arg537His (P/LP)	0.0000068	PopMax<<maxAF	No Evidence	With Caution, consider PVS1_Strong for truncations between aa 480 and 535
TMEM127- related hereditary paraganglioma- pheochromocytoma syndrome	0.00004	0.0004	0.00012	c.245-1G>C (P/LP)	0.000044	PopMAx<BS1	Sufficient Evidence	Yes
TMEM43- related arrhythmogenic right ventricular cardiomyopathy	0.000005	0.00005	0.000015	NA	NA	PopMax<maxAF	NA, pLoF not reported	No
TNNC1- related dilated cardiomyopathy	0.00025	0.0025	0.00075	p.Ala8Val (P/LP)	0.0000069	PopMax<<maxAF	NA, Single pLoF reported as DM	No
TRDN- related catecholaminergic polymorphic ventricular tachycardia TRDN- related long QT syndrome	0.0032	0.032	0.0095	p.Asp18AlafsX (P/LP)	0.00063	PopMax<maxAF	Autosomal Recessive, many pLoF	Yes
TSC1- related tuberous sclerosis	0.000022	0.00022	0.000067	c.2626-3_2626-2del (pLoF)	0.000024	PopMAx<BS1	Sufficient Evidence	Yes
TSC2- related tuberous sclerosis	0.000059	0.00059	0.00018	p.Arg951SerfsX (pLoF)	0.0000074	PopMax<maxAF	Sufficient Evidence	Yes
TTN- related dilated cardiomyopathy	0.001	0.01	0.003	p.Lys25728X (P/LP)	0.00015	PopMax<maxAF	Sufficient Evidence	Yes
TTR- related hereditary transthyretin amyloidosis	0.0019	0.019	0.0056	p.Thr79Ala (P/LP) p.Val142Ile (P/LP&)	0.00039 0.016	PopMax<BA1	NA	No
WT1- related Wilms tumor	0.0000034	0.000034	0.00001	p.Arg374X (P/LP)	0.0000055	PopMAx<BS1	Sufficient Evidence	Yes

NA, not available; "<<", PopVAx < 0.1x maxAF; &, variant is known common variant, reduced penetrance; ^, for genes with sufficient evidence for haploinsufficiency HGVD pLoF are not detailed.