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Updated Recommendation for the Benign Stand Alone ACMG/AMP Criterion

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Abstract

The Clinical Genome Resource (ClinGen) Sequence Variant Interpretation working group set out to refine the ACMG/AMP variant pathogenicity recommendations for standalone rule BA1 (a variant with minor allele frequency (MAF) >0.05 is benign), by clarifying how it should be used and specifying a set of variants that should be exempted from this rule. We cross-referenced ClinVar and ExAC to identify variants for which there was a plausible argument for pathogenicity and the variant exists in one or more population datasets at MAF >0.05. We identified nine such variants that were present in these datasets that may not be benign. The ACMG/AMP criteria were applied to these variants that resulted in four pathogenic and five variants of uncertain significance. We have refined benign rule BA1 by clarifying terms used to describe its use, which databases we recommend using, and assumptions made about this rule. We also recognized an initial list of nine variants for which there was some evidence of pathogenicity even though the MAF was high for these variants. We specify processes whereby individuals can petition ClinGen

for amendments to our variant-specific assertions and the criteria experts should use when setting a numerically lower threshold for BA1 for specific genes.

INTRODUCTION

In 2015 the American College of Medical Genetics and Genomics and the Association of Molecular Pathologists (ACMG/AMP) promulgated recommendations for the assessment of pathogenicity of variants in clinical testing, as applied to Mendelian disorders (Richards et al., 2015). These recommendations have been helpful to the field by codifying and organizing the thinking process that goes on in a clinical testing laboratory to assess the pathogenicity of variants from genetic and genomic testing. The recommendations were recognized as a starting point and it was expected that they would evolve and be refined over time. The NIH-funded Clinical Genome Resource (ClinGen) consortium was formed in 2013 to develop standards and processes for evaluating genes and genomic variation with an eye toward clinical validity and utility (Rehm et al., 2015). The ClinGen consortium has several working groups, one of which is the “Sequence Variant Interpretation” (SVI) working group, whose goal is to refine, extend, and evolve the 2015 ACMG/AMP pathogenicity criteria to further increase their utility to the field (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/). The SVI working group was charged with refining and evolving guidelines from the ACMG/AMP 2015 recommendations and to support a consultation or harmonization function for other groups, such as gene- or disease-specific working groups that may develop approaches to the interpretation of variants.

An early criterion that the SVI working group set out to evaluate and address was the BA1 criterion, the ‘standalone’ benign criterion. The original wording of this criterion was: “Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium”. We propose changing this wording to a more precise formulation and developing a list of variants that merit exceptions to this criterion, that is, variants for which there is at least some evidence of pathogenicity and for which laboratories should consider other types of evidence beyond population frequency.

METHODS

The Sequence Variant Interpretation (SVI) Working Group of ClinGen (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/) , represented by clinical geneticists, bioinformaticians, genetic counselors and clinical laboratory geneticists, has held monthly meetings since 2015 to refine the ACMG/AMG guidelines. The SVI group discussed the BA1 as well as other ACMG/AMP rules at multiple conference calls and in-person meetings. The final modified BA1 guideline was reached after consensus from the members of the entire SVI.

A pilot project to identify the utility of the modified BA1 rule was undertaken. To identify pathogenic variants in the ClinVar database (Landrum et al., 2018) that were >0.05 allele frequency in any of the six major subpopulations, we acquired the ClinVar variant_summary.txt files obtained from the ClinVar ftp site (http://ftp.ncbi.nlm.nih.gov/pub/

clinvar/) in July, 2016. We annotated these variants with allele frequencies from populations in ExAC and then filtered the variants that were >0.05 allele frequency in any of the continental populations in ExAC (http://ftp.broadinstitute.org/pub/ExAC_release, release 0.2). This resulted in 103 variants with at least one pathogenic assertion that were further filtered for classification errors and other criteria described in the Results. A final list of nine variants was manually curated using the ACMG/AMP guidelines ignoring BA1 and BS1 (allele frequency is greater than expected for disorder), and only applying BS2 (observed in healthy individuals inconsistent with disease penetrance) where homozygotes were observed in substantial numbers and the phenotype associated with that variant met the criteria of "full penetrance expected at an early age" following ACMG/AMP guidelines.

RESULTS AND DISCUSSION

The A of the BA1 criterion stands for "standalone". The meaning of "standalone" was not explicitly defined in Richards et al., but the criterion is widely implemented as an exclusionary filter, such that if a variant were to meet that criterion, it could be considered benign without the need for assessing other evidence for or against pathogenicity. We explicitly endorse the concept of this criterion as one that if met by a variant, does not require further evaluation of evidence for or against pathogenicity and can be considered benign based solely on its frequency. However, the working group recognizes that no criterion applies perfectly to all genes, variants, or populations, and there are cases where exceptions can and should be made. To that end, we have clarified the language used to describe BA1 and added some exceptions to its implementation. The proposed updated definition of the criterion is: "Allele frequency is >0.05 in any general continental population dataset of at least 2,000 observed alleles and found in a gene without a gene- or variant-specific BA1 modification." We have specified the datasets that we used for this analysis as the six defined subsets of the ExAC database (African, East Asian, European (Non-Finnish), Latino, and South Asian). It should be noted that although we have excluded Finnish European as a recommended population for allele frequency filtering given that it is a founder population, we did analyze the data and include two variants in our exclusion set that have over 5% allele frequency in the Finnish as described below (Table 1).

We have modified the criterion in Richards et al. in several important ways. We clarified how the population data are to be used. We have made clear that one should use this criterion to assign a variant as Benign if its allele frequency is >0.05 in any one of the six specified datasets when there are at least 2,000 observed alleles at the site in question. We have specified alleles instead of individuals to make it clear that one needs more individuals for a gene on the non-pseudoautosomal portions of the X or Y chromosomes to make an equivalent assessment of frequency. We have listed the datasets we used for this analysis and for which our exception list applies. We appreciate that there are reasons one might set numerically lower allele frequency thresholds for the non-pseudoautosomal portions of the X or Y chromosomes, but we wanted BA1 to be written in a way that applied to the entire nuclear genome. Numerically smaller thresholds may be applied to genes on the non-pseudoautosomal portions of the X or Y chromosomes by expert panels, as described below. We specify "in any one of the specified datasets" to emphasize that there is no need to match the geographic origin of the query case to the genotyped dataset. For example, if the variant

is not on our exception list and an individual with the variant is East Asian and the allele frequency exceeds 0.05 only in Africans, the criterion is satisfied, and the variant can be designated as benign (in contrast, we note that it is important to use geographically-matched data as evidence that a variant is rare). Other population datasets may be used for this purpose, but it is important that these datasets are comprised primarily of unrelated individuals and the 2,000 observed alleles criterion is satisfied. In bottlenecked populations (e.g. Ashkenazi Jewish, or Finnish), it is possible for pathogenic alleles to rise to a high frequency (Martin et al., 2018). Caution should also be exercised if the dataset may include substantial numbers of related individuals (Mitchell et al., 2015) – in that case the dataset should be evaluated by a population geneticist to determine the effective population size. For example, two variants we analyzed, NM_001281724.2 (BTD): c.1336G>C p.(Asp446His) and NM_000017.3 (ACADS): c.511C>T p.(Arg171Trp), were estimated to have >5% MAF only in the Finnish population which is known to have gone through population bottlenecks (Table 1 and Supp. Table 1, citation: Martin et al. , 2018). In this example, using only Finnish population data without controlling for the effective population size may not be appropriate. Population datasets such as ExAC may not necessarily be considered as healthy control datasets in that only severe pediatric disease was excluded and other later onset or milder phenotypes may be present. Pathogenic variants, particularly for adult onset diseases, may be present at appreciable frequencies because of this ascertainment. Laboratories that use other datasets would be responsible for screening those datasets for variants that have an allele frequency >0.05 that may not be benign.

The 0.05 allele frequency threshold was originally selected because the authors of the ACMG/AMP recommendations did not recognize any alleles that were associated with a Mendelian disorder that were higher than this population frequency (Richards et al., 2015). We have recognized the possibility that there may indeed be such variants for more common autosomal recessive disorders. This is especially challenging for disorders like hemochromatosis, where the allele is relatively common, but penetrance is incomplete. To address this issue, we have added to the BA1 criterion the possibility of exceptions, which can be of two possible types. The first is that disease experts may define a gene-specific BA1 criterion that is numerically smaller than 0.05 based on the known prevalence, penetrance, and genetic heterogeneity of the associated diseases (Whiffin et. al, 2017; Gelb et al., 2018; Kelly et al., 2018). The second is that there are a few variants that may exist in continental populations at an allele frequency greater than 0.05 and potentially be pathogenic for a Mendelian disorder. The variants that have this attribute should be nominated to the SVI by the community and will be evaluated and tabulated as a BA1 exception list. This list is initiated here and will be curated and updated by the SVI committee and displayed on the ClinGen web site (www.clinicalgenome.org). This list may be used by laboratories who wish to implement the BA1 0.05 threshold with the datasets specified here as a standalone criterion.

To initiate this process, we performed an analysis using the exome aggregation consortium (ExAC; Lek et al., 2016) to search for variants that exist in a defined population at an allele frequency of >0.05 and for which there was one or more assertions of pathogenicity in ClinVar. We identified 103 variants (Supp. Table 1). These variants were reviewed for several attributes to potentially exclude them from consideration for this exception list. The

following six attributes were used as exclusionary criteria: 1) the variant was better considered a common susceptibility allele or modifier, 2) the gene-disease association was judged to be unproven, 3) the phenotype was better considered a trait, instead of a disease, 4) the variant had very limited evidence which was scored as insufficient by an expert reviewer, 5) the variant was only seen somatically, or 6) the gene is non-coding. Of the 103 variants, 94 met one of these criteria and were not considered for the exception list. Of the nine remaining variants, we applied the ACMG/AMP pathogenicity criteria1, with some modifications (Table 1). First, we did not use criteria BA1 or BS1, as this would be circular reasoning. Second, criterion BS2 was used in a few cases where homozygotes were observed in substantial numbers and the phenotype associated with that variant met the standard of "full penetrance expected at an early age" from the ACMG/AMP recommendations. Of these nine variants, we assessed four as being pathogenic and five as being VUS. Interestingly, two of these variants, MEFV c.1105C>T p.(Pro369Ser) and BTD c.1330G>C p.(Asp444His), were also evaluated in the ExAC marker paper (Lek et al., 2016) and in both cases, their assessments of pathogenicity agreed with ours. We have posted these nine variants on the BA1 exception list, which is posted on the ClinGen web site (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/).

New alleles can be nominated through a submission form on the webpage, for addition to or removal from this list (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/), and expert groups or other interested parties can weigh in to the SVI on the variants that are among the excluded 103 to propose altering their status. Variants may be moved from the 94 that we considered but did not include onto the exception list. Or, one of the variants on the exception list could be 'demoted' if evidence is generated to demonstrate it is benign. Individual laboratories may also choose to add alleles that have a frequency >0.05 to their own in-house exception list if they judge them to be pathogenic. However, in the spirit of sharing testing data, the SVI is eager to learn of any laboratory's assessment of these variants. This initial BA1 exception list should be regarded as interim until a suitable period of time passes for the community to provide input on the list, and necessary adjustments can be made. We are mindful that an exclusionary filter, such as the BA1 criterion, must be robust and we wish to avoid laboratories making pathogenicity errors that are based on inadvertent errors in this process. This list is likely to grow and evolve as large datasets become available from populations not currently well represented in the datasets utilized to date. We do recognize the challenge posed by disorders that lie in the gray area between Mendelian disorders and disorders described as "…multigenic non-Mendelian complex disorders" (definition of scope from the ACMG/AMP recommendations1). Indeed, for some of the variants addressed in the present paper an association with common disease cannot be ruled out. Over time, as approaches for variant interpretation for more common variants are developed, our recommendations for these variants may be supplanted.

As the SVI group is committed in the long-term to shift towards a quantitative Bayesian framework (Tavtigian et al., 2018), we have refined BA1 in such a way that given any conceivable prior probability for a variant, when conditioned with BA1, the posterior probability of pathogenicity would be <0.1% (the definition of Benign; Goldgar et al 2004; Plon et al., 2008). We believe this is consistent with the original intent of the ACMG/AMP recommendations. As this quantitative Bayesian framework has not yet been implemented,

we must use an approximation or heuristic to ensure that the robustness of this criterion is maintained. This will also be an issue for gene-specific (numerically lower than 0.05) BA1 criteria that are developed in the future. Those criteria must demonstrate that the proposed threshold is similarly robust through analyses of known pathogenic variation, as well as detailed and conservative assessment of prevalence and penetrance. Finally, gene-specific criteria must be set so that the frequency threshold represents a valid exclusionary threshold for the most common disorder associated with that gene and that it does not conflict (is not lower than) with the application of BS1.

Frequency-based criteria (BA1, BS1, etc.) are powerful tools to evaluate the pathogenicity of variants. The BA1 criterion allows many common variants to be excluded from time-consuming assessments of the full range of criteria proposed by ACMG/AMP. It is our hope that the refinement of this criterion will increase the confidence in its use and provide a ready method for recognizing some exceptions that do warrant more detailed evaluation. This should contribute to an increase in the consistency of pathogenicity assertions while at the same time reducing its costs and turn-around time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Gene	Variant	Classification	ACMG/AMP Criteria applied (not including BA1 or BS1)	ClinVar ID	ClinGen Allele Registry ID	Chr	Position	Ref	Alt	ExAC Source Pop	ExAC Source Pop MAF	ClinVar disease entry
ACAD9	NM_014049.4: c.-44_-41dupTAAG	VUS	PS3_Supporting; BS2	1018	CA114709	3	128,598,490	C	CTAAG	AFR	0.1261	Deficiency of Acyl--CoA dehydrogenase family, member 9
GJB2	NM_004004.5: c.109G>A (p.Val37Ile)	Pathogenic	PS4; PP1_Strong; PM3_VeryStrong; PS3_Moderate	17023	CA172210	13	20,763,612	C	T	EAS	0.07242	Deafness, autosomal recessive
HFE	NM_000410.3: c.187C>G (p.His63Asp)	Pathogenic *	PS4	10	CA113797	6	26,091,179	C	G	NFE	0.1368	Hereditary hemochromatosis
HFE	NM_000410.3: c.845G>A (p.Cys282Tyr)	Pathogenic *	PS4; PP3	9	CA113795	6	26,093,141	G	A	NFE	0.05135	Hereditary hemochromatosis
MEFV	NM_000243.2: c.1105C>T (p.Pro369Ser)	VUS	PM3; PM5	2551	CA280114	16	3,299,586	G	A	EAS	0.07156	Familial Mediterranean fever
MEFV	NM_000243.2: c.1223G>A (p.Arg408Gln)	VUS	PM3; PM5	2552	CA280116	16	3,299,468	C	T	EAS	0.05407	Familial Mediterranean fever
PIBF1	NM_006346.2: c.1214G>A (p.Arg405Gln)	VUS	PM3; BS2	217689	CA210261	13	73,409,497	G	A	AMR	0.09858	Joubert syndrome
ACADS	NM_000017.3: c.511C>T (p.Arg171Trp)	VUS	PS3_Moderate; PM3; PP3	3830	CA312214	12	121,175,678	C	T	FIN #	0.06589	Deficiency of butyryl--CoA dehydrogenase
BTD	NM_000060.4: c.1330G>C (p.Asp444His)	Pathogenic	PS3; PM3_Strong; PP3; PP4	1900	CA090886	3	15,686,693	G	C	FIN #	0.05398	Biotidinase deficiency

* ACMG/AMP criteria selected does not match the classification as these variants are common low-penetrant variants and the ACMG/AMP guidelines are not designed for this variant type

Detected at >5% MAF only in Finnish population (see text). Genomic coordinates on build GRCh37

AFR: African/African American, EAS: East Asian, NFE: Non-Finnish European, AMR: Latino, FIN=Finnish