Genetics inMedicine

www.nature.com/gim

Check for updates

ACMG STATEMENT

Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG)

David T. Miller[1], Kristy Lee[2], Adam S. Gordon[3], Laura M. Amendola[4], Kathy Adelman[5], Sherri J. Bale[6], Wendy K. Chung[7], Michael H. Gollob[8], Steven M. Harrison[9], Gail E. Herman[10], Ray E. Hershberger[11], Teri E. Klein[12], Kent McKelvey[13], C. Sue Richards[14], Christopher N. Vlangos[15], Douglas R. Stewart[16], Michael S. Watson[17], Christa Lese Martin[18] and ACMG Secondary Findings Working Group[19]*

Genetics in Medicine _####################_; https://doi.org/10.1038/s41436-021-01171-4

Disclaimer: This statement is designed primarily as an educational resource for medical geneticists and other clinicians to help them provide quality medical services. Adherence to this statement is completely voluntary and does not necessarily assure a successful medical outcome. This statement should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinician should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen.

Clinicians are encouraged to document the reasons for the use of a particular procedure or test, whether or not it is in conformance with this statement. Clinicians also are advised to take notice of the date this statement was adopted, and to consider other medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

HISTORY AND FUTURE OF THE ACMG SECONDARY FINDINGS LIST

Guidance from the original American College of Medical Genetics and Genomics (ACMG) Policy Statement on incidental findings in 2013 established that clinical laboratories performing exome or genome sequencing (ES/GS) should report known pathogenic (KP) or expected pathogenic (EP) variants in a defined set of genes considered medically actionable, even when unrelated to the primary medical reason for testing.[1] Subsequently, the ACMG updated the terminology used to describe these types of findings to align with nomenclature recommendations from the Presidential Commission on Bioethical Issues that defined "secondary findings" (SF) as variants that are actively sought in genes that are part of a defined list, as opposed to genomic variants found incidentally or accidentally.[2] In a survey of ACMG members, more than 90% of respondents supported a minimum gene list of SFs that would be updated and refined over time.[3] Recognizing this need, the ACMG Board of Directors (BOD) created the ACMG Secondary Findings Maintenance Working Group (SFWG) in 2014 to define and implement a process for updating the SF list. Shortly thereafter, we established a mechanism for ACMG members to submit nominations to add or remove genes from the list through a nomination form found on the ACMG website.[4] The 2017 update added four new genes, removed one gene, and rejected one nominated gene from inclusion in the list. Adoption of clinical ES/GS since then has expanded even further.[2] The ACMG SFWG and the BOD agree that timely updates to the SF list are increasingly important, which has led to the decision to separate the ACMG SFWG policy update discussed here from the updated ACMG SF gene list. The SFWG will update this general policy statement as needed, but on a less frequent basis, generally every 3–4 years, and the gene list will be updated on an annual basis, with a goal of publishing the updated list each year in January.

Frequency of secondary findings in clinical practice

Since the publication of the original ACMG Policy Statement on SFs, several studies across a variety of research and clinical cohorts have sought to measure the frequency at which SFs are likely to be identified. Although these studies can differ considerably in their ascertainment strategies, variant interpretation methods, and underlying gene lists, reported SF frequencies are consistently in the range of 1–6%.[5–9] Most recently, an estimated medically

[1]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, USA. [2]Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. [3]Department of Pharmacology & Center for Genetic Medicine, Northwestern University, Chicago, IL, USA. [4]Division of Medical Genetics, University of Washington, Seattle, WA, USA. [5]Patient Advocate, Livermore, CA, USA. [6]Bale Genetic Consulting, Chevy Chase, MD, USA. [7]Departments of Pediatrics and Medicine, Columbia University, New York, NY, USA. [8]Division of Cardiology and Department of Physiology, University of Toronto, Toronto, ON, Canada. [9]Broad Institute of MIT and Harvard, Cambridge, MA, USA. [10]Professor Emeritus, Department of Pediatrics, The Ohio State University, Columbus, OH, USA. [11]Divisions of Human Genetics and Cardiovascular Medicine, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA. [12]Departments of Biomedical Data Science & Medicine, Stanford University, Stanford, CA, USA. [13]Departments of Genetics and Family Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA. [14]Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR, USA. [15]Tempus Labs, Inc., Chicago, IL, USA. [16]Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD, USA. [17]Self-employed, Washington, DC, USA. [18]Autism & Developmental Medicine Institute, Geisinger, Danville, PA, USA. [19]American College of Medical Genetics and Genomics, Bethesda, MD, USA. *The Board of Directors of the American College of Medical Genetics and Genomics approved this statement on 22 February 2021. ✉email: documents@acmg.net

actionable SF rate of 2% using the ACMG v.2.0 list was reported in the 49,960-person cohort of the UK biobank.[10] Similarly, a large cohort study ($N = 21,915$), drawn from a diverse group of clinical settings, reported that 2.54% of participants had a pathogenic (P) or likely pathogenic (LP) variant in one of the 59 genes recommended for return on the ACMG v2.0 list.[11] This frequency jumped to 3.02% if additional actionable findings were included, such as homozygosity for the *HFE* p.Cys282Tyr variant and P/LP variants in *PALB2*.[11] In this cohort, SFs associated with cancer susceptibility were the most frequent (1.38%), followed by cardiovascular diseases (0.87%), and lipid disorders (0.50%). This study of primarily European ancestry participants also found no difference in SF frequency among the ACMG SF v2.0 gene–phenotype pairs when stratified by self-reported race/ethnicity, though a difference in frequency among those of European ancestry and those of African ancestry has been reported previously in a smaller study.[5] Given these conflicting results, and the overarching context of disparate overrepresentation of individuals of European ancestry within genomics cohorts, caution should be taken when applying these frequencies to groups with different demographic makeup.[12,13] Future efforts to further refine these frequencies must be conducted in cohorts that reflect diverse human populations.

Stakeholder views on secondary findings

Literature and systematic reviews of studies exploring stakeholder views on SF disclosure find general support for returning actionable results, especially in the context of adult probands and when individual choice is preserved.[14,15] Variability in views based on stakeholder group (e.g., lay person vs. genomics professional) and experience with genetic conditions and genetic testing have also been identified.[14] The authors of these reviews note that interpretation of results may be affected by unclear and variable definitions of the terms "secondary finding" and "actionability" across studies.

Clinicians and researchers in the United States are largely supportive of returning SFs from clinical and research ES/GS (with accredited clinical laboratory confirmation). Clinicians find SF results disclosure similar to traditional results, but with potentially more time required for preparation.[16,17] Among clinical genomics professionals in the UK, most thought that SFs should be restricted to medically actionable, higher penetrance, and serious conditions and favor a cautious approach that continually incorporates relevant evidence.[18] Pediatric genetics and nongenetics health professionals in Canada were generally supportive of returning SFs with a focus on the child's best interests and balancing benefits of potential disease prevention and reduced morbidity against potential harms related to discrimination, autonomy, and stigma.[19] The concept of disclosure based on "benefit to family" has also been proposed as a justification for return of SFs that is independent of any direct benefit to the child.[20]

Results from studies of patients and families exploring the desirability to learn about, and act on, SFs have been mixed. In the United States, SFs have been generally well-received by patients, and some individuals even expressed worry about later experiencing regret if they did not learn these results; however, recommended follow-up and cascade family testing may be lower than typically expected with medically sought diagnostic findings.[21–23] Factors taken into account when considering SF return may be different for adult patients and parents of children having ES/GS. In a small study from Europe, adults with hereditary cancer, cardiac, and/or metabolic conditions tended to express more concern about receiving SFs due to potential psychological consequences, while parents of individuals receiving ES/GS as a diagnostic test for a rare disorder tended to have a more favorable opinion about receiving SFs based on potential medical benefits.[24] Similarly, a systematic review of stakeholder views reported that patients with a history of cancer or other genetic diagnoses were more cautious about learning SFs, whereas parents were more likely to request a wide range of SF results for their children.[14] In Canada, parents whose children were undergoing genomic sequencing for diagnostic purposes reported ambivalence about receiving SFs, but also felt obligated to learn of their child's adult-onset SFs in spite of the perceived burden of that knowledge.[25]

Priorities for this policy update

Our working group has built upon the solid foundation of the original policy statement, making adjustments in response to updates in the medical literature and feedback from the community.[1] Below, we compare and contrast SFs with population screening. Based on reported stakeholder views about consent for SFs, we provide updated guidance about considerations for consenting. We address questions about whether SFs should be reported on test types other than ES/GS (e.g., panels). We revise the germline variant classification recommendations to align with ACMG/Association for Molecular Pathology (AMP) variant classification recommendations that were published subsequent to the original policy on SFs.[26] We also discuss inclusion of only a subset of variants within particular genes as compared to including variants identified in the entire gene. Finally, we propose a framework to update the SF list annually, and on a predictable schedule that will facilitate integration into laboratory and clinical workflows.

POLICY UPDATES/CLARIFICATIONS

Secondary findings vs. population and carrier screening

A significant amount of debate continues around the use of the ACMG SF gene list for general population screening outside of its intended use for opportunistic screening as part of clinical ES/GS, and the ACMG has made it clear that the ACMG SF list was not validated for general population screening.[27] However, the ACMG is supportive of continued research and discussions around the factors to consider in population screening programs, such as penetrance and genotype–phenotype correlations to examine the efficacy of using such genomic screening in asymptomatic individuals. As such, two new working groups, the Genomic Screening of Asymptomatic Patients Working Group and the Population Screening Working Group, have been established by the ACMG to further evaluate this area and develop recommendations. The remainder of this policy statement pertains to SF.

Consenting and reporting practices

Ethical considerations continue to apply in the context of sequencing tests that report SFs. The original policy was modified to allow individuals to opt out of receiving SFs.[28] There is some evidence that laboratories vary in applying the "opt out" policy for SFs, such as one study showing that 4 of 12 laboratories applied the policy as an "opt in" mechanism.[29] The opportunity for individuals to make an informed decision and opt out,[28] if desired, at the time of consent continues to be supported by the ACMG SFWG.

The best interest of the child is still prioritized when disclosing risk for adult-onset conditions in minors,[30] and should be considered in the context of ethical considerations such as individual autonomy, beneficence, and nonmaleficence. We also recognize that SFs may be the only opportunity to identify a potentially life-threatening genetic risk factor in the parents. Whether to include SFs in the context of prenatal ES, typically performed for the indication of a fetal anomaly, has not always been clear. More recently, an ACMG points to consider document has suggested that SFs should be discussed with the patient in the prenatal setting as part of the pre-test informed consent

discussion with the option of opting out of the reporting of these variants.[31]

Aspects of patient–provider communication regarding the scope and implications of results from tests with the potential for SF return are also important to highlight. For example, consent could include discussion of applicable laws related to discrimination based on genetic information. The gene–phenotype pairs suggested here are a minimum set of recommendations and individual labs may decide to modify or expand the scope of SF reporting. The consent process should include discussion of the categories of reportable gene–phenotype pairs related to the ACMG SF list.

Evidence is still limited for many genes regarding penetrance estimates for individuals with P/LP variants in the absence of a personal and/or family history of the condition. Consideration should be given to the clinical context during results disclosure, and when making related medical management recommendations. Conversely, communicating the clinical significance can also be challenging when no SFs are identified.[32] Also, the possibility of identifying somatic mosaicism as a SF should be considered and communicated during consent and results disclosure conversations.[33–35] It is expected that results such as apparent somatic variants would be followed up by genetic counseling, and possibly follow-up diagnostic testing, to clarify the medical implications of the result. We note that the clinical relevance of identifying somatic variants in asymptomatic/presymptomatic individuals is still unclear and can be further compounded when the proband is of an older age and more likely to have variants associated with clonal hematopoiesis of indeterminate potential (CHIP) as is the case with *TP53*. Additionally, there is the possibility of a prior cancer and or cancer treatment, which can lead to somatic mosaicism.

Policy recommendations.

- The SF list is intended as a "minimum list" of actionable secondary findings.
- Providing the opportunity for an informed decision and opt out, if desired, at the time of consent should continue to be the standard for secondary findings.
- The option to receive SFs should be offered regardless of the age of the patient. The best interest of the child should still be prioritized when disclosing risk for adult-onset conditions in minors.
- The option to opt out of SFs should also be presented to the individual in the context of prenatal ES/GS.
- The consent process should include discussion of the categories of reportable gene–phenotype pairs related to the ACMG SF list.
- Thoughtful consideration of the context of a positive SF result during results disclosure, and when making related medical management recommendations, is necessary.
- If laboratories report apparent somatic mosaicism, the consent process should address this.
- Pre-test and post-test genetic counseling should be provided to any person receiving SF results in order to discuss the types of possible results, limitations of testing, and medical implications of any results.

Test types for reporting of secondary findings

The earlier ACMG policy statements were intended as recommendations for reporting on a minimum gene list related to ES/GS.[1,2] Neither policy statement explicitly considered the concept of panel testing, including virtual panels with testing using an ES platform and reporting out a select list of genes. One example of this type of virtual panel is an "exome slice," which is a custom selection of a small number of genes to be reported from an ES platform as the basis for generating sequence data. Some have advocated for reporting of ACMG SF genes tested on virtual panels and exome slices.[36–39]

The working group discussed whether SF should be reported from virtual panels and exome slices during an official meeting in April 2019, and again at the time of manuscript preparation in 2020, and had the following perspective:

1. Consent: Documentation of informed consent may not be obtained as routinely for targeted tests and panel tests as compared to ES/GS. Nongeneticist providers may not be familiar with the elements of informed consent for genetic testing. This may result in individuals receiving SFs without having been provided an adequate informed consent process, or it may delay access to panel testing (e.g., in order to arrange a separate consent visit).
2. Additional workload:
   a. Reporting SFs based on panels and virtual panels would create challenges in the laboratory by increasing the workload beyond what is reimbursed. For example, although a standard exome capture kit may collect data from ACMG SF genes, utilizing that information requires additional technical and clinical review steps by highly skilled and trained individuals. A policy to provide SFs from virtual panels could easily increase the workload related to SFs severalfold.
   b. Reporting SFs based on panels and virtual panels would create challenges in the return of results. Some panels may have good technical coverage for the entire list of ACMG genes, whereas others may only include the cancer genes, or the cardiovascular genes, and therefore would not have the complete "minimum list." This could lead to misunderstanding about whether an individual has been tested for the full list.

Among the recommendations in the initial ACMG statement was their call for a national registry for SF testing and the outcomes in those identified on the presumption that over time some SFs would meet the performance characteristics that justify their becoming primary targets of genetic screening.[1] In the absence of such a registry, the number of SFs recommended to be reported in ES/GS testing has grown, with few being removed from the list based on experience in providing the results. Further, the expectation of incrementally increased reimbursement for inclusion of SFs has not been realized.

We also note that some laboratories are beginning to incorporate mitochondrial DNA analysis into clinical ES, the results of which can be complicated and burdensome to interpret (e.g., due to issues of heteroplasmy), and are thus currently beyond the scope for return as SFs. Additionally, the recommendation to return SF only applies in the clinical setting. The decision to return SFs in a research setting is left to the research team and relevant local institutional review board (IRB).

Policy recommendations.

- The SF recommendations continue to apply to the situation in which a clinician orders ES/GS for a specific clinical indication, as outlined in the original policy statement.[1]
- The original intent of the SF reporting process focused on clinical ES/GS. Reporting of SFs is not expected as part of ordered tests that are not genome-wide, such as virtual gene panels or exome slices. However, if SF are reported from a virtual panel or exome slice, it should be done to the same specifications as would be done on ES (e.g., informed consent, full SF gene list).
- Findings from mitochondrial DNA sequencing are outside the scope of the current ACMG SF list.

- The ACMG recommendation to return SFs only applies in the clinical setting. Researchers, in consultation with their local IRB, should decide on the appropriateness of return of SFs for their study.

Policies related to technical detection of variants

Certain types of genetic variation can be difficult or impossible to identify with current standard ES/GS, and that may affect the decision about whether to include a gene in the SF list. In the context of SFs, laboratories are asked to report opportunistic information that is available secondary to the initial reason for performing a diagnostic test. Some actionable genes may be difficult to assess by ES/GS, so the sequencing data may have suboptimal analytical sensitivity for some genes. Such genes may also present challenges for the laboratory in using orthogonal methods for confirmation, as discussed below.

Poor candidates for secondary findings, due to concerns about analytical validity, include:

a. Genes with one or more associated pseudogenes, and genes with homologous sequences predisposing to recombination and gene-conversion events. Complex genomic architecture may complicate detection of variants from ES/GS. One example is *CYP21A2* related to congenital adrenal hyperplasia (OMIM 201910). Laboratories should not be expected to develop a specific orthogonal test to determine if observed variants are in a gene versus a pseudogene when reporting a SF. Therefore, the SFWG will not recommend inclusion of genes on the SF list when homology precludes the ability to perform orthogonal confirmation.
b. Many genes/variants related to pharmacogenetic (PGx) phenotypes. While there is likely clinical utility in using PGx test results in drug therapy decisions in specific situations, there are significant difficulties for the laboratory to report these variants from standard ES/GS testing. This problem arises from three issues: (1) many of the clinically relevant variants reside in intronic regions that are not captured using current standard reagents, and/or are repeat polymorphisms that are not well-resolved by ES; (2) for some genes and variants there is still controversy regarding genotype-driven pharmacotherapy; (3) PGx genotyping often requires testing multiple positions/regions and types of variation within the same gene, complicating the analysis and reporting (e.g., *CYP2D6* which includes single-nucleotide polymorphisms [SNPs] and a duplication).
c. Genes where intragenic exon-level copy-number variants (CNV) (deletion or duplication) is a frequent cause of disease. Such genes can also be problematic for many laboratories to identify using standard ES. For example, approximately 20–25% of cases suspected of having a P variant in *MSH2*, but with negative *MSH2* sequencing, have an *EPCAM* exonic deletion, and would be missed by laboratories that cannot assess CNVs from their ES data.[40] This can lead to misunderstanding about whether causative variants have actually been assessed as part of SFs.

Policy recommendations.

- The SF list should only include genes where it can be anticipated that the majority of clinically relevant variant(s) are routinely detectable on standard clinical ES/GS, and can be confirmed by standard orthogonal methods.
- Laboratories should not be expected to develop a specific orthogonal test to determine if observed variants are in a gene versus a pseudogene when reporting a SF.
- Although we understand that some genes/variants are excluded from the list due to technical limitations of ES that may not be limitations of GS, we do not recommend a minimum list that differs between ES and GS.

Positive predictive value (PPV)

The SF list is targeting the identification of variants with high PPV by selecting genes such that reporting of P/LP variants in those genes has a high chance of indicating the presence of clinical risk. Of course, reduced penetrance must be taken into account. At the level of analytical validity, we want to avoid situations, such as the presence of pseudogenes, that would affect the PPV at the analytical level. In terms of clinical validity, we also want to minimize the situation of having a low PPV due to low penetrance. This latter point also distinguishes SF from newborn screening (NBS) results where clinical sensitivity is paramount. The SF list is not a replacement for a clinically indicated diagnostic or screening test.

Actionability

The actionability of a gene–phenotype pair needs to be considered in the context of several factors related to the intervention and evidence base. Although not a requirement for the SFWG, in many cases we consider the results of curations performed by the ClinGen Actionability Working Group related to selection of genes for the ACMG SF list.[41,42] Generating consensus scores on actionability that account for both effectiveness and the nature of the intervention can be challenging. Thus the intervention which might be more effective in eliminating the harm (e.g., risk-reducing surgery) gets a lower overall actionability score compared to the intervention which is less effective in eliminating the harm but is also less burdensome and invasive (e.g., breast cancer surveillance). In addition, the available knowledge base among disorders varies significantly, with some disorders having substantial evidence of clinical actionability (e.g., hereditary breast and ovarian cancer), while others have limited evidence (e.g., hereditary paraganglioma–pheochromocytoma syndrome).

Policy recommendations.

- The SF list will continue to be a minimum list of genes that are focused on results that are considered medically actionable by criteria defined by current standards of care.

Variant classification based on ACMG/AMP policy

The original policy statement stated that reporting should include "only variants that have been previously reported and are a recognized cause of the disorder or variants that are previously unreported but are of the type that is expected to cause the disorder."[1] The list of genes indicated reporting of KP or EP variants. In this policy update, we have incorporated the variant classification terms to reflect current norms. Prior to recommending this change, we reviewed the available literature pertaining to the question of how closely the category of LP reflects "expected pathogenic" in actual practice.

The ACMG/AMP guidelines for variant classification defined "likely" in the LP category to be a 90% confidence threshold for the pathogenicity of a given variant–phenotype pair based on the underlying evidence. Although little data were available at the time to validate this theoretical threshold, analyses of variant reclassification in the time since the publication of these guidelines indicate that current usage of the LP category is in line with this original threshold. For example, one study interrogated all variant interpretation records submitted to ClinVar over the period

of January 2016 to July 2019 to determine the proportion of LP variants that had been reclassified. This analysis showed that only 4,501 ClinVar variants (0.8%) were reclassified; of these, 91.9% moved to a more certain category while 8.1% moved to a less certain category (i.e., downgraded). Of the 796 LP variants that were reclassified during this three and a half year period, 83.8%-99.1% of LP classifications were upgraded to P, depending on categorization or inclusion of LP to VUS reclassifications.[43]

We also considered that a primary goal of reporting SFs is to call attention to the presence of actionable variants that could result in a severe medical event. The current data suggest that reporting of LP variants as a whole would more often achieve this goal, as the risk of failing to report a truly actionable LP variant outweighs the risk of infrequently reporting an LP variant that might later be downgraded. In the interest of setting a conservative threshold for reporting novel variants as SFs, the SFWG considered the idea of only reporting LP variants that are predicted loss-of-function (i.e., a PVS1 point could be awarded), but preferred to maintain consistency with the existing rules for variant interpretation.[26] We also note that current ACMG/AMP guidelines set a high bar for novel missense variants to be classified as LP. We also acknowledge that the recommendation to include LP variants will necessitate classification of novel missense variants in SF genes, increasing the burden on laboratories. The balance of this burden against the duty to report truly actionable SFs is a driving force behind the SFWG's philosophy to constrain reporting of SFs to a minimum list of genes with strong consensus evidence supporting pathogenicity and actionability.

Based on these data and considerations, the SFWG determined that the likelihood that a variant reported as LP would be later reclassified to VUS is relatively low, and to update the policy to recommend reporting of all LP and P variants for the diseases and genes on the SF list. In the balance, reporting variants that reach a level of LP or higher is more likely to result in providing information that is actionable, and less likely to result in a "false positive." Although a small proportion of variants returned as LP in these genes may be downgraded with future evidence, the SFWG consensus is to err on the side of reporting these variants, so that future decision-making surrounding these variants can involve the clinicians, patients, and participants directly impacted by these decisions as variant evidence evolves. However, we do note the current lack of standardized recommendations addressing the frequency and burden of variant reclassification, with hopes that this updated SFs policy will encourage the development of reclassification standards.

Policy recommendations.

- Unless otherwise specified in the ACMG v3.0 SF list, all variants that are classified as P or LP according to ACMG/AMP standards and guidelines should be reported as SFs. Describing variants using the terms 'KP' and 'EP' is obsolete in the context of SFs.
- Variants classified as VUS, LB, and B should not be returned in the context of SFs.
- This policy recommendation should not be considered retroactive to testing performed prior to adoption of the SF v3.0 list, and laboratories should not be expected to revise clinical reports based on earlier versions of the list in order to add variants that did not previously meet the reporting threshold.

Scope of genes/variants for reporting as secondary findings

A specific variant as a secondary finding. The minimum gene list originally mandated by ACMG was intended to be composed of genes conferring phenotypes that have the characteristics described in Table 1.

Table 1. Criteria for considering genes for the SF list.

Variable	Favorable	Unfavorable
Technical (detection at gene level)	Relevant variants routinely detectable on standard clinical ES/GS (without requiring customization of the laboratory methods)	Technical limitations prevent/severely limit the ability to detect the majority of P/LP variation (e.g., would require detection of noncoding variants, construction of haplotypes, or detection of CNVs that are not part of routine ES/GS workflow)
Additional clinical or laboratory testing (excluding orthogonal sequencing test)	Additional approaches for a clinical (e.g., diagnostic imaging) and/or laboratory (e.g., biochemical test) diagnosis are available once the SF is reported	Additional approaches for a clinical and/or laboratory diagnosis are not available.
Clinical presentation	Clinically silent prior to high morbidity or mortality. Onset of signs and symptoms are acute, and presence of risk may be unknown to the individual prior to onset of the symptoms or medical event. Preventative measures and/or treatments are available and/or individuals with P variants might be asymptomatic for a long time. Onset may occur in children and/or adults	A condition that presents with signs and/or symptoms in early stages of the disease that, even if they are nonspecific, should prompt a diagnostic workup, potentially to include genetic testing.
Morbidity and mortality	Higher morbidity and/or mortality, and/or disorders in which earlier detection reduces long-term mortality	Lower morbidity and/or mortality
Penetrance	Conditions with higher lifetime penetrance	Conditions with lower, uncertain, or unknown lifetime penetrance
Actionability	A medical intervention is available (e.g., medical or surgical intervention, surveillance). Also, cost of intervention, and patient access, is not considered.	Lifestyle changes (e.g., avoidance of smoking), are not considered medical interventions as defined here. Extreme burden of intervention may be considered unfavorable.

We consider both childhood- and adult-onset phenotypes. Disease prevalence is not a deciding factor (actionable conditions are considered regardless of rarity). The existence of clinical practice guidelines is not considered. Genes are included when the ACMG Secondary Findings Maintenance Working Group (SFWG) reached a consensus.
CNV copy-number variant, *ES/GS* exome or genome sequencing, *P/LP* pathogenic/likely pathogenic, *SF* secondary findings.

In keeping with the original ACMG policy, the SFs list will generally continue to include entire genes for reporting. We also acknowledge that there are situations where the medical actionability is related to a specific variant, or class of variants, rather than the whole gene. An example of a specific reportable variant is homozygosity for *HFE* p.Cys282Tyr. Data from larger studies with lower likelihood of ascertainment bias suggests that penetrance for severe liver disease is 10–24% in male homozygotes, and that penetrance is driven by the p.Cys282Tyr variant, and not other variants in *HFE*.[44,45] These data supported the decision to include homozygosity for this variant on the ACMG v3.0 SF list.[46] As another example, *TTN*-truncating variants (*TTN*tv) achieve a PVS1 point using the ACMG/AMP classification system, and are a common genetic cause of dilated cardiomyopathy, whereas missense variants in *TTN* very rarely cause disease.[47,48] In addition, *TTN* is the largest known human gene, and a requirement to evaluate every missense variant in *TTN* would be burdensome and currently have low clinical utility. The inclusion of individual variants, or a class of variants, for return in a given gene strikes a balance between optimizing the identification of actionable genetic information for the benefit of the patient and the burden for reporting by diagnostic laboratories.

Policy recommendations.

- The SFs list will continue to include P/LP variants, primarily from entire genes, for reporting, and we will also consider situations where the actionability is related to a specific variant, or class of variants.

Including genes based on positive predictive value and penetrance. The original version of the ACMG SF recommendations did not define a penetrance threshold, but stated that the list was generally intended to be "a minimum list that is weighted toward conditions for which penetrance may be high."[1] The SF list is targeting the identification of variants with high PPV by selecting genes such that reporting of P/LP variants in those genes has a high chance of indicating the presence of clinical risk. Of course, reduced penetrance must be taken into account. At the level of analytical validity, we want to avoid situations, such as the presence of pseudogenes, that would affect PPV. In terms of clinical validity, we also want to minimize the situation of having a low PPV due to low penetrance. This latter point also distinguishes SF from NBS results where clinical sensitivity is paramount.

European SF recommendations (cancer only, excluding pediatric-onset genes) stratified penetrance estimates, with the risk of developing the phenotype >40% weighted most highly.[49] The SFWG is mindful of the potential burdens from the return of lower penetrance P/LP variants on individuals receiving the results, and the health-care system in general, although some studies suggest that any negative impact is minimal, and cost to the health-care system is modest.[21] Also, our goal of maintaining a minimum list argues against inclusion of lower penetrance gene–phenotype pairs.

Penetrance may be age-, phenotype-, sex-, gene-, and environment-dependent; stochasticity likely also plays a role. Penetrance is also variable among different genes causing the same phenotype, and among variants within those genes, in part due to difference in genomic background. Penetrance for many germline cancer risk genes,[50] and noncancer genes,[51] are overestimates because of their ascertainment from families affected by the disorder. For many genes, penetrance estimates will decrease over time with the availability of data sets that are larger and consist of more diverse populations, and are consequently less susceptible to ascertainment bias. Thus, whenever possible, we used lifetime penetrance estimates derived from larger cohorts that were sequenced regardless of phenotype (i.e., ascertained by genotype). We also considered penetrance in the context of other variables, such as severity of phenotype and availability of an intervention, precluding our ability to set a strict penetrance threshold. Finally, we incorporated information from the ClinGen Actionability WG reports into our assessment, and the ClinGen Actionability scoring system includes prevalence of clinical manifestations in the metric. This represents a second way that the SFWG accounted for penetrance.

Policy recommendations.

- We recommend avoiding inclusion of gene–phenotype pairs with uncertain or lower penetrance estimates to reduce the number of individuals receiving a SF since this could necessitate surveillance among many individuals where most would never develop disease.
- Preference will be given to gene–phenotype pairs with a higher likelihood that the individual will manifest the phenotype associated with that gene.
- Selection of gene–phenotype pairs will also consider the availability and burden of surveillance/intervention recommendations.
- Information about penetrance will ideally be collected from databases inclusive of diverse populations.

SF list versioning. The SFWG has agreed on nomenclature to support versioning of the SF list, reflecting both major and minor revisions. Major revisions could be necessary due to substantive changes in process and/or in the number or type of gene–phenotype pairs recommended for reporting. A major revision will be denoted by changing the version number to the next integer (v3.0, v4.0, etc.). Minor revisions reflect addition or removal of one or a few genes or variants and will be denoted by changing the number after the decimal point (v3.1, v3.2, etc.). The ACMG BOD has approved this versioning nomenclature.

Policy recommendations.

- We recognize the tendency in the community to refer to the SF list based on the number of genes, but recommend referencing the list using the version numbering system to reduce confusion.
- Laboratories should not be expected to revise an already issued clinical report for the purpose of updating the report to include genes/variants that pertain to a version of the SF list that is more recent than the version of the SF list that was current at the time of clinical testing.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The ACMG SFWG process for the nomination and review of genes for addition to, or removal from, the SF list takes several key factors into consideration to optimize the benefit to patients, while weighing the burden on laboratories and clinicians for reviewing and disclosing these results (Fig. 1). As highlighted above, nominated genes should be medically actionable, have a clear phenotype associated with disease-causing variants, have serious medical implications for at least one of the phenotypes associated with the gene, and be associated with a highly penetrant phenotype. The various types of data collected and evaluated by the SFWG for a nominated gene are included in Table 1.

It is important to note that the ACMG SF gene list is intended to be a minimum list for the return of SFs that have a high likelihood for reducing morbidity and mortality, and not an inclusive list for any genetic result that could be actionable. We recognize that laboratories may choose to add additional genes to the ACMG SF minimum list.

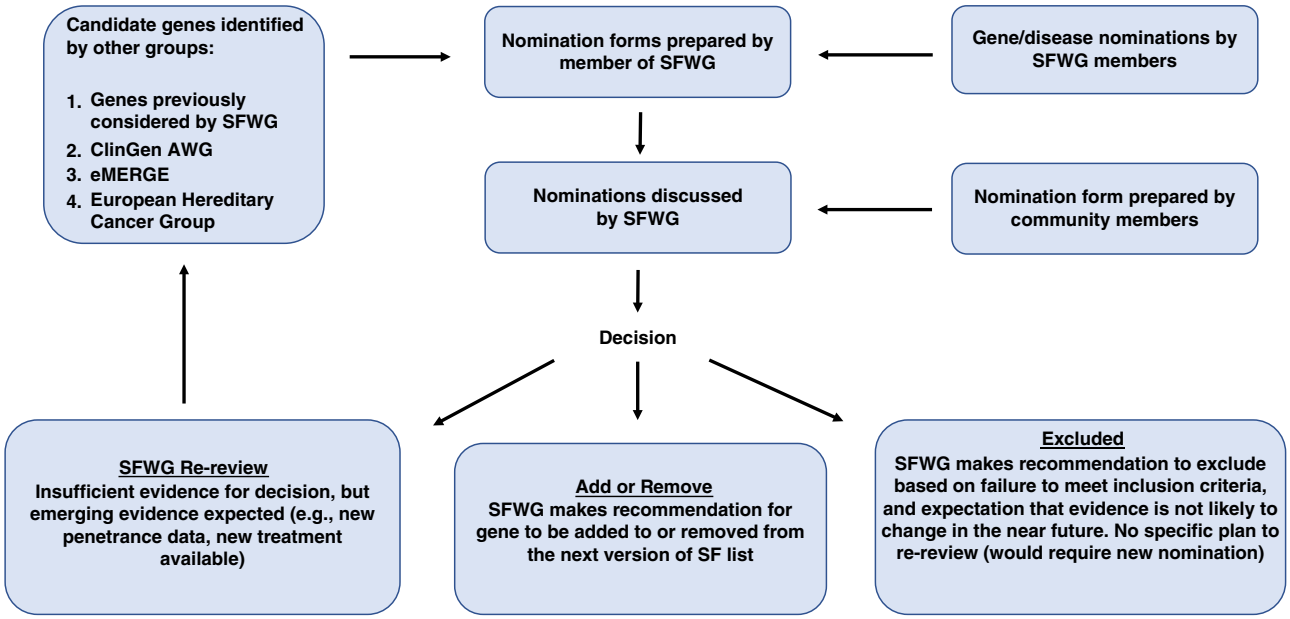

Fig. 1 Evaluation process for genes nominated for addition to, or removal from, the American College of Medical Genetics and Genomics (ACMG) secondary findings SF list. This diagram summarizes the ACMG Secondary Findings Maintenance Working Group (SFWG) process for nomination and consideration of genes potential inclusion on the v3.0 SF list. The SFWG referred to lists from other groups assessing actionability of genes along with gene-phenotype pairs considered for previous versions of the SF list. Candidate genes were triaged by SFWG subgroup members to prioritize genes based on the strength of available evidence, and nomination forms were created for those prioritized gene-phenotype pairs. All completed nomination forms, either from SFWG members or the community, were discussed by the full SFWG. All gene-phenotype pairs were assessed based on the criteria described in Table 1.

Considerations for future additions to the ACMG SF list that broaden the scope of the current list continue to be topics for discussion, including pharmacogenomic variants, and conditions that have insidious onset or are hard to diagnose. The SFWG encourages the submission of gene nominations for consideration that are outside of the current focus of the SF gene list to capitalize on opportunistic genomic screening to detect disease earlier or prevent it from ever occurring, offering increasing benefits as part of patient care.

Looking forward, the SFWG encourages continued nominations from the ACMG community, but now also invites other professional organizations to engage in the nomination process. In addition, to better inform our evaluation process, we encourage research efforts to assess the medical, legal, ethical, and/or economic impact of SFs. We also anticipate that the availability of genotype-first data from an increasing number of large population-based studies will help to inform our future review processes by providing more unbiased estimates of penetrance. Therefore, we recommend that data be collected, aggregated, and openly shared to support future iterations of the SF list and inform decisions of whether primary genetic screening for any seem appropriate.

Finally, the SFWG recommends that the ACMG SF gene list be updated annually, with intervening updates if any critical changes are identified that could impact patient care. If new information emerges about any gene on the list, ACMG members, or other healthcare professionals, are encouraged to submit that information to the SFWG for immediate consideration. Given the rapid evolution of information related to gene-phenotype relationships and advances in therapy, it is expected that genes that were not approved for addition to the list could be candidates for future addition with new data.

In conclusion, the SFWG encourages community feedback in the form of nomination of genes. We present this policy to clarify our process for the review of genes and variants for inclusion/removal from the ACMG SF list. We will continue to evaluate the factors that influence our decisions, as outlined in Table 1, and inform our updates with an evidence-based approach whenever possible. We will plan to update this policy as needed, and give careful consideration to all stakeholders, most importantly the individuals who may benefit from learning about actionable genetic information.

Received: 26 March 2021; Revised: 26 March 2021; Accepted: 26 March 2021;

REFERENCES

1. Green, R. C. et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet. Med.* **15**, 565–574 (2013).
2. Kalia, S. S. et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet. Med.* **19**, 249–255 (2017).
3. Scheuner, M. T. et al. Reporting genomic secondary findings: ACMG members weigh in. *Genet. Med.* **17**, 27–35 (2015).
4. ACMG. Secondary findings nomination form. https://www.acmg.net/PDFLibrary/Secondary-Findings-Panel-Nomination-Form.pdf (2021).
5. Amendola, L. M. et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res.* **25**, 305–315 (2015).
6. Yang, Y. et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA.* **312**, 1870–1879 (2014).
7. Retterer, K. et al. Clinical application of whole-exome sequencing across clinical indications. *Genet. Med.* **18**, 696–704 (2016).
8. Schwartz, M. L. B. et al. A model for genome-first care: returning secondary genomic findings to participants and their healthcare providers in a large research cohort. *Am. J. Hum. Genet.* **103**, 328–337 (2018).
9. Dewey, F. E. et al. Distribution and clinical impact of functional variants in 50,726 whole-exome sequences from the DiscovEHR study. *Science.* **354**, aaf6814 (2016).
10. Van Hout, C. V. et al. Exome sequencing and characterization of 49,960 individuals in the UK Biobank. *Nature.* **586**, 749–756 (2020).

11. eMERGE Clinical Annotation Working Group. Frequency of genomic secondary findings among 21,915 eMERGE network participants. *Genet. Med.* **22**, 1470–1477 (2020).
12. Popejoy, A. B. et al. The clinical imperative for inclusivity: Race, ethnicity, and ancestry (REA) in genomics. *Hum. Mutat.* **39**, 1713–1720 (2018).
13. Popejoy, A. B. et al. Clinical genetics lacks standard definitions and protocols for the collection and use of diversity measures. *Am. J. Hum. Genet.* **107**, 72–82 (2020).
14. Mackley, M. P., Fletcher, B., Parker, M., Watkins, H. & Ormondroyd, O. Stakeholder views on secondary findings in whole-genome and whole-exome sequencing: a systematic review of quantitative and qualitative studies. *Genet. Med.* **19**, 283–293 (2017).
15. Delanne, J. et al. Secondary findings from whole-exome/genome sequencing evaluating stakeholder perspectives. A review of the literature. *Eur. J. Med. Genet.* **62**, 103529 (2019).
16. Darnell, A. J. et al. A clinical service to support the return of secondary genomic findings in human research. *Am. J. Hum. Genet.* **98**, 435–441 (2016).
17. Wynn, J. et al. Clinical providers' experiences with returning results from genomic sequencing: an interview study. *BMC Med. Genomics*. **11**, 45 (2018).
18. Ormondroyd, E. et al. "Not pathogenic until proven otherwise": perspectives of UK clinical genomics professionals toward secondary findings in context of a Genomic Medicine Multidisciplinary Team and the 100,000 Genomes Project. *Genet. Med.* **20**, 320–328 (2018).
19. Szego, M. J. et al. Views from the clinic: Healthcare provider perspectives on whole genome sequencing in paediatrics. *Eur. J. Med. Genet.* **62**, 350–356 (2019).
20. Wilfond, B. S., Fernandez, C. V. & Green, R. C. Disclosing secondary findings from pediatric sequencing to families: considering the "Benefit to Families". *J. Law Med. Ethics.* **43**, 552–558 (2015).
21. Hart, M. R. et al. Secondary findings from clinical genomic sequencing: prevalence, patient perspectives, family history assessment, and health-care costs from a multisite study. *Genet. Med.* **21**, 1100–1110 (2019).
22. Roche, M. I. et al. Factors influencing NCGENES research participants' requests for non-medically actionable secondary findings. *Genet. Med.* **21**, 1092–1099 (2019).
23. Robinson, J. O. et al. Psychological outcomes related to exome and genome sequencing result disclosure: a meta-analysis of seven Clinical Sequencing Exploratory Research (CSER) Consortium studies. *Genet. Med.* **21**, 2781–2790 (2019).
24. Houdayer, F. et al. Secondary findings from next generation sequencing: psychological and ethical issues. Family and patient perspectives. *Eur. J. Med. Genet.* **62**, 103711 (2019).
25. Anderson, J. A. et al. Parents perspectives on whole genome sequencing for their children: qualified enthusiasm? *J. Med. Ethics*. **43**, 535–539 (2017).
26. Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).
27. ACMG Board of Directors. The use of ACMG secondary findings recommendations for general population screening: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **21**, 1467–1468 (2019).
28. ACMG Board of Directors. ACMG policy statement: updated recommendations regarding analysis and reporting of secondary findings in clinical genome-scale sequencing. *Genet. Med.* **17**, 68–69 (2015).
29. O'Daniel, J. M. et al. A survey of current practices for genomic sequencing test interpretation and reporting processes in US laboratories. *Genet. Med.* **19**, 575–582 (2017).
30. Ross, L. F., Saal, H. M., David, K. L. & Anderson, R. Technical report: ethical and policy issues in genetic testing and screening of children. *Genet. Med.* **15**, 234–245 (2013).
31. Monaghan, K. G., Leach, N. T., Pekarek, D., Prasad, P. & Rose, N. C. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **22**, 675–680 (2020).
32. Sapp, J. C. et al. Evaluation of recipients of positive and negative secondary findings evaluations in a hybrid CLIA-research sequencing pilot. *Am. J. Hum. Genet.* **103**, 358–366 (2018).
33. eMERGE Consortium. Harmonizing clinical sequencing and interpretation for the eMERGE III Network. *Am. J. Hum. Genet.* **105**, 588–605 (2019).
34. Batalini, F. et al. Li-Fraumeni syndrome: not a straightforward diagnosis anymore-the interpretation of pathogenic variants of low allele frequency and the differences between germline PVs, mosaicism, and clonal hematopoiesis. *Breast Cancer Res.* **21**, 107 (2019).
35. Cao, Y. et al. A clinical survey of mosaic single nucleotide variants in disease-causing genes detected by exome sequencing. *Genome Med.* **11**, 48 (2019).
36. Biesecker, L. G. Secondary findings in exome slices, virtual panels, and anticipatory sequencing. *Genet. Med.* **21**, 41–43 (2019).
37. Biesecker, L. G. Response to Mendelsohn and Sabbadini. *Genet. Med.* **21**, 763 (2019).
38. Esplin, E. D., Haverfield, E., Yang, S., Aradhya, S. & Nussbaum, R. L. Secondary findings on virtual panels: opportunities, challenges, and potential for preventive medicine. *Genet. Med.* **21**, 1250–1251 (2019).
39. Biesecker, L. G. Response to Esplin et al. *Genet. Med.* **21**, 1252–1253 (2019).
40. Rumilla, K. et al. Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated Lynch syndrome cases. *J. Mol. Diagn.* **13**, 93–99 (2011).
41. Hunter, J. E. et al. A standardized, evidence-based protocol to assess clinical actionability of genetic disorders associated with genomic variation. *Genet. Med.* **18**, 1258–1268 (2016).
42. Webber, E. M. et al. Evidence-based assessments of clinical actionability in the context of secondary findings: Updates from ClinGen's Actionability Working Group. *Hum. Mutat.* **39**, 1677–1685 (2018).
43. Harrison, S. M. & Rehm, H. L. Is 'likely pathogenic' really 90% likely? Reclassification data in ClinVar. *Genome Med.* **11**, 72 (2019).
44. Gallego, C. J. et al. Penetrance of hemochromatosis in HFE genotypes resulting in p.Cys282Tyr and p.[Cys282Tyr];[His63Asp] in the eMERGE Network. *Am. J. Hum. Genet.* **97**, 512–520 (2015).
45. Grosse, S. D., Gurrin, L. C., Bertalli, N. A. & Allen, K. J. Clinical penetrance in hereditary hemochromatosis: estimates of the cumulative incidence of severe liver disease among HFE C282Y homozygotes. *Genet. Med.* **20**, 383–389 (2018).
46. Laberge, A. M. Recommending inclusion of HFE C282Y homozygotes in the ACMG actionable gene list: cop-out or stealth move toward population screening? *Genet. Med.* **20**, 400–402 (2018).
47. Roberts, A. M. et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Sci. Transl. Med.* **7**, 270ra6 (2015).
48. Haggerty, C. M. et al. Genomics-first evaluation of heart disease associated with titin-truncating variants. *Circulation.* **140**, 42–54 (2019).
49. Pujol, P. et al. Guidelines for reporting secondary findings of genome sequencing in cancer genes: the SFMPP recommendations. *Eur. J. Hum. Genet.* **26**, 1732–1742 (2018).
50. Begg, C. B. On the use of familial aggregation in population-based case probands for calculating penetrance. *J. Natl. Cancer Inst.* **94**, 1221–1226 (2002).
51. Wright, C. F. et al. Assessing the pathogenicity, penetrance, and expressivity of putative disease-causing variants in a population setting. *Am. J. Hum. Genet.* **104**, 275–286 (2019).

ACKNOWLEDGEMENTS

We thank members of the original ACMG Incidental Findings Working Group for their groundbreaking efforts to begin the important process of identifying and reporting actionable genetic variants based on genome-scale sequencing. We thank former members of the Secondary Findings Maintenance Working Group for building upon the foundation of the original effort.

COMPETING INTERESTS

S.J.B. is a contractor to GeneDx, a subsidiary of OPKO, through Bale Genetic Consulting, LLC. W.K.C. is a member of the scientific advisory board of Regeneron Genetic Center. D.T.M. has received honoraria from Ambry Genetics and PreventionGenetics LLC. D.R.S. is supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics of the National Cancer Institute (Rockville, MD), and also performs contract clinical telehealth services for Genome Medical, Inc. in accordance with relevant NCI ethics policies. The other authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to ACMG.