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ARTICLE

Tracking updates in clinical databases increases efficiency for variant reanalysis

Lele Li[1,*], Xia Tian[1], Vaughan Woodzell[2], Richard A. Gibbs[1], Bo Yuan[1], Eric Venner[1,2]

[1]*The Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX;* [2]*Codified Genomics, Houston, TX*

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ABSTRACT

Purpose: Variant interpretation, guided by American College of Medical Genetics and Genomics guidelines, can inform clinical decision-making. However, interpretations may change over time for a variety of reasons. Periodic reanalysis of previous variant interpretations is important to ensure that reported genetic findings remain accurate according to current knowledge.
Methods: We performed automated filtering by comparing ClinVar variants available in August 2020 with those from August 2021 to screen for potential reanalysis candidates from 3 projects. These variants were subsequently interpreted based on the American College of Medical Genetics and Genomics/Association for Molecular Pathology variant interpretation guideline or ClinGen revised gene-specific guidelines if applicable.
Results: Our method annotated 241 unique variants requiring reanalysis, from 3 projects containing 3,832,210 previously interpreted variants, including those filtered automatically. Among these 241 variants, 43 variants changed interpretation, including 55.81% ($N = 24$) with upgraded and 44.19% ($N = 19$) with downgraded classifications. An efficiency study showed that our strategy increased the reanalysis efficiency and saved reviewing time.
Conclusion: We demonstrated an effective high-throughput method, initiating from external data updates, to achieve variant reanalysis in a clinical laboratory. This filtering method reduced the number of variants that need to be reanalyzed, screened potential variants, and saved time and cost for clinical laboratories.

Introduction

Genetic testing plays an increasingly vital role in customizing clinical treatment and therapy. Personal and family testing can predict the risk of disease and suggest potential preventative actions. Accurate and evidence-based pathogenicity interpretations provide the means for clinical laboratories to interpret and report genetic variation.[1]

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*Correspondence and requests for materials should be addressed to Lele Li, Human Genome Sequencing Center, Baylor College of Medicine, One Baylor Plaza, Alkek N1419, MS: BCM226, Houston, TX 77030-3411. *Email address:* lelel@bcm.edu

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The results of genetic interpretation however are not static, but dynamic. Various factors may lead to changes in interpretation. For example, additional case data may supplement evidence for pathogenicity of a given variant.[2] Moreover, variants have been found in additional data in the Exome Aggregation Consortium (ExAC)/Genome Aggregation Database (gnomAD), leading to leaning-benign classification.[2] Additionally, new studies may better resolve a variant functional impact of disease.[3] Besides, interpretation standards are continually improved.[4,5] There are additional reasons that may cause reclassification, such as ethnicity differences,[6] the discovery of novel genes associated with such diseases,[7] the additional/evolving phenotypic presentations of patients that were not previously documented,[8] the updated version of annotation software and improved pipeline,[9-11] different genome coordinates and annotation resources,[12,13] and so on. Thus, it is essential to keep variant interpretation up to date through periodic reanalysis to inform patient care.

The 3 clinical sequencing projects involved in this study are collaborative projects involving a large number of regions and populations. The National Institutes of Health All of Us (AoU) Research Project aims to pair genetic information with electronic health record data from more than 1 million people in the United States, with varying ages, regions, gender, education, and ethnicities.[14] In the Electronic Medical Records and Genomics (eMERGE) project, the electronic medical record of 109 genes and about 1551 single-nucleotide variants were studied.[15,16] The HeartCare Project at Baylor College of Medicine Human Genome Sequencing Center (HGSC) assessed genes that are related to the risk of cardiovascular disease, including 158 genes and 709 participating individuals.[17] The large size of this combined data set makes the selection of variants more precise and the reinterpretation results based on this pool are more accurate and objective.

It is important to identify the variants that most require reassessment. If too many variants are selected for reanalysis, it will cause reviewers to spend too much time and increase service costs. If too few variants are selected, key variant information will not be updated, which will further lead to the delay of the patients' treatment, or the patients undergoing unnecessary treatment. Identification of variants to reassess is further complicated when evaluating data from different clinical laboratories, which can use different methods or parameters in their initial ascertainments. For example, variants can be selected based on the allele frequencies observed in patients versus those in the general population, or observed in multiple individuals in the general population but not in patients.[18] Laboratories may focus on variants in a specific time frame,[19-21] in panels related to a certain kind of diseases or pathways such as genes related to cancers or cardiovascular diseases,[19-23] or with a certain American College of Medical Genetics and Genomics (ACMG) classification, such as variants of uncertain significance (VUS).[19,21,24-26] Variants may also be picked among patients who can be recontacted to provide the family history to study the cosegregation of disease.[24,26] To address this complexity, we developed a new and efficient screening method to select potential variants for reanalysis.

Here, we reanalyzed variants from the AoU Research Program,[27,28] eMERGE III,[29] and HeartCare projects[17] updated during August 2020 to August 2021, applying a systematic method of reinterpretation. We also discussed reasons for the change of reported findings during reanalysis. By comparing our filtering method, which is based on changes in ClinVar, with other methods from the perspective of the number of variants needing reinterpretation, the percentage of reclassified variants, and the time being spent, we demonstrate the yield and efficiency of our approach.

Materials and Methods

Filtering of variants to reanalyze

The reanalysis procedure is described in the flowchart in Figure 1. In our current projects, we only report the variants with pathogenic (P)/likely pathogenic (LP) classification and not the variants with VUS/likely benign (LB)/benign (B) classification. To filter variants that need reanalysis, we searched an internal "VIP" database of genomic variation to identify variants that had new ClinVar assertions since the last database update. The VIP database was established by clinical reporting laboratories for the eMERGE III network.[29-31] It currently serves as the internal database for the Baylor College of Medicine HGSC Clinical Laboratory and is updated as new samples are processed. It contains variant information, including gene name, transcript, inheritance, genomic position, Human Genome Variation Society (HGVS) nomenclature, and allele frequency in the sample and in general population.

First, we only recorded a variant as having been affected by the update if it was changed from P/LP to VUS/LB/B, or vice versa, or if it had new P/LP or B/LB assertions since August 2020. For each variant, the internal database will display information such as the number of VUS/LB/B and P/LP ClinVar submissions in the last update and the current update. Second, out of that set of affected variants, we selected only those with new assertions that disagreed with the overall interpretation, such as a new pathogenic assertion for a benign variant or a new benign assertion for a pathogenic variant. Upgrade refers to changing from the original interpretation to a new classification that is more inclined to pathogenic, such as VUS to LP or LP to P. Downgrade is defined as the classification moves to the nondeleterious direction, such as P to LP or LP to VUS.

According to the above description, variants fitting the following 2 types of criteria were marked as needing to be reanalyzed. First, when the variant's VIP category and previous ClinVar category are both VUS, and the current ClinVar updates have VUS and P/LP assertions, reanalysis is required in case additional evidence upgrades the classification. Second, when the VIP category of the variant is

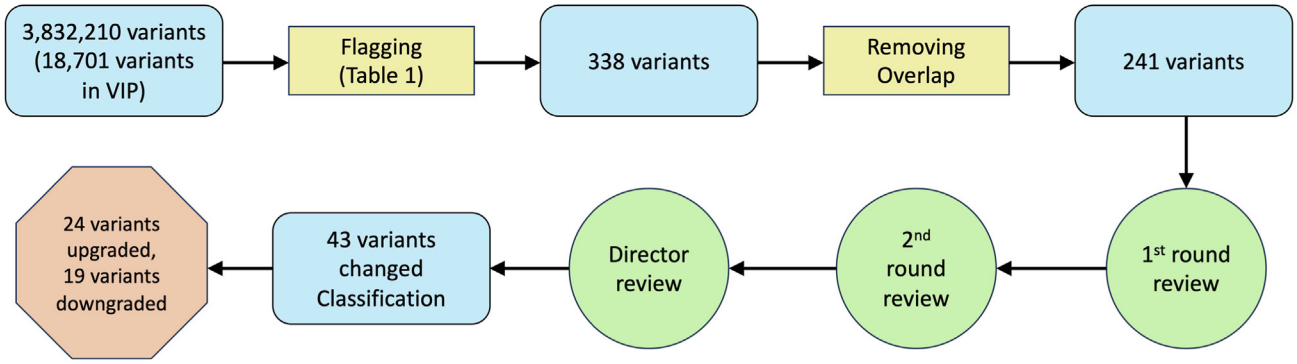

Figure 1 **Flowchart of the reanalysis process.** A total of 3,832,210 variants, including 18,701 from the VIP database, went through a filter that flagged the variants with the standard in Table 1. Three hundred thirty-eight variants were flagged. After removing the overlapped variants that showed up in more than 1 project, there were 241 variants. These variants proceeded to 2 rounds of review before a director decided that the variants were finalized. After interpretation, 43 variants changed classification, among which 24 variants were upgraded, and 19 were downgraded.

P/LP, and the previous ClinVar category and the current ClinVar update are both VUS, reanalysis is required because the latest information may downgrade the former VIP classification. We provided a comprehensive breakdown of each scenario in Table 1 with discussion on the flagged situations.

We also tracked "VUS-Leans LP" variants, which refer to variants displaying evidence of pathogenicity; however, the final score does not meet the threshold of LP based on the "Rules for Combining Criteria to Classify Sequence Variants" of ACMG variant interpretation guideline.[1] These variants are reported as VUS in the final report, as well as the tables in this article. We then asked the participating clinical sites to provide additional clinical evidence for these variants. In some cases, this allowed a VUS to be reclassified as LP, either by applying case-based evidence (AMP/ACMG subcategory PS4) or because of a strong match between the patient's phenotype and the highly specific clinical feature for the gene in question (PP4).

Table 1 Standard for filtering variants that need to be reanalyzed

VIP Category	Previous ClinVar	Current ClinVar Update	Flag for Reanalysis?
VUS	VUS assertion	VUS assertion	No
VUS	VUS assertion	VUS assertion LP/P assertion	Yes
VUS	VUS assertion	VUS assertion LB/B assertion	No
VUS	VUS assertion LB/B assertion	VUS assertion	No
VUS	VUS assertion LP/P assertion	VUS assertion	No
LP/P	VUS assertion	VUS assertion LP/P assertion	No
LP/P	VUS assertion	VUS assertion	Yes

VUS in the table may represent VUS, LB, or B.

B, benign; *LB*, likely benign; *LP*, likely pathogenic; *P*, pathogenic; *VUS*, variants of uncertain significance.

Reanalysis process

The variant review was conducted following the ACMG guidelines.[1] In addition, some genes have special guidelines from published literature or ClinGen, such as *MYH7* (HUGO Gene Nomenclature Committee [HGNC] gene ID: 7577),[4] *LDLR* (HGNC gene ID: 6547),[32] *FBN1* (HGNC gene ID: 3603),[33] *PTEN* (HGNC gene ID: 9588),[34] *TP53* (HGNC gene ID: 11998),[35] and *RYR1* (HGNC gene ID: 10483).[36]

Reviewers conducted 2 rounds of curation (first review and second review) before an ACMG board-certified director decided that the variants were finalized. Sanger confirmations were performed to finalize variants that needed upgrade. Downgraded variants were finalized without extra steps.

Update of reports

When a reclassification occurred, it triggered an update to the clinical report. Amendment reports were sent to respective collaborators.

In this article, we used GRCh37 (Genome Reference Consortium human genome build 37) as the genome build. The variant nomenclatures in this article have passed the VariantValidator check.[37]

Results

Extent of variant reclassification by reanalysis

This reanalysis was carried out for reports from 3 projects and focused on the variants with updates in ClinVar from August 2020 to August 2021. Among 3,832,210 total variants (3,545,401 variants from AoU, 58,842 variants from HeartCare, and 227,967 variants from eMERGE), of which 3,789,887 were unique variants, we screened 338 variants

Table 2 Statistics for variants number in 3 projects

Variants Number Overview	All of Us	HeartCare	eMERGE	Total	Unique (Remove Overlap)
All unique variants number	3,545,401	58,842	227,967	3,832,210	3,789,887
VIP (HGSC internal database) variants number	10,304	1847	6550	18,701	18,437
Variants reviewed number	156	20	162	338	241

from 54 genes for reanalysis (156 variants from AoU, 20 from HeartCare, and 162 from eMERGE), of which 241 were unique variants (Table 2). Forty-three variants changed interpretation, spanning 22 genes (Table 3), among which 55.81% ($n = 24$) had upgraded classification and 44.19% ($n = 19$) had downgraded. When considering the total unique variants that underwent initial filtering, the percentage of variant classification changes was 0.0011% (43/3,789,887). In the internal VIP database, which included 18,701 variants from these 3 projects (10,304 variants from AoU, 1847 from HeartCare, and 6550 from eMERGE), of which 18,437 were unique, the change rate was 0.23% (43/18,437).

Using our reanalysis filtering system, the change rate for the variants we reanalyzed was 17.84% (43/241). Considering that it is not feasible to conduct full reinterpretation of every variant among the 3,789,887 unique variants included in these projects, our screening system effectively narrowed down the range of variants that may potentially change, reduced labor, and saved time and cost.

When performing variant interpretation, the amount of time required can vary depending on the difficulty of the variant. One thing that adds considerable time to the interpretation process is reviewing publications related to that variant. When a variant has not been previously reported in the literature, the interpretation process can be completed relatively quickly. Therefore, it is referred to as an "easy" variant. On the contrary, variants that are published in literature, which need extensive investigation, are referred to as "hard" variants. Based on the statistics of our past review, the time required to interpret an "easy" variant is about 2 minutes. The time required to interpret a "hard" variant or a null variant that needs a write-up is 62 minutes. In total, these projects contain 3,789,887 unique variants. It is impossible for any laboratory to reinterpret every variant presenting in their databases. By using our filtering system, the 241 unique variants screened possess extensive literature, and all require a detailed and time-consuming review. Therefore, the time required to review these 241 variants was 14,942 minutes (62 × 241) (approximately 31 days).

Of the 43 variants in which the interpretation changed, 24 out of 43 variants (55.81%) that had been formerly classified as VUS at our center were upgraded to LP/P (22 VUS → LP, 2 VUS → P). Eighteen in 43 variants (41.86%) formerly classified as LP/P were downgraded to VUS (9 LP → VUS, 9 P → VUS). One out of 43 variants (2.33%) was downgraded from P to LP (Figure 2). The 43 variants reclassified include 35 missense variants, 1 synonymous variant, 2 nonframeshift deletion variants, 3 frameshift deletion variants, and 2 splicing site variants.

Table 4 summarizes the statistics of the variant changes. Some genes such as *LDLR* or *RYR1* have published new special guidelines in recent years.[32,36,38,39] Many institutions reanalyzed with the new guideline and submitted the updated interpretation results to ClinVar, resulting in many changes in ClinVar that were detected by our reanalysis filtering system. This also reminds us that for genes with new specific guidelines, it is quite necessary to reanalyze them with the most updated guideline.

Overall, our method effectively narrowed down the range of variants that need to be reanalyzed, effectively filtered variants that have the potential to change the classification, and saved time for reviewers.

Reasons for reclassification

The clinical lab rule change

The general guidelines are subject to continuous improvements based on the latest findings. In addition to general use, each clinical laboratory has internal policies that are gene based or disease based.[40] These guidelines change occasionally, which leads to the reclassification of variants.

In the 2015 ACMG guidelines,[1] PM2 was described as "Absent from controls (or at extremely low frequency if recessive) in the Exome Sequencing Project, 1000 Genomes, or ExAC." The application of PM2 and related frequency assessment criteria have been discussed before and the category assignment can be different according to disease panels and method of calculating frequency.[40-42] A new rule approved on September 4, 2020, by ClinGen stated that this absence/rarity evidence has been downgraded from a moderate strength level (PM2) to a supporting strength level (PM2_supporting)[43] because rare variants are found frequently and PM2 criterion was over weighted in the 2015 ACMG guideline.[44,45] Because this "_supporting" evidence was not used previously, the original interpretation may be changed during the reanalysis.

For genes with ClinGen guidelines, the gene-specific PM2 cutoff in the guidelines should be utilized. Otherwise, to not overassign PM2, we select a cutoff based on the most stringent ClinGen guideline for genes we report, which is *PTEN* (allele frequency < 0.00001).[34] If the variant allele frequency falls below the threshold, the PM2_supporting criterion should be applied. In addition, it should be noted that the ideal cutoff is related to disease prevalence and penetrance.

Table 3 List of all the reclassified variants

Gene_ Symbol	HGNC Gene ID	Inheritance	HGVS_ Transcript	HGVS_ Protein	HGVS_Genomic _GRCh37	GRCh37 _CHR	GRCh37 _POS	Variant Type	Previous Interpretation (2020 August)	New Interpretation (2021 August)	Reason	Update Type
ATM	HGNC:795	AD/AR	NM_000051.4: c.6115G>A	NP_000042.3:p. (Glu2039Lys)	NC_000011.9:g. 108186757G>A	11	108186757	Missense	VUS	Lpath	New evidence	Upgrade
ATM	HGNC:795	AD/AR	NM_000051.4: c.9022C>T	NP_000042.3:p. (Arg3008Cys)	NC_000011.9:g. 108236086C>T	11	108236086	Missense	VUS	Path	New evidence	Upgrade
BRCA1	HGNC:1100	AD/AR	NM_007294.4: c.604del	NP_009225.1:p. (Gln202Lysfs Ter32)	NC_000017.10:g. 41247929del	17	41247928	Frameshift	Lpath	VUS	PVS1 reevaluation	Downgrade
BRCA2	HGNC:1101	AD/AR	NM_000059.4: c.7529T>C	NP_000050.3:p. (Leu2510Pro)	NC_000013.10:g. 32930658T>C	13	32930658	Missense	VUS	Lpath	New evidence	Upgrade
BRCA2	HGNC:1101	AD/AR	NM_000059.4: c.8177A>G	NP_000050.3:p. (Tyr2726Cys)	NC_000013.10:g. 32937516A>G	13	32937516	Missense	VUS	Lpath	New evidence	Upgrade
CFTR	HGNC:1884	AD/AR	NM_000492.4: c.1125A>C	NP_000483.3:p. (Leu375Phe)	NC_000007.13:g. 117182078A>C	7	117182078	Missense	Path	Lpath	Clinical lab rule change	Downgrade
CFTR	HGNC:1884	AD/AR	NM_000492.4: c.2417A>G	NP_000483.3:p. (Asp806Gly)	NC_000007.13:g. 117232638A>G	7	117232638	Missense	Path	VUS	Clinical lab rule change	Downgrade
CFTR	HGNC:1884	AD/AR	NM_000492.4: c.2735C>T	NP_000483.3:p. (Ser912Leu)	NC_000007.13:g. 117243663C>T	7	117243663	Missense	Path	VUS	Clinical lab rule change	Downgrade
CFTR	HGNC:1884	AD/AR	NM_000492.4: c.3205G>A	NP_000483.3:p. (Gly1069Arg)	NC_000007.13:g. 117251700G>A	7	117251700	Missense	Path	VUS	New evidence	Downgrade
CFTR	HGNC:1884	AD/AR	NM_000492.4: c.3209G>A	NP_000483.3:p. (Arg1070Gln)	NC_000007.13:g. 117251704G>A	7	117251704	Missense	Path	VUS	Clinical lab rule change	Downgrade
CFTR	HGNC:1884	AD/AR	NM_000492.4: c.358G>A	NP_000483.3:p. (Ala120Thr)	NC_000007.13:g. 117171037G>A	7	117171037	Missense	Path	VUS	New evidence	Downgrade
CFTR	HGNC:1884	AD/AR	NM_000492.4: c.601G>A	NP_000483.3:p. (Val201Met)	NC_000007.13:g. 117175323G>A	7	117175323	Missense	Path	VUS	Clinical lab rule change	Downgrade
CHEK2	HGNC:16627	AD	NM_007194.4: c.190G>A	NP_009125.1:p. (Glu64Lys)	NC_000022.10:g. 29130520C>T	22	29130520	Missense	VUS	Lpath	New evidence	Upgrade
DSC2	HGNC:3036	AD/AR	NM_024422.6: c.1034T>C	NP_077740.1:p. (Ile345Thr)	NC_000018.9:g. 28662935A>G	18	28662935	Missense	Lpath	VUS	Clinical lab rule change	Downgrade
DSP	HGNC:3052	AD/AR	NM_004415.4: c.5671_ 5674del	NP_004406.2:p. (Glu1891Argfs Ter37)	NC_000006.11:g. 7583166_ 7583169del	6	7583159	Frameshift	VUS	Lpath	PVS1 reevaluation	Upgrade
KCNE1	HGNC:6240	AD/AR	NM_000219.6: c.292C>T	NP_000210.2:p. (Arg98Trp)	NC_000021.8:g. 35821641G>A	21	35821641	Missense	Path	VUS	Inaccurate previous classification	Downgrade
LDLR	HGNC:6547	AD/AR	NM_000527.5: c.1575T>G	NP_000518.1:p. (Asp525Glu)	NC_000019.9:g. 11224427T>G	19	11224427	Missense	Lpath	VUS	New specific guideline	Downgrade

(continued)

Table 3 Continued

Gene_ Symbol	HGNC Gene ID	Inheritance	HGVS_ Transcript	HGVS_ Protein	HGVS_Genomic _GRCh37	GRCh37 _CHR	GRCh37 _POS	Variant Type	Previous Interpretation (2020 August)	New Interpretation (2021 August)	Reason	Update Type
LDLR	HGNC:6547	AD/AR	NM_000527.5: c.2113G>C	NP_000518.1:p. (Ala705Pro)	NC_000019.9:g. 11231171G>C	19	11231171	Missense	VUS	Lpath	New specific guideline	Upgrade
LDLR	HGNC:6547	AD/AR	NM_000527.5: c.232C>T	NP_000518.1:p. (Arg78Cys)	NC_000019.9:g. 11213381C>T	19	11213381	Missense	Lpath	VUS	New specific guideline	Downgrade
LDLR	HGNC:6547	AD/AR	NM_000527.5: c.626G>A	NP_000518.1:p. (Cys209Tyr)	NC_000019.9:g. 11216208G>A	19	11216208	Missense	VUS	Lpath	New specific guideline	Upgrade
LMNA	HGNC:6636	AD/AR	NM_170707.4: c.992G>A	NP_733821.1:p. (Arg331Gln)	NC_000001.10:g. 156105747G>A	1	156105747	Missense	VUS	Path	New evidence	Upgrade
MSH2	HGNC:7325	AD/AR	NM_000251.3: c.488T>C	NP_000242.1:p. (Val163Ala)	NC_000002.11:g. 47637354T>C	2	47637354	Missense	Lpath	VUS	Clinical lab rule change	Downgrade
MSH6	HGNC:7329	AD/AR	NM_000179.3: c.3227G>A	NP_000170.1:p. (Arg1076His)	NC_000002.11:g. 48030613G>A	2	48030613	Missense	VUS	Lpath	New evidence	Upgrade
MSH6	HGNC:7329	AD/AR	NM_000179.3: c.3469G>T	NP_000170.1:p. (Gly1157Cys)	NC_000002.11:g. 48032079G>T	2	48032079	Missense	VUS	Lpath	New evidence	Upgrade
MUTYH	HGNC:7527	AR	NM_001048174.2: c.1392+2C>T	NP_001041639. 1:p.?	NC_000001.10:g. 45796852G>A	1	45796852	Splicing	Path	VUS	PVS1 reevaluation	Downgrade
MYBPC3	HGNC:7551	AD/AR	NM_000256.3: c.1591G>A	NP_000247.2:p. (Gly531Arg)	NC_000011.9:g. 47364162C>T	11	47364162	Missense	VUS	Lpath	New evidence	Upgrade
MYBPC3	HGNC:7551	AD/AR	NM_000256.3: c.2429G>A	NP_000247.2:p. (Arg810His)	NC_000011.9:g. 47359115C>T	11	47359115	Missense	VUS	Lpath	New evidence	Upgrade
MYBPC3	HGNC:7551	AD/AR	NM_000256.3: c.3815-1G>A	NP_000247.2:p.?	NC_000011.9:g. 47353433C>T	11	47353433	Splicing	Lpath	VUS	PVS1 reevaluation	Downgrade
MYH7	HGNC:7577	AD/AR	NM_000257.4: c.2573G>A	NP_000248.2:p. (Arg858His)	NC_000014.8:g. 23894084C>T	14	23894084	Missense	VUS	Lpath	New specific guideline	Upgrade
MYH7	HGNC:7577	AD/AR	NM_000257.4: c.2631G>T	NP_000248.2:p. (Met877Ile)	NC_000014.8:g. 23894026C>A	14	23894026	Missense	VUS	Lpath	New specific guideline	Upgrade
MYH7	HGNC:7577	AD/AR	NM_000257.4: c.4145G>A	NP_000248.2:p. (Arg1382Gln)	NC_000014.8:g. 23887443C>T	14	23887443	Missense	VUS	Lpath	New evidence	Upgrade
MYH7	HGNC:7577	AD/AR	NM_000257.4: c.5655G>A	NP_000248.2:p. (Ala1885=)	NC_000014.8:g. 23883216C>T	14	23883216	Synonymous	Lpath	VUS	New specific guideline	Downgrade
MYH7	HGNC:7577	AD/AR	NM_000257.4: c.619A>C	NP_000248.2:p. (Lys207Gln)	NC_000014.8:g. 23900990T>G	14	23900990	Missense	VUS	Lpath	New specific guideline	Upgrade

(continued)

Table 3 Continued

Gene_ Symbol	HGNC Gene ID	Inheritance	HGVS_ Transcript	HGVS_ Protein	HGVS_Genomic _GRCh37	GRCh37 _CHR	GRCh37 _POS	Variant Type	Previous Interpretation (2020 August)	New Interpretation (2021 August)	Reason	Update Type
PCSK9	HGNC:20001	AD	NM_174936.4: c.643C>T	NP_777596.2:p. (Arg215Cys)	NC_000001.10:g. 55518070C>T	1	55518070	Missense	Lpath	VUS	Clinical lab rule change	Downgrade
PMS2	HGNC:9122	AD/AR	NM_000535.7: c.614A>C	NP_000526.2:p. (Gln205Pro)	NC_000007.13:g. 6038830T>G	7	6038830	Missense	VUS	Lpath	New evidence	Upgrade
RYR1	HGNC:10483	AD/AR	NM_000540.3: c.1589G>A	NP_000531.2:p. (Arg530His)	NC_000019.9:g. 38946103G>A	19	38946103	Missense	VUS	Lpath	New specific guideline	Upgrade
RYR1	HGNC:10483	AD/AR	NM_000540.3: c.5183C>T	NP_000531.2:p. (Ser1728Phe)	NC_000019.9:g. 38976478C>T	19	38976478	Missense	VUS	Lpath	New specific guideline	Upgrade
RYR1	HGNC:10483	AD/AR	NM_000540.3: c.7042_7044del	NP_000531.2:p. (Glu2348del)	NC_000019.9:g. 38990289_ 38990291del	19	38990284	Deletion (non frameshift)	VUS	Lpath	New specific guideline	Upgrade
RYR1	HGNC:10483	AD/AR	NM_000540.3: c.947G>T	NP_000531.2:p. (Arg316Leu)	NC_000019.9:g. 38939141G>T	19	38939141	Missense	VUS	Lpath	New specific guideline	Upgrade
SCN1A	HGNC:10585	AD	NM_001165963.4: c.4096G>A	NP_001159435. 1:p. (Val1366Ile)	NC_000002.11:g. 166859170C>T	2	166859170	Missense	VUS	Lpath	New evidence	Upgrade
SCN9A	HGNC:10597	AD/AR	NM_001365536.1: c.5408_5409del	NP_001352465. 1:p.(Ser1803Ter)	NC_000002.11:g. 167055744_ 167055745del	2	167055739	Frameshift	Path	VUS	PVS1 reevaluation	Downgrade
SDHB	HGNC:10681	AD	NM_003000.3: c.269G>A	NP_002991.2:p. (Arg90Gln)	NC_000001.10:g. 17359572C>T	1	17359572	Missense	Lpath	VUS	Clinical lab rule change	Downgrade
VHL	HGNC:12687	AD/AR	NM_000551.4: c.227_229del	NP_000542.1:p. (Phe76del)	NC_000003.11:g. 10183758_ 10183760del	3	10183754	Deletion (non frameshift)	VUS	Lpath	New evidence	Upgrade

AD, autosomal dominant; *AR*, autosomal recessive; *HGNC*, HUGO Gene Nomenclature Committee; *HGVS*, Human Genome Variation Society; *Lpath*, likely pathogenic; *Path*, pathogenic; *VUS*, variants of uncertain significance.

For example, variant *DSC2* (HGNC gene ID: 3036) (NM_024422.6):c.1034T>C, p.(Ile345Thr) has been identified in 2 of 251,066 alleles in the general population by the gnomAD, with allele frequency 0.000007966. It was classified as LP in the previous curation; however, in the reanalysis, the population data were downgraded from PM2 to PM2_supporting. In addition, a functional study has shown that this variant affects the localization of the *DSC2* protein and further investigation will be needed to determine the significance of this mislocalization,[46] resulting in PS3 subcategories not applicable here as well. The final classification was downgraded to VUS.

For genes related to multiple diseases, each project focuses on a certain kind of disease. *RYR1* (HGNC gene ID: 10483) gene variants are associated with various diseases, including central core disease (MIM: 117000), King-Denborough syndrome (MIM: 619542), minicore myopathy with external ophthalmoplegia (MIM: 255320), and malignant hyperthermia susceptibility 1 (MIM: 145600). *RYR1* is inherited in both autosomal dominant and autosomal recessive fashion. Loss-of-function variants are usually associated with autosomal recessive forms of *RYR1*-diseases,[47-49] whereas in the condition of malignant hyperthermia, loss of function is no longer the mechanism for the disease and PVS1 is not applicable.[36] In the All of Us project,[27,28] we report only pathogenic variants related to the disease malignant hyperthermia, which leads to VUS classification for multiple frameshifts, nonsense, and splice site variants. Special attention should be paid to when these variants appear in other projects that focus on different diseases with loss-of-function mechanisms. *CACNA1S* (HGNC gene ID: 1397) gene variants have a similar situation.[50] Therefore, the interpretation of the same variants in different projects may be different, which leads to the reanalysis results change.

New specific guidelines

Differences in interpretation may lead to the mistreatment of disease. It is important to use a universal standard (ACMG guidelines).[1] However, the special guidelines for classifying certain genes are continuously summarized and generated by external institutes, for example, a ClinGen expert panel. These gene-specific guidelines set new criteria that are complementary to the general standards, which may cause variant interpretations to change, such as requiring more case numbers to upgrade to a higher level, a newly defined functional domain, or alternatively providing detailed cutoff recommendations of frequency in the population.

A recent report issued a special guideline for the *MYH7* gene (myosin heavy chain 7) (HGNC gene ID: 7577), which encodes myosin heavy chain beta (MHC-β) that expresses in cardiac and skeletal muscle.[4,51,52] In the new guideline, ClinGen's inherited cardiomyopathy expert panel tested 60 variants, adjusted existing rules, and established new consensus rules.[4] The cutoff for PM2 was set to <0.00004 (0.004%) from large population studies, and the standard for PS4 has also been especially noted: (1) PS4: ≥15 probands, (2) PS4_Moderate: ≥6 probands, and (3) PS4_supporting: ≥2 probands.[4] PM1 hot spot/functional domain was redefined as amino acids 181 to 937 without benign variation.[4] Using these new guidelines, PM1 and PS4_supporting applied to variants NM_000257.4:c.2573G>A, p.(Arg858His) and NM_000257.4:c.2631G>T, p.(Met877Ile) and upgraded them from VUS to LP.

The *LDLR* (HGNC gene ID: 6547) (NM_000527.5): c.1575T>G, p.(Asp525Glu) variant was classified as LP and was reanalyzed according to a new guideline.[32] In this guideline, missense change in amino acids 105-232 (NM_000527.5) in exon 4 that also had a filtering allele frequency <0.02% was considered a mutational hotspot in a well-established functional domain critical to protein function and PM1 was applicable.[32,53] In addition, 60 highly conserved cysteine residues that involved in disulfide bond formation were considered important for protein function.[32,38,39,54] Thus, PM1 no longer applies to the p.(Asp525Glu) variants. Together with the given guidance for allele frequencies required for PM2 (PM2 downgraded to PM2_supporting), this variant was reclassified into VUS.

Many other genes also have special guidelines, such as *FBN1* (HGNC gene ID: 3603),[33] *PTEN* (HGNC gene ID: 9588),[34] *TP53* (HGNC gene ID: 11998),[35] and *RYR1* (HGNC gene ID: 10483).[36]

PVS1 reevaluation

In the general ACMG guidelines, PVS1 applies for null variants (such as frameshift, nonsense, and canonical splice sites) when loss of function is a known mechanism of disease.[1] *BRCA1* (HGNC gene ID: 1100) (NM_007294.4): c.604del, p.(Gln202LysfsTer32), which is located in exon 9 of 23 (also known as exon 8 in NM_007297.4 or NM_007298.3), causes a frameshift and loss of function is a known disease mechanism for this gene.[55-58] However, alternative splicing events existed and revealed hundreds of different *BRCA1* isoforms, among which a naturally occurring isoform without 2 exons (known as exon 9 and exon 10 in literature) was identified in normal blood and breast tissue.[59-61] This in-frame isoform results in the deletion of 41 amino acids and encodes a functional protein that has tumor suppressor activity, called a "rescue model."[62,63] The *BRCA1* (HGNC gene ID: 1100) (NM_007294.4):c.604del, p.(Gln202LysfsTer32) variant occurs in 1 of the 2 exons, leading to the possibility of partial functional activities. Thus, PVS1 does not apply here, and this variant was downgraded from LP to VUS. The same thing happens in *MYBPC3* (HGNC gene ID: 7551) (NM_000256.3):c.3815-1G>A, which is predicted to lead to the skipping of exon 34 that has only 3 amino acids, resulting in the protein extending to a former noncoding exon 35.[64]

It should also be noted that PVS1 does not apply when alternative splice sites or starting sites can be generated. *MUTYH* (HGNC gene ID: 7527) (NM_001048174.2): c.1392+2C>T causes the splice site to change from GC to

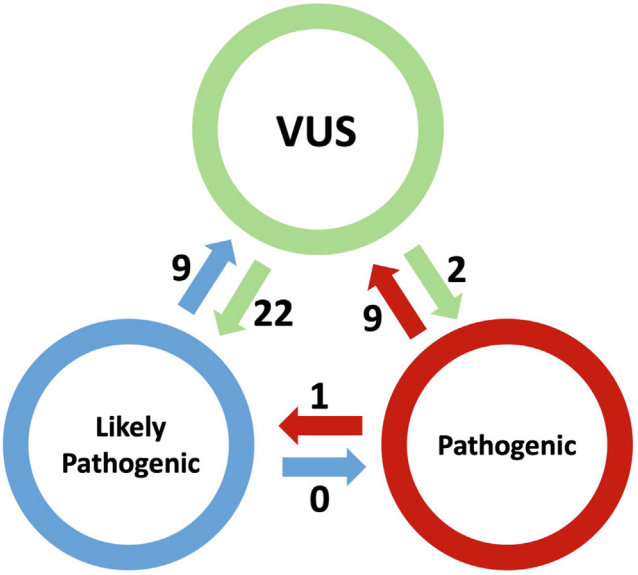

Figure 2 Number of variants changed between each classification. During the reanalysis, 43 variants changed interpretation. Among them, 24 variants formerly classified as VUS were upgraded to LP/P (22 VUS → LP, 2 VUS → P). Nine former LP variants were downgraded to VUS and 9 from P to VUS. One variant was downgraded from P to LP.

GT and restores a stronger consensus splice site, leading to the downgrade of this variant from pathogenic to VUS.

New evidence

More information can be available subsequent to an initial variant review. Newly published cases or new functional studies showing deleterious function can each lead to upgrading of a prior classification. Newly published benign functional studies, or more reports of variants in control populations, may lead to downgrades.

For example, *MYH7* (HGNC gene ID: 7577) (NM_000257.4):c.4145G>A, p.(Arg1382Gln) in eMERGE related to familial hypertrophic cardiomyopathy was initially classified as VUS in March 2019 and changed to LP in October 2021 reanalysis. When initially reviewed, there were not enough affected individuals according to the *MYH7* guideline by ClinGen.[4] After initial review, additional probands allowed us to apply the PS4 ACMG subcategory.[65-67] This variant was discussed extensively in classification concordance research across 9 genomic implementation research studies.[68]

In the process of interpretation, one often encounters variants that are at the borderline of VUS and LP. They are currently classified as VUS but can be upgraded to LP when there is more evidence. People need to pay special attention to these variants because they may change the interpretation due to newly published articles or reported cases. Our internal system has a classification called "VUS-Leans LP" to describe this type of variant. In reanalysis, these variants were screened for interpretation.

Inaccurate previous classification

Because variants are manually reviewed, there is the possibility of misinterpreted evidence or missed data. Periodic reanalysis finds previous errors and corrects them in time. At the same time, analyzing the reasons for previous mistakes can avoid making them again in the future.

KCNE1 (HGNC gene ID: 6240) (NM_000219.6): c.292C>T, p.(Arg98Trp) was initially classified as pathogenic in June 2019 and changed to VUS in October 2021 reanalysis. In the initial review, this variant was seen reported in patients with long QT syndrome or sudden unexpected death[69-72] and segregated with disease.[72] Thus, this variant was evaluated as pathogenic. However, during the reanalysis process, the reevaluation of an old publication and the addition of a newly published article supported that this variant should be better classified as VUS.[73,74] This variant did not segregate with the disease in 1 family affected with epilepsy and/or long QT syndrome, indicating a benign classification.[73] At the same time, a *KCNQ1* exon 2 deletion segregated with the disease, suggesting that the *KCNE1* p.(Arg98Trp) may not be variant that causes the disease.[73] In addition, 2 individuals with this variant are lifelong asymptomatic, suggesting that this variant is associated with a mild form of congenital long QT syndrome.[74] A functional study of this variant showed only a partially reduced current density.[71] Together with the new PM2_supporting rule, this variant was downgraded to VUS in reanalysis.

Among the variants we analyzed, the most common reason for upgrades was "new evidence" (14 variants, 58.33% of total upgraded variants) (Table 5). Newly published cases and functional studies provided additional evidence for interpretation, which can alter the original classification. Another important reason to upgrade was new specific guidelines (9 variants, 37.50% of total upgraded variants). In the new guidelines, many standards and subcategories have new evaluation methods, such as the number of cases and points calculation methods of PS4 subcategories (_strong, _moderate, and _supporting), the evaluation criteria of PS3 in functional research, and the strength level of PP3 in computational evidence support for deleterious effect.

A key factor leading to downgrading is clinical lab rule change (9 variants, 47.37% of total downgraded variants) (Table 5). Because the absence in the population data PM2 changed from a moderate level to a supporting level, the classification of some variants was downgraded. Reevaluation of previous evidence and new specific guideline are also the main reasons for the downgrade.

Discussion

In this report, we generated a systematic filtering method, analyzed the reanalysis statistics in our database, and dissected the reasons that cause reclassification of genetic variants. The reanalysis led to an interpretation result change of 43 variants from the 241 unique variants after filtering.

Table 4 Variants number changed in each gene and genes relation to diseases[a]

Disease Type	Gene	Number of Variants Postfilter	Number of Variants Changed	Percentage of Change	Related Disease and Phenotype MIM Number
Cardiac diseases (17/43 = 39.53%)	*DSC2*	3	1	33.33%	Arrhythmogenic right ventricular dysplasia 11 (MIM 610476)
	DSP	2	1	50.00%	Arrhythmogenic right ventricular dysplasia 8 (MIM 607450)
	KCNE1	1	1	100.00%	Long QT syndrome 5 (MIM 613695); Jervell and Lange-Nielsen syndrome 2 (MIM 612347)
	LMNA	2	1	50.00%	Cardiomyopathy, dilated, 1A (MIM 115200)
	MYBPC3	9	3	33.33%	Cardiomyopathy, dilated, 1MM (MIM 615396); Cardiomyopathy, hypertrophic, 4 (MIM 115197)
	MYH7	11	5	45.45%	Cardiomyopathy, hypertrophic, 1 (MIM 192600)
	LDLR[b]	28	4	14.29%	Hypercholesterolemia, familial, 1 (MIM 143890)
	PCSK9[b]	7	1	14.29%	Hypercholesterolemia, familial, 3 (MIM 603776)
	Total	63	17	26.98%	
Cancer or tumor (13/43 = 30.23%)	*ATM*	6	2	33.33%	Breast cancer, susceptibility to (MIM 114480); ataxia-telangiectasia (MIM 208900)
	BRCA1	10	1	10.00%	Breast-ovarian cancer, familial, 1 (MIM 604370)
	BRCA2	15	2	13.33%	Breast-ovarian cancer, familial, 2 (MIM 612555)
	CHEK2	6	1	16.67%	Breast cancer, susceptibility to (MIM 114480); prostate cancer, familial, susceptibility to (MIM 176807)
	MSH2	4	1	25.00%	Colorectal cancer, hereditary nonpolyposis, type 1 (MIM 120435)
	MSH6	3	2	66.67%	Colorectal cancer, hereditary nonpolyposis, type 5 (MIM 614350)
	MUTYH	7	1	14.29%	Adenomas, multiple colorectal (MIM 608456); gastric cancer, somatic (MIM 613659)
	PMS2	4	1	25.00%	Colorectal cancer, hereditary nonpolyposis, type 4 (MIM 614337)
	SDHB	4	1	25.00%	Paragangliomas 4 (MIM 115310)
	VHL	2	1	50.00%	von Hippel-Lindau syndrome (MIM 193300)
	Total	61	13	21.31%	
Respiratory abnormalities (7/43 = 16.28%)	*CFTR*	19	7	36.84%	Cystic fibrosis (MIM 219700); congenital bilateral absence of vas deferens (MIM 277180)
	Total	19	7	36.84%	
Neurological defects (6/43 = 13.95%)	*RYR1*	22	4	18.18%	Malignant hyperthermia susceptibility 1 (MIM 145600)
	SCN1A	1	1	100.00%	Dravet syndrome (MIM 607208); febrile seizures, familial, 3A (MIM 604403); migraine, familial hemiplegic, 3 (MIM 609634)
	SCN9A	2	1	50.00%	Paroxysmal extreme pain disorder (MIM 167400); erythermalgia, primary (MIM 133020); insensitivity to pain, congenital (MIM 243000)
	Total	25	6	24.00%	

MIM, Mendelian Inheritance in Man.
[a]Genes not listed here have no changed variants in this reanalysis study.
[b]*LDLR* and *PCSK9* are related to cardiac diseases as secondary findings.

55.81% had upgraded classification, and 44.19% were downgraded. Among the variants reclassified, 17 variants (39.53%) are related to cardiac diseases, and 13 variants (30.23%) are related to cancer or tumor (Table 4). A minority of changed variants are associated with respiratory abnormalities and neurological defects (Table 4). Secondary findings were also taken into consideration.[75]

Method of filtering

In our study, we used a newly developed method to filter the variants that need to be reanalyzed by comparing the results submitted in the ClinVar website between August 2021 and August 2020 when we performed our last reanalysis, focusing on newly changed information. This method effectively screened potential candidates that may change during reanalysis. At the same time, the query was conducted in 3 databases containing a large number of patient samples, which made the filtering more comprehensive and not missing potential variants.

We used very detailed screening criteria (Table 1). For example, the change from VUS to LB/B does not affect the doctors' final treatment decision; therefore, there is no need for reanalysis. However, if a variant's classification was VUS in our database before, and the latest ClinVar submitter presents the classification of LP/P, it would be flagged as

requiring reanalysis. Once the VUS is upgraded to LP/P based on the latest information, the patients who have had no therapy requirement before will need to be intervened in the disease through medical means. Similarly, if a patient's variant was previously classified as LP/P in our database, but all up-to-date submissions in ClinVar are VUS, special attention should be paid. Once LP/P downgrades to VUS after reanalysis, the patient does not need to be treated, which can help the patient avoid unnecessary pain.

Method of reclassification

Because methods of different clinical laboratories are dissimilar, reanalysis can also be conducted differently. For example, 1 laboratory focused on 2 of the ACMG classification codes that are related to function (PS3 and BS3) and reclassified these variants with functional study data using automated high-throughput patch clamp devices.[18] Other laboratories emphasized cosegregation of the gene with related diseases (PP1)[24,26] and coordinated the recruitment of other family members by approaching them through phone and email, collected the saliva and tumor samples of relatives, and finally conducted reclassification from the perspective of PP1 by studying the genotypes and phenotypes of other family members.[24,44] Furthermore, some laboratories centered on certain kinds of diseases, such as cardiovascular disease. In addition to the ACMG general guidelines, they also considered the guidelines on specific genes, such as *SCN5A*, *LDLR*, and *MYH7*.[4,22,32,76,77] Moreover, reviewers were mindful of additional clues that might affect interpretation including co-occurrence with other variants, surgical decisions, and so on.[19]

Considering our filtering mechanism is very effective and leads to a significant reduction in the total number of variants needing reanalysis compared with other screening methods, we have more time and labor to focus on these most potential variants. We conducted a thorough interpretation for all these filtered variants, including checking relevant information in related resources, such as ClinVar, gnomAD, Genomenon, PubMed, prediction tools, and the information from our internal database, taking into account the special guidelines of some genes and focusing on literature published recently.

Time spent on reanalysis

Reanalysis can be a time-consuming task depending on the number of projects and variants that need to be reevaluated, whether new guidelines have been published, and the reviewers' experience level. In this study, the time it took for extensive study was calculated based on the statistics of previous interpretations in our center. The time spent in this reanalysis was about 31 days for 1 reviewer (15.5 days for 2 reviewers and 10.3 days for 3 reviewers) and an average of 62 minutes for each hard variant, which is an acceptable amount of time if variants are reanalyzed every year.

Table 5 Statistics of reclassification reasons

Update Type	Reason	Number	Percentage
Upgrade	New evidence	14	58.33%
Upgrade	New specific guideline	9	37.50%
Upgrade	PVS1 Reevaluation	1	4.17%
	Total	24	100.00%
Downgrade	Clinical lab rule change	9	47.37%
Downgrade	PVS1 Reevaluation	4	21.05%
Downgrade	New specific guideline	3	15.79%
Downgrade	New evidence	2	10.53%
Downgrade	Inaccurate previous classification	1	5.26%
	Total	19	100.00%

Reanalysis interval

Reanalysis is necessary for keeping databases updated and providing interpretation that is more accurate for both old and new patients. Updates that are more frequent will definitely lead to results that are more accurate; however, time and labor costs need to be considered. How often should reanalysis be performed? Each clinical laboratory has its own special guideline to balance the information freshness and cost. For example, Chiang et al[21] calculated the suggested time interval for the reassessment by comparing the date of the updated reclassified report with the original test report date. They found that among the 6-year period variants they reviewed, the median and mean time of reclassification were 1 and 1.62 years, respectively,[21] which is consistent with our center 1-year interval workflow.

Other studies also suggested variable intervals based on their research and calculation method, such as the reclassification median time of 39 months for VUS variants follow-ups[78] and median of 298 days in the cosegregation study.[24] Some variants might take longer to update to a new classification such as 4.43 years,[21] and it happened that some VUS variants kept the same classification even after 8 or more years of follow-up.[19] It is highly recommended to reevaluate the original classification periodically to keep the information most up to date.

Limitations

The high percentage of interpretation change among filtered variants (17.84% [43/241] in our reanalysis) suggests that the screening method can efficiently filter potential variants without wasting time on unnecessary variants. Although our screening method has improved the efficiency of reanalysis, it is not perfect and there is still bias. Our screening is based on the ClinVar data. Therefore, variants undergoing

reanalysis must have a ClinVar entry. In addition, the new ClinVar submissions have to be different from the former ones or VIP category to trigger flagging. Variants that do not meet these requirements will be missed. In the future, we will combine screening methods from other laboratories mentioned in this article to make the reanalysis broader and more thorough.

Appropriate follow-up with patients who have VUS variants is necessary for making optimal clinical decisions.[79] Typically, our projects do not return VUS; therefore, the filtering system does not compare the ClinVar VUS and LB/B submissions. A detailed classification of VUS (leans LP or leans LB) would provide results that are even more accurate.

Here, we have shown an automated filtering process triggered by changes in ClinVar. It increased the reanalysis efficiency, screened potential variants, and saved reviewing time. Future directions could focus on improving the filtering process to make it more precise and developing methods to send reclassified reports automatically.

Data Availability

The data sets generated and analyzed during the current study are available from the corresponding author on request.

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Author Information

Data Curation: L.L., X.T.; Formal Analysis: L.L., X.T.; Project Administration: L.L., E.V., B.Y., R.A.G.; Software: E.V., V.W.; Supervision: L.L., E.V., B.Y., R.A.G.; Visualization: L.L.; Writing-original draft: L.L.; Writing-review and editing: L.L., V.W., E.V., B.Y., R.A.G., X.T.

ORCIDs

Lele Li: https://orcid.org/0000-0002-3607-3821
Richard A. Gibbs: https://orcid.org/0000-0002-1356-5698
Bo Yuan: https://orcid.org/0000-0001-7278-5116
Eric Venner: https://orcid.org/0000-0002-3319-6690

Ethics Declaration

Informed consent was not obtained and Institutional Review Board (IRB) or Research Ethics Committee (REC) approval is not required. All the data involved in this paper are genetic variant data that do not involve individual-level information. All the clinical data are deidentified.

Conflict of Interest

Eric Venner is a cofounder of Codified Genomics. All other authors declare no conflicts of interest.

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