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Automated variant re-evaluation is labor-balanced and gives clinically relevant results: Hereditary cardiac disease as a use case

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ABSTRACT

Background: Genetic findings influence clinical care of patients suspected of hereditary cardiac diseases. As additional knowledge arises over time, the classification of genetic variants may change. The labor cost associated with systematic manual reevaluation for reported variants is substantial. We applied an automated variant classifier for reevaluation of previous reported variants to assess how such tools may assist in manual reevaluation.

Methods: Historically (2010–2022), patients (N = 2987) suspected of inherited cardiomyopathies or ion-channel disorders were screened for genetic variants in at least one of up to 114 genes. We had reported 1455 unique variants, of which 742 were among the 14 most relevant genes. In the 14-gene-group, we compared our reported classification to that of an autoclassifier and manually reevaluated variant classification of all variants. Among the remaining genes (N = 100), only variants where the autoclassifier predicted change of clinical impact, such as variant of uncertain significance to likely pathogenic or oppositely, were manually reevaluated.

Results: We identified 9% (66/742) of variants with clinical impact in the 14-gene-group. Of these, 91% could have been identified solely evaluating the 120 variants where the autoclassifier had predicted a change of clinical impact. In the 100 remaining genes, a change of clinical impact was identified in 3% (22/713) after manual reevaluation.

Conclusion: Using an autoclassifier reduces the workload to identify variants likely to have a change in variant class with clinical impact. Hence, we recommend using such tools to identify the variants most relevant to manually reevaluate to improve patient care.

1. Introduction

Molecular genetic diagnostics has evolved enormously in the past decade. As a result, genetic testing is offered to an increasing number of patients in an increasing number of genes as part of the diagnostic workup. Variant interpretation is a key element in clinical genetic reports as the classification and conclusions in the reports are used to guide treatment, patient monitoring, and tracing relatives.

Since 2015, germline genetic variants are classified according to the international variant classification system provided by American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP classification) (Richards et al., 2015; Westphal et al., 2022). Using this system, variants are classified as benign (Class 1, C1), likely benign (C2), uncertain (C3), likely pathogenic (C4), or pathogenic (C5). This evaluation system is considered the gold standard for variant classification in rare hereditary disease. Though stringent, the classification may still leave the clinician unsure of the result in a clinical context, particularly with regards to C3 variants. In recognition, the comprehensive variant interpretation guideline by the British Association for Clinical Genomic Science (ACGS) also includes guidelines for the content in clinical variant reports, e.g., discussions of whether to report C3 variants, recommendations for

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reevaluation of reported variants, as well as highlighting that a laboratory system enabling variant reclassification is crucial (Ellard et al., 2020).

Variant classification is not carved in stone (Ellard et al., 2020). New knowledge of genetic variants continues to arise as more information is shared in public databases such as ClinVar (Landrum et al., 2016, 2018), Decipher (Firth et al., 2009), and UK biobank (Investigators et al., 2021) as well as in publications. Of major impact in recent years is the large work being conducted by ClinGen's Variant Curation Expert Panels (Rivera-Munoz et al., 2018). This adds additional valuable information to the variant classification of already reported variants, as exemplified in recent gene-specific reevaluation studies (Andreis et al., 2023; Hovland et al., 2023; Reuter et al., 2023; Testa et al., 2023) and a recent publication on variant classification principles (Walsh et al., 2024).

Continuous and systematic reevaluation of genetic test results is therefore an increasingly essential issue in modern healthcare. ACGS recommends storing data in a way that allows for reclassification if required. However, they do not suggest re-evaluation *per se*. Instead, careful sharing of reclassified variants to all implicants should take place, if such situations occur (Ellard et al., 2020). The Cancer Variant Interpretation Group UK (CanVIG-UK) has tried to generate a framework for proactive variant reclassification to ease this procedure (Loong et al., 2022). However, they acknowledge that systematic reevaluation is not currently practicable due to lack of automized systems. Also, the recently updated guideline for genetic testing in inherited cardiomyopathies and arrhythmias state that "Periodic review and re-evaluation of previously described variants should be *considered*" (Hayesmoore et al., 2023). Hence, it is not a strict requirement to do regular variant reevaluations although stakeholders agree on the need for guidelines concerning variant reinterpretation to standardize the approach across different laboratories (Berger et al., 2022).

Until now, reevaluation of a specific variant in our setting was only by request from the treating clinician. Alternatively, if a previously reported variant was found in a new patient, a reevaluation may result in a reclassification. In these cases, all patient reports containing the variant were rewritten and sent to the referring clinicians. To our knowledge, systematic variant reevaluation is not a routine part of most clinical genetic laboratories. This is partly because the task is very time-consuming. However, with the increasing use of larger gene panels and whole-genome-sequencing (WGS), the number of variants to be considered continues to increase, which further augment the need for systematic reevaluation.

An accumulating number of commercial tools for variant autoclassification appear on the market. They are based on the current ACMG/AMP classification system and use information in public databases about the genetic variant, include recent *in silico* tools for predicting the variants effect on protein function, variant databases, as well as community information derived from users of the specific tool (Alenezi et al., 2023).

Our goal with this study was to explore if and how an autoclassifier can be used to ease systematic variant reevaluation of previously reported variants. As an example, we used variants reported in patients previously analyzed for inherited cardiac diseases.

2. Materials and methods

This single center study was conducted at Department of Molecular Medicine, Aarhus University Hospital, Denmark. The laboratory has analyzed genetic data from patient groups with hereditary cardiac diseases since 2009; the majority referred from Department of Cardiology, Aarhus University Hospital. The laboratory functions as a service provider and 6 months after final reports are written, the laboratory is not allowed to access patient records. Therefore, pedigrees and/or detailed clinical information are provided as pre-test information only and is not updated after referral.

Since 2016, we have used the ACMG/AMP variant classification system (Richards et al., 2015). In our setting, variants classified as C3-5 at the time of testing are reported back to the clinicians. Until 2016, C1 and C2 variants were also recorded in the variant database system although not reported to the clinicians. After 2016, this was rarely done and only in a non-systematic manner.

We identified all patients who were screened for variants in one or more genes associated with inherited cardiac disease between 2010 and 2022. Carrier testing, i.e., patients only tested for variant(s) previously reported in the family, were not included.

Laboratory methods and gene panels have changed over the study period and are outlined in Supplementary Tables S1 and S2. Reported variants were extracted from our laboratory system WinLAB (HD- Support ApS, Denmark). Information about gene, transcript, nomenclature of the reported variant (DNA and protein level), last date of in-house variant assessment, as well as Reported classification was obtained. Data was extracted anonymously without association to patient identity (Supplementary Table 3).

The identified variants were examined in VarSome Premium using its variant REST endpoint for hg38 b y the associated DNA-level HGVS nomenclature and reference sequence (Kopanos et al., 2019). For each variant, the ACMG verdict from VarSome – termed AutoClassification– was compared to the previously Reported classification from our laboratory. One major advantage of VarSome AutoClassification Premium is the access to and presentation of most recent knowledge. A main limitation is that the criteria PS4, PM3, PP4, BP2, and BP5 are not included in the AutoClassification; (for further limitation details, please see (Varsome).

All variants in the 14 genes most commonly associated to non-syndromic cardiomyopathies (*FLNC, LMNA, MYBPC3, MYH7, TNNI3, TNNT2, TTN, DSG2, DSP, PKP2* (Cirino et al., 1993; Hershberger et al., 1993)) and arrhythmias (*KCNH2, KCNQ1, RYR2, SCN5A* (Hayesmoore et al., 2023)) were manually curated irrespective of conformity between Reported and AutoClassified or not. At least two experienced clinical laboratory geneticists curated the variant following the ACMG/AMP 2015 standard system using ClinGen SVI VCEP recommendations for genes where these are available (ClinicalGenomeResource).

We defined classification changes as having clinical impact if the change was from either C3 to C4 or C5 or from C4 or C5 to C3. Variants that changed class after manual reevaluation were classified as a true class-change termed ReClassification.

Among the remaining 100 inherited cardiac disease associated genes, variants were manually reevaluated only when AutoClassified predicted a difference of clinical impact compared to Reported classification.

If the manual evaluation resulted in variant ReClassification, the laboratory system allowed identification of the patient in which the variant was reported, and a revised clinical report was written and sent to the requisitioned clinical department.

According to Danish legislation, the study classifies as a quality assurance study. Therefore, ethical permission was not needed.

All calculations and plots were done using Microsoft Excel ®. All Reclassified variants are submitted to ClinVar)ClinVar submission SUB14672096).

3. Results

Within the study period (2010–2022) a total of 2987 patients were screened for variants in one or more of 114 genes associated with inherited cardiac disease (Supplementary Tables S1 and S2). None of the screened index patients were known to be related. In these screening analyses, we reported 1455 unique variants. Fig. 1 shows a flowchart summarizing the data processing workflow in the study. We used a tool, VarSome, to autoclassify the variants according to ACMG/AMP variant classes C1-C5. Of the 1455 variants, the Reported and AutoClassified variant classification was identical in 721 (49.6%) cases, 547 (37.6%) cases had 1 class difference, 185 (12.7%) had 2 class differences, and 2

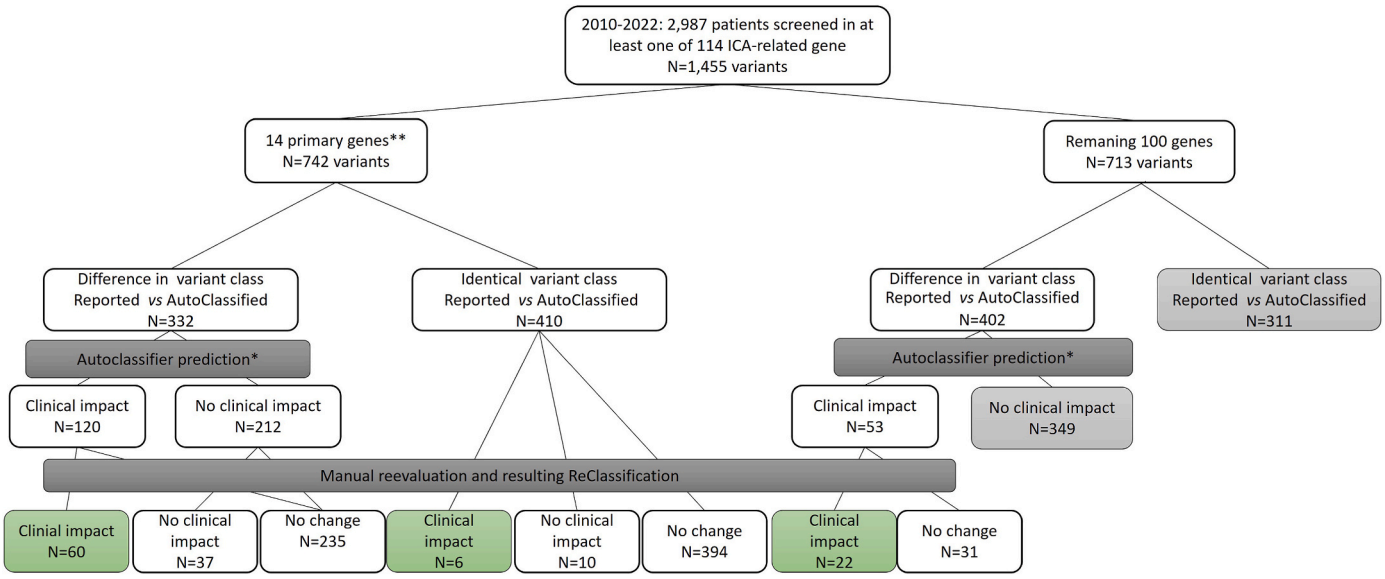

Fig. 1. Flowchart of the variant analysis workflow.
All variants in the 14 genes primary associated to non-syndromic inherited cardiac diseases and all variants in the remaining 100 genes were analyzed separately. Green Background: Re-evaluated variants with clinical impact* Light grey: Variants not manually re-evaluated *Change from C1/C2/C3 to C4/C5 and C4/C5 change to C1/C2/C3 are considered of clinical impact ***FLNC*, *LMNA*, *MYBPC3*, *MYH7*, *TNNI3*, *TNNT2*, *TTN DSG2*, *DSP KCNH2*, *KCNQ1*, *PKP2*, *RYR2*, *SCN5A*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(0.1%) cases had 3 class differences. Of the variants changing 1 class up (Autoclassifier being higher class (Arrow pointing up in Table 1)), 62% (N = 102/165) were changing from C3 to C4. Of those changing one class down, 70% (N = 270/382) were from C3 to C2 (see Table 1, right part).

We manually reevaluated the variant classification of all 742 reported variants in the 14 genes most frequently associated to inherited cardiac disease (Hayesmoore et al., 2023; Cirino Ah, 1993; Hershberger et al., 1993; Cirino et al., 1993–2023; Hershberger and E. Dilated Cardiomyopathy Overview, 1993–2023; McNally et al., 1993–2023; McNally et al., 1993). This corresponds to 51% (742/1455) of the entire pool of variants in the 114 genes associated to inherited cardiac disease. We split the variants into those with difference between Reported and Autoclassified (N = 332; 45%) and those with no-difference (N = 410; 55%).

The autoclassifier identified 120 variants where the predicted

Table 1
Overview of the number of variants with identical (=), lower (↓) or higher (↑) AutoClassification variant class than Reported.

Reported vs AutoClassifier	no of class diff.	14 most relevant genes (N (total = 742)[a]			100 genes (N (total) = 713)[a]			All 114 genes (Ntotal = 1455)[a]			Clinical impact?
		Variants (N)	%	N (sum)	Variants (N)	%	N (sum)	Variants (N)	%	N (sum)	
C1 to C1 (=)	0	51	6.9	410	39	5.5	311	90	6.2	721	
C2 to C2 (=)	0	10	1.3		8	1.1		18	1.2		
C3 to C3 (=)	0	218	29.4		232	32.5		450	30.9		
C4 to C4 (=)	0	39	5.3		4	0.56		43	3.0		
C5 to C5 (=)	0	92	12.4		28	3.9		120	6.2		
C1 to C2 (↑)	1	5	0.67	270	3	0.4	277	8	0.555	547	
C2 to C1 (↓)	1	43	5.8		30	4.2		73	5.0		
C2 to C3 (↑)	1	9	1.2		6	0.84		15	1.0		
C3 to C2 (↓)	1	84	11.3		186	26.1		270	18.6		
C3 to C4 (↑)	1	69	9.3		**33**	4.6		102	7.1		yes
C4 to C3 (↓)	1	8	1.1		**5**	0.68		13	0.89		yes
C4 to C5 (↑)	1	31	4.2		9	1.3		40	2.75		
C5 to C4 (↓)	1	21	2.8		5	0.70		26	1.79		
C1 to C3 (↑)	2		0.0	61		0.0	124	0	0	185	
C2 to C4 (↑)	2	1	0.13		0	0.0		1	0.07		yes
C3 to C1 (↓)	2	17	2.3		110	15.4		127	8.7		
C3 to C5 (↑)	2	41	5.5		8	1.1		49	3.4		yes
C4 to C2 (↓)	2	1	0.13		3	0.42		4	0.27		yes
C5 to C3 (↓)	2	1	0.13		3	0.42		4	0.27		yes
C1 to C4 (↑)	3		0.0	1		0.0	1	0	0	2	yes
C2 to C5 (↑)	3		0.0			0.0		0	0		yes
C4 to C1 (↓)	3	1	0.13		1	0.14		2	0.14		yes
C5 to C2 (↓)	3		0.0			0.0		0	0		yes
C1 to C5 (↑)	4		0.0	0		0.0	0	0	0	0	yes
C5 to C1 (↓)	4		0.0			0.0		0	0		yes

[a] The number in brackets refers to the number of unique reported variants in each gene-group or in total.

change in variant class had clinical impact (defined as C3 to C4 or C5; C4 or C5 to C3; Fig. 1). Upon manual reevaluation, 60 (50%) of these were ReClassified with resulting clinical impact (Table 2A). Of the 410 variants with identical Reported and AutoClassified variant classification (Table 2B), only 16 (4%) variants were ReClassified of which 6 (1.5%) had clinical impact. There was no consistency in gene or variant type among these six variants (data not shown).

Overall, of the 742 variants manually reevaluated in the 14 cardiac disease-associated genes, 66 (8.8%) variants had a variant class change with clinical impact. A total of 91% (60/66) of these 66 changes would have been identified by solely curating the 332 variants in which Reported and AutoClassified differed. The 60 variants were within the group where the autoclassifier predicted a change with clinical impact, i.e., 50% (60/120) of this group had a true reclassification. Sensitivity and specificity for identification of variants with clinical impact were both 91%, see Table 3.

Fig. 2 illustrates the distribution of change in variant class of each of the genes in the inherited cardiac disease-associated 14-gene group. This shows that *TTN* and *RYR2* had the highest number of reported variants. This partly reflects the size of these genes. *TTN* is also the gene with the highest number of reclassified variants; 40 of 62 (65%) variants differed between Reported and ReClassified. These variants are predominantly reclassified from C3 to C4/C5 (38 variants). This was mainly due to the laboratory having a long tradition for reporting nonsense/loss-of-function variants in the A-band of the gene as C3 as opposed to recent recommendations to classify as at least C4 (Morales et al., 2020).

During the study period, we did not see a change in fraction of variants being reclassified, see Supplementary Table S4.

Among the 713 unique variants in the remaining 100 genes, Reported and AutoClassified variant classification was identical in 311 (44 %) cases. In the remaining 402 (55%) variants, where Reported and Autoclassified differed, 53 variants had a change with clinical impact (Table 1, center part). Based on the high sensitivity and specificity predicted from the 14-gene group, we chose only to reevaluate these 53 variants among the 100-gene group. This resulted in ReClassification of 22 variants (42% of the reevalutated variants) with a clinical impact, see Fig. 1 (right). This corresponds to 3% of the 713 variants in the 100-gene group.

In total, we reclassified 88 variants of clinical impact; among the 14-gene group, we identified 60 variants where the autoclassifier identified a variant change of clinical impact and 6 variants where the autoclassifier did not, and among the 100-gene-group, we identified 22 variants with a change of clinical impact. From our variant reporting database, we identified that these variants affected a total of 95 families

Table 3
Punnet square table containing AutoClassified variants with clinical impact compared to the numbers of truly ReClassified upon manual reevaluation among the 14 genes with long-known association to inherited cardiomyopathy or cardiac arrhythmia disease. Sensitivity, specificity positive (PPV) and negative (NPV) predictive values are calculated.

	Reclassified upon manual reevaluation	No Reclassifcation upon manual reevaluation	
Change of clinical impact[a], AutoClassifier	60	60	PPV: 60/(60 + 60) = 50%
No Change of clinical impact[a], AutoClassifier	6	616	NPV: 616/(6 + 616) = 99%
	Sensitivity: 60//60 + 6) = 91%	Specificity: 616/(616 + 60) = 91%	

[a] Clinical impact: Changes from either C3 to C4 or C5 or from C4 or C5 to C3.

Table 2
Among the 14 main genes, the number of variants ReClassified after manual reevaluation of variants where Reported and AutoClassified differed (A) or were identical (B). The grey marking indicates the changes that have clinical impact. Among the 6 variants in Table B, 5 changed from C4 to C3 and were missense or inframe deletion/duplication and one was upgraded from C3 to C4 as a splice variant where functional analysis had become available.

A

		Change by 1 class from…					Change by 2 class from…				
	total	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
Upclassified	69	0	4	45	10	-	0	0	10	-	-
Downclassified	28	-	0	11	4	6	-	-	6	1	0

B

		Change by 1 class from…					Change by 2 class from…				
	total	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
Upclassified	2	0	0	1	1	-	0	0	0	-	-
Downclassified	14	-	0	1	5	7	-	-	1	0	0

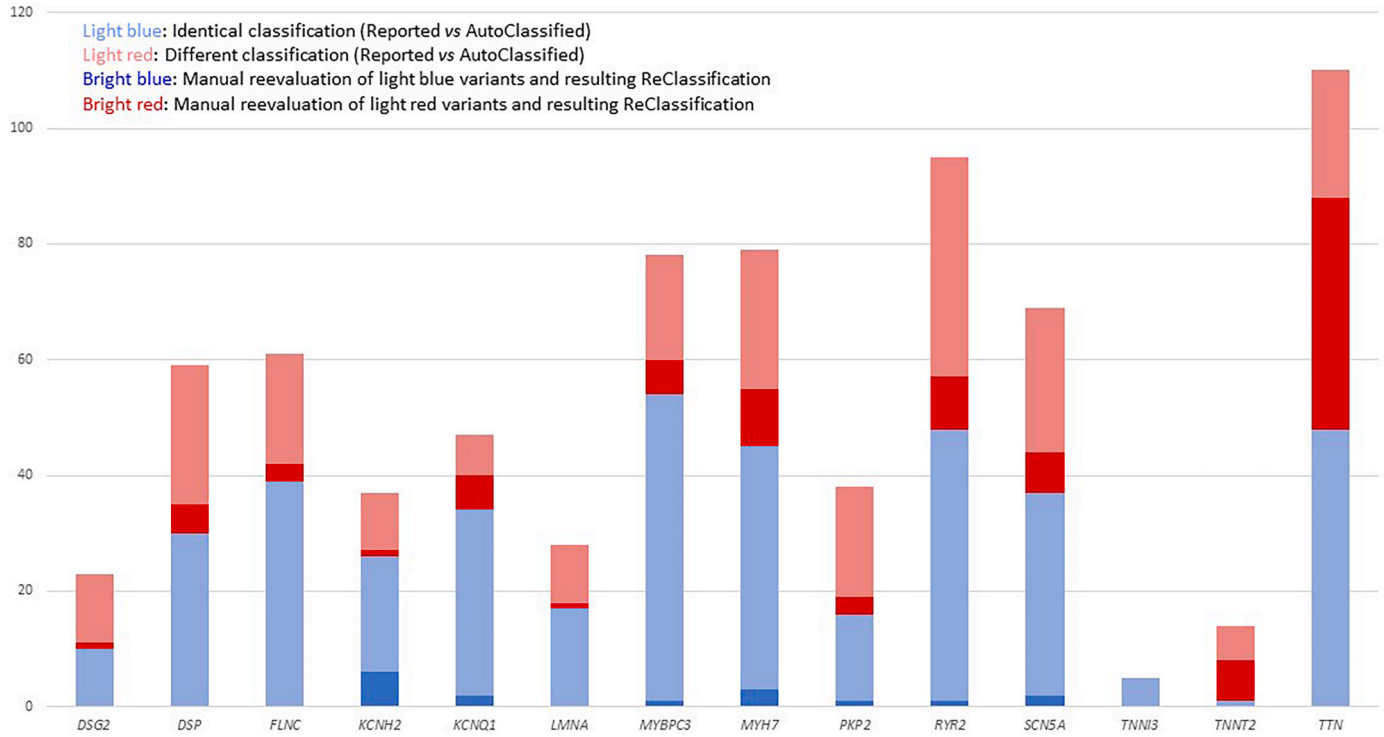

Fig. 2. Distribution of variants among the 14 primary genes, where all reported variants were manually reevaluated.

with a total of 202 family members; 80 families had variants upclassified and 15 had variants downclassified (data not shown). In addition, five reclassified variants with clinical impact were identified in more than one family; these were all A-band variants in *TTN* (data not shown).

4. Discussion

To our knowledge, this is the first study to explore how an autoclassifier can contribute to ease reevaluation of reported genetic variants in patients suspected of hereditary heart disease. In the study period, 1455 variants in 2987 patients were included. Within the two groups of genes, i.e., the 14 most common, and the remaining 100 others, we found that 9% and 3% of the variants, respectively, had a reclassification with clinical impact. The reason for this difference is unknown, but we speculate that the main cause is due to the 14-gene-group containing well-known genes where phenotype-genotype correlation has been known for years, and for which SVIs exists for several of them. For most genes in the 100-gene-group, the phenotype-genotype association is less well defined, e.g. hot-spot-regions or haploinsufficiency are not known.

Importantly, among variants manually reevaluated to have a change of clinical impact in the 14-gene-group, 91% would have been identified if limiting the reevaluation to the 120 variants where a change of clinical impact was predicted. Furthermore, the rate of reclassification was high (50% in the 14-gene-group and 42 % in the 100-gene group) when restricting the analysis only to variants where the autoclassifier had predicted a change with clinical impact. Thereby, we have demonstrated that an autoclassifier is a valuable tool in performing regular systematic reevaluation of variants in clinical laboratories.

A limited number of publications exists concerning the value of variant reevaluation in patients with inherited cardiac diseases. In addition, these are solely based on manual, labor-intensive reevaluations. In a study of 73 patients with dilated cardiomyopathy, no disease-causing variants was identified after reevaluation, but it did result in a downgrading of a large proportion of C3 variants (Westphal et al., 2020). Reevaluation of reported variants in 51 patients with long QT syndrome identified 14% of variants that changed classification from C4/5 to C3 (Martinez-Barrios et al., 2023). In addition, reassessment of variants in 121 patients reported 10 years earlier, resulted in change of classification in 72% of the reported variants, and a significant number changed from C4 to C3 (Campuzano et al., 2020). A different study accessed how variant class changed over time in the ClinVar database focusing on genes associated with cardiac channelopathies and found that variant classification goes towards uncertainty (Rosamilia et al., 2022). These studies, although smaller data set than ours, support that systematic reevaluation will result in reclassification. In our study, we found a higher fraction of upclassified variants compared to previously published work. This may reflect local biases of being too cautious in variant classification. If we exclude the 38 TT N variants, our data to a higher degree resembles that of others.

Our data show that the used autoclassifier has a high precision in finding the variants in need of manual reevaluation. If we limit the manual reevaluation to variants where the autoclassifier indicates changes with clinical impact, we will eventually miss some variants. However, we still find that this strategy yields a good balance between workload and identifying reclassifications of clinical impact.

We found 60 variants which were predicted to have a clinical impact but subsequently did not result in reclassification upon manual reevaluation. The main reason was that the used autoclassifier did not adjust for the gene specific variant interpretation guideline available (ClinicalGenomeResource.). To improve variant classification tools in the future, it is essential that these guidelines are integrated.

Changes in variant interpretation have the potential to alter previously recommended management of patients (Loong et al., 2022; Hayesmoore et al., 2023; Berger et al., 2022). The consequences of changing a variant from C3 to C4 involved 80 families; family members got the opportunity to enter control programs if they carried the variant and be taken out if they did not. Further, the genetic finding can be used in reproductive planning if the family wishes to do so. In our clinical setting, most of the families with reclassified C3 *TTN* variants were already treated as if the variants were C4, but the reclassification of the variants further qualifies predictive genetic testing in family members. In the 15 families with a down-classification from a C4 to a C3 variant,

the family members are now in surveillance programs based on their family heart disease history.

Our goal was to identify variant reclassification with clinical impact. Though, of interest, our data show that the autoclassifier identified a total of 397 variants downgraded from C3 to C1/C2, which corresponds to 47% of all C3 variants in the dataset. Accordingly, a recent large study showed that 80% of C3 variants over time were downgraded to C1/C2 (Chen et al., 2023).

Reasons for not carrying out a systematic variant reevaluation have been associated with limited resource allocation. Our study shows the potential of a systematic reclassification set-up that is feasible to do in a routine clinical setting. We provide an example of how candidate variants most likely to be manually reclassified can be identified with a reasonable balance between workload for manual reevaluation and still finding the majority of variants in need of reclassification of clinical impact. Whether a general autoclassifier or a disease specific database, like BRCA Exchange in hereditary breast- and ovarian cancer (Cline et al., 2018), is the most valuable method to identify variants to be reevaluated must depend on the genes in question and may need local adaptation depending on the design of the local variant databases. Further studies looking into this aspect is warranted. In addition, as variant classification rules align in the years to come, the positive findings from reevaluation may decrease in the future. This may decrease the need for routine automated reevaluation. However, a speculative next-step-scenario still requesting reevaluations in the years to come, is the possibility to automatically (AI-based) include new information from patient and family records in variant reevaluations.

It was not within the scope of this study to evaluate or compare the various autoclassifier tools available today. We highly recommend that the international genetic society can lift this important task and do a large-scale testing of different tools across different clinical and laboratory contexts. The knowledge should then be used to provide best practice guidelines, or at least minimal criteria, for such tools implemented in routine use.

This study does not include an evaluation of how the referring clinicians and patients receive the updated clinical reports. Future studies should investigate that. We also recommend that reevaluation is done in collaboration between the laboratory and clinicians securing a mutual understanding of what to expect from reevaluation. Further, it is advised to inform the patients that a reported variant class is only as good as the data we have available; it may change over time. Long-term, a pro-active approach to reevaluation may allow patients to benefit from the latest research, regardless of the genes involved or the time since the variants were initially observed.

In conclusion, using an autoclassfier to identify variants with high likelihood of a reclassification of clinical impact facilitates a real-time adaptive reclassification approach possible to implement in clinical settings.

CRediT authorship contribution statement

Anne Grosen: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Charlotte K. Lautrup:** Writing – review & editing, Data curation. **Emil Bahsen:** Writing – review & editing, Software, Data curation. **Henrik K. Jensen:** Writing – review & editing, Resources. **Dorte L. Lildballe:** Writing – original draft, Visualization, Resources, Methodology, Formal analysis, Data curation, Conceptualization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2024.104981.

Data availability

Data will be made available on request.

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