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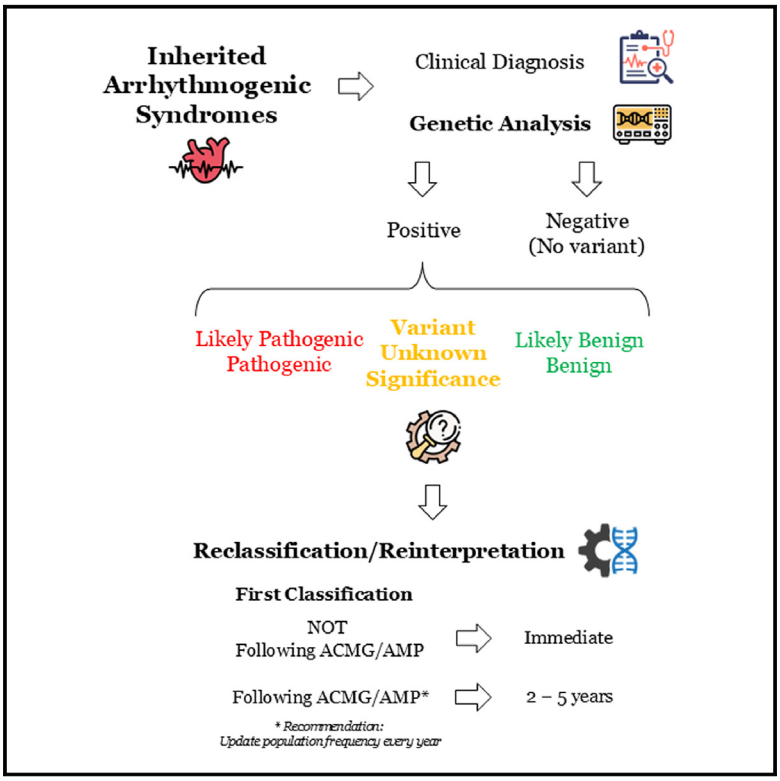
Appropriate time interval to update ambiguous genetic diagnosis in inherited arrhythmogenic syndromes

Graphical abstract



Highlights

- Only a definitive genetic result should be actionable in clinical practice
- The time period of reinterpretation/reclassification should be every 25 years
- Update the population frequency at least once a year, especially if ambiguous
- Legal and ethical responsibilities remain a current matter of argument

Authors

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In brief

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Appropriate time interval to update ambiguous genetic diagnosis in inherited arrhythmogenic syndromes

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SUMMARY

Genetic analysis identified the cause of the disease in inherited arrhythmogenic syndromes. A clinically actionable genetic diagnosis requires an accurate interpretation following the current guidelines. Practically half of the genetic diagnoses remain inconclusive due to the identification of variants of uncertain significance. An update can help shed light on uncertain results. No specific time frame has been set for updating an ambiguous diagnosis. We carried out an analysis of the available reclassification/reinterpretation data concerning genetic diagnosis in inherited arrhythmogenic syndromes. We aim to determine an appropriate interval for updating a conclusive classification. Genetic diagnoses achieved without following current guidelines should be updated immediately. An ambiguous result obtained following the current guidelines should be updated no more than 5 years after the first analysis. There are still questions to be resolved regarding the legal responsibility or who should assume the economic cost of updating a genetic diagnosis.

INTRODUCTION

Inherited arrhythmogenic syndromes (IASs) are rare disorders enclosed in cardiac ion channelopathies (mainly Brugada syndrome [BrS], long QT syndrome [LQTS], short QT syndrome [SQTS], and catecholaminergic polymorphic ventricular tachycardia [CPVT]) and cardiomyopathies (mainly hypertrophic [HCM], dilated [DCM], and arrhythmogenic [ACM]).[1,2] These genetic disorders are characterized by disturbances in electrical conductivity between myocytes, which may occur with or without structural alterations of the myocardium, potentially leading to sudden cardiac death (SCD). Genetic analysis is nowadays an integral part of the IAS diagnostic protocol but should always be performed after a thorough clinical evaluation. However, only a definitive genetic diagnosis is currently actionable in clinical practice for therapeutic decision-making or in forensic field to identify the cause of the death.[3] Indeed, a conclusive genetic diagnosis helps to unravel the origin of the disease in a patient with a conclusive or suspected diagnosis, but it can also clarify the possible cause of an unexpected decease that remains unexplained after performing a complete medico-legal autopsy, known as sudden unexplained death

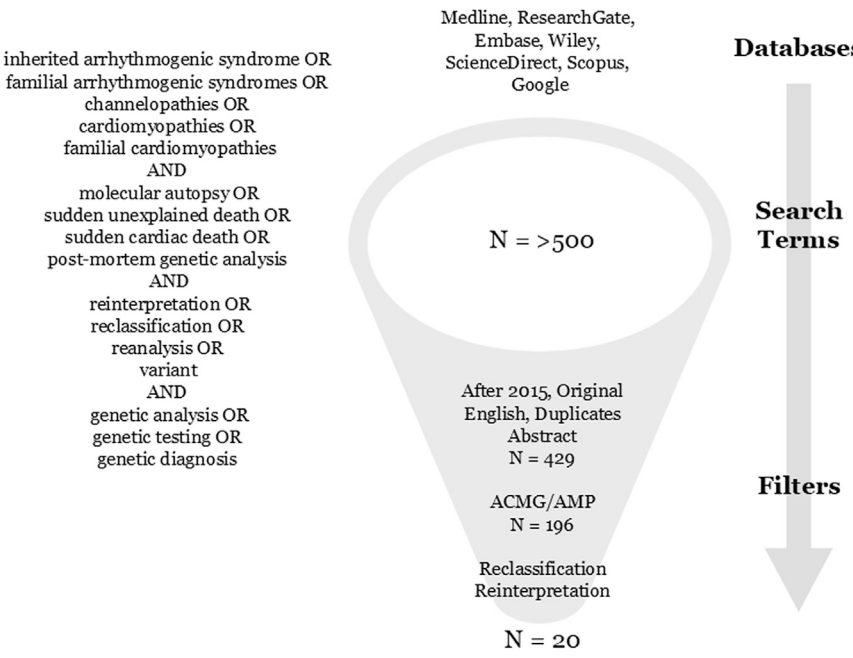

Figure 1. The search flowchart
The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP).

(SUD). Additionally, identifying pathogenic genetic variants associated with IAS facilitates early detection of at-risk family members, who should be also clinically assessed.[4] Some of them may remain asymptomatic but even then are vulnerable to lethal arrhythmogenic episodes due to their genetic predisposition. This early detection enables personalized preventive therapeutic strategies.[1,2]

In 2015, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) published standards and guidelines for interpreting genetic variants based on the available data at the time of classification.[5] These recommendations, which remain the gold standard for variant classification, provide a framework that enhances both accuracy and stringency; it implies that a lack of data or controverse available data leads to a variant of unknown significance (VUS) and thus not actionable genetic diagnosis in clinical practice. This challenge is particularly prevalent for genetic alterations associated with IAS, especially missense variants.[6] This ambiguous role also represents a limitation of molecular autopsy in cases of sudden death without conclusive anomalies explaining the death, as previously mentioned. It is important to highlight that in these postmortem cases that remain unexplained, IAS is suspected as the most likely cause of SUD, especially when it occurs in young population.[7] In these cases, a comprehensive clinical assessment of relatives of mainly young victims results in the diagnosis of IAS in near one-fifth of cases.[4] However, in the criminal justice system, evidence must be obtained beyond any reasonable doubt, and the mere abstract probability of an explanation is not sufficient for legal purposes.

Over time, ambiguous variants may be reclassified as new clinical and experimental evidence emerges, facilitating the identification of deleterious genetic variants that are classified as pathogenic or likely pathogenic (P/LP) according to the ACMG/AMP guidelines.[8–10] This process has important implications for improving prognosis and implementing personalized therapeutic strategies for patients and their relatives.[11] Previous studies reinforce the periodic updating of variants in different genetic diseases.[12,13] Few studies aiming on the reclassification/reinterpretation of variants in IAS have suggested diverse temporal range depending on analyzed disease. However, to date, reclassification timelines remain inconsistent, varying across centers and relying on individual institutional practices, with no standardized framework for result updates. We have comprehensively analyzed all available data as well as our experience in this field to shed light on this current critical gap. This study aims to determine the appropriate time interval for updating ambiguous genetic variants in IAS and provide evidence-based recommendations for reclassification.

RESULTS

Search flow

We identified 20 original studies focused on the reclassification/reinterpretation of genetic variants related to IAS (Figure 1; Table 1). As previously stated, the current guidelines were published in 2015 and have been implemented progressively in the interpretation of genetic diagnoses in laboratories and hospitals worldwide. The earliest reinterpretation study of IAS based on ACMG/AMP guidelines was published in 2019. Most of these studies focused on variants previously classified as VUS or with an ambiguous role in families where a definitive diagnosis of IAS has been made or where IAS was highly suspected. We also conducted a comprehensive analysis of SUD postmortem cases. The percentage of variant reclassification observed varied significantly based on factors such as the year of initial classification, whether the ACMG/AMP guidelines were applied during the initial classification, the year of reclassification or reinterpretation, and whether the cohort analyzed included cases of IAS or postmortem evaluations.

Other genetic alterations such as copy-number variants (CNVs) have been definitively associated with IAS despite being in deleterious ranges of less than 5%.[14–17] To our knowledge, only one study on the reclassification of CNVs have been published.[18] No other studies focused on reinterpretation/reclassification of any other genetic alterations related to IAS have been published so far. Therefore, we have performed an exhaustive analysis of the results reported in each of the 20 studies focused on rare single nucleotide variants (SNVs) or small nucleotide

Table 1. Studies included in our analysis

Study	Cohort	First classification	ACMG/AMP compliance	Reclassification rate
Denham, 2019	BrS (Only *SCN5A*)	Since 2017	No	37%
Bennett, 2019	IAS	2009–2017	No	52%
Mattivi, 2019	*KCNH2* (only deleterious)	2010–2019	No	14%
Campuzano, 2020	IAS SUD	2010	No	71.87%
Quiat, 2020	DCM	2008–2018	No	30%
Westphal, 2020	LQTS	2001–2018	No	14.3%
Cherny, 2020	IAS	2006–2017	No	21.5%
Costa, 2021	ARVC	1998–2015	No	30.94%
		2016–2019	Yes	27.26%
Neubauer, 2021	SUD	2012–2019	No	22.2%
Vallverdú-Prats, 2021	ACM	2016	Yes	30.77%
Davies, 2021	SUD, SCD	2006–2015	No	42%
		2015–2020	Yes	11%
Martinez-Barrios, 2022	*TTN* IAS	2011–2015	No	36.8%
Sarquella-Brugada, 2022	IAS	2016	Yes	18.4%
Novelli, 2022	IAS	2017–2022	Yes	26.6%
Martinez-Barrios, 2023	SUD	2015–2017	Yes	10.52%
Pérez-Serra, 2024	DCM	2016–2019	Yes	12%
Fernández, 2024	IAS	2012–2016	No	52.37%
		2017–2021	Yes	24.22%
Young, 2024	IAS	2004–2015	No	31.5%
		2016–2020	Yes	5.75%
Martin, 2024	IAS, SCD	2016–2022	Yes	32%
Horgan, 2024	HCM, DCM, ACM	2017–2023	Yes	4.8%

ACMG/AMP, American College of Medical Genetics and Genomics and the Association for Molecular Pathology, ACM, arrhythmogenic cardiomyopathy, ARVC, arrhythmogenic right ventricular cardiomyopathy, BrS, Brugada syndrome, DCM, dilated cardiomyopathy, HCM, hypertrophic cardiomyopathy, IAS, inherited arrhythmogenic syndrome, LQTS, long QT syndrome, SCD, sudden cardiac death, SUD, sudden unexplained death.

alterations in genes currently associated with IAS. The results are presented below in chronological order.

Timeline

First reports focused on the period time frame were published in 2019.[19–21] (Figure 2) These three studies reinterpreted rare variants not previously classified according to the ACMG/AMP guidelines. The first study focused on 480 rare variants in the *SCN5A* gene with possible deleterious association with BrS. After applying the ACMG/AMP criteria, only 37% of the coding variants were classified as truly P/LP, mainly null variants. Most of the remaining variants were reclassified as VUS and even 3% were probably benign/benign (LB/B). The main element of reclassification was the improvement in the frequency population available at the time of the update.[19] The second study focused on a cohort of pediatric patients diagnosed with IAS who underwent genetic testing between 2009 and 2017. A total of 23 variants previously classified as VUS without following the ACMG/AMP guidelines were updated and 52% modified their previous function (35% to P/LP and 17% to LB/B). All other variants remained as VUS after the use of items included in the ACMG/AMP guidelines. As in the previous study, the reclassification was primarily influenced by improved population frequency data.[20] The third study published in 2019 focused on 337 previously reported rare variants in the *KCNH2* gene, which did not follow the ACMG/AMP guidelines. The update included population databases, *in silico* tools, and *in vitro* functional studies downgrading 6.5% to LB/B. All other reanalyzed rare variants were confirmed as VUS following the ACMG/AMP guidelines. Similar to earlier studies, the reclassification was carried out due to the continuous improvement in population frequencies.[21]

In 2020, four studies addressed the reclassification of variants associated with IAS or unexplained postmortem cases where IAS was suspected as the most likely cause of death (Figure 2). The first study reanalyzed two cohorts: 104 cases with IAS and 17 postmortem cases. Both cohorts were previously classified in 2010, without following the ACMG/AMP guidelines. We can confirm that 71.87% of the variants suffered a modification according to the current ACMG/AMP recommendations (69.23% in IAS and 94.11% in the postmortem cohort). The main reason for the reclassification was to update the population frequencies.[22] The second study was published by Quiat et al.[23] The authors updated previously classified DCM-associated rare variants found in 73 patients who were studied between

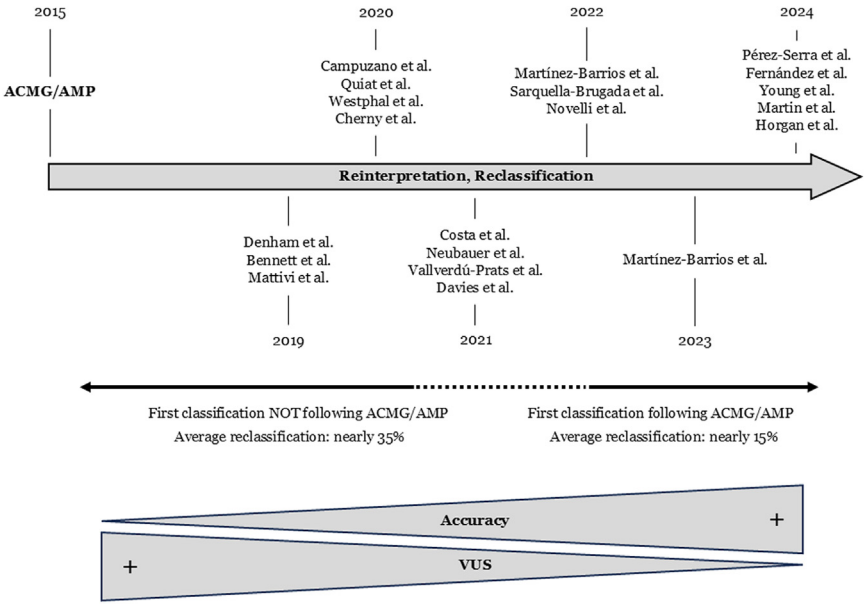

Figure 2. The timeline of the studies included in our analysis

The amount of data available in recent years allows for greater prediction precision that reduces ambiguity. ACMG/AMP, The American College of Medical Genetics and Genomics and the Association for Molecular Pathology; VUS, variant of unknown significance.

2008 and 2018 without following the ACMG/AMP guidelines. VUS reclassification showed a 30% downgrading to LB/B classification, mainly due to the incorporation of updated into general population databases. The third study focused on variants classified between 2001 and 2018 as deleterious in patients with LQTS but not classified according to the ACMG/AMP guidelines. Following reclassification, 14.3% of variants were downgraded from P/LP to VUS, driven by improved population frequency data.[24] The fourth study performed a retrospective genetic update of patients diagnosed with IAS between 2006 and 2017, disregarding the ACMG/AMP guidelines. The reclassification showed that 21.5% of the variants changed their previous classification (7.6% from P/LP to VUS and 0.3% from P/LP to LB/B, 2.7% from VUS to P/LP and 10.6% from VUS to LB/B, 0.3% from LB/B to VUS). This study, like the others, cited updated global population frequency data as the main driver of reclassification.[25]

In 2021, Costa et al. published a study focused on the impact of variant reanalysis on the diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC), currently named ACM.[26] (Figure 2) Nearly half of the variants analyzed had been previously classified without adhering to the ACMG/AMP guidelines, whereas the rest had been classified following the current criteria. After a comprehensive reanalysis, a total of 58.8% of the variants modified their previous role in ACM. Most variants downgraded their previous role, especially those variants classified not following the ACMG/AMP guidelines. This fact caused 10% of patients move from having a definitive diagnosis to a borderline one based on the Task Force Criteria clinical standards.[27] These findings underscore the importance of relying solely on conclusive genetic results obtained using the current ACMG/AMP criteria. In 2021, another study reinterpreted genetic data from 45 SUD cases in which the identified variants had not been previously classified according to the ACMG/AMP guidelines. After updating the data, the percentage of reclassification was 22.2% for P/LP. As we mentioned previously, this is the only study that reports a reclassification of CNVs in IAS; specifically, it modified a 4.4% to potentially deleterious, supporting that these small structural alterations can contribute to cause IAS, even if it is an episode of SUD.[18] The third study published in 2021 focused on the reinterpretation of variants previously associated with ACM. These variants were classified 5 years before their publication, and they all complied with the ACMG/AMP guidelines. The update showed that 30.77% of the variants modified their previous role, mainly due to global population frequencies. More than 80% of the reclassified variants gained certainty, decreasing the number of VUS by 18% and increasing the number of deleterious variants by 5%.[28] In the fourth study, the authors reclassified variants in cases of SUD and SCD; of these, 65% were classified for the first time before 2015 and thus they did not follow the ACMG/AMP guidelines. After the update, 42% of the variants changed their previous roles. In contrast, among the variants previously classified following the ACMG/AMP criteria, only 11% modified their previous role. Reclassified VUS were more likely to be downgraded (73%) to B/LB than upgraded to P/LP (27%). Most reclassifications were driven by updates in global population frequency data, although family segregation analysis was identified as a critical factor in achieving conclusive classifications, especially in cases of SUD/SCD.[29,30]

In 2022, two studies focused on the reclassification/reinterpretation of variants associated with IAS were published (Figure 2). Both studies were conducted by our group.[31,32] The first study conducted a comprehensive reanalysis of rare missense variants in the *TTN* gene that had previously been classified as ambiguous in IAS and that did not follow the ACMG/AMP guidelines. The update showed a modification in 36.8% of the VUS, which were downgraded mainly due to the substantial increase in population frequencies.[32] In the second study, our objective was to update the rare variants associated with IAS originally classified in 2016. A total of 18.4% of the variants changed their classification, decreasing the number of VUS mainly due to the improvement of global frequency data.[31] Finally, also in 2022, Novelli et al. performed a reinterpretation/reclassification of 94 VUS reported between 2017 and 2021 following the ACMG/AMP guidelines. After the analysis, 26.6% of the VUS were reclassified (one was downgraded to LB/B and 24 were upgraded to LP/P). According to the different phenotypes, the reclassification rate

was 45.8% in HCM, 33.3% in DCM, 27.3% in ACM, 12.5% in BrS, and 50% in LQTS.[33]

In 2023, an additional study in this field was conducted in a cohort of SUD cases previously classified according to ACMG/AMP guidelines 5 years earlier (Figure 2). The update showed that more than 10% of rare variants previously classified as VUS reduced their potentially harmful role to LB/B due to improved population frequencies.[34]

Recently, five more studies have been published that performed reinterpretation/reclassification of IAS-associated variants (Figure 2). In the first study, the authors performed a comprehensive update of 125 rare variants previously classified between 2016 and 2019 as VUS following the ACMG/AMP guidelines. All variants were identified in patients with a definitive clinical diagnosis of DCM. The update showed a modification in 12% of the VUS. Specifically, 4% were demoted from VUS to LB due to the increase in MAF compared to the previous classification, and 8% of the VUS were upgraded to LP due to new available data.[35] The second study published in 2024 performed a reclassification of more than 1,500 rare variants associated with IAS cases. The variants were divided into two groups depending on whether the previous classification did not follow the ACMG/AMP guidelines (2012–2016) or, in contrast, followed the current recommendations (2017–2021). The reclassification rate was 52.37% for variants classified until 2016 and 24.22% for variants already classified according to the ACMG/AMP guidelines. Percentage of genetic variants that downgraded or upgraded fluctuates depending on each IAS (upgrade range between 3.7% and 38.89%, and downgrade range between 11.11% and 40%).[36] The third study updated more than 200 variants identified in 517 patients and classified them between 2004 and 2020, mostly before 2015. Using the ACMG/AMP guidelines, 31.5% of variants first classified before 2015 had their function modified (10.34% VUS to LP, 16.09% VUS to LB, and 5.07% % LP to VUS). Regarding variants previously classified according to the ACMG/AMP guidelines, 5.75% modified their previous role (2.3% from VUS to LP and 3.45% from VUS to LB). Most of these modifications were due to the updating of population frequencies.[37] Other studies recently published have focused on the re-evaluation of 53 variants associated with IAS or SCD classified as VUS between 2016 and 2022, all following the ACMG/AMP guidelines. After the update, 32% of the VUS were reclassified (14 variants downgraded to LB/B and three variants upgraded to LP). These modifications were largely attributed to updated population frequency data.[38] Finally, the latest original study has reassessed/reclassified a group of 248 VUS identified in HCM, DCM, and ACM probands over a 5-year period, using the ACMG/AMP guidelines.[39] The authors reported only 1.6% VUS upgraded to LP/P, whereas 3.2% were downgraded to LB/B after reanalysis, suggesting a reclassification every 3 to 5 years to keep pace with evolving evidence.

DISCUSSION

The genetic study is part of the current IAS diagnostic protocols but must always be performed based on clinical suspicion and after a thorough evaluation of the patient. In postmortem cases, autopsy findings should indicate suspicion of IAS as the cause of death before performing a molecular autopsy focused on IAS. The close and continuous interaction between clinicians/forensics and geneticists is crucial because only a conclusive genetic result should be translated into clinical practice.

Reclassification/reinterpretation

Considering all the findings reported so far, variants initially classified before 2015 or without following the ACMG/AMP guidelines exhibit significantly higher reclassification rates than those interpreted under these guidelines. Therefore, we strongly recommend an immediate update of such variants associated with IAS (Figure 3). At present, all genetic diagnoses worldwide should adhere to these guidelines. Regarding the rare variants located in a gene with a definite/strong association to some IAS[1,2] and that have already been interpreted following the ACMG/AMP guidelines, published studies indicate a reinterpretation/reclassification interval ranging between 2 and 5 years (Figure 3). The percentage of modifications relative to the initial classification depends on several factors: the population studied, the gene involved, and the specific IAS diagnosed or suspected, particularly in postmortem cases. In 2023, Landstrom et al. stated that both genetic and clinical follow-up should be integrated to reassess cases with suspected IAS and no conclusive genetic diagnosis. The suggested period was 1 to 3 years despite the specific time should be individualized to the variant and patient.[10] This interval is shorter than ours, as their recommendation also includes patients with inconclusive clinical diagnoses, where closer monitoring is needed to identify phenotypical changes that may lead to a definitive IAS diagnosis. Based on published data, we are partially in agreement with this period only regarding genetic reinterpretation/reclassification. However, we believe that intervals of less than 1 year often yield low reclassification rates, as comprehensive updates to clinical and genetic data, including those required by ACMG/AMP guidelines, take considerable time. Our experience suggests that a reinterpretation/reclassification of a VUS every 1–2 years would likely be a misapplication of resources, according to recent publication.[39] Regarding the limit of 3 years suggested by Landstrom et al.,[10] we agree if clinical follow-up is included; despite, we recommend an annual clinical assessment in our center, especially if there is no definite clinical diagnosis. Focusing on genetic analysis, we limit it to a maximum of 5 years from the first classification, in agreement with a recent study.[40] In a recent study, authors suggest 3 to 5 years of interval for reinterpretation/reclassification of variants previously classified following the ACMG/AMP guidelines.[39] It is important to note that each center should make decisions tailored to its unique circumstances, including patient volume, available personnel, and economic constraints. Regarding this, the ACMG/AMP guidelines stipulate that clinical laboratories should have tailored policies and protocols for initial variant classification, variant-level reassessment, and reanalysis on a case-by-case basis, which should be subject to periodic review and updating, with a recommended interval of at least 2 years.[41]

Among the criteria outlined in the ACMG/AMP guidelines, population frequency data have consistently been the most significant factor in reclassification. This is because in recent years the value of population frequencies has been continually

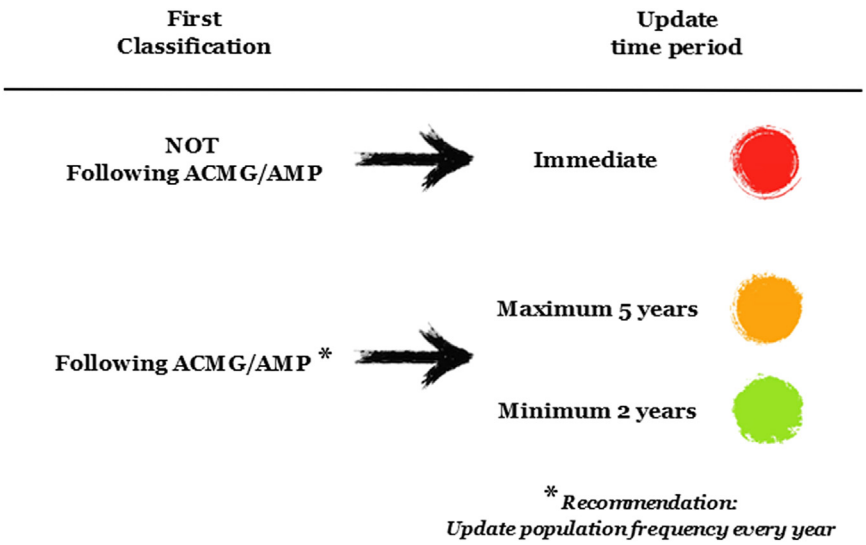

Figure 3. Recommendations for reinterpretation/reclassification of variants

Variants not previously classified following the ACMG/AMP guidelines should be updated immediately. Variants previously classified following the ACMG/AMP guidelines should be updated before 5 years. We recommend an annual update of population frequencies. ACMG/AMP, The American College of Medical Genetics and Genomics and the Association for Molecular Pathology.

updated, so we recommend that this value in the VUS be reviewed at least once a year because it is a quick and cost-effective measure since a significant increase in the frequency of a VUS could rule out it as potentially harmful, especially due to differences in diverse racial and ethnic populations.[42] According to this fact, it is imperative to update data on rare IAS-related variants considering the ethnicity of each patient. To be even more precise, this value of frequency must be adapted to the prevalence in the population of each IAS[1,2] as well as sex/gender differences, as widely accepted in IAS despite the low number of studies available.[43,44]

Another critical consideration is whether updates should focus solely on VUS or also include variants initially classified as P/LP. Recent studies have reported that population frequency updates reduce the number of rare variants with the first damaging role.[39,45–49] This situation is extremely useful for specialists because a downgrade in the pathogenicity of an IAS variant can modify the preventive therapeutic measures recommended to patients/relatives at the time of the first genetic diagnosis. Recent studies suggest that most VUS will be reclassified to LB/B especially due to new population data added to public databases,[50] suggesting that the genetic test must be carried out in a strict and rigorous manner as well as its interpretation. The proportion of P/LP variants that are downgraded is smaller but not negligible.[36,51] This fact is especially relevant if an asymptomatic patient is pharmacologically medicated only to be a carrier of a harmful variant at the time the classification was performed. We suggest that P/LP variants should be reinterpreted/reclassified only if they were not previously classified following the ACMG/AMP guidelines or if new evidence emerges that challenges their previously accepted pathogenicity. We believe that this point should be widely discussed and agreed upon by a committee of experts, as well as the possible legal consequences that it may entail, since a modification that is due solely to new available data is not the same as a classification due to an error due to overestimation of the pathogenicity.

In the forensic field, despite the absence of a stringent regulation that compels to periodically reclassify the variants, the possibility of downgrading variants' significance after reclassification and the intrinsic uncertainty brought by VUS identification could be source of liability for the pathologist who does not include in his/her report specific considerations/recommendations about the potential utility/need to perform regular reclassification in the abovementioned indicated cases or, at least, about the need to refer to specialized genetic centers to evaluate all the possible clinical implications for the first-degree family.[7] Therefore, even in medico-legal reports, we suggest stressing this form of transparency when the variants' significances are discussed.

Variants of unknown significance

Before the publication of the 2015 ACMG/AMP guidelines,[5] ambiguous variants were the Achilles' heel of genetic diagnosis both in clinical and forensic setting. To address this, specific approaches were suggested a decade ago, particularly for HCM cases, recommending periodic reassessments, although no specific time frame was established.[51] The publication of the ACMG/AMP guidelines aimed to provide unified criteria for the classification of variants, reducing ambiguities between laboratories and thus achieving more reliable and unanimous classifications. The application of the ACMG/AMP guidelines was carried out progressively since their publication, but genetic laboratories soon realized that their high number of items did not allow them to achieve a conclusive role in most of the variants, especially if they were rare diseases with incomplete penetrance and variable expressivity, as is the case of the IAS. Due to their rarity, insufficient cases with the same deleterious variant exist to corroborate findings, as most families carry unique causal variant.[3] Furthermore, family segregation is not always available or does not provide conclusive data since incomplete penetrance and variable expressivity are intrinsic characteristics of all IAS. Functional studies clarifying the pathophysiological mechanisms of variants are exceedingly rare, further complicating definitive classification. For this reason, several studies began to be published focusing on specific genes or specific IAS that helped clarify the role that each variant could play, always based on the clinical diagnosis of any channelopathy[52–61] or cardiomyopathy.[8,21,62–65] Therefore, a close and continuous

interaction between clinicians and geneticists is crucial to clarify the role of genetic variants in clinical practice.

In recent years, the number of VUS in IAS pathologies has been progressively reduced thanks to the incorporation of data from clinical studies and basic research. Despite these advances, the percentage of VUS remains high in IAS, leading to suggestions for subclassifications within the VUS group. These subclassifications help recommend potentially harmful or benign classifications, although clinically they are not actionable data because they are not completely conclusive. The subclassifications consist of determining whether the VUS may have a possible deleterious character (VUS-LP or HOT-VUS), benign (VUS-LB or COLD-VUS) or neutral (VUS-VUS or solely VUS), based on the data published up to the date of the subclassification.[66] In general terms, the lack of data (especially population frequencies) suggests a deleterious trend, whereas the existence of contradictory data allows us to adopt positions that are not so deleterious.[32,34,35,39,67,68] Despite this tendency, no VUS can be definitively classified as deleterious or benign unless it strictly adheres to the current ACMG/AMP guidelines. Ongoing advances aim to clarify the role of VUS to make them actionable in clinical practice and alleviate the anxiety of families, even when the precise cause of IAS remains unknown.

Role of clinical assessment in genetic analysis

Clinical assessment is fundamental prior to perform a genetic analysis focused on genes currently associated with IAS, providing essential phenotypic context for variant interpretation. A genetic analysis in patients without a completely established clinical diagnosis of an IAS, or at least with a high suspicion of IAS, will, in most cases, lead to an ambiguous result in the genetic classification or even a possible misinterpretation of the variants identified. Currently, the genetic diagnosis of IAS through the ACMG/AMP recommendations[5] provides a structured framework for variant classification, including a list of items but clinical data remain as the main point to take into consideration. In addition, variable expressivity and incomplete penetrance lead to an increased burden of VUS.[1,6,69] This issue is particularly challenging for missense VUS, whose functional consequences are harder to predict.[70] To address these challenges, recent studies have suggested that integrating IAS-specific phenotype and clinical data into variant adjudication can help reduce VUS ambiguity.[56,60] In addition, a periodic review in multidisciplinary work teams may further improve variant classification and help reduce the number of uncertain variants.[1,2] The experience of our multidisciplinary group recognizes that a definitive clinical diagnosis is a crucial factor in the reinterpretation of rare variants, especially if missense. Therefore, a phenotype-based approach should improve genetic reanalysis, ensuring that test results match the patient's clinical presentation.

Clinical implications of variant reclassification

The clinical implications of variant reclassification in IAS risk stratification and management are specific of each IAS-gene/variant and in most cases are still under discussion.[2] Modifications of a previous classification may lead to revise medical surveillance. From a clinical decision-making perspective, although VUS should not guide patient management due to no conclusive role, their reclassification to a deleterious role can influence genotype-specific counseling as well as therapeutic measures. Conversely, reclassification to no deleterious role can prevent unnecessary interventions and medical surveillance. Additionally, psychological well-being is an important consideration, as reclassification of a VUS to a no deleterious variant may alleviate anxiety in patients/families as well as clinicians. Furthermore, reclassification can alter family screening recommendations, affecting cascade genetic testing and guiding risk assessment in relatives. Consequently, a multidisciplinary approach, ensuring ongoing communication between clinicians and geneticists, as well as timely genetic counseling to patients, is essential to optimize patient care.

Legal/ethical responsibilities

Periodical reinterpretation/reclassification of variants included in a genetic diagnosis is crucial, especially when a genetic diagnosis remains ambiguous/inconclusive. Currently, there is no legal obligation to perform reinterpretation or reclassification of genetic data, nor clarity on who holds responsibility for updating genetic diagnoses or recontacting patients and families, if necessary.[71,72] The European Society of Human Genetics (ESHG) suggests that patients could be recontacted for updates that may have clinical or personal utility.[73] The ACMG/AMP recommends shared responsibility among patients, laboratories, and clinicians, with patients encouraged to reach out to their practitioner for updates,[74] as also recently suggested by the Canadian College of Medical Geneticists (CCMG).[75] We propose that informed consent forms should explicitly address the issue of data updates and provide an approximate time frame for future reinterpretation or reclassification.

Contact with patients and their families should always be facilitated by a specialized clinician, as genetic modification may require adjustments to the clinical treatment currently in place.[76,77] Each patient and their relatives should be thoroughly informed about the genetic diagnosis and its implications before undergoing the test. Psychosocial impact of a reinterpretation/reclassification has not been extensively studied in IAS patients but is dependent on several factors, such as downgrading/upgrading of the reinterpretation/reclassification, medical management (if any), and patient understanding.[78] Therefore, the results should be explained individually and in detail by a specialized team until each genetic carrier of the variant fully understands what it means to carry that variant.[74] However, it is important to note that although there is no legal obligation for healthcare providers to recontact patients or families, there remains an ethical responsibility rooted in the principles of the Hippocratic Oath.[40] That being said, even in absence of an explicit legal indication, since the consistent evidence supporting the need of periodical reclassification, failing to comply could be still considered as a breach of duty (and then a source of liability) in some countries. The profile of forensic expert is diverse under this perspective, because he/she has only the duty to perform the required analysis (and, consequently, flag any critical issue connected to its results) but, because of the specific nature of the mandate, he/she cannot be considered obliged to reclassify variants (and, even if the forensic expert decides to do so, it should be carefully evaluated if he/she still has the right to do so, being the availability of the

genetic data and the possibility to process them restricted to the forensic analysis/scope and not to future processes).

Economic cost

Other important point that has not yet been discussed in depth is who should bear the economic cost of this reinterpretation/reclassification. Legislation in each country should establish baseline recommendations, accounting for the diversity of healthcare systems worldwide. At this point, a distinction must also be made between genetic analyses performed within the framework of public healthcare systems and those conducted by private laboratories. As mentioned earlier, these points should be included in the patient's informed consent form before genetic analysis. To date, there is a lack of comprehensive data addressing the economic aspects of reinterpretation and reclassification.[40] The issue can be even more complex in specific contexts like the forensic one, where the public authority can be represented by public prosecutors, who pay for the analyses but cannot sustain the economic cost of future reclassifications.[7] We believe it is essential to consider these additional costs into account when calculating the final economic value of a genetic analysis to ensure optimal patient care.

Limitations of the study

In our study there are limitations that we would like to mention. They consist of not carrying out an exhaustive reinterpretation/reclassification regarding each IAS, as well as each gene or even the type of VUS. Regarding the pathology, the studies analyzed showed a similar percentage of reinterpretation/reclassification in all IAS, without variation in the time of reinterpretation/reclassification whether it is cardiac channelopathy or cardiomyopathy. Concerning genes, the number of VUS is greater in mean genes associated with each IAS as well as the percentage of reinterpretation/reclassification. This is not unexpected due to different phenotypes associated with some of these genes and, in consequence, proportionally more variants reported in these major genes in comparison to minority genes. At this point it is important to highlight that minor genes are usually reported in few families and sometimes with "phenotype-like," so obtaining conclusive role of VUS seems to be difficult. In the case of the type of VUS, those grouped as radicals (*nonsense, indels, or frameshift*) have more definitive classifications following the ACMG/AMP guidelines, and it is especially the *missense* ones that remain as VUS due to increased difficulty to ascertain based on consequences for protein function; thus, there is proportionally more percentage of reinterpretation/reclassification in *missense* variants. These aspects are very specific, so we believe that large number of studies are necessary in this way to obtain definite answers for each one of these above-mentioned items. A possible solution to address this gap could be to create specific international registries where this information can be shared and thus progress in obtaining a conclusive deleterious or benign role for a variant previously considered ambiguous can be more quickly updated.

Conclusions

Currently, the genetic diagnosis of IAS must comply with the ACMG/AMP guidelines and always be guided by a thorough prior clinical assessment. However, limited data availability or conflicting results often lead to ambiguity in variant classification, hindering their practical application in clinical settings. The continuous improvement of clinical and genetic data implies that the interpretation of each variant may vary over time, mainly those that remain with an ambiguous meaning. While the reinterpretation and reclassification of variants are broadly accepted practices, the optimal time frame for performing these updates remains uncertain and requires further investigation, particularly in the context of IAS. The data obtained from several studies published so far suggest a time window of no more than 5 years, with a minimum time to be determined depending on the IAS analyzed, although always following the ACMG/AMP guidelines. These recommendations must be adapted in each patient depending on the diagnosis/suspected IAS, conclusive/ambiguous genetic diagnosis, and available personal/economic resources. Population allele frequencies that are continually updated with increasing precision provide a straightforward and cost-effective opportunity to reassess variants. We recommend reviewing the frequency of variants in IAS at least once a year, especially those that remain ambiguous, as well as a period of reclassification of variants every 2 to 5 years, including all new evidence available. Responsibilities regarding who should perform and financially support these updates remain undefined. Genetic consent should include all these aspects, and patients should be fully informed about all these aspects before genetic analysis. Finally, it should be noted that the close and continuous interaction between clinicians and geneticists is crucial to clarify the role of genetic variants in clinical practice.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Oscar Campuzano (oscar@brugada.org).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The published article and supplemental information include all data generated and analyzed during this study.
- No code is generated in this paper.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, G.S.-B., R.T., and O.C.; data curation and formal analysis, G.S.-B., E.M.-B., J.C., C.D.-L., N.D.-E., P.C., I.Z., S.C., S.G., A.O., F.C., N.B., and O.C.; writing—original draft, G.S.-B., A.G., E.M.-B., and O.C.; writing—review and editing, all authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

All authors declare no conflicts of interest to disclose.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- METHOD DETAILS
- QUANTIFICATION AND STATISTICAL ANALYSIS

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STAR★METHODS

METHOD DETAILS

Comprehensive systematic research of studies published in several databases (Medline, ResearchGate, Embase, Wiley, ScienceDirect, Scopus, and Google) since 2015 (year of publication of the ACMG/AMP guidelines[5]) until December 2024 was performed. Specific terms of the search were: (inherited arrhythmogenic syndrome OR familial arrhythmogenic syndromes OR channelopathies OR cardiomyopathies OR familial cardiomyopathies) AND (molecular autopsy OR sudden unexplained death OR sudden cardiac death OR post-mortem genetic analysis) AND (reinterpretation OR reclassification OR reanalysis OR variant) AND (genetic analysis OR genetic testing OR genetic diagnosis). More than 500 articles were collected. After removing duplicates, abstracts and studies published in English, a total of 429 were reviewed. Original studies were selected based on their use of variant classification following the ACMG/AMP guidelines. At this point, 196 articles were selected. Data focused on the reclassification (downgrade or upgrade) and potential reinterpretation (with or without clinical implications) of rare genetic variants associated with IAS were exhaustively analysed. After this last filter, only 20 original studies were included in our analysis (Figure 1).

QUANTIFICATION AND STATISTICAL ANALYSIS

To ensure objectivity, all investigators discussed the extracted data, and a consensus was reached to minimize bias.