Methods in the process of re-evaluation of variants in cardiomyopathy

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Abstract

Cardiomyopathy is a heart disease which affect individuals mostly by genetic causes. These mutation needs to be classified. In last years, major changes are made in the classification process. Therefore re-evaluation is necessary. This is already done however, there is no clear structure of the methods, procedures and selection process. This paper provides common methods that are used and also give an advice which structure should be used. Four stages are described: Genetic analysis, Selection process, data collection and clinical translation. Genetic analysis is done in two ways: A disease specific gene panel or whole-exome sequencing. Selection is currently based on the exclusion of mutation which are classified as benign and an allele frequency above 1%. Also, there is an exclusion of individuals which have an incomplete phenotype. Two other possible ways are based on prioritisation of mutations that were classified before 2015 and missense mutations. Data collection is of three different sets of data: population data, functional data and predictive data. Population data is retrieved via GnomAD database, functional data is found using PubMed or by a research in their own laboratories. Predictive data is acquired by multiple lines of evidence on the level of gene, protein and splicing. Clinical translation has a problem in the explanation of the effect of a reclassification. Individuals which have a downgrade sometimes stop with their medication. However, these patients are still suffering from cardiomyopathy.

Introduction

Cardiomyopathy is a disease which affects the heart muscles. There are 5 types: Hypertrophic cardiomyopathy, in this type the wall of the left ventricle is thickened; dilated cardiomyopathy, in this type the left ventricle is enlarged; arrhythmogenic cardiomyopathy, here muscle tissue is replaced by fat and scar tissue; restrictive cardiomyopathy is characterized by stiffening of the left ventricular wall; and noncompaction cardiomyopathy, which is characterized by muscle bundles extending in the left ventricle. Cardiomyopathy can be caused by environmental factors like alcohol abuse, drug abuse, and also some infections like, hepatitis C. However, cardiomyopathy is mostly caused by genetic factors. This means that this disease can be inherited. Only in hypertrophic cardiomyopathy, already 1400 mutations in 13 genes are identified (Das et al. 2019). This makes it important to correctly classify mutations.

Many genes have been classified in the past. However, in recent years there are some important developments which makes it necessary to re-evaluate these mutations. The developments are:

- Guidelines for classification have been made in 2015 by the American College of Medical Genetics (ACMG). Before, these criteria were not established appropriately, every laboratory had their own criteria which led to different conclusions between laboratories.
- In recent years whole exome/genome sequencing became much cheaper which makes it more viable to use in practice. This leads to more extensive genetic analysis. Genetic analysis in earlier days didn't amplify all genes that have known mutations that are linked to

cardiomyopathy, which opened the door for false classification. Nowadays, due to whole exome/genome sequencing, it is possible to do more excessive genetic analysis and find the correct mutation for pathogenicity.
- There is more knowledge in which genes are associated with cardiomyopathy. This can lead to the discovery of new genes or a known gene that is not associated with cardiomyopathy.
- Allele frequencies are also much more known nowadays, which lead to more evidence for the classification.
- Today, there are more prediction possibilities.

These developments ensure that re-evaluation is paramount to obtain the correct classification. Researchers are already re-evaluating. However, currently there is no general logistics to do re-evaluation. Important is that every laboratory should have a very similar method to ensure a valid classification, and that there would be no differences between laboratories.
Also, selection is important. Considering the number of mutations which needs re-evaluation, a proper selection should be made to ensure that mutations are re-examined first, which clinically would have the most significant change and also have a big change that the classification would be adjusted.
This shift can possibly have major effects especially on family members which may carry the same mutation. For example, carrying a pathogenic mutation would have an effect on unaffected family members. This can be mental health issues, living with a mutation that may cause a disease can be difficult to live with. Furthermore, possibly these individuals are already prescribed medication or rules they have to follow. If this mutation is not pathogenic, it could have a big effect on mental health and could cause a decrease in medicine use, which is good for the individual's health and also decreases costs to governments.
This paper provides common procedures and methods to conduct re-evaluation. Systematic searches were conducted to gather information about the methods and procedures for re-evaluation. In addition, advantages and disadvantages of different methods are stated to provide a possible structure to use for re-evaluation.

Methods in re-evaluation

The process of re-evaluation was divided in four stages: Genetic analysis; selection; predictive, functional and population data; and clinical translation. It is important to know that often some of these stages happens simultaneously. For this time, they are discussed separately to clearly describe the methods that were used. First, the guidelines for classification are discussed to know which data is needed. For every process, common procedures and methods are provided.

Guidelines ACMG
In 2015, the ACMG provided guidelines to ensure every classification process is done under the same criteria and therefore provide the most reliable results. The ACMG uses four important types of data: population data, computational and predictive data, functional data, and segregation data (2).

First, population data is the frequency of the prevalence of mutations. Criteria for pathogenicity is that the mutation should be rare or absent in a population. Also, the prevalence of the mutation being significantly higher in affected individuals than controls is seen as evidence for pathogenicity. Stand-alone evidence for benign is that the frequency of the mutation is higher than five percent. If the allele frequency is not greater than five percent but higher than expected for a certain disease, it would still be evidence for benign. This number is altered for different diseases.

Second, computational and predictive data, this data is not based on actual studies. Via computer programs and algorithms, the structures of new proteins can be predicted and the alteration of that structure if an amino acid changes. Mutation can alter single amino acids but also multiple by deletion and insertion of a nucleotide. It is also possible by a change in splice site, exons or introns can be inserted or deleted in comparison to the original protein. The conservation is also important information as it tells something about the importance of an amino acid and thus a prediction if this amino acid would change. A criterion for pathogenicity is that the mutation makes the same amino acid change as an earlier called pathogenic mutation. Also, when there is a missense mutation which shortens the protein on a non-repeat location. Evidence for a benign mutation is that the mutation has no impact on the gene product or a deletion/insertion in an area with no known function. Moreover, prediction programs are often used that predict the impact of the mutation on a gene or product. There can be no impact or a deleterious effect. No impact is evidence for benign and deleterious is evidence for pathogenicity. The same prediction should be found in multiple lines of prediction programs.

Third, functional data. In contrast to computational and predictive data, this should be found in well-established studies. Function of proteins is studied using the mutated variant and control groups. By comparing these results, it is possible to conclude whether the mutation has a significant effect on function. Not only the protein is important but also the gene itself and the different steps in transcription and translation that eventually create the product. Evidence for pathogenicity is that the mutation has a damaging effect on the gene or product. In addition, a mutation on a mutational hot-spot or the active site of an enzyme without benign variation is evidence for pathogenicity. In most cases the location of the mutation is found using genetic analysis. For this region, functional studies need to be found what the overall function is within the protein. Benign evidence is in turn that the mutation has no damaging effect on the product.

Finally, segregation data is based on co-segregation of the mutation in families and if possible, between families. Co-segregation is the inheritance of multiple genes which are closely located. This explains something about the relation between patients. In a research, surrounding markers are sequenced and compared in the patients and unaffected. In the case that there is a lot of similarity in markers, at least for that part of DNA, individuals are related. How further individuals are related but still have a similarity of markers, there is an increase in strength of evidence for pathogenicity. The ACMG uses the number of meiosis to give an indication in the strength of evidence. Basically, this is how often the mutation and the surrounding genes is transferred to the next offspring.

Genetic analysis

In recent years, a lot of new variants are linked to cardiomyopathy. In the time of initial classification in these patients, it is possible that these variants were not included in the gene panel. Therefore, most of the time, new genetic analysis is required, to exclude other variants that can be present in patients. In the case of the presence of other pathogenic mutations, it is unlikely that the initial variant that was addressed as the pathogenic mutation in these patients is the actual culprit. For selection, the earlier genetic analysis can be used. For complete re-evaluation, another analysis is needed to have up to date data that contains all genes that are involved in cardiomyopathy. Once genetic analysis shows that there are no other genes involved, next steps in the re-evaluation process can be done. Researchers use two different methods for genetic analysis: A disease specific gene panel, and whole exome sequencing.

Disease specific gene panel
Most of the researches that did re-evaluation used this technique. A gene panel consist only of some genes which a researcher wants to test. In the case of cardiomyopathy, only the genes that are sequenced are related to cardiomyopathy. Advantages of using this technique is that it is cheaper and takes less time. In the future, this data can lack information because new genes were founded which are involved in cardiomyopathy (3).

Whole exome sequencing
On the contrary, in whole exome sequencing, all genes which encode for a protein are sequenced. Only the genes that are related to cardiomyopathy are further investigated. This is a perfect tool in cases where there is no clear pathogenic mutation or no variation even. Whole exome sequencing can also be used to look at genes that are not yet linked to cardiomyopathy. The difference in comparison to a gene panel is that all necessary genetic data is available from now on. This means that when new genes are found that are involved in cardiomyopathy, no new genetic testing is necessary. Only the filter of what genes are investigated needs to be altered (3).

Examples in practice
Quiat et al. (2020) had a study duration of 10 years. There was an increase in the number of genes that were tested, 5 to 62. This shows the evolution of next generation sequencing, it has become much cheaper and faster, this makes it more available to use in practice (4).

In a reanalysis study of Campuzano et al. (2020) whole exome sequencing was used. They studied patients with different inherited arrhythmogenic syndromes from ten years ago. New genetic testing was done. But also, new predictive, functional and population data. On all patients, the same technique was used. Using computational filters, the right genes could be studied for each syndrome. 71.8% of the mutation had a change in classification (5).

Das et al. (2018) reassessed 136 probands with hyper cardiomyopathy that were tested between 2000 and 2012. As genetic analysis they used a gene panel of at least 7-10 of the most associated genes to hypertrophic cardiomyopathy. Later on, new genes were added which were also associated to hypertrophic cardiomyopathy and genes causing phenocopies. In addition, extensive segregation data was collected and pedigrees were made. New predictive, functional, and population was also collected. 54 variants were found, of which 5 had a reclassification (1).

In a study of Bennett et al. (2019), no genetic testing is used. This study included children with inherited arrythmia syndromes. This can be different diseases like, long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, Brugada syndrome but also cardiomyopathy. However, patients with cardiomyopathy were excluded because not enough genetic information is available for these patients. This research is an example of the possibilities using whole-exome sequencing. Genetic testing isn't necessary anymore. All data is available in their own databases (6).

Selection process
Selection is an important step in re-evaluation. There are limited resources and many mutations to re-evaluate. It is important to first re-evaluate mutations which are likely to change in classification and needs to be clinically significant. When these are re-evaluated, others can be done. Currently a problem is stated by Costa et al. (2021). They stated that of their results, only 41.3% of all variant that were investigated, was clinically relevant (7). Next to the present selection, more selection

criteria should be made. Different selection processes are found in earlier researches. The selection process that were done in earlier researches are based on: benign and incomplete phenotype.

Selection based on benign
Campuzano et al. (2020) excludes mutations that are classified as benign and had a global frequency higher than one percent. This frequency is strong evidence for benign and therefore the chance that these mutations are classified wrongly is small. Therefore, this study, excluded these patients because there is no different classification expected. They first collected all population data before starting with genetic analysis. This way, they saved time and manpower (5). Das et al. (2018) even excluded all benign classified mutations (1).

Selection based on incomplete phenotype
Quiat et al. (2020), which did research in patients with dilated cardiomyopathy, excludes patients with secondary dilated cardiomyopathy or incomplete clinical documentation. Dilated cardiomyopathy can also arise from earlier myocardial insults. This can be left ventricular failure or another cardiomyopathy. In these cases, dilated cardiomyopathy is secondary. This is excluded, because it is important that individuals have a very similar phenotype that is provoked by the mutation. If this is not the case it is very unlikely that a mutation will be classified differently. The patients were found using the diagnostic Fyler code for dilated cardiomyopathy. This code is different in different diseases, using this code, it is fairly simple to filter a database on disease (4).

Clinotator
There is an absence in selection in most cases. Therefore, there is looked at solution to focus more on the more important mutations. One of these solutions is the use of an algorithm that can filter mutations based on specific information. An example is Clinotator. Clinotator works on ClinVar, which is a database that is used as resource for clinical variant interpretation. Clinotator use this information to generate several metrics and to determine strength and consistency of the evidence supporting the classification. It is weighted on significance type, date of submission and submitter. Outdated or incomplete classifications can be filtered. This can be used to rank mutations which are the most outdated or incomplete and need re-evaluation. In this way a systematic selecting model is provided which can be used by every researcher. The algorithm can filter on the submitter, in that way a laboratory can select their own submitted data. In most cases laboratories are only interested in their own classification, this makes that possible (8).

Costa et al. (2021) had no selection. This study was a re-evaluation of variants in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) and included all patient with definite, borderline or possible ARVC to their study. Next to the re-evaluation, they also did a statistical study to examine which mutations are more likely for an alteration in classification. Firstly, mutation that had a classification before 2015 were more likely to change than mutations after 2015. This is due to introduction of universal guidelines by the ACMG. Next to that, the difference between missense mutations and other type mutations was assessed. Missense mutation was more frequently reclassified (66,7% vs 42,3%) and also more clinically relevant (50,0% vs 23,1%). These data describe two more possible ways to prioritize. Clearly, other mutations need to be re-evaluated eventually but according to these data, mutations before 2015 and missense should be prioritized. Researchers can do selection based on these properties (7).

Segregation data

Genetic analysis is done in relatives in order to find possible co-segregation. Genetic analysis in relatives is not that extensive as in the patients themselves. Genetic analysis in patients already rule out most genes as there is no mutation. This would make it unnecessary and costly to do extensive genetic analysis on relatives. This is also done in a study of Quiat et al. (2020), where they performed gene specific testing, only the gene where the mutation was found is sequenced in family members and also around this gene to have enough markers that can be used. In the case the mutation is not present in affected family. New genetic testing needs to be done, for example using whole exome sequencing to find a pathogenic mutation (4).

Predictive and population data

Predictive data is most of the times available in databases. Also, software programs are often used, for example Alamut in the study of Das et al. (2018). Next to that the conservation is always evaluated. This includes genomic evolutionary rate profiling like PhyloP scores. For mutations which changes an amino acid, multiple prediction programs are used. Examples are: Polyphen2, Grantham score and Sorting intolerant from tolerant score. Population data is investigated in all researches using the GnomAD database. This database should be used according to the guidelines of the ACMG in 2015 (1).

Functional data

Functional data is available for a lot of mutations, these can be found using published literature. PubMed is an often-used database. In some cases, laboratories do their own functional studies. For example, the Erasmus Medical centre uses three ways to do functional studies. The first one is analysis of the RNA, protein or pathway activity. Also, diagnostic testing of the pathogenicity of variants of uncertain significance. This is done in in vitro models. Lastly, the zebra fish is used to asses the pathogenicity of multiple diseases like, ciliopathy, cardiomyopathy and neural migration disorders. (9)

Clinical translation

In the process of re-evaluation, also feedback is needed towards the patients. A study of Wong et al. (2018) describes this feedback and also describes ways of improvements. First the reactions towards a re-evaluation are described. For an upgrade in classification often relief was expressed. This relief arose from the utility of the results. The results are not very important for the patients itself but have a big impact for family members as genetic testing can be offered to them and can be used for reproductive planning. On the other hand, the reaction for the proband is neutral. The disease still affects patient's life and doesn't change with the new classification.

Many patients who received a downgrade also expressed relief. However, this was because of a misunderstanding that the disease would be less likely genetic and so family members won't be at risk. This also effects decision-making, as some patients stopped with their medication. Some patients also expressed negative feelings. This was because there was no explanation anymore for their condition. An uncertain significance was also perceived as worrisome as it is unknown what the mutation meant to themselves and also family.

Perception of the re-evaluation process was good. Especially the idea that researchers are still busy with them. However, some patients expressed disappointment due to unmet expectations. These could be inappropriate expectations following pre-test counselling to expectations about the follow-up process after re-evaluation.

Improvements were stated by patients in communication. The majority of patients thinks re-contacting should be done by the health providers. As patients don't think it is reasonable to call once a while to see if there are any updates. The delivery overall is good, the news is explained by their multidisciplinary team who patients trust (10).

Discussion

To summarize, the most important findings are in the genetic analysis. In recent years there is a shift from the use of a disease specific gene panel to whole-exome sequencing. Relatives however are still analysed using a gene panel. The selection process that are currently done are based on benign and incomplete phenotype. This is not enough to make re-assessment a productive process as the findings has a clinical significance of 41.3%. Costa et al. (2021) found that mutation before 2015 and missense mutations has a higher rate of reclassification and more of the results are clinically significant (7). Also, an algorithm could be used to filter mutations on lack of data, date and author (8). Population data was found using the GnomAD database (2). Functional data was found using PubMed or by their own laboratory (9). Predictive programs were further used to obtain computational lines of evidence (1). Clinical translation overall is received well in patients. There is a problem in the understanding of a downgrade in classification. Patients feel relieved and sometimes stop taking their medication. Contact with their doctor is sporadic. Patients don't want to 'harass' their doctor by calling them (10).

In literature an evolution was found where in earlier days, a gene panel was used but because whole exome sequencing has become much cheaper, this method is more and more used (4). Advantages of a gene panel is that it is cheaper and on short term it is also more productive. However, on the long-term whole exome sequencing has a big advantage over a gene panel. Because possibly, in the future new re-evaluation will be necessary (3). By the use of whole exome sequencing no new genetic analysis is needed because all information is present. In the case of new variants in other genes that are known, the filter of what genes are analysed just needs to be adjusted. Where by the use of a gene panel, a new genetic analysis would be needed. Whole-exome sequencing has brought more knowledge in the understanding of genes associated to cardiomyopathy, and thereby more patients and especially their relatives eventually get a clearer diagnosis. Genetic analysis in relatives is done the same in every research already. Only the gene where the variant was found is sequenced in family. Only this gene is sequenced because in the patient already the whole genetic analysis was done to see which gene is responsible for the clinical picture. Also, some markers around the specific gene are sequenced to investigate if there is co-segregation.

As said before, the selection process is not good enough. A percentage of 41.3% (Costa et al. 2021) is simply too low in a field of research where patients are involved (7). The exclusion of benign variants with a high allele frequency is a good start and also an incomplete phenotype gives less change of getting a reclassification. However, more criteria can be used to give some mutations priority. As mutations before 2015 are more likely to get a reclassification and also missense mutations. Earlier classification can be sorted on time of submission and research should start with the ones older than 2015. Patient's files should clear if the diagnosis cardiomyopathy is complete and there is no question of a secondary disease, in case there is an incomplete phenotype, these would be excluded. After population data, allele frequencies are known and again some mutations can be excluded. Genetic analysis would show which mutation is the possible genetic cause of the clinical image and missense mutations should get priority and later on other types of mutations. Instead of manually sifting through all data, an algorithm could be used. This would filter on date (already seen as good

selection criteria) but also if there is lack of data. This has an advantage over manually as it takes less time. Currently no such selection tool is used in practice. Butler et al. (2018) described the use of Clinotator as selection tool but no further research was done on the effectiveness of this tool. This should be done as it has some advantages over manual sifting through the earlier data (8).

In the collection of population, there are already clear guidelines by the ACMG. The GnomAD database should be used. In last years, much more allele frequencies are known and therefore more reliable classification can be done. Also, the use of one database makes it even more reliable as there would be no differences between laboratories (2).
For predictive data, very similar programs and scores are used. Conservation is important, an example is the phyloP score. Also, programs which predict change on protein level for example the Grantham score. Lastly, there could also be changes on gene level and splicing. Using Alamut such a program is available (1). Important, according to the guidelines of the ACMG, is that multiple lines of evidence are used. This means that on all three levels a prediction program should be used (2). The reliability will be higher when more prediction programs are used. It is still in silico data and therefore just a prediction, not absolute science.
Functional data, for researchers it is important to use up to date data and a functional research that meet all requirements. This data can be retrieved via literature but some laboratories do their own research to specific variants in cardiomyopathy (9).

Clinical translation is eventually the most important step of re-evaluation. Patients need to have the best care that is possible. Therefore, the genetic cause of the clinical picture should be known. Thereafter, a good explanation of the results should be done and what effect it has on the patient itself and their family. It shows that there is some confusion in what results means. This should get more attention. An example is when patients stop with their medication when their mutation is downgraded. However, these individuals still suffer from cardiomyopathy and need their medication. In a few cases patients describes inappropriate expectations, to avoid this, there should be a good explanation that the research could lead to nothing, this is mostly described in patients with a variant of uncertain significance. Lastly, it is stated that communication have a positive effect on patients. Just to know researchers are still busy with them. But that as it may, patients feel unreasonable to call once a while. This leads to doubt. According to patients, this should be done by their own team. Individuals know this team and also trust them (10).

Conclusion

To conclude, the re-evaluation process was divided in four steps: selection, genetic analysis, data collection and clinical translation. The first step in the process is to re-asses the patient for symptoms and start with diagnosing the correct disease. In case that there is an incomplete phenotype, patients should be excluded to the re-analysis. Furthermore, a selection should be based on the year of the original classification, variants that were classified before 2015 should get priority. Later on, a genetic analysis is done using whole-exome sequencing. By the use of this the variant can be found again, only this time it is a complete analysis so, the variant that is evaluate is now much more reliable. The shift from a gene panel to whole-exome sequencing shown by Quiat et al. (2020), also show in practice that whole-exome sequencing is more useful nowadays. In case the mutation that was found is a missense mutation, it should be prioritized as the chance that the result would have a clinical significance is the biggest. Population data is then searched for (using the GnomAD database), in case that the same mutation was already classified as benign and the allele frequency is bigger than one, the reclassification doesn’t need more evidence as the chance that there will be a change is very low.

Variants that are left are further assessed by gathering predictive and functional data and also segregation data needs to be retrieved to obtain all evidence for a benign or pathogenic mutation. This order is the most efficient and also make sure that there would be an increase in productivity as there should be less variants assessed that will not have a clinical significant effect. Lastly, the results need to be explained to the patients. According to the feedback that was given in the study of Wong et al. (2019). the advice is that it should be done by their own doctor and this doctor should emphasize the effect of this reclassification as there were problems in this part earlier.

References

1. Das K J, Ingles J, Bagnall RD, Semsarian C. Correction: Determining pathogenicity of genetic variants in hypertrophic cardiomyopathy: importance of periodic reassessment. Genet Med. 2019 May;21(5):1264. doi: 10.1038/gim.2018.21. Erratum for: Genet Med. 2018 Apr;16(4):286-93. PMID: 31048818.
2. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5. PMID: 25741868; PMCID: PMC4544753.
3. Mazzarotto F, Olivotto I, Walsh R. Advantages and Perils of Clinical Whole-Exome and Whole-Genome Sequencing in Cardiomyopathy. Cardiovasc Drugs Ther. 2020 Apr;34(2):241-253. doi: 10.1007/s10557-020-06948-4. PMID: 32080787.
4. Quiat D, Witkowski L, Zouk H, Daly KP, Roberts AE. Retrospective Analysis of Clinical Genetic Testing in Pediatric Primary Dilated Cardiomyopathy: Testing Outcomes and the Effects of Variant Re-evaluation. J Am Heart Assoc. 2020 Jun 2;9(11):e016195. doi: 10.1161/JAHA.120.016195. Epub 2020 May 27. PMID: 32458740; PMCID: PMC7428992.
5. Campuzano O, Sarquella-Brugada G, Fernandez-Falgueras A, Coll M, Iglesias A, Ferrer-Costa C, Cesar S, Arbelo E, García-Álvarez A, Jordà P, Toro R, Tiron de Llano C, Grassi S, Oliva A, Brugada J, Brugada R. Reanalysis and re-evaluation of rare genetic variants associated with inherited arrhythmogenic syndromes. EBioMedicine. 2020 Apr;54:102732. doi: 10.1016/j.ebiom.2020.102732. Epub 2020 Apr 5. PMID: 32268277; PMCID: PMC7136601.
6. Bennett JS, Bernhardt M, McBride KL, Reshmi SC, Zmuda E, Kertesz NJ, Garg V, Fitzgerald-Butt S, Kamp AN. Re-evaluation of Variants of Uncertain Significance in Children with Inherited Arrhythmia Syndromes is Predicted by Clinical Factors. Pediatr Cardiol. 2019 Dec;40(8):1679-1687. doi: 10.1007/s00246-019-02203-2. Epub 2019 Sep 18. PMID: 31535183.
7. Costa S, Medeiros-Domingo A, Gasperetti A, Akdis D, Berger W, James CA, Ruschitzka F, Brunckhorst CB, Duru F, Saguner AM. Impact of Genetic Variant Reassessment on the Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy Based on the 2010 Task Force Criteria. Circ Genom Precis Med. 2021 Feb;14(1):e003047. doi: 10.1161/CIRCGEN.120.003047. Epub 2020 Nov 24. PMID: 33232181.
8. Butler iii RR, Gejman PV. Clinotator: analyzing ClinVar variation reports to prioritize re-evaluation efforts. F1000Res. 2018 Apr 13;7:462. doi: 10.12688/f1000research.14470.2. PMID: 29862020; PMCID: PMC5941247.
9. Erasmus MC. (2021). Klinische Genetica Laboratorium. Erasmusmc.nl. https://www.erasmusmc.nl/nl-nl/patientenzorg/laboratoriumspecialismen/klinische-genetica

10. Wong EK, Bartels K, Hathaway J, Burns C, Yeates L, Semsarian C, Krahn AD, Virani A, Ingles J. Perceptions of genetic variant re-evaluation in patients with inherited cardiac disease. Eur J Hum Genet. 2019 Jul;27(7):1134-1142. doi: 10.1038/s41431-019-0377-6. Epub 2019 Mar 21. PMID: 30903112; PMCID: PMC6777462.
11. Harper AR, Bowman M, Hayesmoore JBG, Sage H, Salatino S, Blair E, Campbell C, Currie B, Goel A, McGuire K, Ormondroyd E, Sergeant K, Waring A, Woodley J, Kramer CM, Neubauer S, Farrall M, Watkins H, Thomson KL; HCMR Investigators. Reevaluation of the South Asian MYBPC3Δ25bp Intronic Deletion in Hypertrophic Cardiomyopathy. Circ Genom Precis Med. 2020 Jun;13(3):e002783. doi: 10.1161/CIRCGEN.119.002783. Epub 2020 Mar 12. PMID: 32163302; PMCID: PMC7299222.
12. Ye JZ, Delmar M, Lundby A, Olesen MS. Reevaluation of genetic variants previously associated with arrhythmogenic right ventricular cardiomyopathy integrating population-based cohorts and proteomics data. Clin Genet. 2019 Dec;96(6):506-514. doi: 10.1111/cge.13621. Epub 2019 Aug 19. PMID: 31402444.
13. Hershberger RE, Givertz MM, Ho CY, Judge DP, Kantor PF, McBride KL, Morales A, Taylor MRG, Vatta M, Ware SM. Genetic Evaluation of Cardiomyopathy-A Heart Failure Society of America Practice Guideline. J Card Fail. 2018 May;24(5):281-302. doi: 10.1016/j.cardfail.2018.03.004. Epub 2018 Mar 19. PMID: 29567486.