ClinVar database of global familial hypercholesterolemia-associated DNA variants: On behalf of the ClinGen FH Variant Curation Expert Panel

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TITLE

ClinVar database of global familial hypercholesterolemia-associated DNA variants: On behalf of the ClinGen FH Variant Curation Expert Panel

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ABSTRACT

Accurate and consistent variant classification is imperative for incorporation of rapidly developing sequencing technologies into genomic medicine for improved patient care. An essential requirement for achieving standardized and reliable variant interpretation is data sharing, facilitated by a centralized open-source database. Familial Hypercholesterolemia (FH) is an exemplar of the utility of such a resource: it has a high incidence, a favorable prognosis with early intervention and treatment, and cascade screening can be offered to families if a causative variant is identified. ClinVar, an NCBI-funded resource, has become a primary central repository for clinically relevant variants of Mendelian disease, including FH. Here we present the concerted efforts made by the Clinical Genome Resource, through the FH Variant Curation Expert Panel and global FH community, to increase submission of FH-associated variants into ClinVar. Variant-level data was categorized by submitter, variant characteristics, classification method and available supporting data. In order to improve interpretation of FH-associated variants, areas of weakness in ClinVar submissions were identified and addressed. These include the need for detailed and reliable data, submission of supporting variant-level data, retrospectively and prospectively, and making data submission an ongoing effort. Working together to provide thorough, reliable evidence-based variant interpretation will improve the care of FH patients.

Keywords: Familial Hypercholesterolemia; Clinical Genome Resource; Variant Interpretation; ClinVAr

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

1. INTRODUCTION

Familial hypercholesterolemia (FH) is an autosomal codominant disorder, characterized by elevated low-density lipoprotein (LDL) cholesterol levels causing premature atherosclerotic cardiovascular disease when left untreated. FH affects an estimated 1 in 250 individuals worldwide (Akioyamen et al., 2017), and is considered to be the most frequent monogenic disorder encountered in clinical practice. Since the 1970s, a vast number of potentially pathogenic DNA variants have been identified in FH patients, primarily within *LDLR* (the gene encoding the LDL receptor), and more recently in other genes involved in LDL metabolism: *APOB* and *PCSK9* (genes encoding apolipoprotein B and proprotein convertase subtilisin/kexin type 9, respectively). Characterizing the genetic etiology of FH has improved our understanding of disease pathophysiology and associated risks, in addition to improving patient management (Defesche et al., 2017; Goldstein & Brown, 2009).

Determination of genetic variant pathogenicity has direct implications for clinical care and family-based ("cascade") screening, and is improved when there is information on variants from multiple independent sources which can be shared among curators. This data-sharing culture is not new among the FH field; for years the Leiden Open Source Variation Database (LOVD) has served as a publicly available FH-variant repository, hosting 1707 unique *LDLR* variants as of 2016 (Leigh et al., 2017). However, ClinVar, an NCBI-funded resource, has since emerged as the primary centralized database for archiving clinically relevant variants for many Mendelian diseases, including FH. ClinVar facilitates a comprehensive approach to both the consolidation and presentation of patient and molecular data, and includes a multitude of interconnected resources to aid in improving variant interpretation (Harrison et al., 2016).

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Prior to 2016, there were 331 total (278 unique) FH-associated variant submissions in ClinVar. Here, we present the recent efforts made by the Clinical Genome (ClinGen) Resource consortium, along with various global FH researchers to update the number and characterization of FH variants hosted by ClinVar to aid in the accurate knowledge of FH variants. Specifically, we break down the number of FH variants now hosted on ClinVar by gene, location, type, and classification; in addition to providing variant-level characterizations. We then discuss the implications learned from these variant-level and aggregate results.

2. METHODS

2.1. ClinGen FH Variant Curation Expert Panel

The ClinGen FH Variant Curation Expert Panel (FH VC-EP) is composed of >20 members (Supplementary Table 1). Members were selected on the basis of achieving a balanced representation of expert clinicians, clinical laboratory diagnosticians, researchers, and genomic medicine specialists. An emphasis was also placed on global representation, with members from the United States, Brazil, United Kingdom, Netherlands, France, Portugal, Czech Republic, Spain, Israel, Australia and Canada. The FH VC-EP is part of the ClinGen Cardiovascular Clinical Domain Working Group.

2.2. Variant submission to ClinVar

Starting in 2016, several sources were recruited for consolidation of FH-associated variants into ClinVar. These efforts were facilitated by the FH Foundation working with ClinGen leadership to convene a session of interested parties, including members of the FH VC-EP at

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the 2016 international *FH Summit* in Dallas and 2017 in Miami. First, FH VC-EP members

began submitting FH-associated variants and variant-level data from their respective internal

databases to ClinVar. We then encouraged global colleagues to submit internally stored FH-

associated variants, with a focus on the largest remaining sequencing centers from various

countries and jurisdictions. Further, we facilitated variant transfer from existing centralized

databases, namely LOVD (https://databases.lovd.nl/shared/genes/LDLR).

Submitters followed a standard protocol for submission. They were required to

register their organization/center on the ClinVar Submission Portal

(https://submit.ncbi.nlm.nih.gov/clinvar/). Following ClinVar approval, variant submissions

were performed using the Submission Template spreadsheet

(https://www.ncbi.nlm.nih.gov/clinvar/docs/submit/). Submitted variants required

standardized annotation (HGVS expression or chromosomal coordinate change), associated

condition, interpretation of clinical and/or functional significance, interpretation criteria,

collection method (clinical testing or research), allele origin (germline or somatic), and

individual affected status. A wide range of additional variant-level data types were optional

for inclusion, such as number of variant observations, ethnicity and/or geographic origin of

the individual, cosegregation/family data, functional data, phenotypic information, and/or

normolipidemic screening results.

2.3. ClinVar variant analysis

Following submission efforts, ClinVar Miner (https://clinvarminer.genetics.utah.edu/) was

used to extract variant-level data from the ClinVar database for *LDLR*, *APOB*, and *PCSK9*.

Variants that did not have a submitted disease association of "Familial hypercholesterolemia"

or accepted alternative term were removed manually. Specifically, 201 *LDLR*, 423 *APOB*,

and 119 *PCSK9* variants (743 in total) with the following submitted disease associations were

removed from the analysis: "Familial hypobetalipoproteinemia" (n=221), "Hypercholesterolemia, autosomal dominant, type B; Hypobetalipoproteinemia, familial, 1" (n=156; entry of two opposing conditions per single individual), "Low density lipoprotein cholesterol level quantitative trait locus 1" (n=3), "hypocholesterolemia" (n=2), "Hypobetalipoproteinemia, familial, 1" (n=2), "Early-onset coronary artery disease (CAD)" (n=2; removed as other dyslipidemias/morbidities can lead to CAD), "Hypobetalipoproteinemia" (n=1), "C0950123: Inborn genetic diseases" (n=1), "not specified" (n=191), and "not provided" (n=164).

3. RESULTS

3.1. Global ClinVar submission

Prior to ClinGen efforts, there were 242 (193 unique) *LDLR*, 63 (59) *APOB*, and 26 (26) *PCSK9* variant submissions present in ClinVar. The number of FH-associated variants now residing in the ClinVar database is summarized in Table 1: 4973 (2314 unique) in *LDLR*, 580 (353) in *APOB*, and 355 (216) in *PCSK9*. Additionally, there are 201 *LDLR*, 423 *APOB*, and 119 *PCSK9* variant submissions that do not have a disease association of FH and were removed from analysis. A total of 30 centers from 13 different countries have submitted FH-associated variants to ClinVar. Submitting center totals are listed per gene in Table 2.

3.2. FH-associated variant characteristics

Unique FH-associated variants present on ClinVar are categorized by type for *LDLR*, *APOB*, and *PCSK9* in Table 3. Missense variants are the most prevalent unique variant type in each

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of the three genes, followed by frameshift variants in *LDLR*, and synonymous variants in both *APOB* and *PCSK9*. Relative proportions of each variant type are shown in Figure 1.

Not all FH-associated variants present on ClinVar are considered to be disease-causing. With the exception of 198 variant submissions, submitting centers provided a pathogenicity classification for their variants, which can be found summarized by gene in Table 4. Unique variants are categorized by classification reported in Table 5; 57.9% (1670 of 2883) of these variants have been classified by submitters as pathogenic or likely pathogenic (or both, in cases of multiple submissions for the same variant), 15.5% (448 of 2883) have been classified as a variant of unknown significance (VUS) and 10.4% (299 of 2883) have been classified as benign or likely benign. The remaining 13.1 % of variants (379 of 2883) have conflicting classifications using a three-tier system.

3.3. Variant classification methods

A wide range of criteria have been used to classify FH-associated variants present on ClinVar. These include the general ACMG/AMP guidelines, specified guidelines adhering to the ACMG/AMP framework, and a number of independent methods. Most variants with multiple submissions have been classified using various different criteria (Figure 2). The specific criteria used by each submitter are listed in Supplementary Table 2. The most used method was ACMG/AMP framework classification, followed by the Association for Clinical Genetic Science (ACGS) guidelines used in all LOVD transferred variants. A large number of variants (n=865) with classifications did not have indication of criteria used (Table 6).

3.4. Variant-level data

Some variants (n=1972 unique, 3435 submissions) were submitted with some kind of supporting variant-level data. This included information on patient clinical features, if there

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was family history of disease, the number of variant alleles or number of families with the variant identified, number of families with observed segregation, if it was an incidental finding and note of any related functional studies published (Table 7). However, information of co-segregation was only submitted to ClinVar for eight variants; and phenotype data was only submitted for 490 unique variants, in 1043 submissions. Functional studies were reported for 334 unique variants (437 submissions), the majority submitted as literature review by a single group.

4. DISCUSSION

Data sharing through a centralized open-source database is an essential component of achieving accurate and consistent interpretation of variants identified during the course of genetic testing. The ClinGen call for submission of FH-associated variants to ClinVar from different global laboratories resulted in an increase of 10 times the number of unique variants reported during the past years. This was only possible due to a common effort and willingness to share internal data by FH experts and diagnostic companies, who are familiar with the importance of having as much information as possible to classify FH variants. The effort was also facilitated by the FH Foundation, a patient-led research and advocacy organization, to convene these experts for in person discussions on the importance of the project and optimization of variant submission processes. Therefore, this effort demonstrates the power of collaboration across patient-groups, academic labs, commercial labs and scientific funding bodies.

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An extensive range of FH-associated variants are now present on ClinVar to aid with variant interpretation. The relative proportions of variants and variant-types per gene were on par with what is expected for this disorder, and are similar to what has been previously reported (Chora, Medeiros, Alves, & Bourbon, 2017; Leigh et al., 2017). However, there are more known FH-associated variants identified in *LDLR*, *APOB*, and *PCSK9* than previously thought. The FH literature has continued to cite a historical number of ~2000 FH-associated variants identified worldwide; however, this has now become outdated, as here we present ~2900.

It is noteworthy that a number of variants with multiple submissions may include instances of "double counting"; a few FH centers represented here have submitted a proportion of their variants to both the LOVD database (in the past) and ClinVar. While the exact number of these variants is presently unknown, we plan to remove such cases in the near future. Secondly, the number of unique CNVs in *LDLR* (140; 98 deletions and 42 duplications) may be underestimated quite considerably. There have been 271 total CNV submissions, yet only 12 have defined breakpoints. This is a result of commonly applied detection methods such as MLPA (Wang, Ban, & Hegele, 2005), or more recently NGS depth of coverage analysis (Iacocca et al., 2017), which are limited to exon-level resolution. CNV submissions in ClinVar have thus largely been grouped by affected exons, but the likelihood of each breakpoint being identical in these "unique" CNV types is questionable. Previous breakpoint analysis has shown there are multiple unique CNV events which lead to the deletion of the 5'UTR–Exon 1 in *LDLR* (Hobbs, Leitersdorf, Goldstein, Brown, & Russell, 1988), and the same may be true for other *LDLR* CNV types.

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Only 10.7% of classified variants in *LDLR* have been considered as VUS by ClinVar submitters, compared to 55.2% and 39.9% VUS in *APOB* and *PCSK9*, respectively, suggesting potential pathogenicity is much more difficult to evaluate in *APOB*/*PCSK9* compared to *LDLR*. Because a loss-of-function in *LDLR* is a known disease mechanism of FH, any clearly deleterious variant-type in *LDLR* can be considered pathogenic. However, only very specific variants in *APOB* and *PCSK9* lead to FH. In *PCSK9*, causative variants must induce a gain of function in the encoded protein, and in *APOB*, causative variants must allow the production of the protein, but need to specifically alter the binding affinity to LDLR (known LDL binding domains are located within *APOB* exons 26 and 29). Generally, any null variant type in these genes will lead to hypocholesterolemia, and thus are not expected to be identified in FH patients. This leaves most candidate *APOB* and *PCSK9* variants, missense or synonymous, difficult variant types to interpret. Further, some *APOB* variants have been shown to have low penetrance, adding another level of difficulty in interpreting variants in this gene (Alves, Etxebarria, Soutar, Martin, & Bourbon, 2014). Accordingly, it is unwarranted to confidently classify variants as pathogenic in *APOB* and *PCSK9* without performing functional studies, leaving many of them as VUS.

Perhaps most importantly, this effort has revealed that many different variant classification methods are being used. This is problematic, allowing for potential differences in the way two different centers interpret the same variant. Indeed, we saw 379 variants with conflicting classifications, ~15% of variants in each gene. Use of ACMG/AMP Guidelines aims to achieve greater standardization and consistency in variant interpretation (Richards et al., 2015). As we saw here, many FH research and diagnostic groups have adopted this new standard. However, the ACMG/AMP guidelines were designed to be generalizable to all Mendelian disorders, and ambiguities leave potential for differences in the application of various criteria among users, culminating in inconsistent classifications. To this end, there are

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114 unique variants with conflicting classifications in cases where all submitters have cited the ACMG/AMP guidelines.

Beyond a degree of inherent subjectivity, the current ACMG/AMP guidelines do not adequately address FH. In a separate study, ACMG/AMP classification of a large subset of FH-associated variants resulted in a large proportion of VUS (42% of *LDLR* as well 90% in *APOB* and 92% in *PCSK9*) (Chora et al., 2017). Cases of misclassifications when compared against known pathogenic/benign variants were also found. One of ClinGen's key goals is the standardization of gene/disease-specific adjustments to the ACMG/AMP guidelines to address these issues, and to use these specified guidelines to provide a high level of confidence in ClinVar variant classifications. Following a rigorous step-wise process that includes completion of a pilot study and development of a sustained variant curation and discrepancy resolution plan, the FH VC-EP will submit an application for Expert Panel status to the ClinGen Clinical Domain Working Group Oversight Committee for review and final approval. Once approval is obtained, the ClinGen FH VC-EP will curate and classify variants at an Expert Panel review level (3-star, high-confidence), with the ultimate goal of reviewing and/or reclassifying all 2883 unique FH-variants on ClinVar using the newly formed FH-specified ACMG/AMP criteria.

To continue improving the interpretation of FH-associated variants, especially in the context of applying FH-specified ACMG/AMP criteria in the near future, the current state of ClinVar submissions indicate a number of issues to be addressed.

First, the detail in submissions needs to be improved. Many *LDLR, APOB, PCSK9* variants were submitted without a disease association, rendering them of little value to curation efforts. Others were submitted with both hyper/hypo associations, and some had potentially wrong disease associations – for example, deleterious/null variants in

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APOB/*PCSK9* submitted with a disease association of FH, but likely not identified in an FH patient, which may be a case of misreporting. This highlights the need for greater attention to detail, as well as the need for reliable data.

Second, richer supporting variant-level data must be submitted. Although FH centers were very successful in reporting variants, the same cannot be said concerning additional supporting variant-level data. Only eight variants had information about cosegregation, and patient phenotype descriptions were nearly nonexistent (e.g., no cholesterol values were presented and only a few had data on cardiovascular disease). The large majority of submitters did not report variant functional studies, although this is an important step for pathogenicity assessment, and are publicly available for more than 300 variants. The ACMG/AMP framework awards points to functional-level data, co-segregation data, normolipidemic data, and number of observations/unrelated patients with each variant, but if this information is kept stored in internal databases, this will ultimately have a major negative impact on accurately re-classifying all ClinVar variants. Patient ethnicity would also be useful data, but was unreported.

All submitters should include this variant-level data for retrospective and prospective variant submission. Ideally, submissions should include a short case summary of phenotypic and genetic testing results for each individual, such as untreated LDL-C, the genes tested, and any other variants found in the patient at that time. As an illustrative case, consider a patient who presents with an LDL-C value typical of heterozygous FH and has a candidate variant in both *LDLR* and *APOB*. If the *LDLR* variant is clearly pathogenic (suggested by previous aggregate evidence) then this case-level information adds evidence to support the *APOB* variant being benign (if no other evidence is available to suggest otherwise). When these two variants are submitted separately without the testing scenario context, other users may interpret the *APOB* variant as a VUS or perhaps pathogenic if they find only this variant in

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their patients and see it has been previously reported on the database. This sort of contextual interpretation is undoubtedly performed internally by diagnostic laboratories but is currently not part of any variant submissions, despite it being readily accessible at the time of submission.

Third, data submission needs to be an ongoing effort. Although most of the world's largest laboratory repositories for FH variants have now submitted to ClinVar, there are still a few significant populations remaining; including Italy, Norway, Germany, Israel and Japan. Efforts are underway to encourage outstanding centers to submit their variants, and it is imperative this is achieved prior to the reclassification of all variants using FH-specified ACMG/AMP criteria in order to ensure diverse representation is accounted for in the specification of these criteria. Further, FH-associated variants are likely being identified on an exponential scale as NGS panels are becoming increasingly implemented in routine FH diagnosis (Iacocca & Hegele, 2017), a trend surely to continue as sequencing costs continue to plummet and awareness of this disorder broadens. Thus, real-time submission of variant data must be an ongoing focus for all centers, due to the potential implications this data may have on ACMG/AMP-algorithm-derived variant classifications.

5. CONCLUSION

Efforts of data sharing, and reliable variant interpretation, are extremely important to improve the care of FH patients. Since FH is so prominent in the population, and as educational efforts continue, more health care/family physicians can be expected to order genetic testing. As such, FH-associated variant submissions to ClinVar are likely to continue to increase. This will also increase the use of ClinVar as an essential resource for variant interpretation, with

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the goal to reach the largest number of 3-star variants and its corollary in terms of acceleration of the molecular diagnosis of FH, ultimately affecting patient management and cascade screening. ClinGen will continue to encourage data sharing and communication between clinical and research FH experts in order to improve variant curation and harmonize FH diagnosis across the world.

Conflict of interests: AC has received honoraria from Amgen SAS and Alexion Pharma France SAS, and is currently receiving a grant from Alexion Pharma France SAS. RS has received honoraria related to consulting, lectures, and research activities from Amgen, Astra Zeneca, Akcea, Biolab, Esperion, Kowa, Merck, Novo-Nordisk, Pfizer, and Sanofi/Regeneron. RAH has received honoraria for membership on advisory boards and speakers' bureaus for Aegerion, Akcea/Ionis, Amgen, Gemphire, and Regeneron/Sanofi. MB received project grants from Sanofi/Regeneron, PRAXIS, and Alexion Pharmaceuticals.

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7. TABLES

Table 1. Number of variants submitted to ClinVar by gene.

	LDLR	*APOB*	*PCSK9*	Total
All variants submitted to ClinVar	5174	1003	474	6651
Variants detected in FH patients	4973	580	355	5908
Unique variants detected in FH patients	2314	353	216	2883

Table 2. Centers that submitted variants to ClinVar associated with FH.

Submitting Centers	Country	*LDLR*	*APOB*	*PCSK9*	Total
LDLR-Leiden Open Source Variation Database, British Heart Foundation	UK	1670	-	-	1670
Laboratory of Molecular Diagnostics, Vascular Medicine, Academic Medical Centre, University of Amsterdam	Netherlands	686	25	46	757
Centre of Molecular Genetics, Obesity and Dyslipidemias Unit, Pitié-Salpêtrière University Hospital	France	414	1	19	434
Cardiovascular Research Group, National Institute of Health Dr. Ricardo Jorge	Portugal	276	53	70	399
Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute	Canada	202	137	30	369
Clinical Services Laboratory, Illumina	USA	97	180	85	362
Molecular Medicine of Metabolic Diseases Unit (U4M), University of Lille, Regional Hospital Center	France	344	-	-	344
Spanish Familial Hypercholesterolemia Foundation	Spain	320	10	1	331
Laboratory of Genetics and Molecular	Brazil	201	63	16	280

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Cardiology, University of São Paulo					
Molecular Genetics Laboratory, Centre for Cardiovascular Surgery and Transplantation	Czech Republic	197	-	-	197
Invitae	USA	156	-	40	196
Cardiovascular Genetics Laboratory, PathWest Laboratory Medicine WA	Australia	152	-	-	152
Color Genomics	USA	23	65	25	113
Other	USA Germany, Finland, India, South Korea	235	46	23	304

Centers which have submitted >100 FH-associated variants are listed; remaining centers are grouped in "Other".

Table 3. Unique variants submitted to ClinVar with association with FH by gene and type of variant.

Variant Type	*LDLR*	*APOB*	*PCSK9*
3'UTR	77	9	40
5'UTR	54	4	18
Frameshift	446	12	1
In-frame indels	88	5	6
Intronic	77	3	26
Splicing	169	24	13
CNV (deletion)	98	-	1
CNV (duplication)	42	-	-
Missense	1012	218	82
Nonsense	160	4	1

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Synonymous	82	74	28
Others	9	-	-
Total	2314	353	216

UTR, untranslated region; indel, insertion or deletion variant; in-frame indels = smaller than 1 exon; intronic = variants after +/-15 nucleotides (nts) in the intron; splicing = variants known to affect splicing + variants within +/-15 nts in the intron; CNV, copy number variation; CNVs = 1 whole exon or more; others, variants whose type could not be ascertained with the information provided

Table 4. Clinical significance of all variant submissions associated with FH, regardless of criteria used for classification (*all variants, all criteria*)

Clinical significance	*LDLR*	*APOB*	*PCSK9*
Benign	205 (4.1%)	57 (9.8%)	88 (24.8%)
Likely benign	312 (6.3%)	97 (16.7%)	54 (15.2%)
Uncertain significance	526 (10.6%)	254 (43.8%)	132 (37.2%)
Likely pathogenic	1525 (30.7%)	10 (1.7%)	15 (4.2%)
Pathogenic	2351 (42.3%)	42 (7.2%)	42 (11.8%)
Not provided	*54 (1.1%)*	*120 (20.7%)*	*24 (6.85)*
Total	4973	580	355

Table 5. Clinical significance of unique variants associated with FH, regardless of criteria used for classification. Multiple submissions are considered for classification concordance. *(Unique variants, 1 or more submitters, all criteria)*

Clinical significance	*LDLR*	*APOB*	*PCSK9*
Benign/Likely benign	200 (8.7%)	44 (15.1%)	55 (26.8%)
Uncertain significance	182 (7.9%)	171 (58.6%)	95 (46.3%)
Pathogenic/Likely pathogenic	1614 (70.2%)	30 (10.3%)	26 (12.7%)
Conflicting classification	303 (13.2%)	47 (16.0%)	29 (14.2%)
Not provided	*15*	*61*	*11*
Total	2314	353	216

Conflicting classification = Benign/Likely benign + Uncertain significance; or Pathogenic/Likely pathogenic + Uncertain significance; or Benign/Likely benign + Pathogenic/Likely pathogenic

Table 6. Criteria used for unique variant classification

Criteria used for classification	*LDLR*	*APOB*	*PCSK9*	Total
ACMG/AMP Guidelines	1144	127	99	1370
ACMG/AMP Framework	295	194	120	609
ACGS Guidelines	1669	-	-	1669
Independent methods	186	26	9	221
No criteria	793	25	47	865

ACMG/AMP Guidelines, American College of Medical Genetics/Association for Molecular Pathology guidelines (Richards et al., 2015); ACMG/AMP Framework, criteria following the ACMG/AMP framework; ACGS, Association for Clinical Genetic Science Guidelines; Independent methods, criteria provided not based on ACMG/AMP or ACGS frameworks; No criteria, classification given but the criteria used was not provided.

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Table 7. Number of unique variants with each variant-level data type available at ClinVar.

Variant-level data submitted as evidence*	*LDLR*	*APOB*	*PCSK9*
Variant alleles/number of families with variant	1885	26	11
Clinical features/Family history	490	0	0
Incidental finding	344	0	0
Functional study	293	19	22
Number of families with observed segregation	8	0	0

*Labels are pulled directly from ClinVar

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8. FIGURE LEGENDS

Figure 1. Relative proportions of unique variants submitted to ClinVar with association with

FH by gene and type of variant.

Figure 2. Number of unique variants classified (*n*=2796) using different sets of criteria. For

87 unique variants, no classification was submitted. ACMG/AMP Guidel, American College

of Medical Genetics/Association for Molecular Pathology guidelines (Richards et al., 2015);

ACMG/AMP Framew, criteria following the ACMG/AMP framework; ACGS, Association

for Clinical Genetic Science Guidelines; Independent, criteria provided not based on

ACMG/AMP or ACGS frameworks; No criteria, classification given but the criteria used was

not provided.

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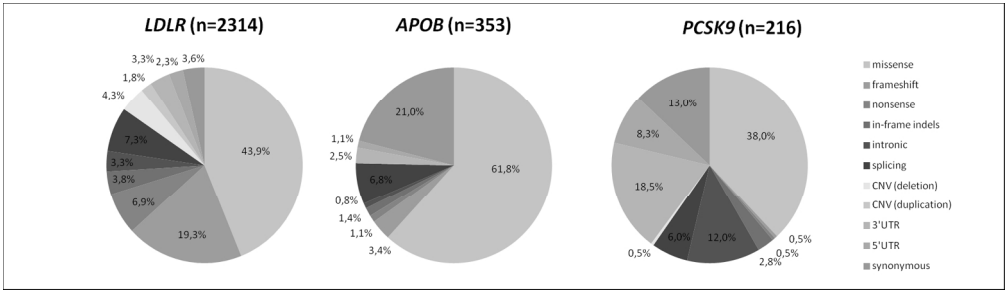
Figure 1 in greyscale

316x91mm (150 x 150 DPI)
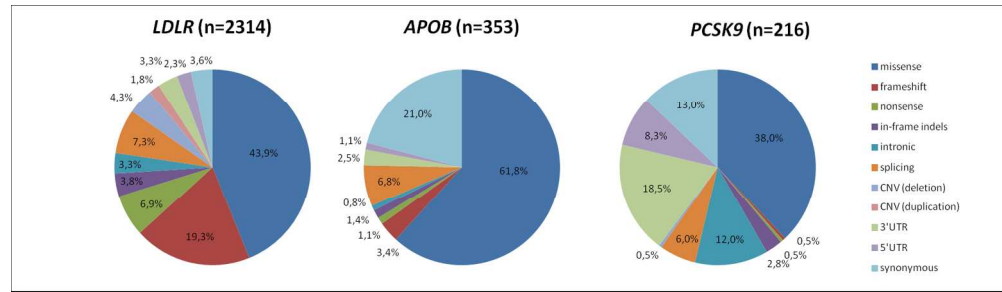
Figure 1 in color_for online only

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Figure 2 in greyscale

318x306mm (96 x 96 DPI)

Figure 2 in color_for online only

318x306mm (96 x 96 DPI)

SUPPLEMENTARY MATERIAL

Supplementary Table 1. ClinGen Familial Hypercholesterolemia Expert Panel members

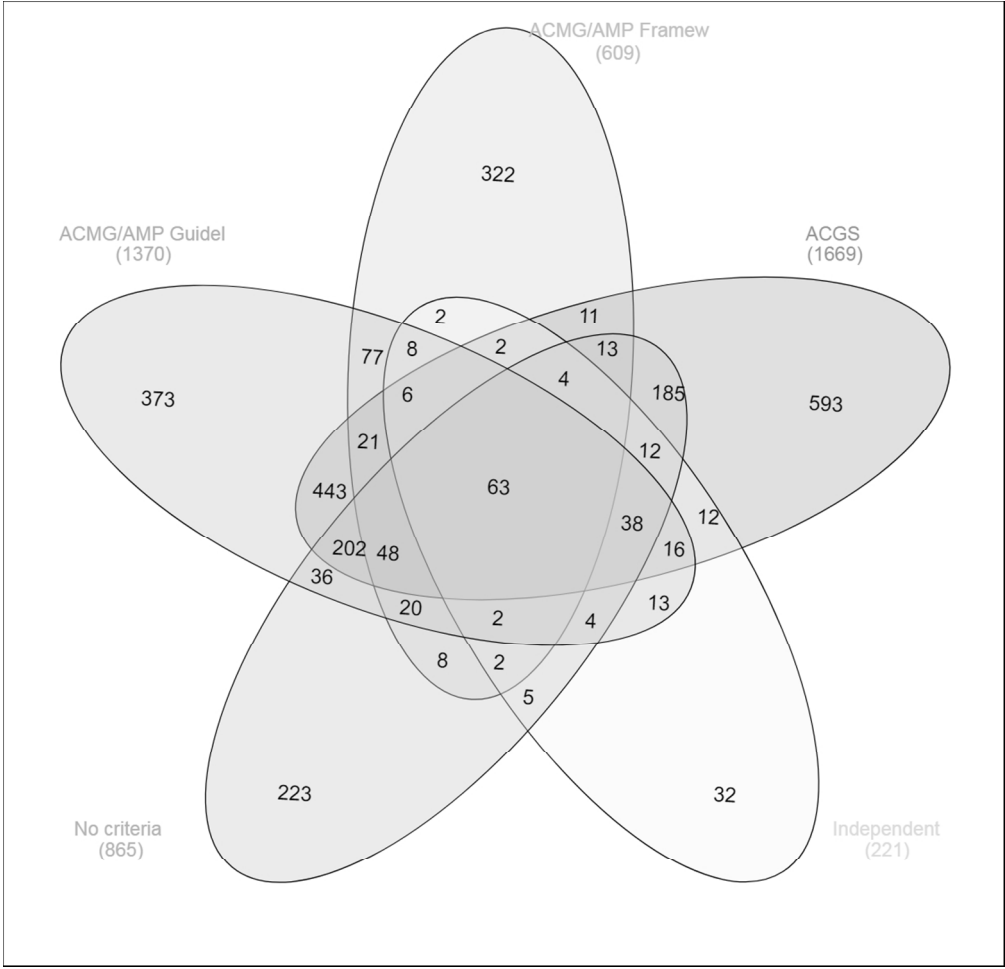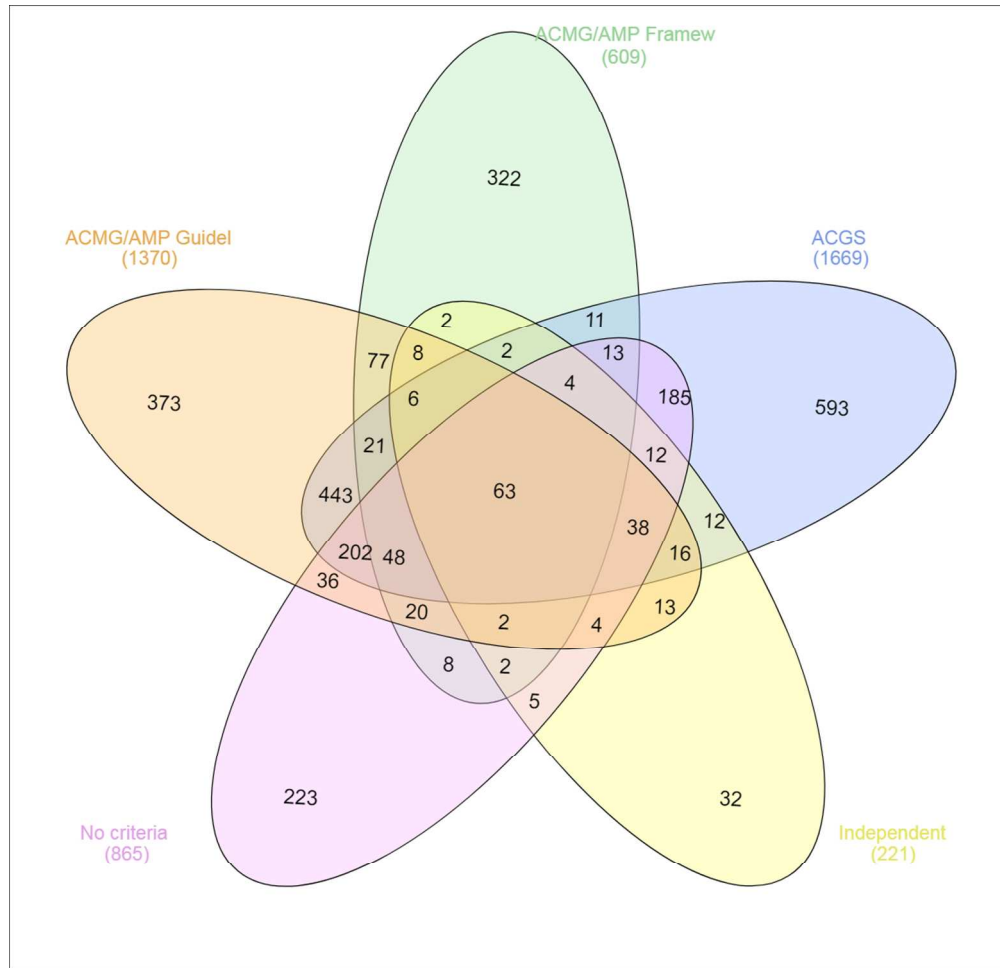| Member List			
Name	**Institution**	**Area and Type of Expertise**	**Role**
Joshua Knowles, MD, PhD	Stanford University	Clinician/Researcher	Executive Leader
Mafalda Bourbon, PhD	Instituto Nacional de Saúde	Research/Laboratory Director	Executive Leader
C. Lisa Kurtz, PhD	University of North Carolina, Chapel Hill	Researcher	Coordinator
Robert Hegele, MD	Robarts Research Institute	Clinician / researcher	Executive Committee
Eric Sijbrands, MD, PhD	Erasmus University Rotterdam	Clinician	Executive Committee
Alain Carrie, MD, PhD	Pitié-Salpêtrière Hospital	Laboratory Director	Executive Committee
Joep Defesche, PhD	Academisch Medisch Centrum	Laboratory Director	Executive Committee
Tomas Freiberger, MD, PhD	Centre for Cardiovascular Surgery and Transplantation	Researcher/ Laboratory Director	Executive Committee
Sarah Leigh, PhD	Genomics England	Researcher	Executive Committee
Amanda Hooper, PhD	PathWest Laboratory Medicine WA	Clinical Scientist	Expert
Steve E Humphries, PhD	University College London	Clinician/Researcher	Expert
Amit Khera, MD	Broad Institute	Clinician/Researcher	Expert
Michael Murray, MD	Geisinger	Director of Clinical Genomics	Expert
Jean-Pierre Rabes, MD, PhD	Hôpital Ambroise Paré	Laboratory Director/Clinician	Expert
Daniel Rader, MD	University of Pennsylvania	Scientific Director/Clinician	Expert
Raul Santos, MD, PhD	InCor, São Paulo University	Clinician	Expert
Marianne Stef,	Progenika	Laboratory	Expert

PhD			
Marina Cuchel, MD, PhD	University of Pennsylvania	Clinician/Researcher	Expert
Mariko Harada-Shiba, MD	Japan	Clinician	Expert
Margaret Chen, PhD, FACMG	GeneDx	Laboratory Director	Expert
Ronen Durst	Hadassah Hebrew University Medical Center	Clinician	Expert
Pedro Mata	Fundacion Hipercolesterolemia Familiar	Clinician	Expert
Joana Chora	Instituto Nacional de Saúde	PhD student	Curator
Michael Iacocca	Robarts Research Institute	MSc student	Curator
Lukas Tichy	University Hospital Brno, Czech Republic	Molecular geneticists	Curator

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Supplementary Table 2. Criteria for variant classification used by each submitting center.

Submitting Centers	Country	Criteria
Centre of Molecular Genetics, Obesity and Dyslipidemias Unit, Pitié-Salpêtrière University Hospital	France	ACMG/AMP Guidelines (Richards et al., 2015)
Cardiovascular Research Group, National Institute of Health Dr. Ricardo Jorge	Portugal	ACMG/AMP Guidelines (Richards et al., 2015)
Molecular Medicine of Metabolic Diseases Unit (U4M), University of Lille, Regional Hospital Center	France	ACMG/AMP Guidelines (Richards et al., 2015)
Spanish Familial Hypercholesterolemia Foundation	Spain	ACMG/AMP Guidelines (Richards et al., 2015)
Laboratory of Genetics and Molecular Cardiology, University of São Paulo	Brazil	ACMG/AMP Guidelines (Richards et al., 2015)
Molecular Genetics Laboratory, Centre for Cardiovascular Surgery and Transplantation	Czech Republic	ACMG/AMP Guidelines (Richards et al., 2015)
Color Genomics	USA	ACMG/AMP Guidelines (Richards et al., 2015)
Knight Diagnostic Laboratories, Oregon Health and Sciences University	USA	ACMG/AMP Guidelines (Richards et al., 2015)
Phosphorus	USA	ACMG/AMP Guidelines (Richards et al., 2015)
Molecular Diagnostics Laboratory, Nemours Alfred I. duPont Hospital for Children	USA	ACMG/AMP Guidelines (Richards et al., 2015)
Institute of Medical Genetics and Genomics, Sir Ganga Ram Hospital	India	ACMG/AMP Guidelines (Richards et al., 2015)
Soonchunhyang University Medical Center	South Korea	ACMG/AMP Guidelines (Richards et al., 2015)
Clinical Services Laboratory, Illumina	USA	ICSL Variant Classification (ACMG/AMP framework; (llumina Clinical Services Laboratory, 2016))

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Invitae	USA	Invitae Variant Classification: Sherloc (ACMG/AMP framework; (Nykamp et al., 2017)
Laboratory Corporation of America	USA	LabCorp Variant Classification Specifications (ACMG/AMP framework; (Laboratory Corporation of America, 2015))
Division of Human Genetics & Genomic Diagnostics, Children's Hospital of Philadelphia	USA	DGD Variant Analysis Guidelines (ACMG/AMP framework; (Division of Genomic Diagnostics & The Children's Hospital of Philadelphia, 2015))
Cardiovascular Biomarker Research Laboratory, Mayo Clinic	USA	Mayo Cardiovascular Biomarkers Research Laboratory *LDLR* variant Interpretation Criteria (ACMG/AMP framework; (Kullo Laboratory, 2015))
Blueprint Genetics	Finland	Blueprint Variant Classification (ACMG/AMP framework; (Blueprint Genetics, 2016))
LDLR-Leiden Open Source Variation Database, British Heart Foundation	UK	ACGS Variant Guidelines (Wallis et al., 2013)
Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute	Canada	Independent method; Submitters publication (Wang et al., 2016)
Clinical Sequencing Exploratory Research, University of Washington	USA	Independent method; Literature (Amendola et al., 2015)

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Institute for Integrative and Experimental Genomics, University of Luebeck	Germany	Independent method; Submitter's publication (Braenne et al., 2016)
Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine, Harvard Medical School	USA	Independent method; Submitter's publication (Duzkale et al., 2013)
SNPedia	USA	Independent method; Literature (Khera et al., 2016)
Laboratory of Molecular Diagnostics, Vascular Medicine, Academic Medical Centre, University of Amsterdam	Netherlands	None
Cardiovascular Genetics Laboratory, PathWest Laboratory Medicine WA	Australia	None
Online Mendelian Inheritance in Man (OMIM)	USA	None
GeneReviews	USA	None
Bioscience Institute for Medical Diagnostics, Sonic Healthcare	Germany	None
GenomeConnect	USA	None

ACMG/AMP, American College of Medical Genetics/Association for Molecular Pathology; ACGS, Association for Clinical Genetic Science