HHS Public Access

Author manuscript

Hum Mutat. Author manuscript; available in PMC 2019 November 01.

Published in final edited form as:

Hum Mutat. 2018 November ; 39(11): 1631–1640. doi:10.1002/humu.23634.

ClinVar database of global familial hypercholesterolemia-associated DNA variants

Michael A. Iacocca[1,*], **Joana R. Chora**[2,3,*], **Alain Carrie**[4,5], **Tomas Freiberger**[6], **Sarah E. Leigh**[7], **Joep C. Defesche**[8], **C. Lisa Kurtz**[9], **Marina T. DiStefano**[10], **Raul Santos**[11], **Steve E. Humphries**[12], **Pedro Mata**[13], **Cinthia Jannes**[11], **Amanda J. Hooper**[14], **Katherine A. Wilemon**[15], **Pascale Benlian**[16], **Robert O'Connor**[17], **John Garcia**[18], **Hannah Wand**[19], **Lukas Tichy**[20], **Eric J. Sijbrands**[21], **Robert A. Hegele**[1], **Mafalda Bourbon**[2,3,**], and **Joshua W. Knowles**[15,19,**] **the ClinGen FH Variant Curation Expert Panel**

[1]Robarts Research Institute, Western University, London ON, Canada [2]Instituto Nacional de Saude Doutor Ricardo Jorge, Lisbon, Portugal [3]BioISI, University of Lisbon, Portugal [4]Assistance Publique-Hôpitaux de Paris (APHP), Hôpitaux Universitaires Pitié-Salpêtrière / Charles-Foix, Molecular and Chromosomal Genetics Center, Obesity and Dyslipidemia Genetics Unit, Paris, France [5]Sorbonne Université, Inserm, Institute of Cardiometabolism and Nutrition (ICAN), Hôpital de la Pitié, Paris, France [6]Centre for Cardiovascular Surgery and Transplantation, Masaryk University, Brno, Czech Republic [7]Genomics England, London, United Kingdom [8]Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands [9]Department of Genetics, University of North Carolina, Chapel Hill NC, USA [10]Harvard Medical School, Harvard University, Boston MA, USA [11]Instituto do Coração, São Paulo, Brazil [12]Centre for Cardiovascular Genetics, University College of London, United Kingdom [13]Fundacion Hipercolesterolemia Familiar, Madrid, Spain [14]PathWest Laboratory Medicine, University of Western Australia, Perth, Australia [15]FH Foundation, Pasadena CA, USA [16]University of Lille, CNRS, CHU Lille, UMR 8199 – EGID (Integrative Genomics and Metabolic Diseases Modeling), Lille, France [17]Color Genomics, Burlingame CA, USA [18]Invitae Corporation, San Fransciso CA, USA [19]Stanford University, Palo Alto CA, USA [20]Centre of Molecular Biology and Gene Therapy, Masaryk University, Brno, Czech Republic [21]University Medical Center, Erasmus University, Rotterdam, Netherlands

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

[*]joint first authors. [**]joint senior authors. **Correspondance:** Mafalda Bourbon, PhD, Unidade de I&D, Grupo de Investigação, Cardiovascular, Dept. Promoção da Saúde e Prevenção de Doenças Não Transmissiveis, Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal, Tel.:(351) 217 508 126 / 217 508 130, mafalda.bourbon@insa.min-saude.pt.

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.

intervention and treatment, and cascade screening can be offered to families if a causative variant is identified. ClinVar, an NCBI-funded resource, has become the primary repository for clinically relevant variants in 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. To further reform interpretation of FH-associated variants, areas for improvement in variant submissions were identified and addressed; these include a need for more detailed submissions and submission of supporting variant-level data, both retrospectively and prospectively. Collaborating to provide thorough, reliable evidence-based variant interpretation will ultimately improve the care of FH patients.

Keywords

Familial hypercholesterolemia; Clinical Genome Resource; Variant interpretation; ClinVar

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).

Interpreting the clinical significance of genetic variants is challenging (Bland et al., 2018), and has direct implications for diagnosis and family based “cascade” screening. While improvements in variant interpretation may aid in the implementation of genetic testing into more routine FH clinical care, this is enhanced when there is information on variants from multiple independent sources which can be shared among laboratories. 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).

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 and interpretation 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 (Supp. Table S1). 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 the 2016 international *FH Summit* in Dallas, USA, and 2017 in Miami, USA. 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: "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). Variant consequences were determined manually from DNA and protein level variant information and confirmed using the Mutalyzer Name Checker batch tool v.2.0.28 (Leiden University Medical Center, Netherlands; https://mutalyzer.nl/).

3. RESULTS

3.1. Global ClinVar submission

Prior to 2016, there were 242 (193 unique) *LDLR*, 63 (59) *APOB* and 26 (26) *PCSK9* variant submissions present in ClinVar. In a concerted effort to increase this number, the ClinGen FH VC-EP encouraged the submission of FH-associated variants by colleagues and sequencing centers on a global scale. As a result, the number of FH-associated submissions now residing in the ClinVar database increased ~ 18-fold and is summarized in Table 1. 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 and shown by location across all exons in Figure 1. Missense variants are the most prevalent unique variant type in each of the three genes, followed by frameshift variants in *LDLR*, and synonymous variants in both *APOB* and *PCSK9*. In *LDLR* 18% of all unique variants are located in exon 4, in *APOB* 41% are in exon 26 and 15% in exon 29 and in *PCSK9* 19% are in the 3'UTR region. Figure 2 illustrates the relative proportions of variant classifications for each variant type.

Variants submitted to ClinVar range from benign to pathogenic or can be submitted without an assertion; with the exception of 198 FH-associated variant submissions, submitting centers provided a pathogenicity classification for their variants, found summarized by gene in Table 4. Unique variants are categorized by classification 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 American College of Medical Genetics and Genomics / Association for Molecular Pathology (ACMG/AMP) guidelines (2015), 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 3). The specific criteria used by each submitter are listed in Supp. Table S2. 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 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 total 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 essential to achieve accurate and consistent interpretation of variants identified during the course of genetic testing. Through the concerted efforts of the ClinGen FH VC-EP, 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, and demonstrates the power of collaboration across patient-groups, academic labs, commercial labs and scientific funding bodies.

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 are consistent with 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, with ~2900 presented here, this has now become outdated.

It is noteworthy that a number of variants with multiple submissions may include instances of “double counting”; a few FH centers 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, efforts are underway to remove such cases. Secondly, the number of unique CNVs in *LDLR* (142; 100 deletions and 42 duplications) may be underestimated quite considerably. There have been 273 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. *LDLR* CNV submissions in ClinVar have thus largely been grouped by affected exon(s), 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.

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 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, which pose challenges to interpretation. 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.

This effort has also revealed that many different variant classification methods are being used, which is problematic since non-standardization can lead to different interpretation of identical variants. Indeed, we saw 379 variants (~15% of variants in each gene) with conflicting classifications. 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, yielding inconsistent classifications. For instance, 114 unique variants have conflicting classifications despite all submitters having 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% in *LDLR,* as well as 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.

Current ClinVar submssions point to specific issues that need to be addressed imminently in order to further improve the interpretation of FH-associated variants, especially in the context of applying FH-specified ACMG/AMP criteria.

First, clinical details accompanying a submission need to have minimum standards. Many *LDLR, APOB* and *PCSK9* variants were submitted without a disease association, rendering them of little value to curation efforts. Others were submitted with both hyper/hypocholesterolemia associations, and some had potentially incorrect disease associations – for example, deleterious/null variants in *APOB*/*PCSK9* submitted with a disease association of FH.

Second, richer supporting variant-level data must be submitted. Although FH centers successfully reported numerous 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 data on lipid profiles or cardiovascular disease). The large majority of submitters reported no functional studies for detected variants, although this is key to pathogenicity attribution, 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 the number of observations/unrelated patients with each variant; if this information is kept stored in internal databases it 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 supporting variant-level data for retrospective and prospective variant submission. Ideally, submissions should include a short summary of phenotype and genetic testing results for each individual, such as untreated LDL-C, the genes tested, and any other variants detected in the patient's sample. 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 two such variants are submitted separately outside the testing context, others might interpret the *APOB* variant as a VUS or perhaps even as pathogenic if it is the only variant ascertained in their patient and see it has been previously reported on the database. Such contextual interpretation is undoubtedly performed internally by diagnostic laboratories but is currently

not part of any variant submission process, despite it being readily accessible at the time of submission.

Third, data submission needs to be ongoing. Although most of the world's largest laboratory repositories for FH variants have now made submissions to ClinVar, a few important populations remain outstanding; including Italy, Denmark, 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 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 sure to continue as sequencing costs continue to plummet and awareness of FH broadens. Thus, real-time submission of variant data must be a 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 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. The ClinGen FH VC-EP 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Illumina Clinical Services Laboratory; JRC acknowledges her PhD fellowship funded by the Science and Technology Foundation (SFRH/BD/108503/2015). TF was supported by the Ministry of Health of the Czech Republic, Grants nr. 16–29084A and 15–28277A (all rights reserved). SEH acknowledges funding from the British Heart Foundation for support for the UCL LOVD database (BHF PG08/008) and from the NIHR UCLH BRC.

Grant Sponsors: ClinGen is primarily funded by the National Human Genome Research Institute (NHGRI), through the following three grants: U41HG006834, U41HG009649, U41HG009650.

6. REFERENCES

Akioyamen LE, Genest J, Shan SD, Reel RL, Albaum JM, Chu A, & Tu JV (2017). Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. BMJ Open, 7, e016461.

Alves AC, Etxebarria A, Soutar AK, Martin C, & Bourbon M (2014). Novel functional APOB mutations outside LDL-binding region causing familial hypercholesterolaemia. Human Molecular Genetics, 23, 1817–1828. [PubMed: 24234650]

Amendola LM, Dorschner MO, Robertson PD, Salama JS, Hart R, Shirts BH, … Jarvik GP (2015). Actionable exomic incidental findings in 6503 participants: challenges of variant classification. Genome Research, 25, 305–315. [PubMed: 25637381]

Bland A, Harrington EA, Dunn K, Pariani M, Platt JCK, Grove M, & Caleshu C (2018). Clinically impactful difference in variant interpretation between clinicians and tetsing laboraoties: a single-center experience. Genetics in Medicine, 20, 369–373. [PubMed: 29240077]

Blueprint Genetics. (2016). A guide to understanding variant classification Retrieved April 11, 2018, from https://submit.ncbi.nlm.nih.gov/ft/byid/nxpnxkpc/variant_classification_wp_vara41-03.pdf

Braenne I, Kleinecke M, Reiz B, Graf E, Strom T, Wieland T, … Schunkert H (2016). Systematic analysis of variants related to familial hypercholesterolemia in families with premature myocardial infarction. European Journal of Human Genetics, 24100, 191–197.

Chora JR, Medeiros AM, Alves AC, & Bourbon M (2017). Analysis of publicly available LDLR, APOB, and PCSK9 variants associated with familial hypercholesterolemia: application of ACMG guidelines and implications for familial hypercholesterolemia diagnosis. Genetics in Medicine

Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, & Wierzbicki AS (2017). Familial hypercholesterolaemia. Nature Reviews Disease Primers, 3, 17093.

Division of Genomic Diagnostics, & The Children's Hospital of Philadelphia. (2015). DGD Variant Analysis Guidelines Retrieved April 11, 2018, from https://submit.ncbi.nlm.nih.gov/ft/byid/q5qzurm4/dgd_variant_analysis_guidelines.docx

Duzkale H, Shen J, Mclaughlin H, Alfares A, Kelly M, Pugh T, … Lebo M (2013). A systematic approach to assessing the clinical significance of genetic variants. Clin Genet, 84, 453–463. [PubMed: 24033266]

Goldstein JL, & Brown MS (2009). The LDL receptor. Arteriosclerosis, Thrombosis, and Vascular Biology, 29, 431–8.

Harrison SM, Riggs ER, Maglott DR, Lee JM, Azzariti DR, Niehaus A, … Rehm HL (2016). Using ClinVar as a Resource to Support Variant Interpretation. Current Protocols in Human Genetics, 89, 816.1–8.16.23.

Hobbs HH, Leitersdorf E, Goldstein JL, Brown MS, & Russell DW (1988). Multiple crm-mutations in familial hypercholesterolemia. Evidence for 13 alleles, including four deletions. The Journal of Clinical Investigation, 81, 909–17. [PubMed: 3343347]

Iacocca MA, & Hegele RA (2017). Recent advances in genetic testing for familial hypercholesterolemia. Expert Review of Molecular Diagnostics, 17, 641–651. [PubMed: 28524730]

Iacocca MA, Wang J, Dron JS, Robinson JF, McIntyre AD, Cao H, & Hegele RA (2017). Use of next-generation sequencing to detect LDLR gene copy number variation in familial hypercholesterolemia. Journal of Lipid Research, 58, 2202–2209. [PubMed: 28874442]

Khera AV, Won H-H, Peloso GM, Lawson KS, Bartz TM, Deng X, … Kathiresan S (2016). Diagnostic Yield and Clinical Utility of Sequencing Familial Hypercholesterolemia Genes in Patients With Severe Hypercholesterolemia. Journal of the American College of Cardiology, 67, 2578–2589. [PubMed: 27050191]

Kullo Laboratory. (2015). Kullo Lab Assertion Criteria Retrieved April 11, 2018, from https://submit.ncbi.nlm.nih.gov/ft/byid/RJ4WMVct/Kullo_Lab_Assertion_Criteria_01072016.pdf

Laboratory Corporation of America. (2015). LabCorp: Variant Classification Specifications Retrieved April 11, 2018, from https://submit.ncbi.nlm.nih.gov/ft/byid/pttb9itm/labcorp_variant_classification_method_-_may_2015.pdf

Leigh S, Futema M, Whittall R, Taylor-Beadling A, Williams M, den Dunnen JT, & Humphries SE (2017). The UCL low-density lipoprotein receptor gene variant database: pathogenicity update. Journal of Medical Genetics, 54, 217–223. [PubMed: 27821657]

llumina Clinical Services Laboratory. (2016). ICSL Variant Classification Retrieved April 11, 2018, from https://submit.ncbi.nlm.nih.gov/ft/byid/4jQgNGYk/ICSL_Variant_Classification_20161018.pdf

Nykamp K, Anderson M, Powers M, Garcia J, Herrera B, Ho Y-Y, … Topper S (2017). Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. Genetics in Medicine: Official Journal of the American College of Medical Genetics, 19, 1105–1117. [PubMed: 28492532]

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, … ACMG Laboratory Quality Assurance Committee. (2015). 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. Genetics in Medicine, 17, 405–423. [PubMed: 25741868]

Wallis Y, Payne S, Mcanulty C, Bodmer D, Sister-mans E, Robertson K, … Devereau A (2013). Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics, (September), 16.

Wang J, Ban MR, & Hegele RA (2005). Multiplex ligation-dependent probe amplification of LDLR enhances molecular diagnosis of familial hypercholesterolemia. Journal of Lipid Research, 46, 366–372. [PubMed: 15576851]

Wang J, Dron JS, Ban MR, Robinson JF, McIntyre AD, Alazzam M, … Hegele RA (2016). Polygenic Versus Monogenic Causes of Hypercholesterolemia Ascertained Clinically. Arteriosclerosis, Thrombosis, and Vascular Biology, 36, 2439–2445.

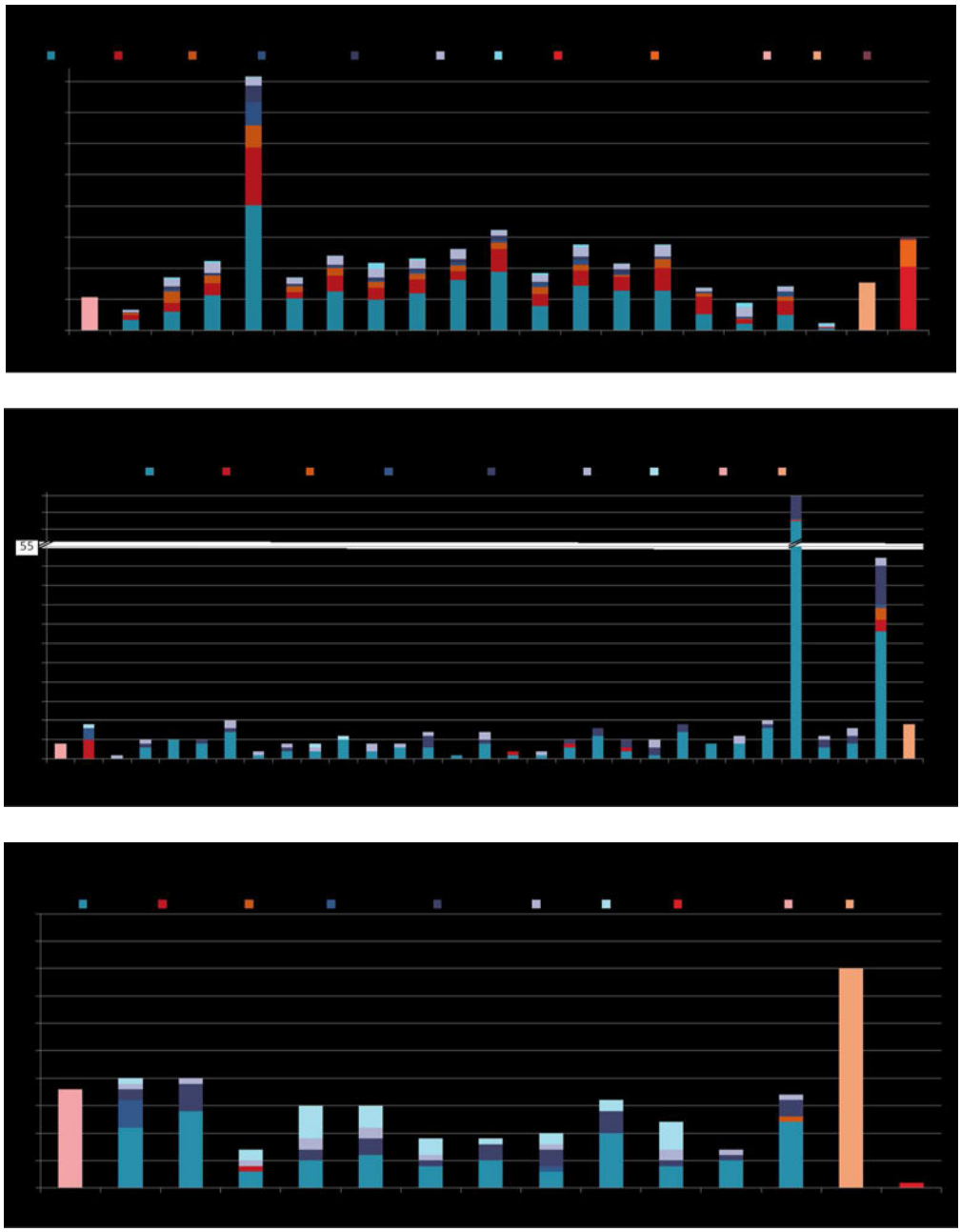
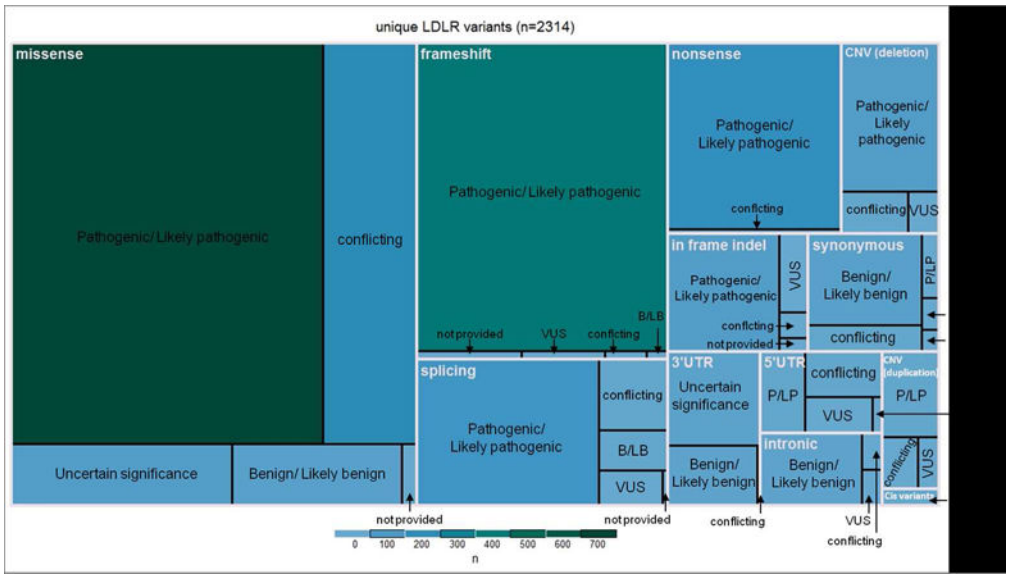
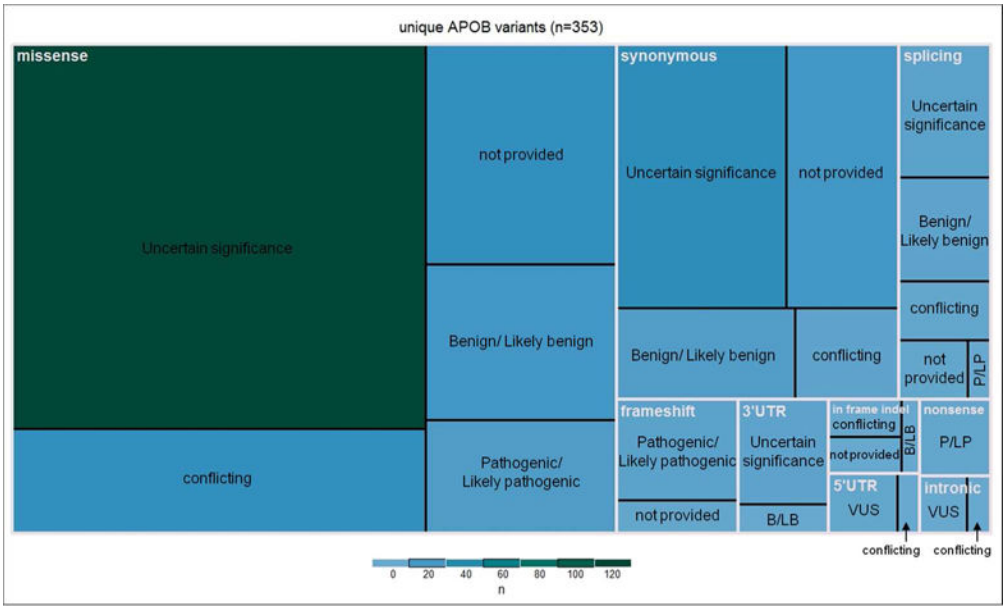


Figure 1.

Unique FH-associated variants in ClinVar by exonic location and type of variant. Variants in introns are represented in the closest exon. NA, not applicable (variants spanning more than one exon); double variant, single submission with two variants in the same allele; intronic, variants outside +/−15 nucleotides (nts) of intron/exon border; splicing, variants known to affect splicing or variants within +/−15 nts of intron/exon border; indel, insertion or deletion variant; UTR, untranslated region; CNVs, copy number variation.





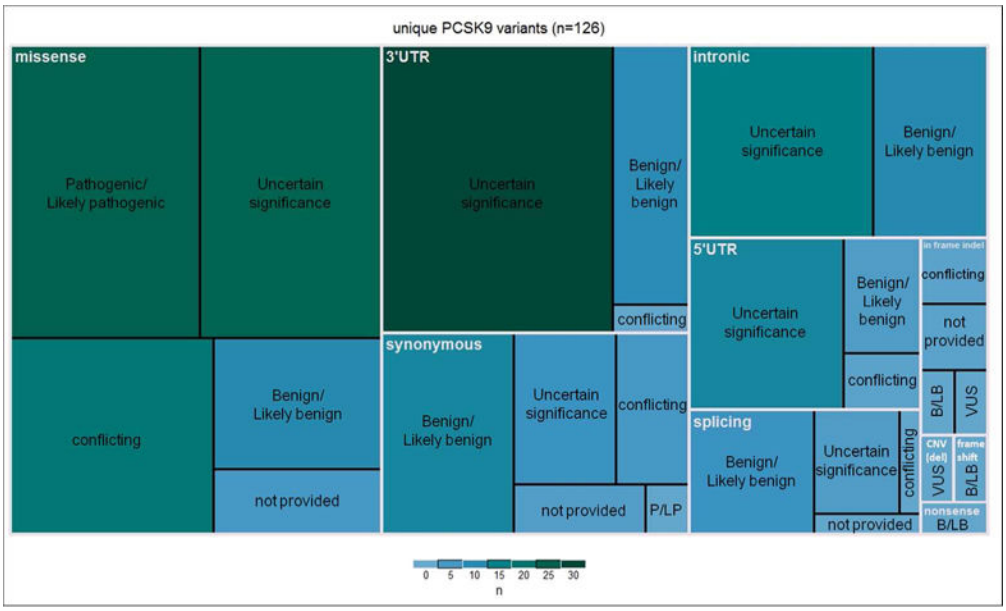


Figure 2.

Treemap partition of unique FH-associated variants in ClinVar conveying the relative proportions of variant classifications for each variant type. Conflicting classifications are only considered for variants with multiple submissions, defined by the following discordances: Benign/Likely benign + Uncertain significance; Pathogenic/Likely pathogenic + Uncertain significance; Benign/Likely benign + Pathogenic/Likely pathogenic. Double variant, single submission with two variants in the same allele; intronic, variants outside +/−15 nucleotides (nts) of intron/exon border; splicing, variants known to affect splicing or variants within +/−15 nts of intron/exon border; Indel, insertion or deletion variant; CNVs, copy number variation; UTR, untranslated region; P/LP, Pathogenic/Likely pathogenic; B/LB, Benign/Likely benign; VUS, variant of Uncertain Significance.

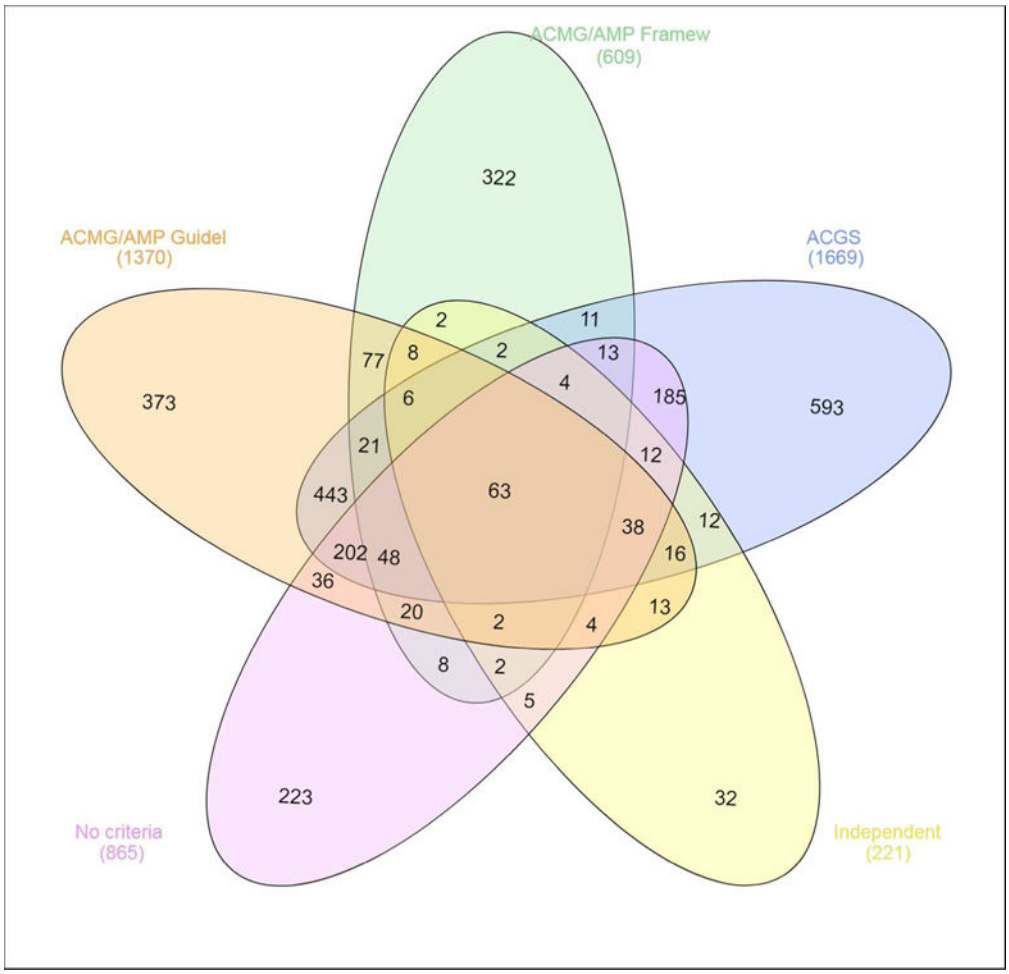


Figure 3.

Number of unique variants (n=2796) classified by different sets of criteria. For 87 unique variants, no classification was submitted. ACMG/AMP Guidel, American College of Medical Genetics and Genomics/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.

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 FH-associated variants to ClinVar.

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 Cardiology, University of São Paulo	Brazil	201	63	16	280
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 FH-associated variants submitted to ClinVar by gene and type of variant.

Variant Type	*LDLR*	*APOB*	*PCSK9*
3'UTR	77	9	40
5'UTR	54	4	18
Frameshift	430	12	1
In-frame indels	87	5	6
Intronic	48	3	26
Splicing	198	24	13
CNV (deletion)	100	-	1
CNV (duplication)	42	-	-
Missense	1011	218	82
Nonsense	179	4	1
Synonymous	83	74	28
Double variants	5	-	-
Total	2314	353	216

UTR, untranslated region; indel, insertion or deletion variant; in-frame indels = smaller than one 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 = one whole exon or more; double variants (single submission of two variants on same allele) include: 3 double missense, 1 inframe indel + frameshift, 1 inframe indel + missense

Table 4.

Clinical significance of *all* FH-associated variant submissions in ClinVar, independent of criteria used for classification.

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 (6.8%)*
Total	4973	580	355

Table 5.

Clinical significance of *unique* FH-associated variants in ClinVar, independent of criteria used for classification.

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 classifications (considered for variants with multiple submissions): Benign/Likely benign + Uncertain significance; or Pathogenic/Likely pathogenic + Uncertain significance; or Benign/Likely benign + Pathogenic/Likely pathogenic.

Table 6.

Criteria used for FH-associated variant classifications in ClinVar.

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 and Genomics/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.

Table 7.

Number of unique variants with each variant-level data type available in 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 extracted directly from ClinVar