European Journal of Medical Genetics 63 (2020) 103831

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Familial hypercholesterolemia: A complex genetic disease with variable phenotypes

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ARTICLE INFO

Keywords:
Familial hypercholesterolemia
Phenotypic variability
Genetic heterogeneity
Pathogenicity evaluation
Modifier factors
FH phenocopies
Lp(a)

ABSTRACT

Familial hypercholesterolemia (FH) is the most frequent genetic disease and is characterized by elevation of LDL-cholesterol that accumulates in tissues leading to premature atherosclerosis and sometime tendon xanthomas. Main causes of FH are pathogenic variants in the genes encoding the LDL receptor (*LDLR*), its ligand - the apolipoprotein B (*APOB*) - or Proprotein Convertase Subtilisin/Kexin Type 9 (*PCSK9*). Rarer causes include variants in genes encoding apolipoprotein E (*APOE*) and the signal-transducing adaptor family member 1 (*STAP1*).

Genetics of FH is extremely complicated by 1. high heterogeneity, 2. presence of variant clusters and 3. phenotypic variability. In fact, a great variability was observed among patients with the same genetic status: an overlap of LDL-cholesterol levels was observed between heterozygous patients (HeFH) and homozygous FH patients, as well as some HeFH showed a normal lipid profile. A correct pathogenicity evaluation is the first step to correctly define the genetic status helping to identify the variants which really cause the FH.

Several phenotypic differences were observed among HeFH patients carrying different variant types (null or defective) or variants in different affected genes. Patients with a null variant in *LDLR* gene showed higher LDL-cholesterol levels and higher risk for coronary artery disease than patients with a defective variant. Pathogenic variants in several lipid-related genes causing different dyslipidemias were found among FH patients acting as both modifying factors (worsening the phenotype) and confounding factors (needing a differential diagnosis to be discriminated from FH). This review aims at depicting the complex genetic basis of FH.

1. Introduction

Familial hypercholesterolemia (FH) is the most frequent genetic disease with an estimated prevalence of about 1:250 according to a recent meta-analysis (Akioyamen et al., 2017). The main features of FH include a severe elevation of LDL-cholesterol (LDL-c) that accumulates in tissues leading to premature atherosclerosis-based cardiovascular diseases and, less frequently, to tendon xanthomas or corneal arcus. The disease is inherited by an autosomal dominant transmission of pathogenic variants in genes encoding proteins related to the LDL receptor (LDLR) metabolism (Defesche et al., 2017) (Table 1). Most of FH-causative variants have been reported in the *LDLR* gene, in the *APOB* gene, encoding the only apolipoprotein included in LDL, and in the *PCSK9* gene encoding the Proprotein Convertase Subtilisin/Kexin Type 9, a protein inducing LDLR degradation. More recently, two additional genes have been recognized as causative of FH, the *APOE* and *STAP1*, encoding for the apolipoprotein E and the signal-transducing adaptor family member 1, respectively. Another type of FH is autosomal recessive hypercholesterolemia (ARH) caused by variants in the gene encoding the LDLR adaptor protein 1 (*LDLRAP1*) (Benito-Vicente et al., 2018a). The key features of FH genetics are the following:

1. Genetic heterogeneity. A very high number of FH causative variants in several genes has been reported: > 2,000 in *LDLR*; 32 in *APOB*, 23 in *PCSK9*; 1 in *APOE* and 4 in *STAP1* (Defesche et al., 2017).
2. Variant clusters. The prevalence of a single or a few FH-causative variants is particularly high in some geographic regions and in some cases a founder effect was also demonstrated (Bertolini et al., 2017).
3. Phenotypic variability. A wide range of LDL-c levels and clinical features has been reported among patients carrying an FH causative variant at heterozygous status. A similar variability was observed among HoFH patients (Cuchel et al., 2014).

The presence of variant clusters could be useful to reduce the

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https://doi.org/10.1016/j.ejmg.2019.103831
Received 29 August 2019; Received in revised form 1 November 2019; Accepted 21 December 2019
Available online 25 December 2019
1769-7212/

Table 1
Genetic causes of familial hypercholesterolemia and other diseases consisting in high cholesterol levels.

Gene	Protein	MIM	Pathogenicity mechanism/Notes
FH autosomal dominant			
LDLR	LDL receptor	606945	It encodes for the only one receptor that binds APOB. The most frequent cause of FH.
APOB	Apolipoprotein B	107730	It encodes for the only one apolipoprotein included in LDL. Causative variants produce a protein unable to bind LDLR.
PCSK9	Proprotein convertase subtilisin/kexin type 9	607786	It encodes for a protein that down-regulates LDLR levels. Only variants leading to Gain of Function are causative of FH
APOE	Apolipoprotein E	107741	It encodes for an apolipoprotein included in VLDL, IDL, chylomicron remnants and HDL. Only one variant was described as causative of FH, the p.Leu167del (ClinVar VCV000126456.1) that increase the affinity of APOE towards its receptors causing a down-regulation of LDLR.
STAP1	Signal-transducing adaptor family member 1	604298	It is the most recently identified cause of FH. Unknown molecular mechanism.
FH Autosomal recessive			
LDLRAP1	Low density lipoprotein receptor adaptor protein 1	605747	It encodes for the adaptor protein linking LDLR and clathrin during vesicles endocytosis. It is a very rare cause of FH, with high frequency in specific geographic regions.
FH phenocopies - autosomal recessive			
ABCG5 and *ABCG8*	ATP-binding cassette, subfamily g, member 5 and member 8	605459 and 605460	They encode for transporters responsible of biliary excretion of plant sterols. These genes are causative of sitosterolemia, a disease characterized by high cholesterol levels and xanthomas.
LIPA	Lipase A, lysosomal acid	613497	It encodes for a lysosomal lipase that hydrolyze cholesterol esters and triglycerides inside the cell. Pathogenic variants are causative of lysosomal acid lipase deficiency (LALD - also known as Cholesteryl ester storage disease and Wolman disease), characterized by high levels of cholesterol and transaminases and low HDL-cholesterol levels
CYP27A1	Sterol 27-hydroxylase	606530	It encodes for an enzyme responsible of bile acids production from cholesterol. Pathogenic variants are causative of cerebrotendinous xanthomatosis, a disease characterized by mildly elevated cholesterol levels and xanthomas.

genetic screening required to identify an FH causative variant, whereas the other genetic features may have a negative impact on genetic diagnosis. If genetic heterogeneity can be overcome by next generation sequencing (NGS) allowing to simultaneously screen several genes at increasingly lower costs, phenotypic variability can make it hard to identify patients, causing a delay in treatment (Nordestgaard et al., 2013).

Phenotypic variability can consist in 1. the lack of LDL-c increase and cardiovascular complications observed in some heterozygous FH (HeFH) patients (incomplete penetrance); and 2. the overlap of lipid profile between HeFH and Homozygous FH (HoFH). The phenotypic variability of LDL-c levels can be explained by both the great relevance of additional genetic variants (including the common ones) in genes related to lipid metabolism and the different impact of FH-causative variants (Santos et al., 2016). As to the variability of cardiovascular complications associated with FH, the role of different risk factors (which are in some cases influenced by additional genetic traits) as phenotype modifiers should also be taken into account.

The identification of the FH-causative variant has great relevance in view of the application of cascade screening searching for the causative variant in other family members and increasing the number of diagnosed patients, above all the youngest ones who mostly benefit from early treatment (Knowles et al., 2017).

Recently, the different phenotypes associated with the presence of a genetic variant causative of FH have aroused great interest. This review aims at reporting literature evidence of the impact of different FH-causative variants on lipid profile and FH clinical features. These data may allow for a better interpretation of the genetic screening results during clinical management of FH patients.

2. Clinical and genetic diagnosis

Two main criteria are used to identify FH patients, the Simon Broome (1991) and the Dutch Lipid Clinic Network (DLCN) criteria (Nordestgaard et al., 2013). Both LDL-c levels and clinical symptoms, such as xanthomas, corneal arcus and premature cardiovascular disease, in the patient and in his relatives are considered in the above-mentioned criteria. In particular, in the Simon Broome criteria the presence of increased total or LDL-c levels in the proband is the basic requirement for diagnosis (total cholesterol $\geq$ 7.5 mmol/l or LDL-c $\geq$ 4.9 mmoL/l in an adult – total cholesterol $\geq$ 6.7 mmol/l or LDL-c $\geq$ 4 mmoL/l in a child under 16 years). To make a definite diagnosis also tendon xanthomas should be present in the patient or a 1st or 2nd degree relative. The presence of an FH-causative variant is a stand-alone criterion to make a definite diagnosis of FH.

To make a possible diagnosis, one of the two following elements should also be present: 1. family history of myocardial infarction before 50 years in a 2nd degree relative or below 60 in a 1st degree relative; 2. family history of increased total cholesterol: total cholesterol $\geq$7.5 mmol/l or $\geq$6.7 mmol/l in a child under the age of 16.

According to the DLCN criteria, a score is assigned for each feature present in the patient or in the family as reported in Table 2 (Nordestgaard et al., 2013). Similarly to the Simon Broome criteria, the DLCN criteria take into account the lipid profile of the patient and his relatives together with personal and familiar clinical complications. Using the score system, the different features can contribute to the diagnosis according to the corresponding score. In particular, the presence of an FH-causative variant gives a high score, although this is not sufficient to make a diagnosis of definite FH.

Among these two criteria, only the Simon Broome criteria can be used in children due to the use of specific cholesterol thresholds. As to children, the European Atherosclerosis Society also proposed to decrease the LDL-c threshold to 130 mg/dL if a previous diagnosis of FH was made in a parent (Wiegman et al., 2015).

3. Pathogenicity assessment: a first step towards defining genetic status

The definition of a variant pathogenicity is crucial to correctly understand the genetic status of a patient, for instance to differentiate between patients without mutations and HeFH patients or between HeFH and HoFH. As clinical and genetic FH does not match perfectly, a correct definition of an FH-pathogenic variant is essential for determining the prevalence of genetic FH (Akioyamen et al., 2017; Nordestgaard et al., 2013).

Some old studies report rare variants in FH-causative genes as

Table 2
Dutch Lipid Clinic Network criteria for diagnosis of familial hypercholesterolemia.

Family history	
First-degree relative with known premature (< 55 years of age in men, < 60 years of age in women) coronary heart disease or first-degree relative with known low-density lipoprotein (LDL) cholesterol > 95th percentile by age and sex for country	1 point
First-degree relative with tendon xanthoma and/or arcus cornealis or children < 18 years of age with LDL cholesterol > 95th percentile by age and sex for country	2 points
Clinical history	
Patient has premature coronary heart disease (< 55 years in men; < 60 years in women)	2 points
Patient has premature cerebral or peripheral arterial disease (< 55 years in men; < 60 years in women)	1 point
Physical examination	
Presence of tendon xanthoma	6 points
Presence of arcus cornealis in a patient < 45 years of age	4 points
LDL cholesterol	
≥8.5 mmoL/L (≥326 mg/dL)	8 points
6.5–8.4 mmoL/L (251–325 mg/dL)	5 points
5.0–6.4 mmoL/L (191–250 mg/dL)	3 points
4.0–4.9 mmoL/L (155–190 mg/dL)	1 point
Genetic testing	
Presence of a causative variant	8 points

Interpretation	
Score > 8 points	Definite FH
Score 6–8 points	Probable FH
Score 3–5 points	Possible FH
Score 0–2 points	Unlikely FH

pathogenic only because these are found in FH patients and are absent in a few hundred controls. To date, genetic testing has become more affordable in terms of the laboratory procedures and costs, but data interpretation has become more complex due to the large amounts of generated data. Bioinformatics can help the data interpretation but remain only pathogenicity predictions (Camastra et al., 2015).

The great number of variants identified during genetic screenings by NGS and the different procedures used in each laboratory to establish their pathogenicity has made it mandatory to provide guidelines for a correct interpretation of genetic variants (Richards et al., 2015). However, some criteria should be adjusted to each specific feature of this genetic disease. For FH-putative variants, the pathogenicity criterium based on the absence in NGS databases, such as Genome Aggregation Database (gnomAD); Exome Variant Server (EVS) and 1000 genomes, cannot be applied rigorously. Since FH has a frequency of about 1:250, it is likely that this frequency was also present among the subjects included in these very large sequencing studies. In fact, many functionally characterized FH-causative variants are present in NGS databases, although with a very low frequency; as it is the case of the *LDLR* variant c.1646G > A, p.(Gly549Asp) that is present in gnomAD in 6 heterozygous subjects with an allele frequency of 2.39e-5 and is reported as pathogenic\likely pathogenic in ClinVar (access number VCV000003698.2) and other papers (Bertolini et al., 2013).

Much interest has recently been drawn to the pathogenicity assessment of the putative FH-causative variants. Chora et al. re-evaluated the publicly available *LDLR*, *APOB* and *PCSK9* variants which are supposed to be associated with FH (Chora et al., 2018) and reported an adaptation of the ACMG criteria to the specific FH genetic features. As to the functional tests - that according to the ACMG guidelines are considered a strong pathogenicity criterion provided that reliable assay is performed - the authors considered two different levels of evidence associated with different types of functional studies. Laboratory procedures used in functional assays are critical to demonstrate a molecular defect caused by FH variants (Benito-Vicente et al., 2018b; Di Taranto et al., 2015a; Etxebarria et al., 2014). For example, as to functional assays of *LDLR* variants, if incubation of cells bearing the variant with fluorescent LDL is performed for a short time, not all the defect classes can be correctly identified as pathogenic (Di Taranto et al., 2015a).

The integration of genetic screening and functional assays could help avoiding any misinterpretation of genetic data as, for instance, the case of a patient who had been identified as bearing a rare variant in *LDLR* and a rare variant in *PCSK9*, but only the last variant was shown to be functionally relevant (Di Taranto et al., 2017). The first assay of *LDLR* variants was performed on patient fibroblasts in the era of Goldstein and Brown (1974), an assay which requires tissue retrieval from patients. Also for *APOB* variants, the first functional assays were performed on fibroblasts incubated with LDL isolated from the patient bearing the *APOB* variant or controls (Pullinger et al., 1995). To date, these assay can be performed on hepatic cell lines, such as HepG2, to avoid the use of primary human fibroblasts (Motazacker et al., 2012). However, functional defects of ApoB variants toward LDLR binding are sometime found in conflict with clinical evidences of hypercholesterolemia (Benn et al., 2005; Rabes et al., 2000), probably due to incomplete penetrance. The integration of functional and clinical evidences should be the key point in variant evaluation.

The patient context in which each variant was found, i.e. patient phenotype, presence of other genetic variants, data about co-segregation of the variant with the phenotype, are additional elements improving pathogenicity evaluation, although frequently missing in variant databases. Patients registries can be a useful tool to integrate clinical and genetic data (Averna et al., 2017). These data were reported in the LOVD database for the *LDLR* gene (Leigh et al., 2017). The recent update of the ClinVar public database aims at better curation addressing all the submitted FH-associated variants (Iacocca et al., 2018a), thereby encouraging each center to submit variants together with the collected pathogenicity evidences. The analysis of the ClinVar database highlights that the number of FH-associated variants is higher than previously estimated and that many of these, mainly in the *APOB* and *PCSK9* genes, remain of uncertain significance or are reported with conflicting evidences (Iacocca et al., 2018a). Unfortunately, the strict application of the pathogenicity guidelines leads to the classification of many variants as being of “uncertain significance”, hampering a clear interpretation of genetic results.

4. Different variant types causing FH

The pathogenicity of each FH-causing gene is determined by different mechanisms: for *LDLR*, the mechanism is the total or partial loss of function (LOF) of the variant protein; for *APOB*, it is necessary that the protein loses the LDLR binding capacity but not its ability to assemble VLDL; for *PCSK9* the mechanism mainly consists in a gain of function (GOF) of the protein increasing LDLR degradation, but a duplication of the whole gene was also reported (Benito-Vicente et al., 2018a; Iacocca et al., 2018b).

For *LDLR*, variants were often divided into null variants and defective variants based on the complete or partial LOF of the encoded protein, respectively. Null variants are the total disruptive variants leading to a completely modified or absent protein and include nonsense variants, change of initiation codon, frameshift variants, splicing alterations and large deletions involving one or more exons (Richards et al., 2015). Of course, null variants cannot be considered a pathogenicity mechanism for *APOB* and *PCSK9* because their LOF usually leads to hypocholesterolemia (Tarugi and Averna, 2011). Defective variants are usually missense changes, which are the most frequent FH-causing variants in *LDLR*, and small in-frame insertions or deletions. However, all nucleotide changes, including synonymous, missense, small in-frame insertion/deletion and deep-intron variants should be considered for a possible splicing alteration, as already reported (Ho

et al., 2015; Reeskamp et al., 2018). If these silent or mild variants are actually causative of splicing alteration, they should be considered null variants.

Several papers reported the association of higher levels of LDL-c in heterozygous patients with a null variant compared to patients with a defective variant (Bertolini et al., 2013; Khera et al., 2016; Rubba et al., 2017). In agreement, functional characterization of heterozygous cells carrying null variants usually detected a residual LDLR activity of about 50%, whereas, higher residual activities were observed in defective variants (Romano et al., 2010, 2011). Anyway, patients with a pathogenic variant, independently of the variant type, showed a worse lipid profile than patients without identified pathogenic variants (Rubba et al., 2017; Wald et al., 2016).

A recent study on more than 14,000 subjects has reported that the increase of LDL-c associated with the presence of a LOF variant was about 120 mg/dL (Khera et al., 2016) and this was also associated with an Odds Ratio (OR) for coronary artery disease (CAD) of 9.5 respect to patients without variants. In case of pathogenic missense variants, the LDL-c increase was about 40 mg/dL and the OR for CAD was 3.5.

Why genetic screening should be performed if it might be sufficient to measure LDL-c and assess its related cardiovascular risk? The answer can be found in the above-mentioned study by Khera et al. Among the subjects stratified for LDL-c levels the authors observed higher OR for CAD in subjects with a FH-causative variant than in subjects without causative variants. In particular, in patients with LDL-c > 220 mg/dL the OR for variant-positive subjects was 25.8 respect to 7.7 for variant-negative subjects. On the other hand, in subjects with LDL-c lower than 130 mg/dL, the authors found an OR for CAD equal to 2.2 for variant-positive versus variant-negative subjects (Khera et al., 2016). All these data highlight the relevance of genetic testing for FH-causative variant also for prognostic evaluation both in patients with high and low LDL-c levels. The difference between patients with and without causative variants, as well as that between patients with defective and null variants have been identified in children that usually do not show other cardiovascular risk factors.

Besides LDL-c levels and CAD, null-variant carriers showed a worse phenotype than defective-variant carriers also for additional clinical features of FH, such as low HDL-cholesterol and prevalence of xanthomas in adults and children (Bertolini et al., 2013; Chaves et al., 2001; Di Taranto et al., 2019; Rubba et al., 2017). Furthermore, the presence of carotid plaque in adults (Bertolini et al., 2013; Rubba et al., 2017) or of increased carotid intima-media thickness in children was also observed in patients with a null variant compared with patients with a defective variant (Guardamagna et al., 2009).

However, some other studies did not reveal any difference between patients with different variant types, probably due to the low number of studied patients (Minicocci et al., 2017; Vilades Medel et al., 2013). Some missense variants were even associated with very high LDL-c levels, higher than several null variants as reported in 1600 FH patients belonging to several variant clusters (Bertolini et al., 2017).

Genetic status was also examined in relation to different responses to lipid-lowering treatment: in some cases the presence of a null-variant was associated with a poor response to statin therapy (Chaves et al., 2001; Rubba et al., 2017; Santos et al., 2014; Vilades Medel et al., 2013), whereas in others instead no difference was found (Choumerianou and Dedoussis, 2005; Mata et al., 2011).

5. Different genes, different phenotypes

Several observations suggested differences of lipid profile and cardiovascular features between patients carrying pathogenic variants in the different FH-causative genes. Due to the low frequency of *APOB* and *PCSK9* variants, only few studies reach a significant sample to perform reliable comparisons. In an Italian population, patients with *APOB* pathogenic variants showed lower LDL-c levels and delayed clinical onset of coronary heart disease than patients with *LDLR* pathogenic variants (Bertolini et al., 2013). In a large genetic screening in U.S., the patients with *LDLR* pathogenic variants showed higher levels of LDL-c than patients with both *APOB* or *PCSK9* pathogenic variants, whereas no differences were observed between patients with *APOB* and *PCSK9* variants (Abul-Husn et al., 2016). Also OR for premature CAD was higher in patients with *LDLR* variants (in particular for null variants) than in patients with *APOB* or *PCSK9* variants (Abul-Husn et al., 2016). However, we should consider that no null variants in *APOB* and *PCSK9* genes were identified as a cause of FH. A different result was observed by Hopkins: carriers of a pathogenic variant in *PCSK9* showed a worse phenotype than both patients with pathogenic variants in *APOB* and patients with defective variants in *LDLR*, whereas no differences were found with patients carrying a null *LDLR* variant (Hopkins et al., 2015).

The only FH-pathogenic variant in the *APOE* gene is the in-frame deletion p.Leu167del - ClinVar VCV000126456.1 - (Awan et al., 2013; Marduel et al., 2013), previously reported as causative of sea-blue histiocytosis and familial combined hyperlipidemia (FCH). A Spanish screening of 288 FH patients without pathogenic variants in the three major genes revealed a 3.1% prevalence of the p.Leu167del variant (Cenarro et al., 2016), whereas in the Italian screening of more than1,000 FH patients only one carrier was found (Pirillo et al., 2017). To date no comparisons between patients with this variant and patients with pathogenic variants in the three other genes have been performed.

The *STAP1* gene is the most recent and the rarest genetic cause of FH. Carriers of pathogenic variants in *STAP1* show a lipid profile less severe than *LDLR* carriers and similar to *APOB* carriers (Fouchier et al., 2014).

To date, the search for variants related to FH in ClinVar reported the following results: 2580 variants in *LDLR*, 896 variants in *APOB*, 351 variants in *PCSK9*, 16 variants in APOE and only 1 variant in *STAP1* (database accessed 10-25-2019). However, the number of variants classified as pathogenic or likely pathogenic is lower. Notably, the only one variant in *APOE* demonstrated as causative of FH (p.Leu167del - VCV000126456.1) is missing among the FH related variants, because is reported as causative of sea-blue histiocytosis (last update 2014). Much more attention should be paid by expert researchers to the addition of newly identified variants together with patient data to the ClinVar database in order to obtain a complete and accurate database useful for the interpretation of genetic data. These data would be useful also to improve the pathogenicity evaluation of variants, that according to ACMG guidelines, would benefit from multiple evidences of variant association with the phenotype and from detailed clinical and biochemical information about the patient and his family.

The idea that severe hypercholesterolemia could be due to the contemporary presence of multiple common variants in different genes was the basis for the polygenic hypothesis: hypercholesterolemia in FH patients without pathogenic variants can be due to a high genetic risk score (GRS), i.e. a score based on the presence of selected polymorphisms (Futema et al., 2015; Talmud et al., 2013). Several GRS have been considered based on different polymorphisms (Futema et al., 2015; Natarajan et al., 2018; Talmud et al., 2013; Wang et al., 2016). GRS was slightly higher in patients without pathogenic variants than in those with pathogenic variants (Futema et al., 2015) and showed a high prevalence in patients with slightly increased LDL-c levels than in those with very high LDL-c levels (Wang et al., 2016). The polygenic basis attracted a lot of interest because it could explain a considerable percentage of cases with severe hypercholesterolemia, although its effect size is considerable lower than the presence of a pathogenic variant (Natarajan et al., 2018). In addition, although some differences can be observed in large populations, the GRS calculation cannot not be considered a diagnostic tool. In fact, high GRS does not segregate with high LDL-c within affected families (Sjouke et al., 2016b).

6. Modifying and confounding factors

Bedsides the gene and the variant type, FH variable phenotype can

depend on several modifier factors that also include variants in several lipid-related genes, as well as variants causing different genetic dyslipidemias. Other patient characteristics could also lead to incomplete penetrance, i.e. absence of the typical FH phenotype despite the presence of a causative variant. Incomplete penetrance was observed several times in FH patients (Garcia-Garcia et al., 2011; Nordestgaard et al., 2013; Ruotolo et al., 2014) and should be considered during cascade screening.

Tada et al. recently defined as "oligogenic FH" the contemporary presence of an FH-causing variant and a deleterious variant in the *ABCG5/8*, *LDLRAP1* and *APOE* genes, responsible for sitosterolemia, ARH and different dyslipidemias, respectively (Tada et al., 2018). Patients with oligogenic FH showed higher LDL-c than patients with FH caused by a single pathogenic variant in traditional genes. They found a considerable number of patients with both oligogenic FH and with variants only in these accessory genes, suggesting that these genes should be included in NGS panels for FH screening. However, these patients only carried these variants at heterozygous status and cannot be considered affected by sitosterolemia or ARH.

The presence of several variants in genes traditionally associated with triglyceride levels could alter the phenotype of FH patients leading to a mixed hyperlipidemia. Historically, the presence of high triglyceride levels excluded the diagnosis of FH and, if a familiar inheritance of high triglyceride and/or cholesterol was evident, familial combined hyperlipidemia (FCH) was diagnosed. Actually, some patients with a clinical diagnosis of FCH carried pathogenic variants in the *LDLR* gene (Civeira et al., 2008; Di Taranto et al., 2015b; Staiano et al., 2013). This subgroup of FCH patients was recently described as a separate class of FH patients with hypertriglyceridemia (Masana et al., 2019).

Sitosterolemia and lysosomal acid lipase deficiency (LALD – caused by variants in *LIPA* gene) are autosomal recessive diseases considered phenocopies of FH, i.e. affected patients show a phenotype similar to FH and some cases need a differential diagnosis by genetic screening (Sturm et al., 2018). In different large screenings, it was demonstrated that several suspected FH patients without pathogenic variants in causative genes carried pathogenic variants in the *LIPA* gene, although sometimes at heterozygous status (Chora et al., 2017; Sjouke et al., 2016a; Vinje et al., 2018). In the Portuguese study, LALD was diagnosed in 3 of the 492 studied patients (Chora et al., 2017).

LALD is usually suggested by high LDL-c levels together with low plasma levels of HDL-cholesterol. This condition was also found in a FH patient with a genetic diagnosis of both FH and lecithin:cholesterol acyltransferase deficiency, due to variants in the LCAT gene (Pisciotta et al., 2005).

Cerebrotendinous xanthomatosis CTX is an autosomal recessive dyslipidemia caused by alteration of the *CYP27A1* gene that leads to xanthomas although LDL-c levels are usually moderately increased (Di Taranto et al., 2016; Schaefer et al., 2000). Although differential diagnosis can be easily achieved by biochemical evaluations including the profile of plasmatic sterols, the presence of CTX pathogenic variants at heterozygous status was revealed among FH patients (Corral et al., 2018). In addition, the presence of pathogenic variants causing both FH and CTX was observed in 2 patients suffering from extreme xanthomatosis (Huijgen et al., 2012). However, these rare lipid diseases would benefit from the inclusion in newborn screening to be diagnosed very early (DeBarber et al., 2018; Lukacs et al., 2017) and from prenatal diagnosis (Bona et al., 1989; Maruotti et al., 2013).

In the next future, a wide application of NGS using extended gene panels including all these lipid-related genes will reveal the real frequencies of FH patients carrying variants in other genes and will explain the different phenotypes improving the clinical identification of patients.

Lipoprotein(a) – Lp(a) – is a modified LDL with additional atherogenic properties due to its capacity to bind oxidized phospholipids and to competitively inhibit plasminogen activity (Boffa and Koschinsky, 2019). Since the amounts of cholesterol contained in Lp(a) contribute to the amounts of LDL-c measured or calculated by the Friedewald formula (Langlois et al., 2018), high Lp(a) levels can be the cause of high LDL-c levels, the key feature considered for FH diagnosis. The presence of very high levels of Lp(a) can be the only responsible for FH-clinical picture (high LDL-c and premature cardiovascular disease) in absence of FH-causative variants and can constitute a factor modifying genotype-phenotype correlations (Langsted et al., 2016). Higher Lp(a) levels were observed in patients with a probable/definite diagnosis of FH than in patients with unlikely FH, but after redefining FH diagnosis based on LDL-c levels corrected for Lp(a) levels, fewer patients were included in the probable/definite diagnosis group and no differences of Lp(a) levels were observed (Langsted et al., 2016). These results highlight the importance of Lp(a) levels as a confounding factor in FH diagnosis. A formula to correct for Lp(a) contained cholesterol was proposed (Kinpara et al., 2011).

High Lp(a) levels are also an independent cardiovascular risk factor, so that the measurement of Lp(a) levels is strictly recommended in FH patients (Catapano et al., 2016) to detect an additional risk source. Alonso et al. reported that the cardiovascular disease-free time in FH patients carrying a null variant was lower if Lp(a) levels were > 50 mg/dL respect to levels < 50 mg/dL; the same difference was present in FH patients carrying a defective variant (Alonso et al., 2014).

7. Conclusions

Genotype-phenotype correlations in FH are particularly difficult due to the different genetic causes and to the presence of variants in several lipid-related genes that modify the lipid profile and the consequent cardiovascular complications. Genetic screening will benefit from the utilization of NGS panels that will allow to identify the contemporary presence of variants causing different diseases. At the same time, an accurate pathogenicity evaluation of identified variants should become mandatory and should not overlook the patient characteristics.

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