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Significance of Lipoprotein(a) in Familial Hypercholesterolemia Patients Comprehensive Literature Review

(title)

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LIST OF ABBREVIATIONS AND ACRONYMS

ACC	American College of Cardiology
AHA	American Heart Association
ANGPTL3	Angiopoietin-like protein 3
Apo(a)	Apolipoprotein(a)
ApoB100	Apolipoprotein B100
ARH	Autosomal recessive hypercholesterolemia
ASCVD	Atherosclerotic cardiovascular diseases
ASO	Antisense oligonucleotides
AVC	Aortic valve calcification
AVS	Aortic valve stenosis
BCFH	British Columbia Familial Hypercholesterolemia Cohort
BQ	Beta quantification
CAD	Coronary artery diseases
CETP	Cholesteryl ester transfer protein
CVD	Cardiovascular disease
DLCNS	Dutch Lipid Clinics Network Score
EAS	European Atherosclerosis Society
EMA	European Medical Agency
ESC	European Society of Cardiology
FAMCAT	Familial Hypercholesterolemia Case Ascertainment Tool
FH	Familial hypercholesterolemia
HDL	High- density lipoproteins
HDL-C	High-density Cholesterol
HeFH	Heterozygous familial hypercholesterolemia
HMG-CoA reductase	3-Hydroxy 3-methylglutaryl-Coenzyme A reductase
HoFH	Homozygous familial hypercholesterolemia
IDL	Intermediate density lipoproteins
K-IV	Kringle IV domain
K-IV2	Kringle IV type 2 domain
K-V	Kringle V domain
LA	Lipoprotein apheresis
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LDLRAP1	Low-density lipoprotein receptor adaptor protein 1
Lp(a)	Lipoprotein(a)
Lp(a)-C	Lipoprotein(a) cholesterol
MACE	Major adverse cardiovascular events
MEDPED	Make Early Diagnosis to Prevent Early Death
mRNA	Messenger Ribonucleic acids
NPC1L1	Niemann-Pick C1-Like 1 cholesterol transporter
OxPls	Oxidized phospholipids
PAD	Peripheral artery diseases
PCSK9	Proprotein convertase subtilisin/kexin type 9
RISC	RNA-induced silencing complex
SB	Simon-Broome criteria algorithm
siRNA	Small interfering RNA
SNR	Single nucleotide repeats
TC	Total cholesterol

1. SUMMARY

Lipoprotein(a) and Familial Hypercholesterolemia represent a small group of dyslipidaemias commonly found in patients. Both seem to influence premature atherosclerotic cardiovascular diseases in different ways. Within recent years more and more interest was gained in the exact role of Lipoprotein(a) and also its contribution to the overall cardiovascular risk. In order completely understand the connections it became crucial to understand how Familial Hypercholesterolemia is diagnosed and also treated and how Lipoprotein(a) might influence this process. Also the lack of clear laboratory cut-off values and specific therapies further complicate the general awareness and worsen the outcomes of patients presenting with high Lipoprotein(a) levels. To bring more clearance to this new and evolving field and also evaluate local patient data, the following comprehensive literature review and analysis of a case series was conducted.

2. KEYWORDS

Familial hypercholesterolemia, lipoprotein(a), LDL cholesterol, lipoprotein(a) cholesterol, atherosclerotic cardiovascular disease, aortic valve stenosis, LDL receptor, diagnosis, cascade screening, lipid phenotyping, Friedewald formula, therapeutic strategy, Dutch Lipid Network Criteria, pharmacotherapy

3. INTRODUCTION

3.1 LIPOPROTEIN(A)

Lipoprotein(a) [Lp(a)] is an atherogenic low-density lipoprotein particle, similar to Low density Lipoprotein (LDL), that contains apolipoprotein B100 (ApoB100) of an LDL particle covalently bound to an additional apolipoprotein(a) [apo(a)] by disulphide bonds.(1–4) Apo(a) is a highly repetitive structure, genetically determined by *LPA gene*, and consisting of two kringle domains, IV (K-IV) and V (K-V) (5,6). K-IV has 10 subtypes with the kringle IV type 2 (K-IV2) domain being highly variable and it expresses up to over 40 copies of K-IV2 alleles per gene. Kringle is a loop of a protein, responsible for the interactions among proteins, enzymes, membranes and other structures (7,8). [For comparison of different Lp(a) molecules see Annex 2] This variability in apo(a) contributes up to 70% of Lp(a) variations within the human genome. Furthermore there is an inverse correlation between the number of K-IV2 domains and the plasma levels of Lp(a) where low numbers (< 23) (9) of K-IV2 expression represents small apo(a) molecules and thus high numbers of Lp(a) in serum. It can be explained

by the fact that larger isoforms of apo(a) can be degraded intracellularly in hepatocytes, whereas smaller isoforms remain within the circulation ultimately leading to an increase in Lp(a) values. In total there are more than 500 genetic variants of *LPA gene* associated with different effects on Lp(a) concentrations and more than 90% of the Lp(a) concentration is genetically determined by a variability in the *LPA* locus.(9,10) But Lp(a) levels were also linked to *APOE, CETP* and *APOH* loci mutations.(9,11,12) According to the recent European Atherosclerosis Society (EAS) consensus statement "Lp(a) is the most prevalent monogenetic lipid disorder globally, with prevalence of Lp(a) > 50 mg/dL estimated at >1.4 billion people" and patients with elevated levels of Lp(a) remain significantly underdiagnosed. (9,13) Up to 20-30% of the general population having levels higher than the current recommended threshold for CVD (>30mg/dL or > 75nmol/L).

Increased levels of Lp(a) were observed especially in Chinese, White, South Asian and even more significantly in Black individuals.(9,14,15) Like LDL cholesterol (LDL-C), Lp(a) is now recognized as an independent causal risk factor for the development of Atherosclerotic cardiovascular diseases (ASCVD) (16) and as recent studies demonstrated also as a novel risk factor for aortic valve calcification (AVC), especially in high concentrations. Here it was concluded that in individuals between 45-54 years of age a marked increase in micro- and macrocalcifications of the aortic valve could be clinically noticed. (9,17) Even in the presence of low LDL-C values, an increase in ASCVD was observed in individuals with high Lp(a) concentrations. Also the prevalence of an increased probability for stroke or peripheral artery diseases (PAD) became obvious. Surprisingly multiple studies were able to show that very low levels of Lp(a) are in fact an independent risk factor for Type 2 diabetes mellitus development.(9,18) Moreover Lp(a) has shown to have a proinflammatory and also pro-atherosclerotic properties that could be related to oxidized phospholipids (OxPls) carried by Lp(a) inducing inflammation.(3,19) Currently there is a lack of global consensus in defining a threshold value for elevated Lp(a) in clinical practice. (5) Here the recent 2022 consensus on Lp(a) from the European Atherosclerosis Society defined a threshold of Lp(a) >50mg/dL or >125nmol/L to rule in an increased cardiovascular risk, which should be referred as benchmark in Europe. Also Lp(a) <30 mg/dL or 75nmol/L could be used to rule out cardiovascular risk. In the "grey zone" between 30-50mg/dL or 75-125nmol/L the general cardiovascular risk should be taken into account.(9,20) Contrary UK guidelines recommend a threshold of > 90nmol/l. (3) [For an additional scheme of Lp(a), see Annex 1].

3.2 FAMILIAL HYPERCHOLESTEROLEMIA

Familial hypercholesterolemia (FH) is among the most common genetic disorders in humans, where a very atherogenic metabolism is the key clinical aspect. The dominant clinical finding in FH is a lifelong elevation in the levels of circulating Low-density Lipoprotein cholesterol (LDL-C) which inevitably leads to premature development of atherosclerotic cardiovascular diseases (ASCVD). The overall prevalence of FH was estimated to be 1 in 311 individuals, rather similarly distributed among children (1:364) and adults (1:303).(21) Genetically it can be distinguished between two different types of FH: heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH). Here by far the most common variant is HeFH with an estimated prevalence of up to 1 in 250 individuals in the general population. It is inherited in an autosomal dominant or codominant pattern.(1,22,23) On the other hand HoFH appears way less often in the general population and its prevalence is estimated to be one 1 in 160,000-300,000. Rarely HoFH can be inherited in a recessive way. (1,24)

In general the FH prevalence was found to be 10-fold higher in patients with ischemic heart diseases and up to 23-fold higher among patients with hypercholesterolemia. The true global prevalence however remains unknown due to missing screening programs in 90% of countries. (22)

If highly elevated levels of LDL-C are left untreated they lead to premature ASCVD development, as early as during the childhood or adolescence in HoFH or in the third to forth decade of life in HeFH. Currently four main genetic defect were identified to be the underlying factor in FH, of which loss of function mutations in the LDL-receptor (*LDLR*) gene were found to be causative in about 90-95%. Here up to now more than 1,700 *LDLR* mutations were identified, which are generally classified into five categories: absence of biosynthesis (class 1); interfering with maturation/transportation of the LDL receptor (LDLR) to the Golgi apparatus (class 2); reducing binding affinity of the LDLR to LDL (class 3), altering internalization of the receptor-ligand complex (class 4) and preventing normal LDLR recycling (class 5). (1,23,25,26) *LDLR* mutations can be additionally classified into “null” mutations, where less that 2% of normal LDLR activity is observed. These null mutations are associated with severe forms of FH, where homozygous LDLR null-null mutations marked the most severe clinical courses.(24) Another form of LDLR mutations are “defective” mutations, where up to 25% of normal LDLR functions can be observed. With about 5% of all FH cases, mutations in Apolipoprotein B100 (apoB100) constitute the second most common causative genetic mutations. ApoB100 is an important component of LDL and serves as a ligand in binding to

LDLR. There are several loss of function *APOB* mutations, but only a few were associated with FH; this condition might also be called "familial defective apolipoprotein B".(1,24) The third most common causative mutations were found in proprotein convertase subtilisin kexin type 9 (PCSK9) and appeared to be a gain of function mutation lowering the abundance of LDLR on the cellular wall in many different ways and ultimately leading to increased LDL-C concentrations. They accounted for roughly 1% of FH cases.(1,27,28) The least common type of genetic mutations, which were only observed in HoFH phenotype patients, was caused by variations in LDLR adaptor protein 1 (*LDLRAP1*), but in this special circumstances it can be also called autosomal recessive hypercholesterolemia (ARH), inherited in a recessive way. (1) However it needs to be mentioned, that even the presence of causative mutations in LDLR, APOB or PCSK9 are not always linked with the clinical occurrence of FH. (29,30)

Clinically FH is mostly assessed using different scoring systems, incorporating phenotypical and laboratory characteristics and sometimes additional genetic testing results. Among the most commonly used scoring systems are the Dutch Lipid Clinics Network Score (DLCNS)(31), the UK Simon-Broome criteria algorithm (SB) and the US Make Early Diagnosis to Prevent Early Death (MEDPED) as well as the newer Familial Hypercholesterolemia Case Ascertainment Tool (FAMCAT). Here the DLCNS remains the mostly used algorithm in Europe and the only one suitable for children according to European Society of Cardiology guidelines (ESC). (1,22)

Table 1. Dutch Lipid Clinic Network criteria (DLCN) for FH

Criteria	Points
Family history	
First-degree relative with premature coronary heart disease, OR First-degree relative with LDL-C >95th percentile by age and gender for country	**1**
First-degree relative with xanthoma and/or arcus cornealis, OR Children <18 years with LDL-C >95th percentile by age and gender for country	**2**
Clinical history	
Patient with premature* coronary heart disease	**2**
Patient with premature* cerebral or peripheral vascular disease	**1**
Physical examination	
Tendinous xanthomata	**6**
Arcus cornealis prior to age 45 years	**4**
DNA analysis	
Functional mutation in *LDLR, APOB* or *PCSK9 gene*	**8**
Diagnosis (diagnosis is based on the total number of points obtained)	
Definite Familial Hypercholesterolemia	**>8**
Probable Familial Hypercholesterolemia	**6-8**
Possible Familial Hypercholesterolemia	**3-5**
Unlikely Familial Hypercholesterolemia	**<3**

* Premature= <55 years in in men, <60 years in women

Table 2. Modified Simon Broome FH diagnostic criteria (UK) (32,33)

Point	Criteria
A	DNA mutation
B	Tendon xanthomas on patient or 1st or 2nd-degree relative
C	Family history of myocardial infarction <50 years in 2nd-degree OR <60 years in 1st-degree relative
D	Family history of total cholesterol >7.5 mol/L in 1st/2nd-degree relative
E	Total cholesterol > 7.5 mmol/L (adult) or >6.7 mol/L (age <16 years)
F	LDL-cholesterol >4.9 mol/L (adult) or >4.0 mol/L (age <16 years)
	Definite FH: Hypercholesterolemia as defined in points E/F plus A. Probable FH: Hypercholesterolemia as defined in points E/F plus B Possible FH: Hypercholesterolemia as defined in points E/F plus either C or D

In general the prevalence of ASCVD among confirmed FH patients was observed to be three times higher, compared to the general population in the SAFEHEART study.(34) Other studies like the Copenhagen General Population Study were also able to demonstrate a much higher prevalence of coronary artery diseases (CAD) among FH affected individuals.(35) Furthermore in 2016 Khera et al. stated, that among FH patients with causative mutations a 4-fold increased risk for the development of CAD was seen.(36)

3.3 PECULARITIES OF LP(A) IN FAMILIAL HYERCHOLESTEROLEMIA

Within the recent years, scientific interest was shifted slightly away from focussing solely on familial hypercholesterolemia and more interest was gained in the role of Lipoprotein(a) in the pathogenesis, diagnostics, screening and also treatment of patients with a high ASCVD risk.

3.3.1 DIAGNOSTIC PECULARITIES

Particularly with regard to a correct diagnosis, among scientists, there is large debate in the recent years, if all patients clinically diagnosed with FH were diagnosed correctly? The key assumption behind this question is that Lp(a) contributes significantly to the interpretation of LDL-C values received from laboratories.(1,37,38)

In order to understand this discussion it is crucial to know that the diagnosis of FH mostly relies on the concentration of LDL-C in serum, which can be determined in several different ways. In nearly all available methods for measuring LDL-C, Lp(a) cholesterol [Lp(a)-C] contributes

to it. Furthermore estimations of LDL-C remain the mostly used methods, over direct LDL-C measurement with automated chemistry analyzers and also over the current gold standard: beta quantification (BQ) after ultracentrifugation. In all these methods LDL-C as well as cholesterol from Intermediate density lipoproteins (IDL) and cholesterol within Lp(a) are measured.(38,39) Historically the most common determination of LDL-C by far is the Friedewald equation [LDL-C= TC – HDL-C – (TG/5)]. The limitations of this formula mainly lie in the fact that its only applicable if triglycerides are less than 4.52mmol/l. At a value higher direct measurement is used. Another commonly used equation to estimate LDL-C is the Martin-Hopkins formula [LDL-C=TC – HDL-C – TG/novel factor], where the novel factor is variable and based on patient characteristics. It is derived from the Friedewald formula, but more accurate if LDL-C is lower than 1,8 mmol/L or TG higher 4,5 mmol/L. (40)

In most clinical settings a direct Lp(a)-cholesterol measurement is not routinely available and the correct determination of Lp(a)-C remains challenging. Lp(a) itself is mostly determined directly using automated latex enhanced immunoassay, detecting the apo(a) moiety, as for example Quantia Lp(a) assay (Abbot laboratories). Here monoclonal antibodies are used in a turbidimetric immunoassay for an estimation of Lp(a) in either serum or plasma. This test uses the agglutination reaction in an Architect autoanalyzer C16000 (Abbot Diagnostics). Crucial in this technique is that it is not influenced by the isoform size of Lp(a). As mentioned before, due to the large variability in K-IV2 Lp(a) size can vary greatly. In order to develop comparable results there are special calibrations “with World Health organization-approved, IFCC reference standard apo(a) with [apo(a)] with 21 kringle 4 repeats for standardization of Lp(a) (IFCC/SRM 2B)”(37,41) The results can be given either in mass units (mg/dL) or, as preferred by 2022 Lp(a) consensus, in molar units (nmol/L). Results in mass units were found to be less accurate because of the many apo(a) isoform sizes in Lp(a). Moreover a great heterogenicity among Lp(a) size also leads to heterogenicity among its cholesterol content. Here it is mostly estimated that depending on the molecular size, cholesterol content ranges from 30-45% of total Lp(a).(9,38,39) This technique seems even less precise, taking into account that the cholesterol content in Lp(a) can reach up to 74%, according to newer data. (42) An exact measurement of Lp(a)-C is now only possible in certain laboratories, not available to the vast majority in everyday practices. Here after the separation of Lp(a), densitrometric measurements of the cholesterol staining in gel electrophoresis can directly determine Lp(a)-C independently of variability in Lp(a) size.(38,43) Much more often used is another approach, where after exact determination of Lp(a) using immunoassays the assumption, that approximately 30-45% of

LCL-C have their origin in Lp(a) is used. So the amount of Lp(a)-C is gathered by subtracting 30% of an individual's Lp(a) total mass from LDL-C values. Here a calculation would be: $LDL\text{-}C_{corrected} = LDL\text{-}C - [Lp(a) \times 0{,}30]$ or $LDL\text{-}C_{corrected} = LDL\text{-}C - [Lp(a) \times 0{,}45]$. These calculations are based on a study from Kinpara et al. in 2011.(44) To sum it up a clear and direct measurement of Lp(a)-C is not uniformly possible, further complicating the diagnostics.

In several recent studies the thesis of inaccurate LDL-C measurement was independently proven. Already in 2016 a prospective cohort study by Langsted et al., based on the Copenhagen General Population Study, concluded that one quarter of all clinical diagnosed FH patients might be due to high Lp(a) levels. Enrolled in this were 46,200 individuals in which LDL-C was estimated using the Friedewald formula, except when total triglycerides were higher than 4mmol/L. Here a direct LDL-C measurement was performed. In order to measure Lp(a) levels turbidimetric assays were used and the amount of Lp(a)-C was estimated to be between 30-45%, as described before.(44) Clinical diagnosis of FH were done using DLCN, SB or MEDPED criteria, which were adapted to exclude genetic testing results. It could be concluded that for DLCN 23% fewer participants were classified as "possible FH" after adjusting LDL-C values for Lp(a). A comparable trend was seen when using SB criteria with 24% decrease. So the conclusion was drawn, that those differences in diagnosis were attributed to the presence of Lp(a)-C.(29) Another more recent study by Fatica et al., published in 2020, marked one of the biggest and longest observations. A total of 31,215 samples were analysed over a period of 15 years, evaluating the contribution of Lp(a)-C on general LDL-C and the effects on phenotypical FH classification using different scoring systems. In this study LDL-C was either calculated using one of the 3 equations, or directly measured by beta quantification. In contrast to other studies here Lp(a)-C was densitometrically determined and then subtracted from LDL-C values. This has the advantage, that no estimations were needed, but rather a direct measurement and subtractions were possible; leading ultimately to a much higher accuracy. Here an average contribution of Lp(a)-C in LDL-C of 26% (range: 13-50%) could be observed. Also it was seen that with rising LDL-C the Lp(a)-C contribution seemed to decrease, but with increasing LDL-C concentrations in the same time an increase in numbers of samples with measurable Lp(a)-C was recognized. Here among the highest values of LDL-C, 38% of samples showed detectable Lp(a)-C values, where it were only 7% in the lowest LDL-C values. After subtracting the contribution of Lp(a)-C from LDL-C, the authors then found that in fact it had clinical consequences in the classification of FH. A total of 940 subjects (3% of all participants and 11% of participants with measurable Lp(a)-C) had to be reclassified using

DLCNs criteria into lower FH categories. 241 subjects even were not among FH patients anymore, due to adjusted LDL-C values below the threshold values. When using SB criteria an estimated reclassification rate even was as high as 40%. The study then concluded that "when considering only the subjects with measurable Lp(a)-C, the average down-classification rates of all four methods of LDL-C determination was 47.0% for LDL-C between 190 and 249 mg/dL, 49% for LDL-C between 250 and 329 mg/dL, and 32% for LDL-C > 330 mg/dL."(38)

Another, yet smaller cross-sectional study conducted in 2019 by Chan et al. with a cohort of 907 adult patients, came to comparable results: Here LDL-C was estimated using the Friedewald equation, but with values above 4.5mmol/L LDL-C was directly measured (as compliant with current guidelines)(16). Lp(a) was yielded by an automated immunoassay calibrated according to WHO standards. LDL-C was adjusted by subtracting 30-45% (44) of an individual's Lp(a) total mass from plasma LDL-C. The phenotypical classification of FH was done using either DLCNS or SB criteria. Of those 907 patients 330 had elevated Lp(a) concentrations of >0.5g/L. Among the reclassified individuals, a majority was not carrier of a pathogenic mutation for FH. 74 patients being classified as FH by DLCNS and 207 by SB criteria were reclassified as "unlikely FH" after adjusting LDL-C values for Lp(a)-C. Interestingly for individuals with very high LDL-C values above 250mg/dL (>6.5 mmol/L) no significant reclassification rates were observed. In the end it was then concluded, that especially among individuals with LDL-C values between 191-250 mg/dL and high Lp(a) values of >1.0g/L adjusting of LDL-C significantly reduced the misdiagnosis of FH using common criteria.

Furthermore it was tested, if *LPA* gene variants had an influence on FH phenotypical diagnosis by LDL-C values. Here Chan et al. supposed, that it is currently not recommendable to include *LPA* gene variants in the diagnosis of FH. Moreover the authors concluded, that current Lp(a)-C measurements by calculation were imprecise and thus there is a need for new more robust direct tests for Lp(a)-C in clinical practice. Chan et al. supported routine Lp(a) measurements among FH patients, not only for ASCVD risk estimation, but also for eventual diagnostic adjustments.(37,42)

Finally the 2022 consensus on Lp(a) by EAS came to the general conclusion that "this panel does not recommend routine correction of LDL-C for Lp(a)-C"(9) with one exception being patients clinically suspected of having FH. Here elevated Lp(a) levels might affect the diagnosis, as pointed out before, and thus regular correction of LDL-C for Lp(a) might be feasible to avoid unnecessary genetic testing or to rule out FH. (9,29,37)

3.3.2 SCREENING

Much debate was seen in recent years about suitable screening algorithms in order to early detect individuals at the highest cardiovascular risk. Traditionally screening was only performed with regard to the efficient diagnosis of FH by LDL-C values in clinical practice. However as shown before, newer evidence suggests, neither the current tests nor the used algorithms seem to be sufficient to properly diagnose FH based on clinical observations. (29,37,38) However in a newer study by Ellis et al. the effectiveness of Lp(a) measurement during cascade screening after an index case of FH was discussed. Here it was seen that about 30% of the relatives with genetically confirmed FH show increased Lp(a).

Different screening programs, searching for elevated Lp(a), were investigated among the SAFEHEART study in Spain. During the investigations, screening was initiated after an index case was genetically defined as FH. Now two separate screening approaches, systematic (cascade screening) and opportunistic screening, were compared. It could be concluded, that using systematic screening 1 new FH case was found for every 1.6 relative screened and 1 case with increased Lp(a) levels could be identified for every 2.4 relatives screened. In comparison during an opportunistic screening approach after an index case, 1 new case of FH was detected for every 1.5 relatives screened and only every 5.8 relatives screened had elevated Lp(a) levels. This increased effectiveness of detection was seen in all FH index cases even in those without elevated Lp(a) levels at diagnosis. Thus the authors recommended to incorporate routine Lp(a) screening into regular cascade screening programs for FH. (37,45,46)

Moreover the authors of the most recent and largest study by Fatica et al. on diagnostic errors in FH patients provided even more scientific evidence, that among all FH suspected individuals routine cascade testing for Lp(a) should be initiated. The aim here is to correctly classify patients with FH and those suffering from Hyperlipoproteinemia(a). The authors even proposed family screening over genetic testing for FH in patients with high Lp(a) as a main priority.(38)

Another approach was chosen in a review, published in 2020 by Masato Hamasaki and Kazuhiko Kotani. Here it was supposed to measure Lp(a) levels in all first visits of FH suspected individuals and only in individuals with high Lp(a) and high LDL-C, the authors suppose genetic testing. (47)

In general, the role of Lp(a) in screening and diagnosing FH remains unclear. Some scientists see enough evidence to incorporate Lp(a) into the diagnostic algorithms for FH. Here Anne Langsted and Borge G. Nordestgaard published in a paper in 2022 the potential of including

Lp(a) in FH diagnosis. According to the fact that about 25% of all patients diagnosed with FH actually show hyperlipoproteinemia(a), they assumed that the *LPA* gene could be seen as second most genetic mutation among FH patients after LDLR mutations. This was even further emphasised by their statement that: "Ideally, we believe that lipoprotein(a) could be included as a cause of FH, and thereby genetic testing for FH should include a plasma lipoprotein(a) measurement and possibly even testing for mutations in the *LPA* gene associated with high lipoprotein(a) levels." (48) Contrary to this approach, Chan et al. proposed in 2019, that their study could not support the idea of using *LPA* gene variants in order to improve diagnostic accuracy in FH patients. (37) In a common statement by National Heart, Lung and Blood Institute, American Academy of Paediatrics, American Heart Association and American College of Cardiology in 2018 saying that universal lipid screening is recommended by children in the ages from 9-11 years old. (30,49)

The recent 2022 EAS consensus statement by Kronenberg et al. recommends measuring Lp(a) levels in all adults at least once a life, best during the very first lipid profile in order to properly assign a patient to a cardiovascular risk group. Children should also early be tested for elevated Lp(a) concentrations, if they had a history of early ASCVD in family members or a history of ischemic stroke. Furthermore cascade testing should be offered if a family member shows increased Lp(a) levels or during FH cascade testing. Additionally Lp(a) cascade screening might be incorporated in diabetes, hypertension and obesity care. (9,45)

3.3.3 INTERACTIONS OF LP(A) AND FH

Both familial hypercholesterolemia and Lipoprotein(a) are considered as independent risk factors for ASCVD for many years, where patients with even one of both conditions were commonly assigned to high-risk or very high-risk groups, depending on clinical circumstances.(16) Keeping this in mind, a patient with both familial hypercholesterolemia and increased levels of Lp(a) constitute a rather unique situation, where two genetic risk factors interact with each other. Yet here an additive risk for ASCVD could even be observed.(7,50) Furthermore studies found, that in such patients, reducing only LDL-C did not reduce the overall cardiovascular risk associated with Lp(a). (5,50) Former studies among patients with FH revealed that Lp(a) levels in those were generally three times higher, compared to the general population. As early as 2000 Kraft et al. already reported that Lp(a) concentrations were found to be twofold higher among HoFH compared to HeFH patients, but even HeFH patients had significantly higher Lp(a) values than the general population. (5,51) Newer

research in 2016 by Langsted et al. concluded after their retrospective study of 46,200 individuals from the Copenhagen General Population Study, the observation of 39-58% higher Lp(a) concentration in individuals with FH. (29) The exact reason for this observations remains debatable, but a recent publication by Trinder et al. in 2020 linked the overall increased cardiovascular disease (CVD) risk among Lp(a) patients to be associated with the genetic polymorphism of LPA single nucleotide repeats (SNRs). Here the expression of rs10455872 and rs3798220 of the LPA genotypes were in particular observed with ASCVD. (7) To find this association the authors used the British Columbia Familial Hypercholesterolemia Cohort (BCFH) as well as participants from the UK Biobank cohort. It was found that the overall prevalence of Lp(a) levels >50mg/dL was present among 35.8% of individuals in BCFH compared to the general European population with about 20% prevalence. Further it was stated that the increased Lp(a) levels were not caused by an impaired clearance by LDL receptor pathway in FH patients, but more likely by an overproduction.

Finally Trinder et al. also adjusted the LDL-C values for Lp(a)-C and saw a 16.6% decrease in patients clinically diagnosed as FH using DLCNS criteria. Contrary here the authors interpreted the results slightly differently from the above mentioned approaches by Langsted or Fatica. Trinder et al. came to the conclusion, that elevated Lp(a) in fact positively increases the likelihood of diagnosing FH, because elevated Lp(a) overall lead to an "FH-like phenotype". (52)

With respect to an overall increased risk for cardiovascular diseases in FH patients with elevated Lp(a) levels, Langsted et al. also observed a 5.3-times risk of myocardial infarction among patients with FH and Lp(a) >50mg/dL. This risk was even further increased up to 9.8-times if FH was diagnosed in individuals with LDL-C values adjusted for Lp(a)-C. (29) Moreover with respect to aortic valve stenosis (AVS) both FH and Lp(a) could be identified as independent causal factors in the past. Here it was especially observed that the development of AVS was seen in HoFH, mostly already in children.(53) Additionally in a recent analysis of the SAFEHEART study Perez de Isla et al. in 2021 observed that Lp(a) and HeFH might conjointly contribute to AVS. Overall HeFH was recognized to be associated with a 5.7fold increase in prevalence of aortic valve replacement due to AVS, compared to the general population. Hereby Lp(a) values <50mg/dL in FH were linked with a hazard ratio (HR) of 4.08. When Lp(a) cut-off levels were set to <30mg/dL a HR of even 4.75 could be seen. The mechanism, by which Lp(a) contributes to AVS include "valvular deposition of oxidized phospholipids, autotaxin-mediated generation of phosphatidic acid, activation of the nuclear

factor-kB inflammatory cascade, and calcification due to induction of alkaline phosphatase“. (54,55)

Nevertheless there is still a large lack of awareness of this conjoint interactions between FH and Lp(a) in the acceleration of ASCVD due to a vast majority of individuals remaining undiagnosed.

3.3.4 TREATMENT CONSIDERATIONS

The treatment of Lipoprotein(a) in familial hypercholesterolemia patients remains challenging, mostly due to the fact that data is missing on the effectiveness of current medications used to treat FH on Lp(a). One key difference in patients with high Lp(a) concentrations compared to other dyslipidaemias is, that Lp(a) is largely influenced by genetics, hence common CVD risk factor management might be ineffective. Especially lifestyle and diet recommendations as well as smoking cessation show to have only minimal effects on Lp(a) levels and thus can be considered as ineffective. Here an exclusion are that a diet low in carbohydrate and high fat, might decrease Lp(a) levels up to 15%.(9,56)

Nevertheless they remain important in the overall context of CVD risk reduction. (30,57) Currently no approved medications exist, to specifically lower Lp(a) levels, but as recent studies demonstrated established drugs also exhibit a partial effect on Lp(a) levels and new drugs are already on the horizon. [Additionally an illustration of different mechanism of actions of lipid lowering drugs, see Annex 3]

1. Statins

Statins, 3-Hydroxy 3-methylglutaryl-Coenzyme A reductase (HMG-CoA reductase) inhibitors, are known in the reduction of LDL-C for many years and build up the backbone of current dyslipidaemia and FH treatment. They act principally in 3 different ways: 1. increasing LDL clearance; 2. decreasing hepatic production and secretion of apoB-containing lipoproteins; 3. upregulate the LDLR expression. (1) By inhibiting HMG-CoA enzyme in the liver that converts HMG-CoA to mevalonic acid, a cholesterol precursor, and ultimately reduce the amount of LDL-C. They are highly effective in lowering LDL-C values and with the use of statins the prevalence of CVD in HeFH was reduced by two thirds, compared to pre-statin area. (58) The efficiency of statins is dose dependent, where high intensity statin therapy can reduce LDL-C >50% from baseline. In FH patients it is generally recommended to initiate high intensity statin therapy as early as possible. Here European Atherosclerosis Society (EAS) and

European Society of Cardiology (ESC) recommend to start stain therapy even in FH children from 8 years of age. (58) Also all adults are recommended to initiate high intensity statin therapy early after FH diagnosis.(3,16,58) The main problem with statin therapy are the occurrence of side effects as mostly reported myalgias or even rarely rhabdomyolysis, leading to large rate of treatment discontinuation. Nevertheless many studies have demonstrated that statin therapy did not lower Lp(a) levels and even in some studies an increase in Lp(a) up to 20%(16,59) was observed during statin therapy. This supported also the thesis, that Lp(a) is degraded by different mechanisms than only the LDLR- as statins only act here.(1,30)

2. Ezetimibe

Ezetimibe acts in the small intestine, by selectively binding to Niemann-Pick C1-Like 1 cholesterol transporter (NPC1L1) and thus inhibits the uptake of cholesterol from nutrition. In FH patients a 16.5% reduction of serum LDL-C was found in the ENHANCE trial, but no differences were seen in carotid artery intima-media thickness. (57,58,60,61) Currently ESC/EAS guidelines recommend to start Ezetimibe therapy additional to statin therapy in FH patients in most cases.(16) The effect of Ezetimibe on LDL-C reduction was proven by many independent studies in the past, however the effect on Lp(a) remains debatable. In 2018 Awad et al. reviewed the effect and came to the conclusion that Ezetimibe leads to a small significant reduction in Lp(a) values compared to placebo. (62) However another review by Sehebkar et al. including 5,188 subjects demonstrated no significant changes in Lp(a) levels with Ezetimibe.(1,30,57,63)

3. PCSK-9 inhibitors

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is responsible to regulate the number of LDLR in hepatocytes. It also binds to the LDL receptor ultimately leading to the LDLR internalization and degradation. PCSK9 inhibitors are monoclonal antibodies, Alirocumab and Evolocumab, preventing this degradation thus leading to an increased LDLR expression and LDL-C reduction by an increased clearance.(64,65) In the RUTHERFORD 2 trial, the efficiency and safety of PCSK9 inhibitors was demonstrated, leading to a 60% reduction in LDL-C. Furthermore in the ODYSSEY FHI and FHII trials lipid lowering therapy with Alirocumab resulted in a 57.9% and 51.4% reduction in LDL-C. (30,57,58,63) Compared to HeFH in HoFH patients, the mean reduction of LDL-C with PCSK9 inhibitors was only 21,2% observed in the TAUSSIG study. This was especially seen in HoFH patients with null-null mutations.(64,66) According to current ACC/AHA guidelines PCSK9 inhibitors can be used

in patients with primary hypercholesterolemia and LDL-C >100mg/dL despite statin and Ezetimibe therapy.(58) Contrasting to this the ESC/EAS dyslipidaemia guidelines from 2019 suggest to use PCSK9 inhibitors if LDL goals are not reached with maximally tolerated statin and Ezetimibe therapy.(16) In studies it was also found that PCSK9 inhibitors reduce Lp(a) levels about 20-30% with a mechanism not fully understood.(67–69) Villard et al. observed that PCSK9 inhibitors increase apo(a) secretion and thus it was concluded that PCSK9 inhibitors rather interfere with Lp(a) synthesis, than its degradation. (70)

Furthermore in the FOURIER trial, a large randomized double-blind placebo trial among 27,564 patients, it was firstly possible to demonstrate that Evolocumab lead to a 7% cardiovascular risk reduction with Lp(a) reductions. Here was also observed that Lp(a) levels were lowered by up to 27% in individuals with hight Lp(a) levels at base line. The biggest Lp(a) reduction was observed among individuals with highest Lp(a) serum values (48,67) Additionally in a pooled analysis of Alirocumab phase III studies by Ray et al. in 2019 “a 12% relative risk reduction in MACE per 25% reduction in Lp(a) in patients” (61) was found. However after adjusting Lp(a)-C for LDL-C, this was no longer viewed as significant because the mean reduction in LDL-C was about 52% in this population. Due to the effect of the study the authors then concluded that no significant association between Lp(a) reduction and incidence of MACE was seen. (1,61) Contrary to this in another analysis of the ODYSSEY OUTCOME trial by O’ Donoghue et al. in 2021 a marked reduction in on MACE risk among patients with LDL-C levels close to 70 mg/dL and increased Lp(a) levels were recognized. It was estimated that this reduction was about 30%.(67) However up to now, PCSK9 inhibitors are not officially registered to reduce Lp(a) levels. (9)

4. Niacin

Nicotinic acid, Niacin, is a B-complex vitamin, which was among the first known medications to effectively reduce LDL-C and triglycerides. Different clinical trials were also able to reveal a decrease of 22.9-31% of Lp(a) levels, by an unknown mechanism. In the AIM HIGH trial it was not possible to demonstrate that Lp(a) reduction was resulting in cardiovascular risk reduction and due to the high and severe side effects of Niacin the general use in Europe was highly restricted. Now it is only approved for the use in patients with clinical hypertriglyceridemia and thus not available for neither FH patients nor for Lp(a) reduction. (9,57,71)

5. Monoclonal antibodies against angiopoietin 3

Angiopoietin-like protein 3 (ANGPTL3) is a protein, whose physiological task is to inhibit the activity of lipoprotein lipase and endothelial lipase, responsible for the phospholipid and triglyceride breakdown. In the Eclipse HoFH trial it was proven that monoclonal antibodies against ANGPTL3, Evinacumab, caused a 43% reduction in LDL levels compared to a control group. (72) Other phase II clinical studies reported similar effects. Currently further studies regarding the safety and efficiency and cardiovascular outcomes are awaited. With respect to Lp(a) recent studies demonstrated a minimal reduction of about 8-10% using Evinacumab.(58) (1)

6. CETP inhibitors

Cholesteryl ester transfer protein (CETP) inhibitors act by blocking the transfer of cholesterol esters from HDL-C to ApoB-containing lipoproteins, exhibited by CETP. Thus the amount of HDL-C is increased and LDL-C is reduced. Many different CETP inhibitors have failed to show sufficient changes in lipid levels in the past. The effect of CETP inhibitors on Lp(a) levels remain highly variable, depending on the compound. Anacetrapib was able to lower Lp(a) levels by about 40,8%(73) The exact mechanism, by which Anacetrapib lowers Lp(a) remains in discussion, but recent publications indicated that the effect is rather attributed to a decreases apo(a) production. Nevertheless no studies have been conducted revealing a reduction in cardiovascular events with the use of CETP inhibitors yet. (57)

7. Antisense oligonucleotides

Nearly all currently available medications in FH and Lp(a) focus more on increasing Lp(a) clearance from the circulation. Here antisense oligonucleotides (ASO) follow a different approach by blocking the assembly of Lp(a) in hepatocytes. ASO are synthetic single stranded nucleic acid sequences that bind to messenger RNA (mRNA), leading to the degradation of mRNA.(57) Mipomersen is a synthetic antisense oligonucleotide that selectively decreases apoB production by interfering with apoB protein translation. As apoB is a part of either LDL-C and also Lp(a), Mipomersen has shown in phase III studies to modestly reduce Lp(a) levels by 26,4% on average. (74) It lead to the conclusion that with ASOs targeted at *APOB* (Mipomersen) no sufficient effect in Lp(a) reductions could be observed. Due to a high occurrence of adverse events, especially hepatic steatosis, the authorization of Mipomersen was refused by the European Medical Agency (EMA). But on the other hand in the US Mipomersen is approved for the treatment of HoFH adults. (57) (1)

Another generation of ASO are now being developed, who are targeted towards *LPA gene* directly in order to sufficiently and sustainably reduce Lp(a) levels. Here the most promising drug Pelacarsen that is composed of single stranded nucleic acids, which complementary bind to *LPA* mRNA within hepatocytes. After binding the enzyme RNase H1 recognizes and cleaves the ASO complex and thus in the end reduces apo(a) production. First pre-clinical studies were able to reduce Lp(a) levels by up to 90% using IONOS-APO(a)$_{RX}$. During subsequent phase I and II clinical studies an effective reduction of 77,8% was observed.(75) After this first success ASO were modified by adding a triantennary N-acetyl-galactosamine (GaINAc3) which further increased their potency. Pelacarsen is one of the improved OSA now being evaluated in phase III clinical studies. In a recent study 286 patients were enrolled and got different dosages of Pelacarsen injections at four week intervals or two week intervals. The result of the study was that Pelacarsen was lowering Lp(a) in a dose-dependent manner up to 67% (76) Another bigger trial currently under investigation is the HORIZON trial, investigating the effect of Pelacarsen on Lp(a) and the impact on MACE in 7,680 participants. Results are expected to be published in 2024. (57) [More about ASOs, see Annex 4]

8. Small interfering RNA

Small interfering RNA (siRNA) are different from ASO in that they are composed of double-stranded RNA molecules, which separate after entering the hepatocyte. One promising drug is Olpasiran. Here the antisense strand is then included into the RNA-induced silencing complex (RISC), targeted at *LPA* and apo(a) mRNA ultimately disrupting apo(a) synthesis. (77,78) SiRNA result in even longer cleavage of targeted RNA, thus less frequent dosing would be required. In phase I studies a mean Lp(a) reduction of 71-97% was observed and currently phase II trials are under investigation. There were no major adverse events up to now reported. (57)

Another approach by siRNA is Inclisiran, where siRNA is slicing the PCSK9 gene in hepatocytes and this way blocks the PCSK9 synthesis. In the ORION-9 phase III randomized control clinical trial HeFH patients received 4 doses subcutaneously leading to a mean 47,9% reduction in LDL-C levels. Moreover it was found that the efficiency of Inclisiran comparable to monoclonal antibodies against PCSK9 in HoFH patients and superior in HeFH. (1) Here it was also found that Inclisiran additionally acts on serum Lp(a) by decreasing serum levels about 17%. Currently phase II study results are awaited. (30,58,78,79)

9. Bile acid sequestrants

Bile acid sequestrants disrupt the enterohepatic circulation and thus decrease the availability of bile acids. This stimulates the liver to produce more bile from cholesterol ultimately reducing LDL-C levels. Among those drugs Colesevelam is now mostly used, a second generation bile acid sequestrant. It has the advantage of a better side effect profile compared to Colestipol (first generation). If Colesevelam is added to statins and Ezetimibe therapy in FH patients, an additional 12% reduction of LDL-C was observed. There are no reported effects on Lp(a) levels. Bile acid sequestrants are now mostly used in pregnancy, as they are among the only approved drugs in pregnant women to date. (58)

10. Bempedoic acid

Bempedoic acid inhibits adenosine triphosphate citrate lyase in the liver and thus "de-novo cholesterol biosynthesis upstream of the enzyme HMG-CoA reductase" (58) the principal action of statins. It upregulates the number and expression of LDLR and thus leads to a reduced LDL-C concentration. (30) Compared to statins there are less side effects, especially related to muscles because it specifically acts only in the liver. It can be seen as a potential alternative drug in statin intolerant patients and has the ability to reduce LDL-C by 21-29%. No effects regarding Lp(a) are available yet.

11. Lipid apheresis

Lipoprotein apheresis (LA) is an invasive, rather old and time consuming procedure which removes ApoB-containing lipoproteins from the circulation. Classically it is performed on a weekly basis, each session lasting about 3 hours.(80) LDL-C levels can generally be reduced between 57-63% depending on if it is performed in HoFH or HeFH patients.(58) Lp(a) levels could be reduced by as much as 70% with most techniques. Also it has to be stressed that there was no sustainable effect seen with rapidly regenerating levels of Lp(a) due to production in the liver. On a time-averaged equation the reduction rate of Lp(a) by apheresis only reached 35%.(78,80,81) An important, not yet understood, consideration of the advantage of LA is that it simultaneously is capable of removing OxPLs. LA is mostly used in adults with HoFH, who are not reaching their LDL-C goal on other treatments. Among the countries more routinely applying LA is Germany, where LA is indicated in adult patients with progressive CVD and isolated Lp(a) levels more than 60mg/dl. In contrast in the US and UK FH and Lp(a) are only among possible indications for LA. (24) Nevertheless independent studies showing an actual effect of LA over a control group are still missing today. (77)

12. Liver Transplantation

Among HoFH children occasionally liver transplantations are performed. Nevertheless no current guidelines recommends these very drastically treatment approaches due to lack of clinical data and serious adverse effects related to lifelong immunosuppression, (58)

4. LP(A) IN LITHUANIAN FH PATIENTS: DATA FROM FH SCREENNG PROGRAMME

The aim of this investigation was to detect Lp(a) values in different FH patients groups and determine a correlation between the occurrence of ASCVD and elevated Lp(a) levels taking into account high LDL-C levels.

4.1 METHODS

The research was approved by Vilnius Regional Bioethics Committee (agreement number: 158200-18/5-1010-538). . [Bioethics Committee arrival, see Annex 7] All patients provided a written informed consent form on participation.

Patients were already pre-selected, according the following characteristics:

Adults with clinically suspected FH (LDL-C ≥5 mmol/L) are referred to specialist lipid centre, where detailed personal and familial anamnesis, physical examination, evaluation of laboratory and instrumental tests are being performed and secondary causes of dyslipidaemia are excluded. The clinical diagnosis of FH was determined according to Dutch Lipid Clinic Network (DLCN) criteria. Patients with DLCN score ≥6 and/or LDL-C ≥6,5 mmol/L are referred to genetic testing. Cascade first-degree relatives screening are initiated if an index-case meets DLCN criteria for definite or probable FH.

The data was collected between 2018 and 2022 (n=347). Measurements of LDL-C concentration in venous blood serum were performed using the Friedewald formula, or directly when triglyceride level was above 5 mmol/L. Lp(a) was gained by a turbidimetric essay. The clinical diagnosis of FH was ascertained using DLCN criteria.

A conversion of Lp(a) values from mass units (mg/dL) into molar units (nmol/L) was done by a conversion factor of 2-2.5, as recommended by the latest 2022 ESC and EAS Consensus.(9)

All statistical analysis was performed using R (v. 4.0.4) program package. The mean, standard deviation (SD), median, first and third quartiles and the available number of observations of the quantitative variables are presented. Categorical variables are presented as the absolute amount and the percentage.

For normality of the quantitative variables the Shapir-Wilks test was used. In order to test the hypothesis for pairwise comparison of the quantitative variables, nonparametric Mann-Whitney U test was used as normality assumption was violated for all of the pairs. To identify relations between two quantitative variables Spearman correlation was used as appropriate.
To make a comparison of which one of the quantitative factors is the best for the [CAD] indication the ROC curves (Youden index) were used, in addition sensitivity and specificity for these values are presented, also AUC with 95% CI included. A p-value less than 0.05 was considered significant.
[For more information, see Annex 5 & 6]

4.2 RESULTS

4.2.1 Results of lipid testing

The exact results of lipid testing is shown in ANNEX 5 & 6. The highest LDL concentrations (n= 343) with a median of 6,32 mmol/L (5,5-7,29 mmol/L). While comparing different medians of the high LDL concentrations among patients in our data that were assigned to the "highest LDL" group (n=343), here a median LDL value of 6,32 mmol/L (5,5-7,29 mmol/L) was found. Among the selected patients the median Lp(a) levels were 9,52 mg/dL (4,00-35,91 mg/dL) in mass units (n=244), or 25,85 nmol/L (11,23-100,65 nmol/L) in molar units (n=230), where in the following analysis only molar units will be used as a reference.

4.2.2 Lp(a) in high LDL-C patients

In patients with high values of LDL cholesterol, defined as LDL-C ≥6,5 mmol/L, the median Lp(a) levels were 31,65 nmol/L (12,25-116,93 nmol/L).

Table 3. Mean and Median Lp(a) concentrations in patients with high LDL-C

LDL-C	n	mean Lp(a) (nmol/L)	sd	median Lp(a) (nmol/L)	Q1	Q3	p-value
<6,5 mmol/L)	112	67,9	93,9	24,95	11,1	90,4	0,300
(≥6,5 mmol/L)	116	85,4	111,4	31,65	12,25	116,925	0,300

4.2.3 Effects of Lp(a) on clinical FH classification

The median age of FH diagnosis among patients within the sample (n=347) was 47 years (37-54 years).
Using the DLCN criteria for the classification and diagnosis of FH among the selected patients among all age categories, 87 (25%) were categorized as definite FH; 155 (45%) as possible FH; 91 (26%) were classified as probable FH and 13 (4%) as unlikely FH.

The median Lp(a) among the different DLCN categories were investigated and compared to each other. The lowest median Lp(a) concentration was seen in those patients categorized as unlikely FH, with 9,6nmol/L (6,1-19,0 nmol/L). Among possible FH patients, a median Lp(a) concentration of 25,9 nmol/L (12,1-96,3 nmol/L) and in probable FH category a median Lp(a) level of 44,2 nmol/L (14,1-121,8 nmol/L) was observed. In definite FH group, the median Lp(a) was 23,1 nmol/L (10,1-77,5 nmol/L). Comparing those categories with their Lp(a) measurements, three statistical significant differences were seen: with a p-value of 0,036 statistical significant differences in median Lp(a) were found between unlikely FH and possible FH patients; between unlikely FH and probable FH (p-value 0,018) and between unlikely FH and definite FH (p-value 0,40). Among all other groups, no statistical significance could be proven. To add, statistical significance (p-value 0,024) between those unlikely FH and all other groups together became obvious. Results are shown in Figure 1.

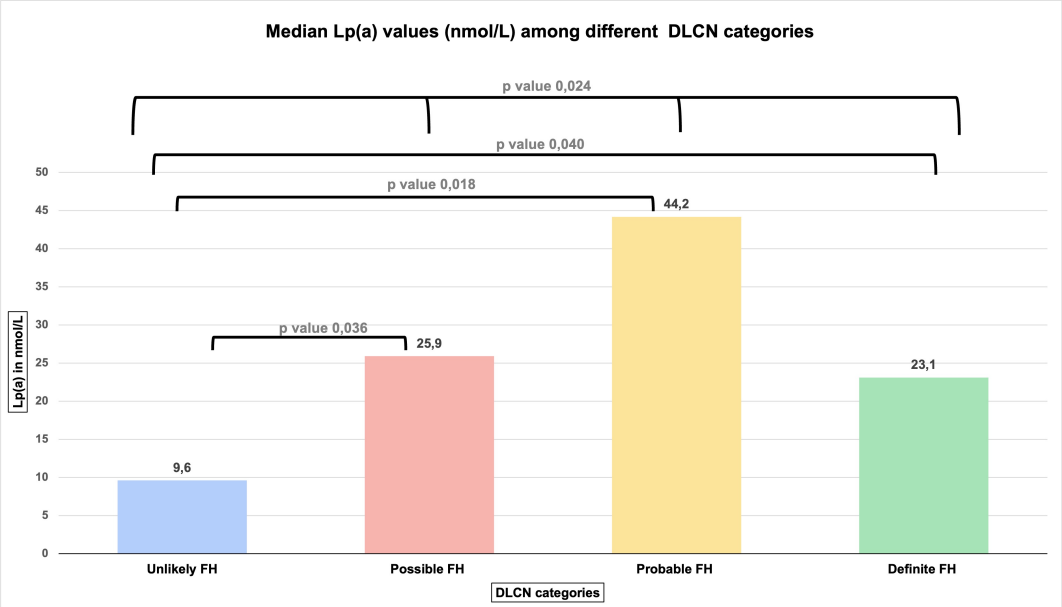

Figure 1 Median of Lp(a) values among different DLCN categories. Only statistically significant differences are shown here by their p-values.

4.2.4 Lp(a) in CAD and stroke patients

Among patients with CAD, median Lp(a) value of 42,5 nmol/L (11,35-188,65 nmol/L) could be recognized, where the median Lp(a) in those without CAD was lower with 22,6 nmol/L (10,08-66,25 nmol/L) (p-value 0,073). In patients with the occurrence of premature coronary artery disease (CAD) or stroke, which is defined as occurrence <55 years in males and <60 years in females, the median Lp(a) concentration was 42,5 nmol/L (12,5-225 nmol/L). The

median Lp(a) value among patients without premature stroke or CAD were 50 nmol/L (12,5-93,75 nmol/L).

According to our study, Lp(a) is better indicator for identifying CAD than the highest LDL-C (>6,5 mmol/L). Despite AUC is sill quite low, it shows indication that patients with higher Lp(a) have CAD with higher possibility. The best threhold for the split would be: if Lp(a) ≥41.80 nmol/L, then there is higher chance to have CAD (Figure 2).

	Variable	specificity	sensitivity	auc	ci_lower	ci_upper	p-value	the best threshold
CAD	Lp(a) (nmol/L)	0,676	0,606	**0,638**	0,528	0,749	0,014	**41.8**
	Highest LDL-C (mmol/L)	0,335	0,804	**0,544**	0,456	0,632	0,332	---
Premature CAD	Lp(a) (nmol/L)	0,511	0,595	**0,459**	0,328	0,589	0,384	---
	Highest LDL-C (mmol/L)	0,468	0,691	**0,550**	0,444	0,655	0,523	---

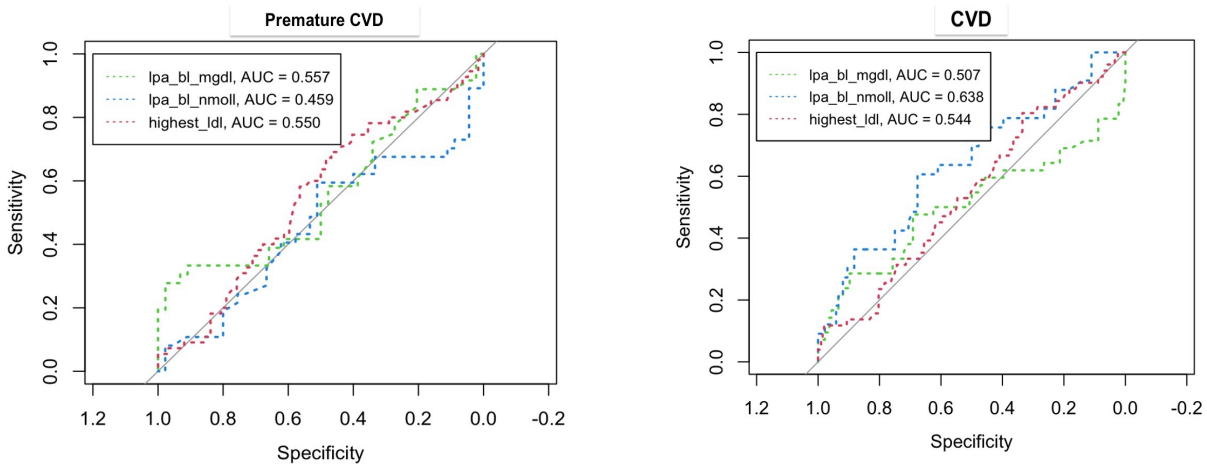

Figure 2 Association between Lp(a) serum levels and premature cardiovascular diseases (CVD) and cardiovascular disease occurrence.

Table 4. Mean and Median Lp(a) in patients with CAD and/or stroke and premature CAD and/or stroke

	CAD / stroke	n	mean	sd	median	Q1	Q3	p-value
Lp(a) nmol/L	No	128	64,63	95,79	22,6	10,075	66,25	0,073
Lp(a) nmol/L	Yes	43	113,00	140,94	42,5	11,35	188,65	0,073
	Premature CAD/ stroke	**n**	**mean**	**sd**	**median**	**Q1**	**Q3**	**p-value**
Lp(a) nmol/L	No	43	69,15	82,18	50	12,2	93,75	0,559
Lp(a) nmol/L	Yes	37	118,65	148,33	42,5	12,5	225	0,559

In the data set a correlation between higher Lp(a) concentrations and the occurrence of CAD, which was statistically significant with a p-value of 0,014. According to the analysis, a cut-off point of >41,80nmol/L could be seen here.

5. DISCUSSION

Due to an increased awareness of hyperlipidaemia and especially increasing interest in Lp(a) within recent years, the general knowledge about the interplay between Lp(a) and FH also gained more and more relevance. Here among the biggest challenges to solve yet, remains a widely available, cost efficient way of directly measuring Lp(a) values in patient samples and by this also increasing the accuracy of FH diagnosis. As mentioned before, it can be expected, that the true numbers of patients falsely diagnosed with FH due to increased Lp(a) levels might be significant and that after a careful re-evaluation of current diagnostic opportunities, the importance of hyperlipoproteinemia(a) might further grow.

As it was even possible to demonstrate a higher likelihood of CAD among patients with increased Lp(a) values among a selected Lithuanian FH patients group, further focus should be on the potentially dangerous interplay between Lp(a), high LDL-C and other CAD risk factors. This observation is also backed up by much larger populational studies, as the Copenhagen General Population study, mentioned earlier. (29) Moreover among Lithuanian population, a tendence of higher Lp(a) levels in patients with CAD became observable with a p-value 0,073. One of the biggest challenges, besides a proper detection of Lp(a) remains the setting of concrete cut off values, to identify those at a higher risk. Here the most recent 2022 Consensus, remains the current gold standard in Europe. Nevertheless, within our analysed patients, a statistical significant, yet weak correlation between higher Lp(a) values and the occurrence of CAD could be observed. Here a more extensive data set would be required to ultimately clarify the observation among FH patients. Surprisingly it became obvious that above Lp(a) values of >41,80 nmol/L (p-value 0,014) a higher chance of CAD development was observed. So that a different cut-off level for Lp(a) for CAD development, of ≥ 41,80 nmol/L, was pointed out. According to the recent 2022 Consensus, such values are much lower, that the proposed 125nmol/L to rule in an increased Lp(a) level. However, the mentioned consensus also states that a value of 41,80 nmol/L falls into a “grey zone”, where other ASCVD risk factors have to be taken into account. (9) Surprisingly with regard to this cut-off value, among our patient sample, it could not be observed, that there was a statistical significant correlation between premature CAD or stroke. In order to validate these findings, larger populational samples would be needed.

Finally after diagnostic uncertainties the lack of Lp(a) specific medications and additionally the lack of effect of current medications and life style adjustments, mark a critical problem in further preventing ASCVD and other complications from high Lp(a) levels. Here the long awaited results of phase II and III studies of promising new drugs (see above) will hopefully bring changes in the near future.

6. CONCLUSIONS AND RECOMMENDATIONS

The exact faith of Lipoprotein(a) in Familial Hypercholesterolemia patients remains unknown up to know, but more and more evidence support the arguments that Lipoprotein(a) is an independent and strong risk factor for the development of coronary artery diseases. Our study observations were able to demonstrate a clear correlation between moderately elevated Lipoprotein(a) levels and the development of coronary artery diseases among 347 patients. Nevertheless screening using current, imprecise, techniques to determine Lipoprotein(a) cholesterol levels, remains one of the biggest initial problems in dyslipidaemia diagnosis. Here better, more precise and cost effective measurements have to be rolled out to the vast majority of clinics.

With regard to effective and precise Familial hypercholesterolemia diagnosis an adjustment of DLCN criteria with respect to Lipoprotein(a) remains highly debatable and further studies are needed. Among previous studies it became obvious that Lipoprotein(a) levels should be measured at least once for every patient, presenting with dyslipidaemia, and that systematic screening for Lipoprotein(a) levels after an index case of Familial hypercholesterolemia is highly effective and cost efficient. The biggest challenges to be solved in the future remain effective long term treatment and thus risk reduction, which will only be possible with the new promising drugs to be introduced in the near future.

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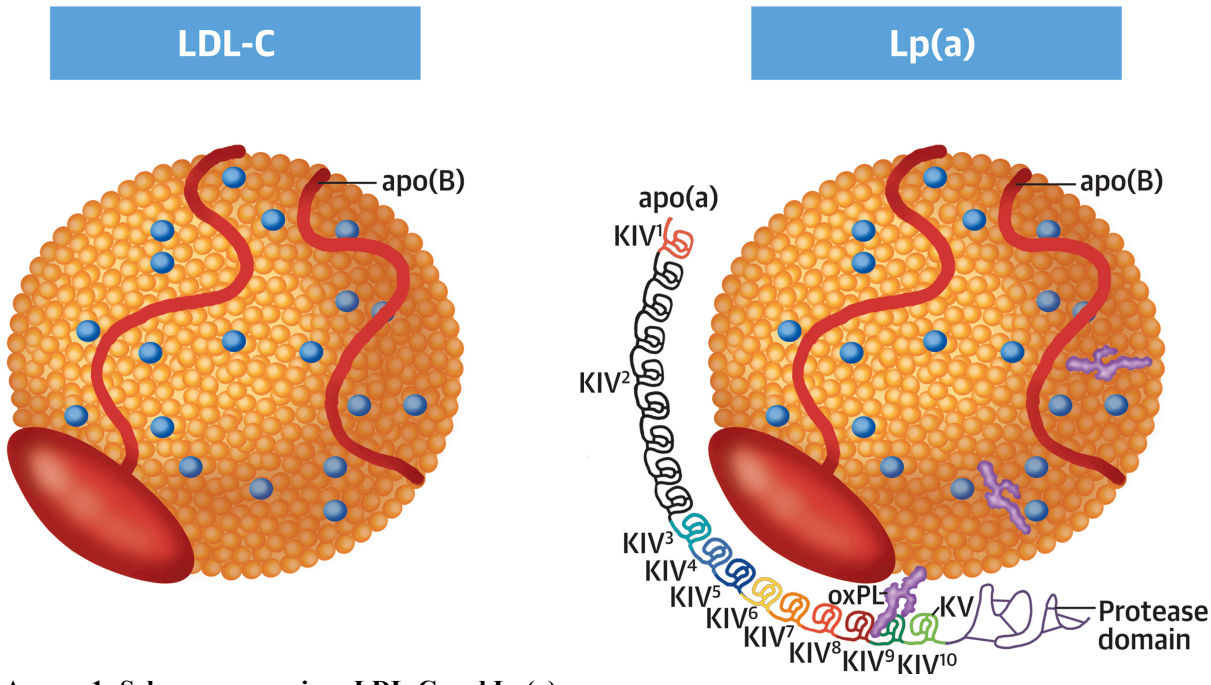
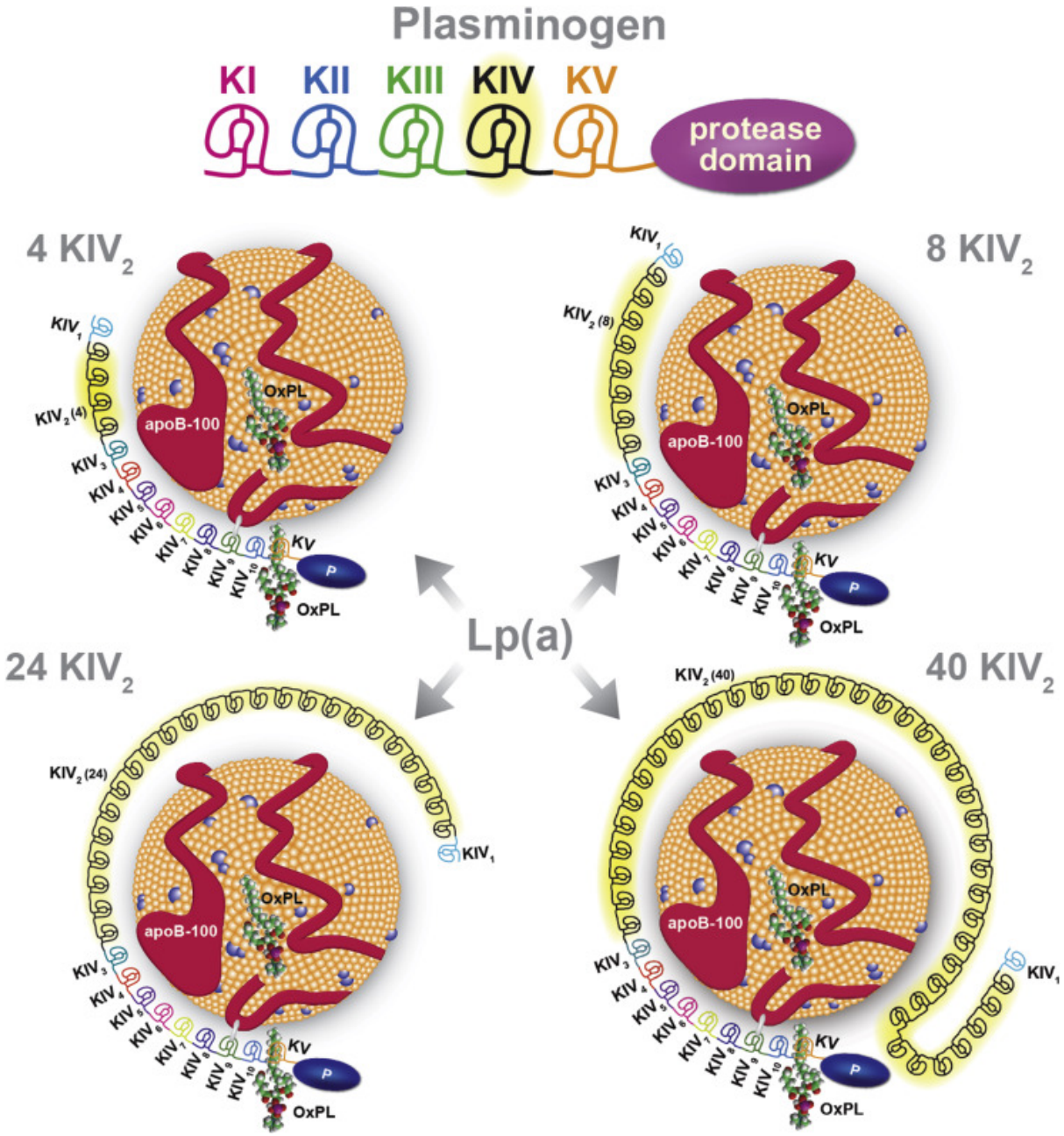
9. ANNEXES



Annex 1: Scheme comparison LDL-C and Lp(a)

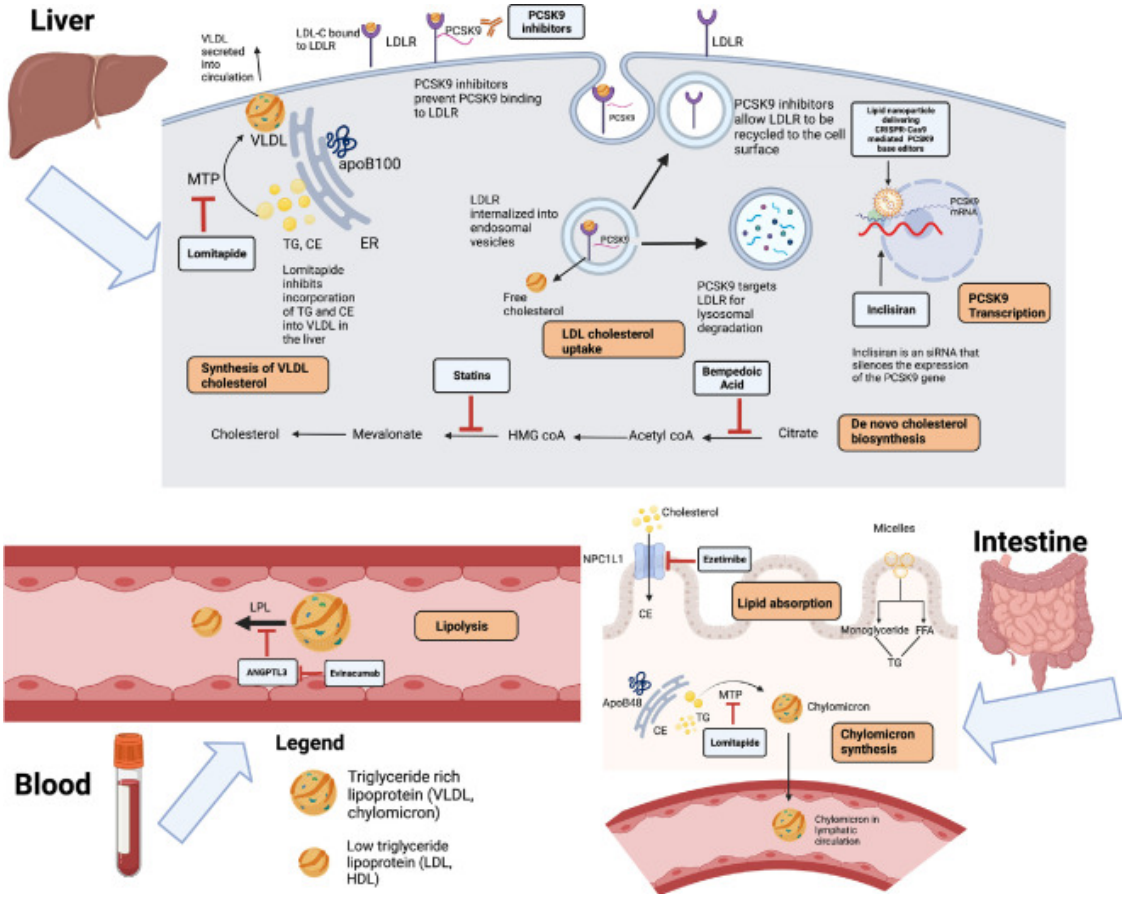
"(Left) Low-density lipoprotein (LDL) particle; (right) lipoprotein(a) [Lp(a)] particle. Apoprotein (apo) B is the scaffolding for lipidation of both lipoprotein species. Lp(a) is an LDL particle that is modified by the covalent

addition of apo(a) to apoB. Apo(a) is comprised of a series of kringles (protein loops; kringle IV [1–10] followed by kringle V) and a protease terminus. The number of repeats in kringle IV type 2 is highly variable person to person, genetically determined, and correlates with serum levels of Lp(a) as well as the magnitude of risk for cardiovascular disease exerted by this lipoprotein. LDL-C 1⁄4 low-density lipoprotein cholesterol."(7)



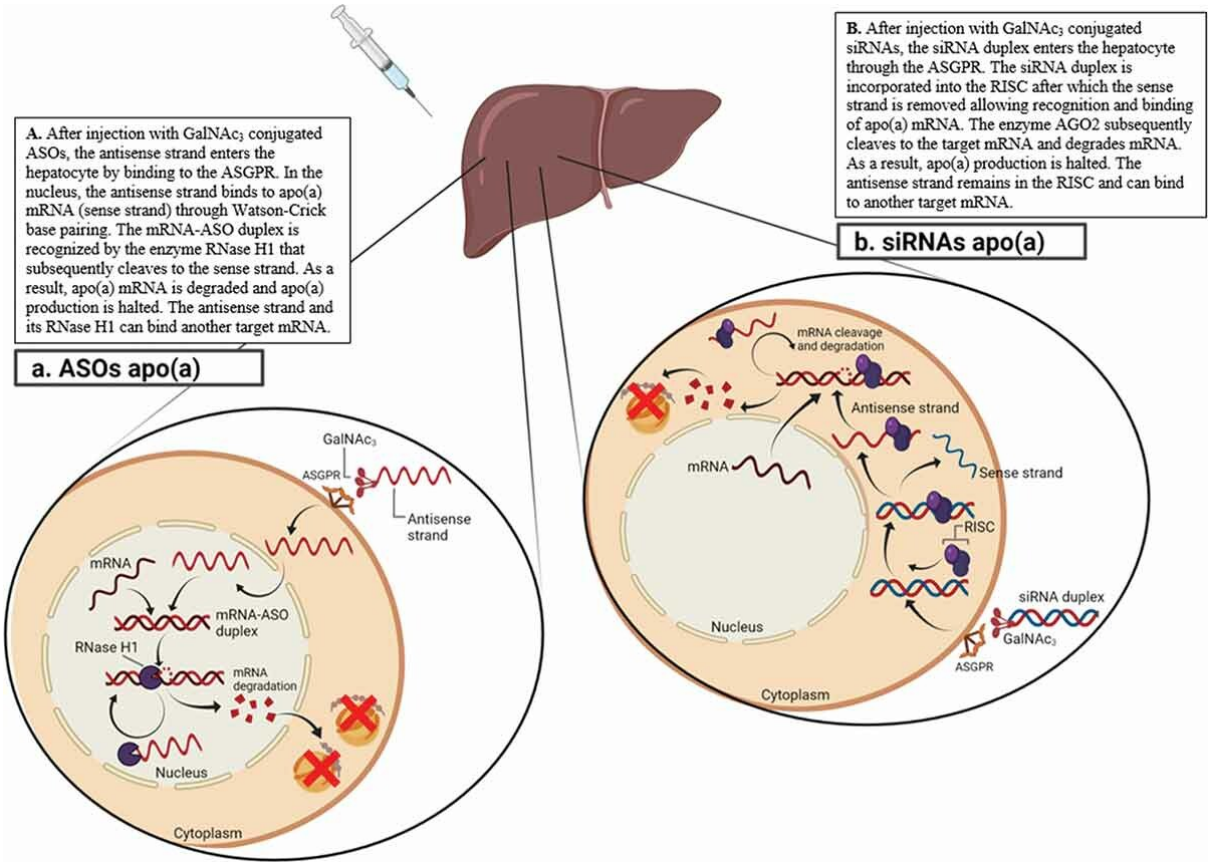
Annex 2: Structural differences and Lp(a) polymorphism

"Lipoprotein [Lp(a)] is composed of apolipoprotein B-100 (apoB-100) covalently bound to apolipoprotein (a) [apo(a)], which is derived from kringle IV (KIV) and KV, and the protease domain of plasminogen. Plasminogen has 1 copy each of KI to KV and an active protease domain. Apo(a) contains 10 subtypes of KIV repeats, composed of 1 copy each of KIV1, multiple copies of KIV2, and 1 copy of KIV3−10, KV, and an inactive protease-like (P) domain. In these examples, apo(a) isoforms of 4, 8, 24, and 40 KIV2 repeats are shown, representing 13, 17, 33, and 49 total KIV repeats. Oxidized phospholipids (OxPL), represented here by 1-palmitoyl-2-oxovaleroyl-sn-glycero-3-phosphocholine (POVPC), are present covalently bound to apo(a), and also dissolved in the lipid phase of apoB-100." (19)



Annex 3: Visualization of different lipid lowering drugs site of action

"Schematic diagram depicting sites of action of various lipid lowering agents. In the liver, cholesterol is synthesized de novo from Acetyl-CoA through several intermediate steps, two of which are inhibited by statins and Bempedoic acid. Esterified cholesterolparticles are combined with triglycerides and Apolipoprotein B100 in the endoplasmic reticulum to form nascent VLDL particles which are later secreted into the bloodstream. By inhibiting MTP, Lomitapide inhibits this process. Uptake of LDL particles in the liver is primarily mediated by the LDL receptor (LDLR). PCSK9 is an enzyme which binds to LDLR and is internalized with it to undergo lysosomal degradation. Monoclonal antibodies targeting PCSK9 inhibit the action of PCSK9, while Inclisiran and CRISPR gene editing techniques inhibit the transcription of the PCSK9 gene. In the intestine, absorbed cholesterol and triglycerides are incorporated into chylomicron particles in the endoplasmic reticulum along with Apolipoprotein B48, which are later secreted into the lymphatic circulation. Ezetimibe inhibits the absorption of cholesterol through the NPC1L1 transporter while Lomitapide inhibits MTP and thus the incorporation of cholesterol and triglycerides into chylomicrons. By inhibiting ANGPTL3, Evinacumab disinhibits lipoprotein lipase, an enzyme that metabolizes triglyceride rich lipoproteins at the vascular endothelial lining. Created with Biorender.com Abbreviations: LDL-C= Low-density lipoprotein cholesterol, LDLR= LDL receptor, VLDL= Very low- density lipoproteins, apboB= Apolipoprotein B, MTP= Microsomal triglyceride transfer protein, TG= Triglyceride, CE= Cholesterol esters, ER= Endoplasmic reticulum, LPL= Lipoprotein lipase, Lp(a)= Lipoprotein (a), FFA= Free fatty acids."(58)



Annex 4: Antisense Oligonucleotide mechanism of action

"Mechanism of action of antisense oligonucleotides (a) and small interfering RNA (b).Figure created with BioRender.com. GalNAc3, triantennary N-acetyl-galactosamine; ASOs, antisense oligonucleotides; ASGPR, asialoglycoprotein receptors; apo(a), apolipoprotein(a); mRNA, messenger ribonucleic acid; siRNAs, small interfering RNAs; RISC, RNA-induced silencing complex; AGO2, argonaute 2" (57)

Variable	n	mean	sd	median	Q1	Q3	norm
Age	347	45,98	12,26	47,00	37,00	54,37	FALSE
Highest LDL-C (mmol/L)	343	6,52	1,68	6,32	5,50	7,29	FALSE
Lp(a) (mg/dL)	244	27,75	39,16	9,52	4,00	35,91	FALSE
La(a) (nmol/L)	230	76,16	103,07	25,85	11,23	100,65	FALSE

Annex 5: Data analysis from Lithuanian FH patients

Age distribution and different Lp(a) levels among participants. n= absolute number of participants, sd= standard deviation, Q= quantile 1, Q3= quantile 3

DLCN category	n	mean Lp(a) (nmol/L)	sd	median Lp(a) (nmol/L)	Q1	Q3	p-value	Norm
probable FH	49	96,2	112,7	44,2	14,1	121,8	0,202	FALSE
definite FH	73	76,7	106,7	23,1	10,1	77,5		
possible FH	96	70,2	98,6	25,9	12,1	96,4	0,995	FALSE
definite FH	73	76,7	106,7	23,1	10,1	77,5		
unlikely FH	11	36,4	62,2	9,6	6,1	19,0	0,040	FALSE
definite FH	73	76,7	106,7	23,1	10,1	77,5		
probable FH	49	96,2	112,7	44,2	14,1	121,8	0,139	FALSE
possible FH	96	70,2	98,6	25,9	12,1	96,4		
probable FH	49	96,2	112,7	44,2	14,1	121,8	0,018	FALSE
unlikely FH	11	36,4	62,2	9,6	6,1	19,0		
possible FH	96	70,2	98,6	25,9	12,1	96,4	0,036	FALSE
unlikely FH	11	36,4	62,2	9,6	6,1	19,0		
probable + possible + unlikely FH	156	76,0	102,0	26,8	11,8	104,8	0,845	FALSE
definite_FH	73	76,7	106,7	23,1	10,1	77,5		
definite + probable + unlikely_FH	133	80,5	106,7	25,3	10,1	108,5	0,767	FALSE
possible_FH	96	70,2	98,6	25,9	12,1	96,4		
definite + possible + unlikely FH	180	70,8	100,2	24,6	10,5	85,4	0,079	FALSE
probable_FH	49	96,2	112,7	44,2	14,1	121,8		
definite + probable + possible FH	218	78,2	104,6	27,5	11,8	102,1	0,024	FALSE
unlikely_FH	11	36,4	62,2	9,6	6,1	19,0		

Annex 6: Data analysis from Lithuanian FH patients

Comparison of Mean and Median Lp(a) values between different DLCN categories. Yellow marked values represent statistically significance ($p < 0,05$). n= absolute number of participants, sd= standard deviation, Q= quantile 1, Q3= quantile 3.

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LEIDIMAS
ATLIKTI BIOMEDICININĮ TYRIMĄ

2018-05-08 Nr.158200-18/5-1010-538

Tyrimo pavadinimas:

Šeiminės hipercholesterolemijos ilgalaikės stebėsenos tyrimas

Protokolo Nr.:	FH01
Versija:	03
Data:	2018 03 21
Informuoto asmens sutikimo forma:	01 2018 02 09
Pagrindinis tyrėjas:	**Žaneta Petrulionienė**
Įstaigos pavadinimas:	VšĮ Vilniaus universiteto ligoninės Santaros klinikos
Adresas:	Santariškių g.2, Vilnius
Leidimas galioja iki:	**2028 04**

Leidimas išduotas Vilniaus regioninio biomedicininių tyrimų etikos komiteto posėdžio (protokolas Nr. 158200-2018/5), vykusio 2018 m. gegužės 8 d. sprendimu.

Pirmininkas 

prof. Saulius Vosylius

Annex 7: Vilnius University Bioethics Committee Approval.