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Individuals with familial hypercholesterolemia and cardiovascular events have higher circulating Lp(a) levels

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BACKGROUND: Cardiovascular disease (CVD) is a major cause of mortality and morbidity. Increased low-density lipoprotein cholesterol (LDL-C) level is its major risk factor. Familial hypercholesterolemia (FH) is a genetic disorder characterized by elevated LDL-C since birth and subsequent premature CVD. There is a heterogeneity in the CVD onset in patients with FH. This is potentially due to the presence of other independent risk factors. Lipoprotein(a) [Lp(a)] is an LDL-like particle and represents a strong risk factor for CVD.

OBJECTIVE: Our objective was to understand the contribution of Lp(a) in the susceptibility to CVD in individuals with genetic diagnosis of FH.

METHODS: We measured Lp(a) levels in 2 independent and well-characterized genetic-FH cohorts: the FH-Gothenburg cohort (n = 190) and the FH-CEGP Milan cohort (n = 160). The genetic diagnosis

was performed by targeted next-generation sequencing (FH-Gothenburg and part of the FH-CEGP Milan cohort), or by Sanger sequencing.

RESULTS: We show that among individuals with genetic diagnosis of FH, those with previous CVD had higher Lp(a) levels. In addition, analyzing the response to the lipid-lowering therapies, we have also shown that statins had the same LDL-C–lowering effect irrespective of the type of FH-causative mutation. However, when we examined the lipid-lowering effect of proprotein convertase subtilisin/kexin type 9 inhibition by antibodies, we observed a trend in a better reduction of the LDL-C level in carriers of nonsense mutations.

CONCLUSION: In conclusion, our results suggest that Lp(a) contributes to CVD onset in individuals with genetic diagnosis of FH. Our finding supports the importance to identify an efficacious therapy to lower Lp(a) in patients with FH to prevent CVD onset or recurrence.

Introduction

Cardiovascular disease (CVD) is a major cause of mortality and morbidity in Western Countries. Increased low-density lipoprotein cholesterol (LDL-C) level is a major risk factor for CVD[1] and thus one of the main target for its prevention.[2]

Familial hypercholesterolemia (FH) is a genetic inherited disorder mainly caused by mutations in genes encoding for the LDL receptor (LDLR), apolipoprotein B (APOB, the main protein of LDL) or proprotein convertase subtilisin/kexin type 9 (PCSK9).[3] FH is characterized by elevated LDL-C since birth and subsequent premature CVD development.[4] Statins represent the pivotal LDL-C–lowering drug in individuals with FH to prevent CVD. However, the efficacy of statins in FH shows high individuals variability.[5] Despite the strong penetrance of the disease, there is a heterogeneity in the onset of CVD in patients with FH. This is potentially due to differences in the response to lipid-lowering therapy or to the presence of other independent risk factors including high levels of lipoprotein(a) [Lp(a)].

Lp(a) (or LPA) is an LDL-like particle synthetized by the liver and consists of an APOB-100 covalently linked to a very large glycoprotein known as apolipoprotein(a). Circulating Lp(a) levels are predominantly controlled by genetic variations on the *LPA* gene[6] and seem not to be changed by diet, physical activity or other environmental factors.[7,8] To date, Lp(a) is not effectively lowered by any approved drugs. However, novel therapeutic drugs able to lower Lp(a) are currently under clinical trials.[9–11]

Lp(a) represents a strong risk factor for CVD.[12] Mendelian randomization studies with genetic variables primarily increasing Lp(a) have shown that high Lp(a) is an independent risk factor for CVD in the general population independently from the other traditional risk factors including LDL-C.[13–15] However, the role of Lp(a) in CVD prevalence in patients with FH is still a matter of debate. Previous studies described higher Lp(a) levels in patients with FH with previous CVD. However, most of these studies were performed in patients with diagnosis of FH established using clinical evidence and not confirmed by genetic

tests.[16–19] Alonso et al in 2014 showed that individuals with genetic diagnosis of FH had higher Lp(a) than their unaffected relatives.[20] They have also shown that among the individuals with genetic diagnosis of FH, those with CVD had higher Lp(a) than those without previous CVD events. Furthermore, they showed that individuals with a nonsense mutation in the *LDLR* had higher Lp(a) and higher CVD incidence than those with missense mutation on the same gene. However, in this very elegant study, the genetic diagnosis of FH was performed using a microarray containing only a selection of mutations in *LDLR* and of different mutations in *APOB*. In addition, only selected mutations (namely the most frequent null and defective mutations) on the *LDLR* were included in the analysis stratified by type of mutation.

Here, to understand the contribution of Lp(a) in the susceptibility to CVD, we performed a comprehensive genetic diagnosis of FH using targeted next-generation sequencing of the main FH-causative genes namely *LDL-R*, *APOB*, and *PCSK9* in individuals from two European Lipid Clinics. We show that among individuals with definite genetic diagnosis of FH, those with previous CVD had higher circulating levels of Lp(a) in these 2 independent cohorts. Analyzing the response to the lipid-lowering therapies, we have also shown that statins had the same LDL-C–lowering effect irrespective of the type of mutation (nonsense vs missense) in both FH study cohorts. However, when we examined the lipid-lowering effect of PCSK9 inhibition by antibodies, we observed a trend in a better reduction of the LDL-C level in carriers of nonsense mutations.

Materials and methods

Study cohorts

The FH-Gothenburg cohort

In the present study, we included 190 adult individuals with genetic diagnosis of FH from the Lipid Clinic, Department of Cardiology, Sahlgrenska University Hospital

in Gothenburg, Sweden. At the time of the first visit, all the individuals underwent a physical and clinical examination. Clinical profile (including lipid profile, liver panel, and other biochemical parameters) was determined in freshly isolated 12-hours fast plasma or serum at the Department of Clinical Chemistry, Sahlgrenska University Hospital, as previously described.[21] LDL-C after lipid-lowering therapy (namely treated LDL-C) refers to the LDL-C level at the last available assessment. The diagnosis of CVD was performed based on the presence of previous myocardial infarction or stroke (self-reported or present in personal reports). Individuals referred to the lipid clinic for genetic diagnosis have had a myocardial infarction > 5 months ahead of the visit and therefore not in an acute state.

All subjects gave their written informed consent to participate in the study. The study was approved by the regional ethics committee of Gothenburg.

The FH-CEGP Milan cohort

We included 168 adult subjects with genetic diagnosis of FH from the Dyslipidemia Center "E. Grossi Paoletti" of ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy. At the time of the first visit, all the individuals underwent a physical and clinical examination. Biochemical profile was determined after at least 1 month of lipid-lowering treatment suspension in freshly isolated 12-hour fast plasma or serum as previously described.[22] LDL-C after lipid-lowering therapy (namely treated LDL-C) refers to the LDL-C level at the last available assessment. Cardiovascular events were defined as previous diagnosis of angina, acute myocardial infarction, stroke, and peripheral arterial disease. All subjects gave their written informed consent to use their clinical data for the study, which was approved by the ethics committee of ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy (approval no 19-022016).

Genetic diagnosis of FH

For the FH-Gothenburg cohort, the genetic diagnosis of FH was performed by targeted next-generation sequencing as previously described.[21] Specifically, DNA was isolated from whole blood. Targeted next-generation sequencing was performed using SEQPRO LIPO RS (Progenika Biopharma, Derio, Spain, http://www.progenika.com/) (for the first 165 samples) or using SEQPRO LIPO IS (Progenika Biopharma, Derio, Spain, http://www.progenika.com/) (the remaining samples). The exons and their flanking regions of *LDLR*, *APOB* (exon 26 and 29), *PCSK9,* and *low-density lipoprotein receptor adaptor protein 1* (*LDLRAP1*), or of *LDLR*, *APOB, PCSK9, LDLRAP1, apolipoprotein E* (*APOE*, exon 4), and *signal-transducing adaptor protein 1* (*STAP1*) were sequenced using SEQPRO LIPO RS or by SEQPRO LIPO IS, respectively.

For the FH-CEGP Milan cohort, the genetic diagnosis of FH was performed by Sanger sequencing (first 90 cases) or by targeted next-generation sequencing (the remaining

samples) within the LIpid TransPort Disorders Italian GEnetic Network (LIPIGEN) project.[23]

Plasma Lp(a) measurements

For the FH-Gothenburg cohort, fasting plasma samples were collected at time of enrollment and stored at −80°C. Lp(a) levels were then measured on all samples simultaneously (on April 2018) by immunoturbidimetric assay (Randox laboratory, United Kingdom) using an ABX Pentra400 (Horiba, Japan) analyzer. The precision of the Randox assay has been determined at 2 levels in an in-house verification: 1) at the 0.19 g/L level, a total coefficient of variance (CV) of 2.4% was determined and 2) at the 0.50 g/L level, a total CV of 1.6% was determined. For the present analysis, Lp(a) measurements below the assay detection limit were considered equal to the lower detection limit (10 mg/dL).

For the FH-CEGP Milan cohort, Lp(a) levels were measured on freshly isolated fasting plasma samples at the time of enrollment (since 2015) using the immunoturbidimetric assay (Tina-quant Lipoprotein (a) Gen.2, Roche Diagnostics, Switzerland) using a Cobas c311 autoanalyzer. All samples of this cohort were measured using the same assay. The precision of the Roche assay has been determined in a total CV of 1.7% for 40.7 nmol/L level and a total CV of 1.2% for 156 nmol/L level.

To evaluate the assay agreement between the different methods, a subset of plasma samples (N = 27) from the Gothenburg cohort, was aliquoted, refrozen, and analyzed both in Milan using the Roche assay and in Gothenburg using the Randox assay. The samples were chosen to get a representation of the whole measurement range. The result from this method comparison is presented in Supplement Figure 1.

Statistical analysis

For descriptive statistic, continuous traits are shown as mean and standard deviation or as median and interquartile range. Categorical traits are shown as number and proportion. For continuous traits, *P*-value was calculated by linear regression unadjusted, or adjusted for age, gender, and BMI when appropriate. Non-normally distributed values were log-transformed before entering the model. For categorical traits, *P*-value was calculated by chi-square or by Fisher-exact test as appropriate. Lp(a) levels in patients with FH for the FH-CEGP Milan cohort stratified by CVD status were compared using one-tailed T-test. For assays agreement evaluation, a Bland–Altman plot was generated. To evaluate correlation between the 2 methods, results from the Gothenburg method were plotted against results from the Milan method, a regression line was fitted, and both the Pearson and the Spearman correlation coefficients were calculated. Statistical analyses were performed using the IBM Statistical Package for Social Sciences (IBM SPSS, version 20.0, Inc. Chicago, IL) and GraphPad

Prism7.02. *P*-values <.05 were considered statistically significant.

Results

Characteristics of the FH-Gothenburg cohort

A total of 190 individuals with genetic diagnosis of FH from the lipid clinic, Department of Cardiology, Sahlgrenska University Hospital in Gothenburg, Sweden (namely FH-Gothenburg cohort) were examined. The characteristics of the participants are shown in Table 1. Briefly, individuals were all adults (mean age 46 ± 15 years) with mean BMI 26 ± 4 kg/m², mean LDL-C level before treatment 299 ± 68 mg/dL, and median current Lp(a) levels 24 (10–56) mg/dL. Fifty-one percent of them were men, 10% had hypertension, 3% had diabetes, and 7% were active smokers. Most of the cohort was under statin treatment (85%), whereas 7% (n = 13) was under PCSK9 mAbs treatment alone or in combination with statin (10/13 were treated with PCSK9 mAbs plus statins, 3/13 with PCSK9 mAbs only). Among the 190 genetic-FH patients, 61 had personal history of CVD events (43%) defined as previous stroke or myocardial infarction. Most individuals from the FH-Gothenburg cohort were carriers of mutation in *LDLR* gene (93.7%, n = 178), 4.7% (n = 9) were carriers of mutations in *APOB*, and only a minority of them were carriers of mutations in *PCSK9* (n = 2) or *STAP1* (n = 1) (1.1 and 0.5%, respectively). Most of the individuals of the FH-Gothenburg cohort were carriers of mutations previously described as FH-causative (80% were carriers of mutations described as pathogenic, 12% possibly and 2% probably pathogenic). In addition to these, we detected 7 new mutations with unknown pathogenicity (not previously described as FH-causative mutations) in 10 individuals (6%). The totality of these mutations was detected on the *LDLR*. Specifically, we identified 2 mutations in splicing sites, 1 in-frame deletion, and 4 different amino acid changes (accounting for the 57% on the individuals with mutation of unknown pathogenicity) (Supplementary Table 1).

Individuals with genetic diagnosis of FH and previous CVD have higher Lp(a) levels in the FH-Gothenburg cohort

To test whether individuals with genetic-FH and previous CVD had higher Lp(a), we examined the differences in the clinical characteristics of the study participants stratified by CVD status. We found that individuals with previous CVD had approximately 2-fold increased Lp(a) levels compared with those without CVD (*P* = .037, Fig. 1A). Importantly, we did not detect differences in the untreated or in the treated LDL-C comparing these 2 groups (Fig. 1B). These data suggest that Lp(a) is a risk factor for

Table 1 Characteristics of patients with FH from Gothenburg

Trait	FH Gothenburg
N	190
Women/Men, n (%)	92/95 (49/51)
Homozygosity, compound heterozygosity, or double heterozygosity, n (%)	7 (4)
Age, y	46 ± 15
BMI, kg/m²	26 ± 4
Xanthomas, n (%)*	21 (21)
Xanthelasmas, n (%)	-
Arcus cornealis, n (%)†	8 (8)
Cardiovascular disease, n (%)‡	61 (43)
Active smoking, n (%)	14 (7)
Hypertension, n (%)	19 (10)
Diabetes, n (%)	6 (3)
Plasma lipids	
Total cholesterol, mg/dL	383 ± 78
Triglycerides, mg/dL	79 (72–141)
LDL-C, mg/dL	299 ± 68
HDL-C, mg/dL	55 ± 16
Treated LDL-C§, mg/dL	141 ± 69
Lp(a), mg/dL	24 (10–56)
Liver panel	
AST, U/L	27 (22–34)
ALT, U/L	32 (23–45)
γ-GT, U/L	-
Total bilirubin, mg/dL	-
Other biochemical parameters	
Creatinine kinase, U/L	112 (76–170)
Uric Acid, mg/dL	-
Glucose, mg/dL	100 ± 12
Insulin, mIU/L	9.2 (6.3–14)
HOMA-IR, U	2.2 (1.7–3.7)
Lipid medications	
Statin use, n (%)‖	105 (85)
PCSK9 mAbs use, n (%)	13 (7)
Gene	
LDL-R	178 (93.7)
APOB	9 (4.7)
PCSK9	2 (1.1)
STAP1	1 (0.5)
Pathogenicity	
N	180
Pathogenic	144 (80)
Possibly pathogenic	22 (12)
Probably pathogenic	4 (2)
Unknown#	10 (6)

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment; PCSK9 mAbs, proprotein convertase subtilisin kexin type 9 monoclonal antibodies.

Data are expressed as mean (±SD) or as number (proportion) as appropriate. Lipid profile was measured before starting any treatment.

*Data on xanthomas available for N = 101.

†Data on arcus cornealis available for N = 95.

‡Data on cardiovascular disease available for N = 141.

§Treated LDL-C at last available assessment.

‖Data on statin use available for N = 123.

#The totality of mutation classified as of unknown pathogenicity were detected on *LDLR*.
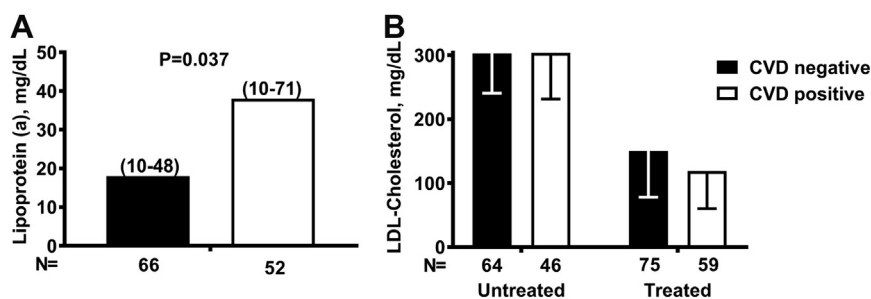
Figure 1   Patients with FH with previous cardiovascular disease events have higher circulating level of Lp(a) in the FH-Gothenburg cohort. Plasma levels of (A) Lp(a) and (B) untreated and treated LDL-C in patients with FH from the Gothenburg cohort stratified by cardiovascular status. Data expressed as median and quartile range or as mean and SD as appropriate. P value has been calculated by linear regression unadjusted. Non-normally distributed traits were log-transformed before entering the model. Lp(a), lipoprotein(a).

CVD in patients with FH even stronger than LDL-C. No differences in age, gender, hypertension, or smoking were found between the 2 groups (Supplementary Table 2). However, genetic-FH individuals with previous CVD had higher BMI (28 ± 5 kg/m^2 vs 26 ± 4 kg/m^2, $P = .031$) and higher prevalence of diabetes (10% vs 0%, $P = .003$). In addition, the proportion of individuals under statin therapy was higher in genetic-FH individuals with CVD than in those without previous CVD.

The response to lipid-lowering therapy does not differ between genetic-FH patients with nonsense or missense mutations in the FH-Gothenburg cohort

To test whether the response to lipid-lowering therapy was different in genetic-FH with nonsense or missense mutations, we firstly examined the absolute and relative LDL-C reduction in the overall FH-Gothenburg cohort stratified by type of mutation. Individuals homozygous for FH-mutations were excluded from the analysis. We found that there were no differences between individuals with nonsense or missense mutations in the absolute nor in the relative LDL-C in the overall FH-Gothenburg cohort (Fig. 2A and B).

Then we examined the absolute and relative LDL-C reduction in individuals after statin or PCSK9 mAbs

therapy separately. We found that there were no differences in the absolute or in the relative LDL-C reduction after statin therapy based on the type of mutation. When we examined the response to the PCSK9 mAbs therapy (alone or in combination with statin), we found a slightly higher absolute and relative LDL-C reduction in individuals with nonsense compared to those with missense mutations suggesting a better response to PCSK9 mAbs therapy in individuals with nonsense mutation. However, this slight difference was not significant. No differences in the anthropometric traits or in CVD, active smoking, hypertension, or diabetes prevalence were found between the 2 groups. However, individuals with nonsense mutation had a more severe phenotype with higher prevalence of tendon xanthomas, and higher total cholesterol and untreated LDL-C (Supplementary Table 3). In addition, the proportion of individuals under statin therapy was higher in carriers of nonsense than in carriers of missense mutation.

Lp(a) is a risk factor for CVD in individuals with genetic diagnosis of FH and the type of FH mutation does not affect the response to statin therapy in an independent genetic-FH cohort

To confirm our finding in the FH Gothenburg cohort, we used an independent genetic-FH cohort from Milan, Italy (namely FH CEGP Milan cohort). A total of 168
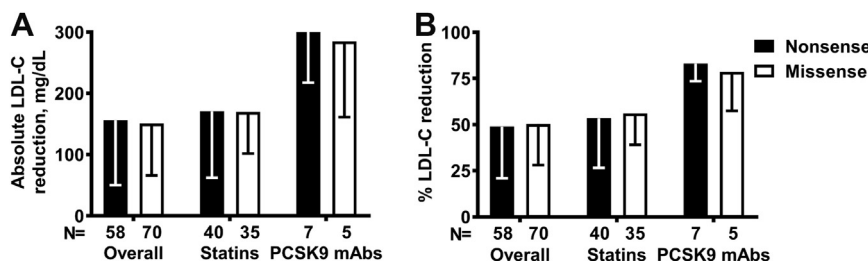

Figure 2   The response to lipid-lowering therapy does not differ between patients with FH with nonsense or missense mutations in the FH Gothenburg cohort. (A) Absolute and (B) relative LDL-C reduction according to type of mutations in patients with FH from the Gothenburg cohort. Data are expressed as mean $\pm$ SD. P value calculated by linear regression unadjusted. A total of 10 FH patients were simultaneously under statin and PCSK9 mAbs treatment. Individuals homozygous for FH mutations have been excluded from the analysis. LDL-C, low-density lipoprotein cholesterol; FH, familial hypercholesterolemia; PCSK9, proprotein convertase subtilisin/kexin type 9.

individuals with genetic diagnosis of FH from Dyslipidemia Center "E. Grossi Paoletti" ASST Grande Ospedale Metropolitano Niguarda were included in the present study. Anthropometric, clinical, and biochemical characteristics of the FH CEGP Milan cohort are shown in Table 2. Briefly, 60% of them were men, 20% had hypertension, and 4% had diabetes. When we compared the 2 independent cohorts, we found that individuals from the FH-CEGP Milan cohort were younger (39 ± 17 years vs 46 ± 15, $P < .0001$), with lower BMI (24 ± 4 kg/m^2 vs 26 ± 4 kg/m^2, $P = .002$), and with a better liver and glucose profile (Supplementary Table 4). However, individuals from the FH-CEGP Milan cohort had a more severe phenotype with higher prevalence of tendon xanthomas and arcus cornealis, and with higher levels of total cholesterol, LDL-C (both untreated and treated LDL-C), and Lp(a). The proportion of individuals under statin therapy and the prevalence of CVD were lower in the FH-CEGP Milan cohort, whereas the number of individuals under PCSK9 mAbs therapy was higher than the FH-Gothenburg cohort. For Lp(a) measurements, we have evaluated the assay agreement between the 2 methods and, lack of uniformity was demonstrated (Supplementary Fig. 1). Nevertheless, both assays performed sufficiently well to demonstrate the association of Lp(a) with CVD in the respective cohorts.

To confirm our finding on the increased Lp(a) levels in genetic-FH with previous CVD, we examined the differences in the clinical characteristics of the FH-CEGP Milan cohort stratified by CVD status. Consistently with our results in the FH-Gothenburg cohort, we found that individuals with previous CVD had approximately 2-fold increased Lp(a) levels compared with those without CVD (one-tailed $P = .045$, Fig. 3A) in the FH-CEGP Milan cohort. Importantly, we did not detect differences in the untreated nor in the treated LDL-C comparing these 2 groups (Fig. 3B).In the FH-CEGP Milan cohort, genetic-FH individuals with previous CVD were older, with a higher BMI, and higher prevalence of hypertension than those without previous CVD (Supplementary Table 5). In addition, HDL level and the proportion of active smokers were lower in individuals with previous CVD than in those without. No other differences in biochemical traits were detected between the 2 groups.

These results support our findings in FH-Gothenburg cohort and confirm Lp(a) as risk factor for CVD in individuals with genetic diagnosis of FH.

To confirm that the response to lipid-lowering therapy does not differ between genetic-FH patients with nonsense or missense mutations, we examined the absolute and relative LDL-C reduction in the FH-CEGP Milan cohort stratified by type of mutation. Individuals homozygous for FH mutations were excluded from the analysis. We found that there were no differences between individuals with nonsense or missense mutations in the absolute nor in the relative LDL-C in the overall FH-CEGP Milan cohort (Fig. 4A and B). The same result was obtained when only individuals under statin therapy were analyzed. When we

Table 2 Characteristics of patients with FH from FH-CEGP Milan cohort

Trait	FH CEGP Milan
N	168
Women/men, n (%)	68/100 (40/60)
Homozygosity, compound heterozygosity, or double Heterozygosity, n (%)	7 (4.2)
Age, y	39 ± 17
BMI, kg/m^2	24 ± 4
Xanthomas, n (%)[*]	95 (59)
Xanthelasmas, n (%)	14 (8)
Arcus cornealis, n (%)[†]	44 (27)
Cardiovascular disease, n (%)	39 (23)
Active smoking, n (%)	24 (14)
Hypertension, n (%)	33 (20)
Diabetes, n (%)	7 (4)
Plasma lipids	
Total cholesterol, mg/dL	394 ± 85
Triglycerides, mg/dL	114 (79–155)
LDL-C, mg/dL	316 ± 82
HDL-C, mg/dL	51 ± 14
Treated LDL-C[‡], mg/dL	162 ± 69
Lp(a), mg/dL	21 (6–51)
Liver panel	
AST, U/L	20 (17–26)
ALT, U/L	20 (15–28)
γ-GT, U/L	16 (12–25)
Total bilirubin, mg/dL	0.63 (0.44–0.93)
Other biochemical parameters	
Creatinine kinase, U/L	109 (75–164)
Uric acid, mg/dL	4.9 ± 1.3
Glucose, mg/dL	87 ± 18
Insulin, mIU/L	-
HOMA-IR, U	-
Lipid medications	
Statin use, n (%)[§]	92 (67)
PCSK9 mAbs use, n (%)	26 (15)
Gene	
LDL-R	168 (100)
APOB	
PCSK9	-
STAP1	-
Pathogenicity	
N	168
Pathogenic	156 (93)
Possibly pathogenic	6 (4)
Probably pathogenic	2 (1)
Unknown[‖]	4 (2)

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment; PCSK9 mAbs, proprotein convertase subtilisin kexin type 9 monoclonal antibodies.

Data expressed as mean (±SD), median and quartile range or as number (proportion) as appropriate. Lipid profile was measured before starting any treatment.

*Data on xanthomas available for N = 162.

†Data on arcus cornealis available for N = 162.

‡Treated LDL-C at last available assessment.

§Data on statin use available for N = 140.

‖The totality of mutation classified as of unknown pathogenicity were detected on *LDLR*.
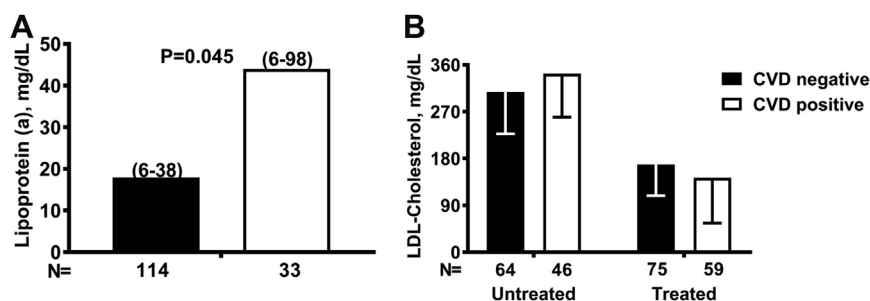
Figure 3 Patients with FH with previous cardiovascular disease events have higher circulating level of Lp(a) in the FH CEGP Milan cohort. Plasma levels of (A) Lp(a) and (B) untreated and treated LDL-C in FH patients from the CEGP Milan cohort stratified by cardio-vascular status. Data expressed as median and quartile range or as mean and SD as appropriate. *P* value has been calculated by one-tailed T-test. Non-normally distributed traits were log-transformed before entering the model. Lp(a), lipoprotein(a); LDL-C, low-density lipoprotein cholesterol; FH, familial hypercholesterolemia.

examined the response to the PCSK9 mAbs therapy, we found a slightly higher and borderline significant relative LDL-C reduction in individuals with nonsense compared with those with missense mutations (Fig. 4B, *P* = .044). The same trend was observed in the absolute LDL-C reduction although not significant. No other differences between the 2 groups were detected except for higher prevalence of active smokers and higher Lp(a) levels in individuals with nonsense than in those with missense mutation (Supplementary Table 6). These results support our finding in the FH-Gothenburg cohort and suggest that the type of FH mutation does not affect the response to statin therapy. However, it seems that individuals with nonsense mutations have a better response to PCSK9 mAbs therapy resulting in a higher relative LDL-C reduction.

Discussion

In this work, we show that individuals with genetic diagnosis of FH and previous cardiovascular events have higher Lp(a) levels than those without CVD in 2 independent cohorts. Lp(a) is an independent risk factor for CVD[24–28] and high levels of this lipoprotein may contribute to the variability of the onset of CVD observed in individuals with FH. Our result is in line with previous finding

describing higher Lp(a) in individuals with genetic diagnosis of FH and previous CVD events.[20,29] In these studies, the genetic diagnosis of FH was performed using microarray containing a selection of mutations in *LDLR* or *APOB*,[30] or by unspecified DNA tests.[29] In the present study, we examined Lp(a) levels in 2 independent cohorts in which the genetic diagnosis of FH has been performed by targeted next generation sequencing or Sanger sequencing. Specifically, in the FH-Gothenburg cohort, we performed genetic diagnosis of FH by screening comprehensively the main FH-causative genes (namely, *LDLR*, *APOB*, *PCSK9*, and *LDLRAP1*, or *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *APOE*, and *STAP1*). In this cohort, 93.7% of the individuals with genetic diagnosis of FH were carriers of mutation on *LDLR*; 4.7% were carriers of mutations on *APOB*; and only a minority of them were carriers of mutations on *PCSK9* or *STAP1* (1.1 and 0.5%, respectively). We identified 2 individuals homozygote for *LDLR* mutation (LDLR p.Gly505Asp, or LDLR p.Glu408Lys), one compound heterozygote (carrying the LDLR p.Ser637Cys and LDLR p.Asn665Lys), and 4 double heterozygote (LDLR p.Cys296*plus PCSK9 p.Arg46Leu; LDLR p.Cys261Phe plus *PCSK9* c.524-4A>G, LDLR c.2390-2A>G plus APOB p.Arg3527Gln, and APOB p.Arg3527Gln plus PCSK9 p.Arg46Leu). Most of the individuals were carriers of mutations previously
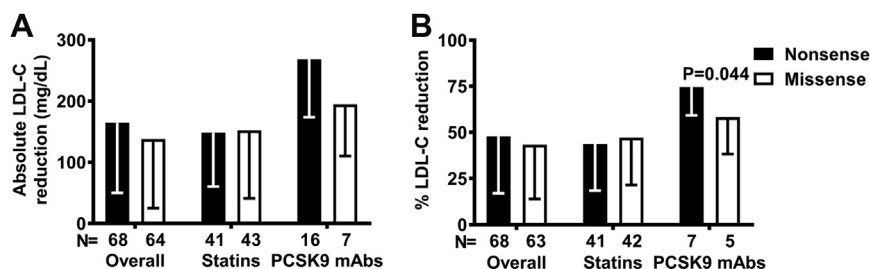

Figure 4 The response to lipid-lowering therapy does not differ between patients with FH with nonsense or missense mutations (with the exception for the PCSK9 mAbs) in the FH CEGP Milan cohort. (A) Absolute and (B) relative LDL-C reduction according to type of mutations in patients with FH from the Gothenburg cohort. Data are expressed as mean ± SD. *P* value calculated by linear regression unadjusted. Individuals homozygous for FH mutations have been excluded from the analysis. PCSK9, proprotein convertase subtilisin/kexin type 9; LDL-C, low-density lipoprotein cholesterol; FH, familial hypercholesterolemia.

described as pathogenic, possibly pathogenic or probably pathogenic (80, 12, and 2%, respectively). In addition, our unbiased approach allowed us to detect 7 novel mutations (not previously described in patients with FH) in 10 (6%) individuals from the FH-Gothenburg cohort. Of the 7 novel mutations, 5 where resulting in an amino acidic change, 1 in an in-frame deletion, and 1 occurred in a splicing site. Pathogenicity of these mutations should be confirmed.

In the FH-CEGP Milan, the genetic diagnosis of FH was performed using Sanger sequencing of *LDLR* gene (the first 90 patients), or by targeted next generation sequencing of *LDLR*, *APOB*, *PCSK9,* and *LDLRAP1*. This difference in sequencing methods in the FH-CEGP Milan cohort does not affect the FH diagnosis reliability. However, the comprehensive screening of the main FH-causative genes increases the diagnosis rate and allows the identification of new possible causative mutations. For these reasons, we switched from Sanger sequencing to next generation sequencing to perform the FH genetic diagnosis also in the FH-CEGP Milan cohort. In this cohort, all the individuals were carriers of mutation on the *LDLR*. Of these individuals, 2 were double heterozygous carrying additional mutations on *APOB* or on *PCSK9* (LDLR p.Arg350* plus PCSK9 p.Asp50Asp; LDLR p.Gly343Ser plus APOB p.Thr3826Met plus PCSK9 p.Ala30Gly; LDLR Cys222_Asp227dup plus APOB Thr3754Ile + Thr2749Ala). In addition, 2 novel mutations in 2 subjects have been detected in the FH-CEGP cohort. One is a duplication of the nucleotide 1125 that results in a frameshift mutation (p.Lys376Glnfs*5), and one is an intronic deletion that affects a splicing site (c.1587-2delA). Pathogenicity of these mutations should be confirmed as well.

In both FH-Gothenburg and Milan cohorts, the diagnosis of CVD was performed based on the presence of previous myocardial infarction or stroke. Lp(a) levels were measured by turbidimetric assays performed using frozen plasma or freshly isolated plasma for FH-Gothenburg and Milan, respectively. We have evaluated the assay agreement between the 2 methods and demonstrated a lack of uniformity. Despite the differences in Lp(a) measurement methods between the 2 cohorts, we detected 2-fold higher Lp(a) levels in FH individuals with CVD than in those without CVD in both cohorts. This finding was consistent in the 2 independent cohorts, suggesting that the final finding is comparable between the 2 cohorts and not affected by the type (frozen or fresh) of samples used for the measurements. However, variation due to assay methodology could affect risk assessment in individual patients, accentuating the need to improve calibration and standardization of Lp(a) assays.[31]

The same 2-fold increase in Lp(a) that we detected in individuals with previous CVD, was detected by Alonso et al in individuals with genetic diagnosis of FH and CVD.[20] Nenseter et al, detected a 3-fold increased Lp(a) levels in individuals with genetic diagnosis of FH susceptible to CVD (with premature CVD) compared with those CVD resistant (later or no CVD).[29] Very recently, Ellis et al showed that the combined presence of high Lp(a) and FH confers 5.3-fold increased risk of premature CAD, compared with a 1.9-fold and 3.2-fold increase due to the presence of the 2 factors separately.[32] All together these findings strongly suggest that Lp(a) is a risk factor for CVD in individuals with genetic diagnosis of FH.

When we looked at other clinical characteristics of the individuals with genetic diagnosis of FH stratified based on the CVD status, we observed that those with previous CVD event had higher classical CVD risk factors, namely diabetes, hypertension, and BMI, but we did not detect any difference in smoking status. This is consistent with a previous report on 1690 individuals with clinical diagnosis of FH in which those with previous CVD events had higher classical CVD risk factors with the exception of smoking.[33]

When we stratified the FH cohorts based on type of mutation (nonsense vs missense mutation), we found that individuals with nonsense mutation had an overall more severe phenotype (namely, higher total cholesterol, and LDL-C) in FH Gothenburg with similar trend in FH Milano. This is consistent with the possible presence of residual LDLR activity in missense mutations, which may mitigate the FH phenotype. In other words, nonsense mutations induce a complete loss of function of LDLR and may result in a more severe phenotype; missense mutations alter LDLR activity without inducing a complete loss of function. This results in a possible presence of residual LDLR activity in missense mutations, which may mitigate the FH phenotype.

In addition, we observed that individuals carrying nonsense mutations had higher Lp(a) levels than those carrying missense mutations in the FH CEGP Milan cohort (with a similar but not significant trend in the FH Gothenburg). It has been proposed that *LDLR* mutations result in high Lp(a) levels with a gene-dosage effect.[34] Alonso et al showed that Lp(a) levels were higher in individuals with *LDLR* null allele than in those with defective allele.[20] In this study, only the most prevalent null and defective mutations on the *LDLR* were included in the analysis. Here we extend these findings in 2 independent cohorts (one from Sweden and one from Italy) by a comprehensive analysis of all the nonsense or missense mutations detected on the sequenced genes. Considering that most individuals from our 2 cohorts were carriers of mutations on the *LDLR*, our results together with previous data support the role of LDLR in Lp(a) circulating levels although the mechanisms remain still known.

When we looked at the response to lipid-lowering therapies, we found the same lowering effect of statins irrespective of the type of mutation in both FH cohorts. The efficacy of statins in FH shows high interindividual variability.[5] It has been proposed that this variability may be a function of the type of FH-causative mutation. Previous studies investigated this hypothesis with conflicting results. Most of these studies, consistently with our results, detected similar response to statins irrespective of the

type of mutation.[35–39] However, other studies described a better response to statins in individuals with more deleterious mutations (null allele) than individuals with milder mutations (defective allele),[40–43] and few studies, on the contrary, reported a better response to statins in individuals with mild mutations compared with individuals with deleterious mutations.[44,45] All these previous studies examined the response to weak statins (mostly fluvastatin and simvastatin) stratified by type of mutation of only the *LDLR* gene. In the present study, we examined the response to stronger statins (atorvastatin and rosuvastatin). In addition, we extended the analysis not only at the *LDLR* mutations but also to all the nonsense or missense mutations detected in *LDLR*, *APOB*, *PCSK9,* and *STAP1* gene.

Moreover, for the first time to our knowledge we examined the lipid-lowering efficiency of PCSK9 inhibition stratified by type of mutation. We observed a trend in a better reduction in the LDL-C in carriers of nonsense mutations under anti-PCSK9 treatment alone (FH-Milan cohort) or in combination with statins (FH-Gothenburg cohort). These results need confirmation on larger numbers of patients treated with PCSK9 inhibition.

In conclusion, our results suggest that Lp(a) contributes to CVD onset in individuals with genetic diagnosis of FH. Our finding supports the importance to identify an efficacious therapy to lower Lp(a) in patients with FH to prevent CVD onset or recurrence.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jacl.2019.06.011.

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Appendix

Supplementary Table 1   Description of the mutations of unknown pathogenicity in the *LDLR* gene from the FH-Gothenburg cohort (Ref Seq NM_000527.4)

N (%)*	Exon	Nucleotide change	Amino acid change	Type
1 (5)	6	c.940_940+14del15	NA	SPLICING
2 (10)	7	c.1012T>G	Cys338Gly	AC
2 (10)	8	c.1174T>C	Cys392Arg	AC
1 (5)	9	c.1187-7C>A	NA	SPLICING
2 (10)	10	c.1514G>A	Gly505Asp	AC
1 (5)	12	c.1754T>C	Ile585Thr	AC
1 (5)	13	c.1871_1873delTCA	Ile624del	IN-FRAME INDEL

Cys, cysteine; Gly, glycine; Arg, arginine; NA, not available; Trp, tryptophan; Asp, aspartate; Ile, isoleucine; Thr, threonine; del, deletion; Gln, glutamine.

*Proportion was calculated on the N = 20 total number of individuals with mutation classified as of unknown pathogenicity.

Supplementary Table 2   Characteristics of patients with FH from the FH-Gothenburg cohort stratified by cardiovascular disease status

Trait	FH Gothenburg		P value
	CVD negative	CVD positive	
N	80	61	
Women/men, n (%)	42/37 (53/47)	25/36 (41/59)	.154
Homozygosity, compound heterozygosity or double heterozygosity, n (%)	4 (5)	2 (3)	.698
Age, y	46 ± 14	49 ± 15	.171
BMI, kg/m^2	26 ± 4	28 ± 5	.031
Xanthomas, n (%)[*]	16 (23)	4 (16)	.573
Xanthelasmas, n (%)	-	-	-
Arcus cornealis, n (%)[†]	4 (6)	3 (14)	.360
Active smoking, n (%)	8 (12)	5 (14)	.757
Hypertension, n (%)	8 (10)	9 (19)	.187
Diabetes, n (%)	0	6 (10)	.003
Plasma lipids, mg/dL			
Total cholesterol, mg/dL	391 ± 69	383 ± 84	.269
Triglycerides, mg/dL	88 (67–142)	115 (95–152)	.339
HDL-C, mg/dL	59 ± 18	51 ± 14	.081
Liver panel			
AST, U/L	26 (23–34)	26 (21–34)	.729
ALT, U/L	33 (24–48)	31 (21–46)	.804
γ-GT, U/L	-	-	-
Total bilirubin, mg/dL	-	-	-
Other biochemical parameters			
Creatinine kinase, U/L	118 (76–176)	123 (82–201)	.952
Uric acid, mg/dL	-	-	-
Glucose, mg/dL	99 ± 10	102 ± 15	.149
Insulin, mIU/L	9.7 (8.4–11)	8.6 (5.3–20)	.272
HOMA-IR, U	2.2 (2.0–2.6)	2.1 (1.2–5.3)	.155
Lipid medications			
Statin use, n (%)[‡]	58 (79)	35 (97)	.014
PCSK9 mAbs use, n (%)	4 (5)	9 (15)	.075

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment; PCSK9 mAbs, proprotein convertase subtilisin kexin type 9 monoclonal antibodies.

Data expressed as mean (±SD), median, and quartile range or as number (proportion) as appropriate. Lipid profile was measured before starting any treatment. For continuous traits, P-value calculated by linear regression adjusted for age, gender, and BMI when appropriate. Non-normally distributed values were log-transformed before entering the model. For categorical traits, P-value calculated by chi-square or by Fisher exact test as appropriate.

*Data on xanthomas available for N = 93.

†Data on arcus cornealis available for N = 88.

‡Data on statin use available for N = 109.

Supplementary Table 3   Characteristics of patients with FH from the Gothenburg cohort stratified by type of mutation

	FH Gothenburg		
Trait	Nonsense	Missense	P value
N	89	99	
Women/men, n (%)	42/47 (47/53)	50/48 (51/49)	.602
Homozygosity, compound heterozygosity or double heterozygosity, n (%)	2 (2)	3 (3)	.247
Age, y	44 ± 14	47 ± 15	.203
BMI, kg/m^2	26 ± 4	26 ± 5	.958
Xanthomas, n (%)[*]	18 (35)	3 (6)	<.0001
Xanthelasmas, n (%)	-	-	-
Arcus cornealis, n (%)[†]	5 (11)	3 (6)	.477
Cardiovascular disease, n (%)[‖]	26 (40)	35 (46)	.471
Active smoking, n (%)	4 (7)	10 (17)	.153
Hypertension, n (%)	11 (16)	8 (11)	.382
Diabetes, n (%)	4 (6)	2 (3)	.435
Plasma lipids, mg/dL			
Total cholesterol, mg/dL	391 ± 84	376 ± 71	.020
Triglycerides, mg/dL	88 (71–124)	106 (77–150)	.276
LDL-C, mg/dL	307 ± 73	293 ± 64	.009
HDL-C, mg/dL	54 ± 15	56 ± 17	.083
Treated LDL-C[‡], mg/dL	142 ± 75	139 ± 64	.575
Lp(a), mg/dL	25 (10–53)	21 (10–53)	.518
Liver panel			
AST, U/L	28 (24–36)	26 (22–32)	.443
ALT, U/L	34 (23–45)	29 (23–45)	.557
γ-GT, U/L	-	-	-
Total bilirubin, mg/dL	-	-	-
Other biochemical parameters			
Creatinine kinase, U/L	124 (76–182)	109 (82–159)	.562
Uric acid, mg/dL	-	-	-
Glucose, mg/dL	99 ± 13	101 ± 10	.867
Insulin, mIU/L	9 (7.3–19.6)	9.4 (5.8–11.5)	.247
HOMA-IR, U	2.3 (1.8–5.6)	2.2 (1.3–2.9)	.596
Lipid medications			
Statin use, n (%)[§]	61 (95)	44 (75)	.001
PCSK9 mAbs use, n (%)	7 (8)	6 (6)	.657

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment; PCSK9 mAbs, proprotein convertase subtilisin kexin type 9 monoclonal antibodies.

Data expressed as mean (±SD), median, and quartile range or as number (proportion) as appropriate. Lipid profile was measured before starting any treatment. For continuous traits, P-value calculated by linear regression adjusted for age, gender, and BMI when appropriate. Non-normally distributed values were log-transformed before entering the model. For categorical traits, P-value calculated by chi-square or by Fisher exact test as appropriate.

*Data on xanthomas available for N = 93.

†Data on arcus cornealis available for N = 88.

‡Treated LDL-C at last available assessment.

§Data on statin use available for N = 109.

‖Data available for N = 65 (Nonsense) and N = 76 (Missense).

Supplementary Table 4   Characteristics of patients with FH stratified by center of recruitment

Trait	FH Gothenburg	FH CEGP Milan	P-value
N	190	168	
Women/men, n (%)	92/95 (49/51)	68/100 (40/60)	.100
Homozygosity, compound heterozygosity or double heterozygosity, n (%)	7 (3.7)	7 (4.2)	.061
Age, y	46 ± 15	39 ± 17	<.0001
BMI, kg/m²	26 ± 4	24 ± 4	.002
Xanthomas, n (%)*	21 (21)	95 (59)	<.0001
Xanthelasmas, n (%)	-	14 (8)	-
Arcus cornealis, n (%)†	8 (8)	44 (27)	<.0001
Cardiovascular disease, n (%)‡	61 (43)	39 (23)	<.0001
Active smoking, n (%)	14 (7)	24 (14)	<.0001
Hypertension, n (%)	19 (10)	33 (20)	.146
Diabetes, n (%)	6 (3)	7 (4)	.996
Plasma lipids			
Total cholesterol, mg/dL	383 ± 78	394 ± 85	.033
Triglycerides, mg/dL	79 (72–141)	114 (79–155)	.184
LDL-C, mg/dL	299 ± 68	316 ± 82	.049
HDL-C, mg/dL	55 ± 16	51 ± 14	.488
Treated LDL-C§, mg/dL	141 ± 69	162 ± 69	.023
Lp(a), mg/dL	24 (10–56)	21 (6–51)	.003
Liver panel			
AST, U/L	27 (22–34)	20 (17–26)	<.0001
ALT, U/L	32 (23–45)	20 (15–28)	<.0001
γ-GT, U/L	-	16 (12–25)	-
Total bilirubin, mg/dL	-	0.63 (0.44–0.93)	-
Other biochemical parameters			
Creatinine kinase, U/L	112 (76–170)	109 (75–164)	.483
Uric acid, mg/dL	-	4.9 ± 1.3	-
Glucose, mg/dL	100 ± 12	87 ± 18	<.0001
Insulin, mIU/L	9.2 (6.3–14)	-	-
HOMA-IR, U	2.2 (1.7–3.7)	-	-
Lipid medications			
Statin use, n (%)‖	105 (85)	92 (67)	<.0001
PCSK9 mAbs use, n (%)	13 (7)	26 (15)	.001

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment; PCSK9 mAbs, proprotein convertase subtilisin kexin type 9 monoclonal antibodies.

Data are expressed as mean (±SD) or as number (proportion) as appropriate. Lipid profile was measured before starting any treatment. For continuous traits, P-value calculated by linear regression adjusted for age, gender, and BMI when appropriate. Non-normally distributed values were log-transformed before entering the model. For categorical traits, P-value calculated by chi-square or by Fisher exact test as appropriate.

*Data on xanthomas available for N = 162 and N = 101 for Milan and Gothenburg cohort, respectively.
†Data on arcus cornealis available for N = 162 and N = 95 for Milan and Gothenburg cohort, respectively.
‡Data on cardiovascular disease available for N = 141 for Gothenburg cohort.
§Treated LDL-C at last available assessment.
‖Data on statin use available for N = 140 and N = 123 for Milan and Gothenburg cohort, respectively.

Supplementary Table 5   Characteristics of patients with FH from the FH-CEGP Milan cohort stratified by cardiovascular disease status

Trait	FH CEGP Milan		P value
	CVD negative	CVD positive	
N	129	39	
Women/men, n (%)	60/69 (47/53)	8/31 (20/80)	.003
Homozygosity, compound heterozygosity or double heterozygosity, n (%)	4 (4)	2 (5)	.999
Age, y	36 ± 17	49 ± 14	<.0001
BMI, kg/m^2	24 ± 5	26 ± 4	.021
Xanthomas, n (%)[*]	69 (56)	26 (67)	.244
Xanthelasmas, n (%)	9 (7)	5 (13)	.33
Arcus cornealis, n (%)[†]	27 (22)	17 (44)	.008
Active smoking, n (%)	20 (16)	4 (10)	.001
Hypertension, n (%)	20 (16)	13 (33)	.019
Diabetes, n (%)	3 (2)	4 (10)	.056
Plasma lipids, mg/dL			
Total cholesterol, mg/dL	386 ± 83	422 ± 86	.257
Triglycerides, mg/dL	102 (76–147)	132 (112–197)	.364
HDL-C, mg/dL	52 ± 15	45 ± 12	.029
Treated LDL-C, mg/dL[‡]	168 ± 60	143 ± 87	.182
Liver panel			
AST, U/L	20 (17–26)	21 (18–27)	.934
ALT, U/L	19 (15–27)	24 (16–37)	.939
γ-GT, U/L	15 (12–22)	22 (15–29)	.516
Total bilirubin, mg/dL	0.62 (0.43–0.94)	0.66 (0.49–0.83)	.969
Other biochemical parameters			
Creatinine kinase, U/L	109 (72–174)	110 (89–154)	.184
Uric acid, mg/dL	4.7 ± 1.3	5.3 ± 1.4	.995
Glucose, mg/dL	85 ± 13	95 ± 28	.219
Insulin, mIU/L	-	-	-
HOMA-IR, U	-	-	-
Lipid medications			
Statin use, n (%)[§]	71 (68)	21 (58)	.281
PCSK9 mAbs use, n (%)	15 (12)	11 (28)	.033

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment; PCSK9 mAbs, proprotein convertase subtilisin kexin type 9 monoclonal antibodies.

Data are expressed as mean (±SD), median, and quartile range or as number (proportion) as appropriate. Lipid profile was measured before starting any treatment.

[*]Data on xanthomas available for N = 162.

[†]Data on arcus cornealis available for N = 162.

[‡]Treated LDL-C at last available assessment.

[§]Data on statin use available for N = 140.

Supplementary Table 6   Characteristics of patients with FH from the FH-CEGP Milan cohort stratified by type of mutation

Trait	FH CEGP Milan		P value
	Nonsense	Missense	
N	85	78	
Women/men, n (%)	38/49 (44/56)	30/49 (38/62)	.457
Homozygosity, compound heterozygosity or double heterozygosity, n (%)	2 (2)	5 (6)	.259
Age, y	39 ± 17	39 ± 17	.990
BMI, kg/m^2	24 ± 4	24 ± 5	.941
Xanthomas, n (%)*	49 (57)	44 (59)	.752
Xanthelasmas, n (%)	7 (8)	7 (10)	.749
Arcus cornealis, n (%)†	24 (28)	20 (27)	.901
Cardiovascular disease, n (%)	24 (28)	15 (19)	.209
Active smoking, n (%)	15 (18)	9 (12)	.009
Hypertension, n (%)	17 (20)	14 (18)	.770
Diabetes, n (%)	4 (5)	3 (4)	.999
Plasma lipids, mg/dL			
Total cholesterol, mg/dL	396 ± 79	386 ± 87	.395
Triglycerides, mg/dL	121 (83–167)	112 (78–141)	.104
LDL-C, mg/dL	318 ± 74	308 ± 85	.411
HDL-C, mg/dL	50 ± 13	52 ± 16	.242
Treated LDL-C‡, mg/dL	159 ± 71	164 ± 67	.724
Lp(a), mg/dL	22 (7.3–57)	16 (5.0–47)	.020
Liver panel			
AST, U/L	19 (17–26)	21 (18–26)	.224
ALT, U/L	18 (14–28)	20 (15–29)	.331
γ-GT, U/L	16 (13–26)	16 (12–24)	.346
Total bilirubin, mg/dL	0.59 (0.42–0.93)	0.66 (0.49–0.93)	.458
Other biochemical parameters			
Creatinine kinase, U/L	108 (75–163)	110 (74–163)	.922
Uric Acid, mg/dL	4.8 ± 1.4	4.9 ± 1.3	.456
Glucose, mg/dL	88 ± 21	87 ± 15	.761
Insulin, mIU/L	-	-	-
HOMA-IR, U	-	-	-
Lipid medications			
Statin use, n (%)§	45 (64)	47 (69)	.549
PCSK9 mAbs use, n (%)	16 (23)	8 (12)	.087

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyltransferase; HOMA-IR, homeostatic model assessment; PCSK9 mAbs, proprotein convertase subtilisin kexin type 9 monoclonal antibodies.

Data are expressed as mean (±SD), median, and quartile range or as number (proportion) as appropriate. Lipid profile was measured before starting any treatment.

*Data on xanthomas available for N = 162.

†Data on Arcus cornealis available for N = 162.

‡Treated LDL-C at last available assessment.

§Data on statin use available for N = 140.