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FULL RESEARCH PAPER
Prevention in practice

Contemporary lipid-lowering management and risk of cardiovascular events in homozygous familial hypercholesterolaemia: insights from the Italian LIPIGEN Registry

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See the editorial comment for this article 'Homozygous familial hypercholesterolemia: the impact of novel treatments', by C. Kennedy, https://doi.org/10.1093/eurjpc/zwae018.

Aims The availability of novel lipid-lowering therapies (LLTs) has remarkably changed the clinical management of homozygous familial hypercholesterolaemia (HoFH). The impact of these advances was evaluated in a cohort of 139 HoFH patients followed in a real-world clinical setting.

Methods and results The clinical characteristics of 139 HoFH patients, along with information about LLTs and low-density lipoprotein cholesterol (LDL-C) levels at baseline and after a median follow-up of 5 years, were retrospectively retrieved from the records of patients enrolled in the LIPid transport disorders Italian GEnetic Network-Familial Hypercholesterolaemia (LIPIGEN-FH) Registry. The annual rates of major atherosclerotic cardiovascular events (MACE-plus) during follow-up were compared before and after baseline. Additionally, the lifelong survival free from MACE-plus was compared with that of the historical LIPIGEN HoFH cohort. At baseline, LDL-C level was 332 ± 138 mg/dL. During follow-up, the potency of LLTs was enhanced and, at the last visit, 15.8% of patients were taking quadruple therapy. Consistently, LDL-C decreased to an average value of 124 mg/dL corresponding to a 58.3% reduction ($P_t < 0.001$), with the lowest value (~90 mg/dL) reached in patients receiving proprotein convertase subtilisin/kexin type 9 inhibitors and lomitapide and/or evinacumab as add-on therapies. The average annual MACE-plus rate in the 5-year follow-up was significantly lower than that observed during the 5 years before baseline visit (21.7 vs. 56.5 per 1000 patients/year; $P = 0.0016$).

Conclusion Our findings indicate that the combination of novel and conventional LLTs significantly improved LDL-C control with a signal of better cardiovascular prognosis in HoFH patients. Overall, these results advocate the use of intensive, multidrug LLTs to effectively manage HoFH.

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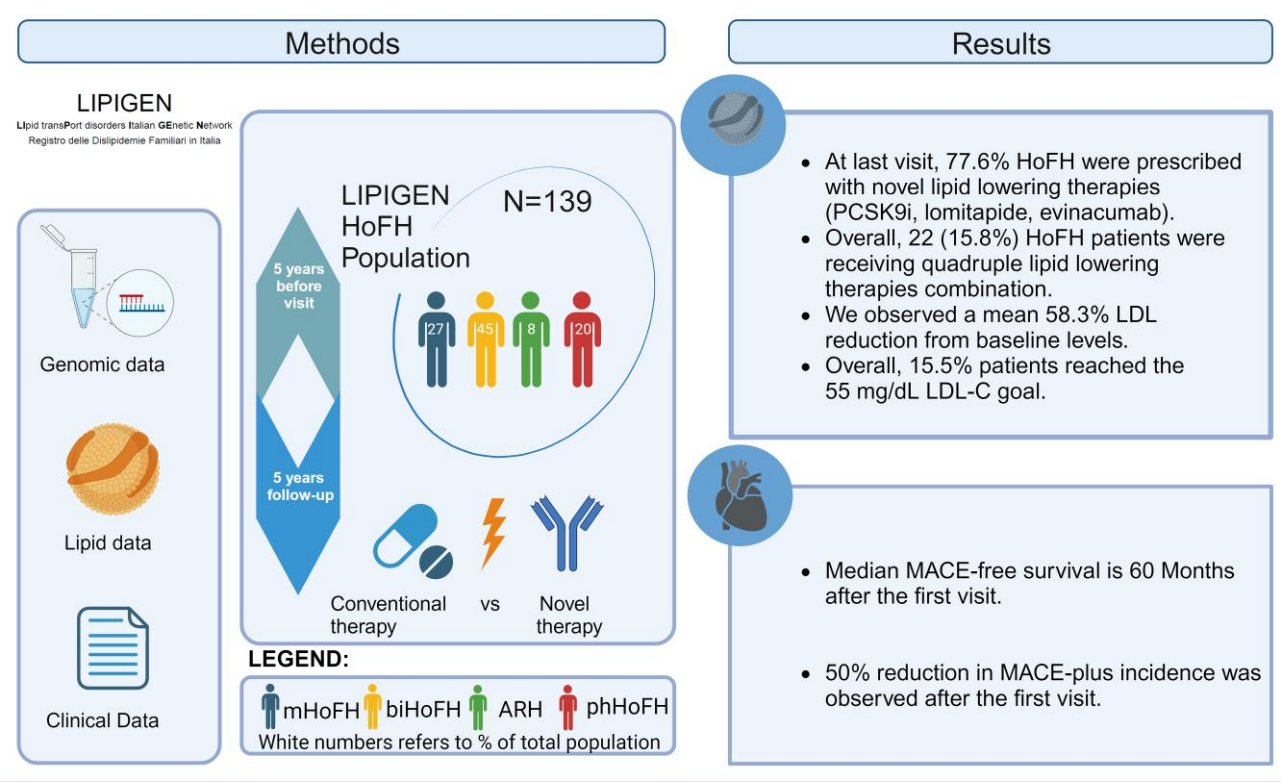
Lay summary Contemporary real-world data from the Italian cohort of patients affected by homozygous familial hypercholesterolaemia demonstrated that the addition of novel, low-density lipoprotein receptor (LDLR)-independent medications to conventional therapies allowed the achievement of unprecedented low-density lipoprotein cholesterol (LDL-C) values with a trend towards a reduction of cardiovascular risk.

Graphical Abstract



Keywords Homozygous familial hypercholesterolaemia • Lipid-lowering therapies • Real-world • PCSK9 inhibitors • Lomitapide • Evinacumab • Cardiovascular risk

Key findings:

- The combination of novel medications with conventional therapies determines a pronounced LDL-C reduction also in homozygous familial hypercholesterolaemia (HoFH) patients carrying the most deleterious mutations.
- The advent of novel drugs ameliorates the prognosis of HoFH patients by improving the control of LDL-C.

Introduction

Homozygous familial hypercholesterolaemia (HoFH) (OMIM #143890) is a rare, genetic form of hypercholesterolaemia affecting ~1 in 300,000 individuals in the population.[1] Typically, HoFH is inherited in a co-dominant fashion and caused by biallelic mutations in genes regulating the activity of low-density lipoprotein receptor (LDLR), which represents the main route of removal of low-density lipoprotein (LDL) particles from the blood.[1] The gene most frequently affected is that coding LDLR (*LDLR*), but HoFH also arises from mutations in *APOB* and *PCSK9* genes.[1,2] The first encodes for the apolipoprotein B (ApoB), the specific ligand for LDLR, whereas the second leads to the production of the protease proprotein convertase subtilisin/kexin type 9 (PCSK9), a plasma protein which reduces the availability of LDLR on the cellular membrane.[1–3] In addition, mutations in the adaptor protein, LDLRAP1, a membrane protein responsible for the proper endocytosis of LDL–LDLR complex by the hepatocyte[4–8] result in the HoFH phenotype with a recessive pattern of inheritance, the so-called autosomal recessive hypercholesterolaemia (ARH).

Independently from the underlying genetic defect, HoFH is phenotypically characterized by increased plasma concentration of LDL that translate in extremely elevated LDL cholesterol (LDL-C) levels (>400 mg/dL) since birth.[1] The lifelong exposure to increased burden of LDL-C leads to an acceleration of the atherosclerotic processes with major atherosclerotic cardiovascular events (MACE) often occurring early in life.[1] Accordingly, guidelines for the management of HoFH recommend early initiation of intensive LDL-lowering intervention to attain LDL-C goals <1.8 mmol/L (<70 mg/dL) in

primary prevention or <1.4 mmol/L (<55 mg/dL) in secondary prevention.[1]

In recent years, the management of HoFH has rapidly changed owing the availability of novel lipid-lowering therapies (LLTs), such as monoclonal antibodies against PCSK9 [PCSK9 inhibitors (PCSK9i)], lomitapide, and, more recently, evinacumab.[1,2,9,10] Lomitapide is a small molecule that inhibits the microsomal triglyceride transfer protein (MTP), thereby inhibiting ApoB lipidation and the secretion of triglyceride-rich lipoproteins from the intestine and liver.[2,11] Evinacumab is a monoclonal antibody that inhibits ANGPTL3,[2,9,12] a known inhibitor of lipoprotein lipase (LPL), the extracellular lipase that processes circulating triglyceride-rich lipoproteins. Familial hypobetalipoproteinaemia type 2 is a genetic condition associated with ANGPTL3 loss-of-function and very-low LDL-C.[13,14] However, the mechanism of LDL-C reduction is still unknown.[9,12] Although these drugs can lead to an additional 50% reduction of LDL-C on top of conventional treatments [e.g. statins, ezetimibe, LDL apheresis (LA)], their use and effectiveness in the real-world clinical practice is not well established. Whether the addition of these medications to the usual care results in a better cardiovascular prognosis or not in HoFH patients is poorly documented. This information is of importance to understand the implementation of therapeutic advances in the management of HoFH and to assess the incremental benefit of these novel LLTs.

To provide an updated evaluation of clinical management and cardiovascular risk of HoFH, we analysed the pattern of LLTs prescriptions, the benefit on LDL-C control, and the occurrence of cardiovascular outcomes in a contemporary cohort of HoFH patients enrolled into the Italian national LIPid transport disorders Italian GEnetic Network-Familial Hypercholesterolaemia (LIPIGEN-FH) Registry.

Methods

Patients' selection

Homozygous familial hypercholesterolaemia patients enrolled into the LIPIGEN-FH Registry were included in the present analysis. The LIPIGEN-FH Registry is an on-going, multicentre, nationwide, observational, registry study of familial hypercholesterolaemia (FH) whose protocol has been reported in detail elsewhere.[4] Briefly, hypercholesterolaemic patients followed-up in the lipid clinic network throughout Italy and suspected to have FH based upon a Dutch Lipid Clinic Network (DLCN) score ≥ 3 were proposed to be included into the registry.[15] After enrolment, patients underwent molecular screening of FH-causing genes (*LDLR*, *APOB*, *PCSK9*, and *LDLRAP1*). Due to the non-interventional nature of the LIPIGEN-FH, none of the enrolled patients received any procedures outside the standard clinical care. The study was approved by ethic committees of participating institutions, and all patients gave their informed consent.

Patients were defined as affected by HoFH based on results of molecular analysis and/or clinical criteria.[1] Criteria of the Medical College of Genetics and Genomics were used to assess pathogenicity of the variants found by molecular analysis.[16] Genotypes were double-checked by investigators (M.C., St.B.), and patients were classified as follows: (i) mHoFH were those carrying two identical pathogenic variants in the same gene, (ii) biHoFH were those showing two different variants in the same gene (one copy each of two different variants, formerly known as compound heterozygotes FH) or variants in two different genes (digenic HoFH, formerly known as double heterozygous FH),[1] (iii) ARH had two copies of the identical variant in *LDLRAP1*, and (iv) phHoFH if the genotype was unknown or if the patient was carrying a single pathogenic heterozygous variant or/and variant of uncertain significance (VUS).[1] If the genotype was unknown or if the patient was carrying heterozygous variant or/and VUS, the recommended clinical criteria for diagnosing HoFH were applied.[1] Among mHoFH, those carrying variants with an estimated ≤2% residual functional activity of LDLR were defined as NULL/NULL, while those showing 2–70% residual functional activity were classified as DEF/DEF.[1] To date, 177 patients with severe hypercholesterolaemia are included into the registry; of these, 139 were classified as HoFH and had available follow-up information; these patients were included in the present analysis. The duration of follow-up was calculated as the time between first (baseline) and the most recent follow-up visit at the lipid centre (last visit), which occurred between July 2005 and March 2022; of note, only 9.4% ($n = 13$) of patients had the last visit prior 2017. The median follow-up duration was 5 years (interquartile range 1–11 years).

Data collection and definition

Individual demographic, clinical, and biochemical characteristics as well as details on LLTs were retrospectively retrieved from medical records by each centre and double-checked by investigators (L.D., M.G., M.C.).

Regarding LLTs, statins, ezetimibe, and LA were classified as conventional whereas monoclonal PCSK9i, lomitapide, and evinacumab as novel therapies. Then, patients' treatments were further categorized into six groups, as follows: (i) *no treatment*, (ii) *statins* (± *ezetimibe*), (*iii*) *apheresis* if the patient was receiving LA with or without statins (± ezetimibe), (iv) *conventional + PCSK9i* if the patient was taking any of the conventional drugs plus PCSK9i, (v) *conventional + lomitapide* if the patient was taking any of the conventional drugs plus lomitapide (among these, eight patients (20.8%) were receiving lomitapide with PCSK9i), and (vi) *conventional + evinacumab* if the patient was taking any drug plus evinacumab [among them, three patients (42.8%) were also receiving PCSK9i, two patients (28.6%) lomitapide, and one patient (14.3%) lomitapide plus PCSK9i).

To gain more information on LDL-C lowering effect, patients receiving novel therapies were further categorized as follows: (i) PCSK9i alone, (ii) lomitapide alone, (iii) lomitapide + PCSK9i, (iv) evinacumab alone, (v) evinacumab + PCSK9i, (vi) evinacumab + lomitapide, and (vii) evinacumab + lomitapide + PCSK9i. These treatments combinations are intended to be in add-on to conventional therapies.

To assess changes in LDL-C, their levels were considered as follows: (i) *untreated* values, corresponding to the highest LDL-C value available in the medical charts while the patient was not receiving any treatment; (ii) *baseline* values, corresponding to those recorded at the first visit; and (iii) *last visit* values, corresponding to those at the last visit at the lipid centre. If available, information on the age of statin therapy initiation and the presence and type of xanthomata were also collected.

Major atherosclerotic cardiovascular events occurring during follow-up were also recorded. Total MACE-plus was defined as a composite of the following: ST segment elevation myocardial infarction (STEMI), non–ST segment elevation myocardial infarction (NSTEMI), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), aortic valve stenosis, aortic aneurysm reparation, stroke, carotid revascularization, peripheral artery revascularization comprehending revascularization of the upper or lower limb arteries, and mesenteric or renal arteries revascularization.[17] Unfortunately, no information was collected on the type of aortic valve disease and, therefore, this information was not included in the analysis.

Statistical analysis

Continuous variables were described by using median and interquartile range [or mean ± standard deviation (SD)] while the categorical variables by frequency counts and corresponding percentages. For comparisons, χ^2 or Fisher's exact tests were used for categorical variables and *t*-test or Mann–Whitney for continuous variables.[8,10]

Logistic regression analysis was used to evaluate the association between several variables and the incident MACE-plus taken as dichotomous outcome (present or absent). Adjustment was made by including in the model the following variables: untreated LDL-C (as a proxy of genotype severity), the presence of history of MACE-plus at baseline, sex, and type of LLTs. Univariate linear regression analysis was used to evaluate the association between several variables and LDL-C levels. Values not normally distributed were *log*-transformed before entering the model.

Incident MACE-plus was described by using Kaplan–Meier survival curves. Curves were analysed in the whole cohort and according to history of MACE-plus. Hazard ratios were assessed to compare survival in groups. Cox proportional hazard model was used to investigate the predictive role of sex, genotype, type of treatment, and history of MACE-plus.

To evaluate whether the enhancement of therapy could have led to an improvement in the cardiovascular prognosis of HoFH patients, the number of MACE-plus observed during follow-up was compared with those recorded in the 5-year period before the baseline visit. This time frame was

Table 1 Baseline characteristics of HoFH patients

n (%)	Whole cohort		Males		Females		*P*-value
	139		71 (51.1%)		68 (48.9%)		
Age at clinical diagnosis, mean ± SD (min–max)	26.2 ± 19.5	(1–76)	25.8 ± 20.6	(1–76)	26.5 ± 18.5	(1–64)	NS
Age at first visit, mean ± SD (min–max)	34.4 ± 20.4	(0–76)	34.4 ± 21.0	(0–76)	34.3 ± 19.8	(1–70)	NS
Genotype							
mHoFH, *n* (%)	37	(26.6)	24	(33.8)	13	(19.1)	NS
ARH, *n* (%)	11	(7.9)	6	(8.45)	5	(7.35)	
BiHoFH, *n* (%)	63	(45.3)	31	(43.7)	32	(47.1)	
PhHoFH, *n* (%)	28	(20.1)	10	(14.1)	18	(26.5)	
Xantomathas, *n* (%)							
Planus	47	(33.8)	20	(28.2)	27	(39.7)	NS
Tuberosus	29	(21.2)	17	(24.3)	12	(17.9)	
Tendineus	83	(61.0)	47	(67.1)	36	(54.5)	
Untreated TC, mg/dL, mean ± SD (min–max)	572 ± 146	(492–1086)	578 ± 152	(258–1023)	564 ± 139	(363–1086)	NS
Untreated LDL-C, mg/dL, mean ± SD (min–max)	490 ± 149	(197–1029)	487 ± 147	(44–800)	494 ± 153	(273–1029)	NS
Baseline TC, mg/dL, mean ± SD (min–max)	406 ± 140	(90–940)	394 ± 155	(90–940)	419 ± 121	(172–866)	NS
Baseline LDL-C, mg/dL, mean ± SD (min–max)	332 ± 137	(44–877)	325 ± 152	(44–877)	340 ± 120	(48–800)	NS
Age at statin start, years, mean ± SD (min–max)	29.6 ± 18.7	(1–69)	28.5 ± 18.4	(2–69)	31.5 ± 19.4	(1–67)	NS
MACE-plus, *n* (%)	46	(33.3)	31	(43.7)	15	(22.4)	0.008
Age of first MACE, years, mean ± SD (min–max)	40.2 ± 11.7	(5–70)	38.8 ± 11.1	(20–65)	43.0 ± 13.0	(21–64)	NS

Data are expressed as mean ± SD, median (IQR), and number (percentage) as appropriate. *P*-value has been provided for comparison between sexes. Data on the history of MACE-plus at baseline were available for 139 patients, and the date of MACE were missing for 5 HoFH patients. Age at clinical diagnosis was missing for 6 patients, and LDL untreated was missing for 26 patients. Complete untreated lipid profiles were available in 106/139 (51 female, 55 male) patients, while complete baseline lipid profiles were available in 126/139 (60 female, 66 male) patients. Age at statin start was missing for 52 HoFH. mHoFH, monogenic homozygous familiar hypercholesterolaemia; biHoFH, biallelic HoFH, phHoFH, phenotypic HoFH; ARH, autosomal recessive hypercholesterolaemia.; LDL-C, low-density lipoprotein cholesterol; MACEs, major atherosclerotic cardiovascular events; SD, standard deviation; NS, not significant.

chosen as it corresponds to the median duration of the follow-up. To this purpose, the annual rates of MACE-plus before and after baseline were calculated as the ratio between the number of MACE-plus counted in each year divided by the sum of patients observed in the reference year by employing a procedure previously reported.[10,18] The annual MACE-plus rates are also reported as average annual rates; the latter one was estimated by calculating the mean of the 5-year annual rates. The cumulative MACE-plus rates were calculated by summing the annual rates reported in the 5 years before and after baseline. The annual rate of MACE-plus was expressed as per 1000 person/year and the cumulative rates of MACE-plus as per 100 person/year.[10,18] The significance of difference between the cumulative annual MACE-plus rates was estimated by using paired *t*-test.

Statistical analyses were performed using the IBM Statistical Package for Social Sciences (IBM SPSS, version 25.0, Inc. Chicago, IL). A *P*-value < 0.05 was considered as statistically significant.

Results

Characteristics of homozygous familial hypercholesterolaemia patients

Clinical and biochemical characteristics of 139 HoFH patients considered in the present analysis are reported in *Table 1*. In the whole cohort, mean age at first clinical diagnosis was 26.2 ± 19.5 years (25.2% of patients being younger than 18 years) and that at baseline was 34.4 ± 20.4 years. Mean level of untreated LDL-C was 491 ± 149 mg/dL, while that at the baseline was 332 ± 138 mg/dL; even though 73.9% of patients were on statins, 55.5% were taking ezetimibe, 18% were on LA, and 10.1% were on PCSK9i (data not shown). The age of initiation of statin therapy was 29.6 ± 18.7 years in the 87 HoFH patients with this information available. At baseline visit, about one-third (33.3%) of patients had already experienced a MACE-plus and this occurred at a mean age of 40.2 ± 11.7 years. No significant differences were found between sexes in the distribution of clinical characteristics except for the prevalence of history of MACE-plus, which was higher among men than women (43.7% vs. 22.4%, $P = 0.008$) (*Table 1*). Men experienced MACE-plus at younger age as compared with women (38.8 ± 11.1 vs. 43.0 ± 13.0 years, respectively), even though the difference was not statistically significant.

In our HoFH cohort, 37 (26.6%) patients were classified as mHoFH and 63 (45.3%) as biHoFH, and 11 (7.9%) were affected by ARH. Among mHoFH, 27 (75%) were predicted to carry defective (DEF/DEF) and 9 (25%) null genotypes (NULL/NULL). Twenty-eight patients (20.1%) were categorized as phHoFH. The complete list of mutations identified is reported in Supplementary material online, *Table S1*.

Clinical and biochemical data of HoFH patients according to genotypes are reported in Supplementary material online, *Table S2*. As expected, mHoFH showed a higher prevalence of xanthomas as well as higher untreated LDL-C levels when compared with the other genotypes ($P_{for\ trend} = 0.001$). Untreated LDL-C values were significantly correlated with genotypes {β 0.383 [95% confidence interval (CI) 28.4–76.2], $P < 0.001$}. Among mHoFH, NULL/NULL patients showed the highest untreated LDL-C levels as compared with DEF/DEF (788.6 ± 129.1 vs. 492.4 ± 109.0 mg/dL) (data not shown). mHoFH patients have the highest prevalence of history of MACE-plus as compared with the other genotypes (51.4% vs. 27.3% in ARH, 27.0% in biHoFH, and 25.0% in phHoFH; $P_{for\ trend} = 0.05$) (see Supplementary material online, *Table S2*). Surprisingly, DEF/DEF patients reported greater prevalence of MACE-plus at baseline as compared with NULL/NULL patients (17% vs. 2%). This could be partially explained

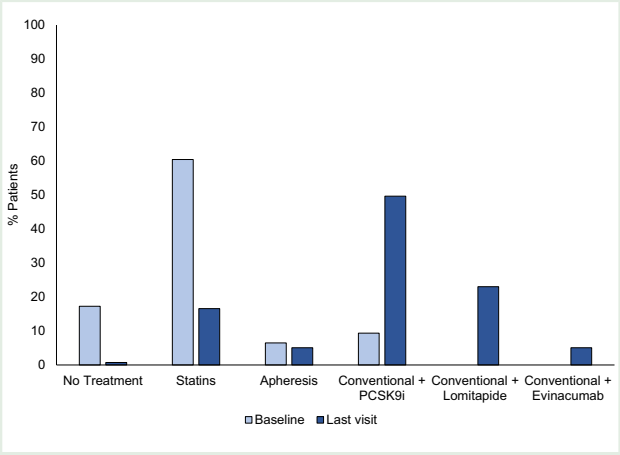


Figure 1 Changes in lipid-lowering therapies during follow-up. Data are represented as percentage. PCSK9i, protease proprotein convertase subtilisin/kexin type 9 inhibitor.

by the significantly earlier initiation of statins in NULL/NULL than in DEF/DEF (4.2 vs. 33.4 years, respectively, $P = 0.008$). Conversely, the mean age of onset of MACE-plus was not significantly different among genotypes (data not shown).

Treatment and low-density lipoprotein cholesterol changes during follow-up

The change in LLTs between baseline and last visit is shown in *Figure 1* and Supplementary material online, *Table S3*. At baseline visit, one-fifth of patients ($n = 24$, 17.3%) were not receiving any medications, but we observed this prevalence dropping to almost zero ($n = 1$) at the last visit. Statins alone or in combination with ezetimibe were prescribed in 62.3% ($n = 84$) of patients at baseline and 16.5% ($n = 23$) at the last follow-up; among these, respectively 63.1% ($n = 53$) and 91.3% ($n = 21$) were also receiving ezetimibe. On the contrary, the proportion of patients on LA (with or without statins) remained stable over time (6.5% vs. 5.0%). The use of LLT combination including PCSK9i, lomitapide, and evinacumab increased remarkably during follow-up (9.4% vs. 77.6% $P < 0.001$). Indeed, PCSK9i were prescribed on top of conventional therapies in 49.6% ($n = 69$) HoFH patients at the last visit vs. 9.4% ($n = 13$) at baseline. Similarly, lomitapide was added in 23.0% ($n = 32$) and evinacumab in 5.0% ($n = 7$) of patients during follow-up.

The distribution of LLT at the last visit in the different genotypes is represented in Supplementary material online, *Figure S1*. Overall, combination treatments including novel therapies were used irrespectively from genotypes, even though the proportion tended to be higher in mHoFH patients. Indeed, all NULL/NULL patients, 89.3% ($n = 25$) of DEF/DEF and 72.7% ($n = 8$) of ARH patients, were receiving novel drugs. Interestingly, while most (>60%) of NULL/NULL and ARH patients were on an association of conventional therapies plus lomitapide, this combination was used in 32.1% ($n = 9$) of DEF/DEF patients. Fifty percent ($n = 14$) of DEF/DEF patients was on PCSK9i. Proprotein convertase subtilisin/kexin type 9 inhibitor was the most used new therapy in both biHoFH and phHoFH, while lomitapide was prescribed in 14.3% of phHoFH patients and evinacumab in 6.3% of patients classified as biHoFH. We found that baseline MACE-plus ($P = 0.033$, $R^2 = 0.54$) and untreated LDL-C ($P = 0.031$, $R^2 = 0.36$), a reflection of severity of underline mutation, were significantly associated with the choice to prescribe novel therapies. When the same model was added with genotypes, these results did not change.

At baseline, mean LDL-C in the whole cohort was 332 ± 138 mg/dL. At the end of follow-up, LDL-C levels were reduced to 124 ± 70.8 mg/dL, corresponding to a 58.3 ± 26.9% decline from baseline and 72.6 ± 16.9% from untreated values (*Figure 2A*). Overall, 46% of the patients achieved an LDL-C level < 100 mg/Dl, but this proportion dropped to 26.4% and 15.5% if the 70 and 55 mg/dL LDL-C goals[12] were considered, respectively (*Figure 2B*). No differences were observed in the LDL-C levels and target achievements between sexes (data not shown). As expected, patients with the NULL/NULL genotype were less responsive to the therapy as demonstrated both by the absolute value of LDL-C achieved at the end of the follow-up and the percentage of achievement of LDL-C goals (*Figure 2C and D*).

Figure 3 describes the efficacy of combination therapy including of novel LTTs. The largest percent LDL-C reductions were observed in patients taking the combination of evinacumab + lomitapide (−66.5%) and evinacumab + lomitapide + PCSK9i (89.5%). Overall, the combination of lomitapide + PCSK9i and evinacumab + lomitapide + PCSK9i resulted in the achievement of an unprecedented LDL-C lowering below 90 mg/dL. It is interesting to note that LLT combinations including lomitapide and evinacumab were used in patients with the higher untreated LDL-C values and these regimens reduced LDL-C up to 104 mg/dL.

Analysis of cardiovascular events

During the entire follow-up, a total of 58 incident MACE-plus have occurred; among these, 13 (32.5%) were recurrent MACE-plus in patients with history of MACE at baseline (*Table 2*). If considering the 5-year follow-up period, a total of 54 incident MACE-plus have occurred in 32 (23.9%) patients.

As expected, the most common MACE component was represented by coronary events (63.7%). The second most common was cerebrovascular events followed by the ultrasound diagnosis of severe aortic valve stenosis or valve replacement (*Table 2*). Four patients died for cardiovascular complications (fatal acute myocardial infarction and acute heart failure) during the follow-up. Coronary events occurred more frequently among mHoFH as compared with the other genotypes (see Supplementary material online, *Table S4*).

The incident MACE-free survival curve during follow-up is reported in *Figure 4*: 50% of HoFH cohort survived MACE-free 60 months (*Figure 4A*). No differences were found between sexes and across genotypes (data not shown), while HoFH patients with history of MACE-plus at baseline showed a significantly lower MACE-free survival as compared with those without history of MACE-plus (217 vs. 355 months; $P = 0.03$) (*Figure 4B*). Consistently, Cox regression analysis demonstrated that the best predictor of incident MACE-plus was the history of MACE-plus (HR = 2.8, $P = 0.010$). Results did not change if we added into the model the type of LLTs.

Because the control of LDL-C improved during follow-up, we tried to find out if these changes translated into a better cardiovascular prognosis. To this aim, we compared the annual MACE-plus rates during a 5-year period before and after initiation of follow-up (*Figure 5*). The annual MACE-plus rate increased from 25.4 in the 5 years before (−5 timepoint) to 138.4 in the year before (−1 timepoint) baseline visit. When the intensification of treatment began, the annual MACE-plus rate declined from 61.5 after the first year (+1 timepoint) to 29.4 at the 5 years timepoint (*Figure 5A*). We also expressed the occurrence of cardiovascular events as cumulative MACE-plus rate curves before and after first visit (*Figure 5B*). From this analysis, it was clear that the intensification of treatment determined a flattening of the cumulative incidence curve, and this effect was estimated to be statistically significant (HR = 0.56; CI 95% 0.12–0.90, $P = 0.0016$). In fact, the average annual MACE-plus rates resulted to be two-fold higher in the 5 years before than that after baseline (21.7 vs. 56.5 1000 patients/year). To determine if intensifying treatment could lead to cardiovascular benefits,

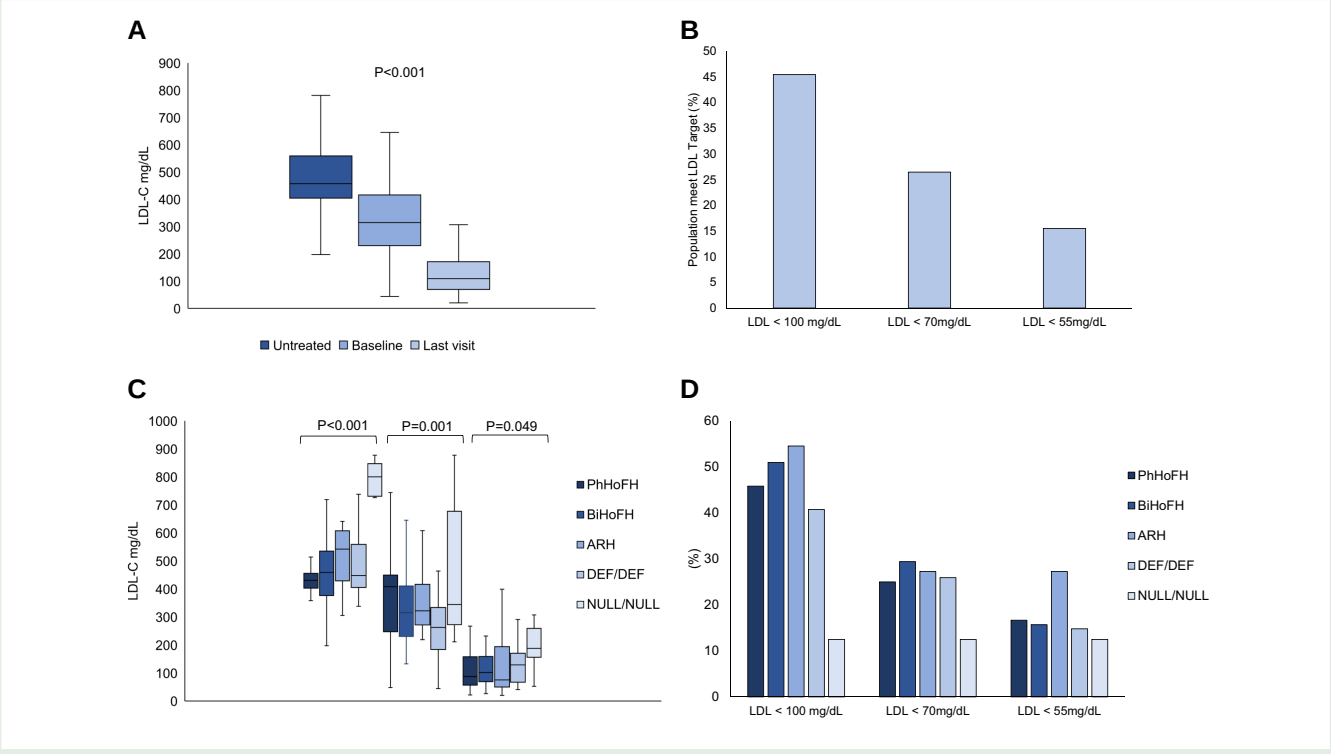


Figure 2 Distribution of untreated and treated LDL-C levels and LDL-C goal achievements at the last follow-up visit in patients with HoFH. LDL-C values (untreated, baseline, and last visit) and the proportion of patients achieving LDL-C goals at the last visit are shown. Data are represented as median (interquartile range) or percentage as appropriate. (*A* and *B*) Data in the whole population. (*C* and *D*) Data according to genotypes. mHoFH, monogenic homozygous familiar hypercholesterolaemia; biHoFH, biallelic HoFH; phHoFH, phenotypic HoFH; ARH, autosomal recessive hypercholesterolaemia; DEF/DEF for monogenic HoFH patients carrying pathogenic variants with predicted LDLR results functional activity between 2% and 70%; NULL/NULL for monogenic HoFH patients carrying pathogenic variants with predicted LDLR results functional activity below 2%; LDL-C, low-density lipoprotein cholesterol.

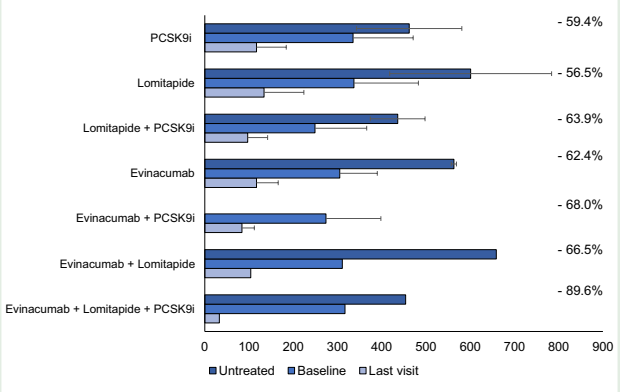


Figure 3 Changes in LDL-C levels during follow-up according to the type of novel LLT combinations. Data are reported as mean and standard deviation. Changes in LDL-C are represented by reporting untreated, baseline, and last visit LDL-C values. On the right side of the graph, the percent reduction of LDL-C from baseline is also represented. The definition of the type of novel LLT is represented as described in Methods. PCSK9i, protease proprotein convertase subtilisin/kexin type 9 inhibitor.

we compared the lifelong MACE-plus risk of these patients with that of the historical LIPIGEN cohort.[5] The contemporary LIPIGEN cohort showed a significantly longer survival free from MACE-plus events compared with the historical cohort (median MACE-plus-free survival 53 vs. 35 years, respectively, HR = 0.42, 95% CI 0.31–0.56, $P < 0.001$). This suggests that the implementation of LLTs delayed the onset of cardiovascular events (see Supplementary material online, *Figure S2*).

Discussion

The main purpose of this real-world study was to describe the use of newer LLTs in HoFH patients and to analyse how these therapies translated into a better LDL-C control. The main findings are the following: (i) at the end of 2022, 77.6% of HoFH patients were receiving at least one novel treatment as add-on to conventional LLTs; (ii) multidrug treatments including novel LLTs allowed the achievement of LDL-C reduction above 80% with the lowest absolute LDL-C levels of 90 mg/dL attained when PCSK9i, lomitapide, and evinacumab were associated together; and (iii) the enhancement of LDL-C lowering efficacy obtained with the use of novel LLTs appeared to significantly reduce the cardiovascular risk of HoFH patients.

Patients included in this analysis were part of the Italian HoFH cohort enrolled into the LIPIGEN Registry, and 67 of them (48.2%) have not been previously reported.[5,8,17] Their clinical and biochemical

Table 2 Total number and types of incident MACE-plus occurred during follow-up in HoFH patients

	Whole cohort ($n = 139$)	Male ($n = 85$)	Female ($n = 64$)
Total MACE-plus, *n*	58	35	23
Coronary events, *n* (%)	37 (63.7)	25 (71.4)	12 (52.2)
Extracoronary events, *n* (%)	3 (5.1)	0 (0)	3 (13.0)
Cerebrovascular events, *n* (%)	8 (13.7)	6 (17.1)	2 (8.7)
Severe aortic valve stenosis, *n* (%)	6 (10.3)	3 (8.6)	3 (13.0)
Cardiovascular deaths, *n* (%)	4 (6.9)	1 (2.9)	3 (13.0)

MACE-plus, major atherosclerotic cardiovascular events plus carotid revascularization, aortic aneurysm stenting, severe aortic valve stenosis and peripheral artery revascularization, or definite thrombosis.

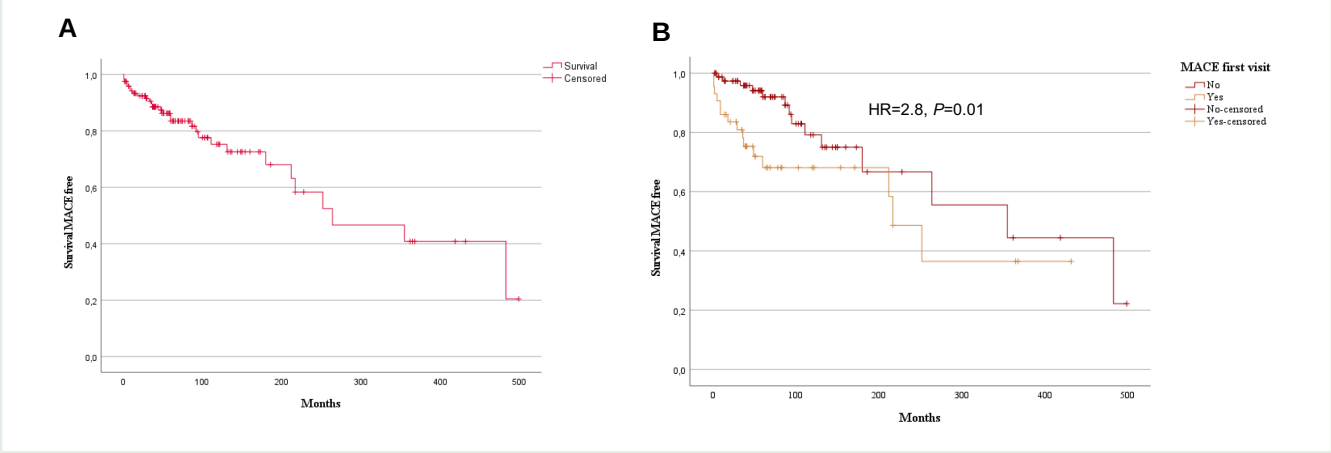


Figure 4 Incident MACE-plus-free survival curves. Kaplan–Meyer curves show incident MACE-free survival. (*A*) Data in the whole cohort. (*B*) Differences in survival among patients with and without medical history of MACE-plus at baseline. MACE-plus, major atherosclerotic cardiovascular event.

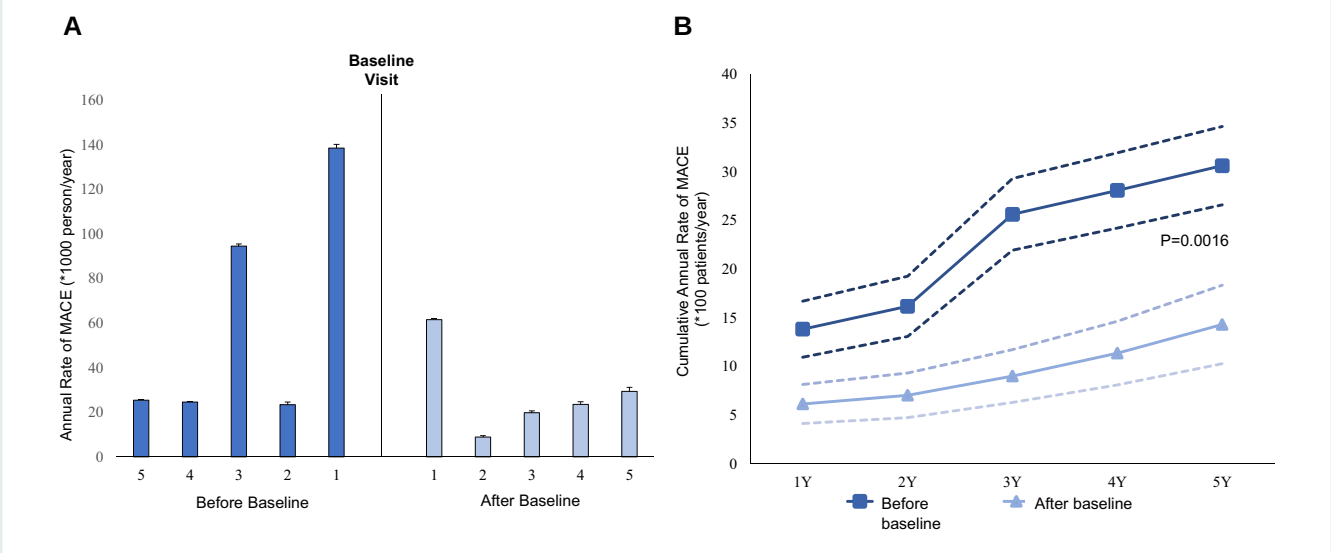


Figure 5 MACE-plus rates before and after baseline. MACE-plus in the HoFH cohort are reported in the 5 years before and after baseline visit. (*A*) Annual rate of MACE-plus calculated as the ratio between the number of MACE-plus collected and the reference observed population (see Methods for details). (*B*) Curves of cumulative annual rates of MACE-plus before and after baseline. Dotted lines represent the standard errors. Paired *t*-test was used to compare the cumulative annual MACE-plus rates before and after baseline.

characteristics well recapitulate the typical features of HoFH. Many patients (79.8%) had a molecularly confirmed diagnosis of HoFH while, in the remaining, the diagnosis was based on clinical criteria.[1] As expected, despite the relatively young age, one-third of patients presented with a MACE-plus at baseline visit and the mean age of first event was 40.2 ± 11.7 years, thus confirming the high cardiovascular risk of these patients.

Reports on how the novel LLTs are entering into the contemporary care of HoFH are very limited. A recent analysis carried out among HoFH patients (16 children and 51 adults with either a clinical or a genetic diagnosis of HoFH) enrolled into the CASCADE FH Registry[19] showed that, at last follow-up visit, PCSK9i, lomitapide, and evinacumab were prescribed in 71.8%, 23.1%, and 15.4% of patients, respectively. These figures are different from those observed in our Italian cohort, where the administration of lomitapide was higher (23.0%, $n = 32$) and that of evinacumab lower (5.0%, $n = 7$). Moreover, in three patients, these two treatments were combined. Albeit new approaches are being used, these data indicate differences between countries and physicians' attitudes to drug selection as well as regulatory limits to prescription. An interesting observation from our survey was that the physicians' decisions to use newer therapies were not guided by the knowledge of HoFH genotype but by the severity of clinical phenotype. Consistently, multivariate analysis highlighted that high LDL-C and the history of MACE-plus were the only significant predictors of the choice of novel therapies.

A crucial aspect related to the use of newer LLTs is how they can improve LDL-C levels in HoFH patients. Again, only little real-world data are available in this regard, especially for those drugs whose mechanism of action does not depend on the activity of the LDL (e.g. lomitapide and evinacumab). In the recently published work by the Italian and European Working group on lomitapide,[10] it was reported in a cohort of 75 HoFH that the add-on of lomitapide reduced, independently of the genotype, the LDL-C by a mean of 60% from baseline. In the open-label extension of the Phase 3 trial, the use of evinacumab in seven Italian HoFH reduced LDL-C from baseline of 54.4%, 48.9%, −49.4%, and −46.8%, at 6, 12, 18, and 24 months after initiation, respectively.[20] Moreover, among the six adults who entered an open-label study with evinacumab while enrolled into CASCADE FH Registry,[19] a 50% LDL-C decrease was reported.[19] These findings are consistent with results from placebo controlled studies.[11,20] However, current guidelines suggested that the treatment of HoFH patients should follow a stepwise approach, starting with conventional LLTs (statins, ezetimibe) and, only then, the addition of novel therapies should be considered, choosing PCSK9i as first-line drug.[1] The effectiveness of this strategy in bringing LDL-C close to the recommended goals is partially known. A recent observation describing the results of the use of PCSK9i in an Italian HoFH population indicated that none of the 11 biHoFH/mHoFH achieved LDL-C goals.[21] Not surprisingly, the limited effectiveness of PCSK9i in lowering LDL-C in HoFH (about 25%) has been already demonstrated by others[22,23] and relates the fact that these drugs fail to be fully effective in the presence of low or absent residual LDLR functionality, a condition that occurs in NULL/NULL or ARH genotypes.[5,22,24] In our cohort, ∼15% of HoFH patients belonged to these categories. Additionally, DEF/DEF HoFH patients had significantly elevated LDL-C levels at baseline (262 mg/dL) despite treatments. This suggests a low likelihood that PCSK9i alone will be effective in achieving LDL-C goals when used in conjunction with conventional therapies.

On the other hand, it has been convincingly demonstrated that the combination of several LLTs can markedly improve the lipid control in HoFH. Tyco *et al.*[17] showed that HoFH patients taking multiple LLTs were those with the highest probability of achieving LDL-C goals, with those taking three or four LLTs achieving LDL-C goals in a percentage ranging from 16.7% to 18.9%. However, in this survey, most patients were treated with conventional LLTs. Yet, also, the analysis of CASCADE FH Registry,[19] where novel LLTs were more frequently used, reported that the lowest LDL-C levels were reached by using a greater number of LLTs. Of note, HoFH patients in the CASCADE FH Registry reached at the end of follow-up an overall LDL-C level of 127 mg/dL, a figure very close to that observed in our cohort. However, the present analysis provided more detailed information showing that the combination of lomitapide and evinacumab allowed to reach LDL-C levels below 90 mg/dL, with the increase to 60% of patients who attained their LDL-C goals. To this regard, it is worth mentioning that in a 52-year-old HoFH woman, the addition of lomitapide and evinacumab determined a remarkable improvement in LDL-C levels, the disappearance of xanthomata, and the regression in atherosclerotic plaques.[25] Altogether, these observations may open a completely new scenario where treatments including LDLR-independent medications should be ideally considered as the first-line choice in the care of HoFH patients with the more severe LDL-C elevation. In this perspective, pharmacoeconomic studies are needed to definitively establish the cost–benefit of these high-cost treatment combinations.

This survey was not designed to primarily test changes in cardiovascular risk as consequence of improvement in LDL-C control. Nevertheless, we have compared the annual rates of MACE-plus preceding and following the implementation of LLTs. By using this approach, we were able to show an overall 60% reduction of MACE-plus rate during the 5-year after the baseline visit as compared with the 5 years before, confirming the potential cardiovascular benefit of increasing the LDL-lowering potency of treatment. This was further supported by the evidence that the curve of increase in the cumulative MACE-plus rate has flattened during follow-up, demonstrating a definite delay in the occurrence of cardiovascular outcome in HoFH patients more aggressively treated. Our observations are in line with the results of investigations demonstrating that the long-term cardiovascular protection of HoFH patients is strongly dependent on the levels of LDL-C obtained with cholesterol-lowering therapy.[23,24] Despite these promising progresses, larger and longer investigations are needed to definitively establish the cardiovascular benefit of the multidrug approach in HoFH.

Limitations

Although significant for the comprehension of changes in the real-world, contemporary treatment of HoFH, our study has several limitations. The nature of the LIPIGEN Registry may have introduced bias in the choice of therapies. In fact, as within the LIPIGEN Registry, the vast majority of HoFH patients was followed in highly specialized, tertiary lipid centres, caring physicians may have a higher propensity to offer more powerful and advanced treatment regimens. Moreover, the new therapies were progressively added during the entire duration of the follow-up and therefore the time of exposure to these drugs was highly variable and difficult to be determined. Nevertheless, the comparisons we performed between baseline and the last visit should provide representative information of the overall change that occurred in the care of these patients. Again, the adherence as well as of the persistence of HoFH patients to different regimens have not been evaluated. Furthermore, the follow-up duration within patients included in the LIPIGEN cohort was variable and there might be dissimilarities in the patient's management in the different LIPIGEN centres. Finally, the number of patients taking multidrug therapies was rather limited, thus reducing the statistical power of baseline vs. follow-up comparisons.

When interpreting data on MACE-plus, caution should be exercised due to potential biases and confounders in the data model used. Additionally, the retrospective nature of the study means that events prior to and following the baseline were collected by reviewing medical charts as recorded by the responsible physicians, without being adjudicated or reviewed by an independent team of researchers.

Furthermore, the text lacks information regarding LLT or concomitant drugs used for cardiovascular protection. It is worth noting that all HoFH patients included in this survey were referred to tertiary lipid clinics by cardiologists or general practitioners, who had already initiated standard LLT in over 70% of the patients in the LIPIGEN cohort. The main prescription during follow-up in specialized centres was novel cholesterol-lowering drugs authorized for the treatment of HoFH. Regrettably, precise information regarding the exact timepoints of the introduction of these new therapies was not available. Therefore, we compared the therapy at the time point when it was deemed to be optimized by the responsible physicians (first and last visit). It should be noted that the comparison of rates of MACE-plus occurrence may be biased by the fact that patients were potentially exposed to high LDL-C levels for a longer time before baseline, since birth, compared with the levels achieved during follow-up[22]. However, the comparison with the historical LIPIGEN cohort supports the data indicating the benefits of innovative therapies over current conventional treatments.

We must acknowledge that another limitation of the study is that there might be several HoFH patients that are not included in the present registry. The LIPIGEN is a registry run by SISA foundation, and the participation to the Lipid Clinic Network is spontaneous. Unfortunately, we cannot provide information on how HoFH patients outside the Lipid Clinic are currently managed, but we could estimate that the population included in this paper represents about the 70% of the Italian HoFH patients.

Conclusions

Homozygous familial hypercholesterolaemia is still an underdiagnosed and undertreated disease. Here, we show that the use of multidrug LLT, also including those drugs acting through LDLR-independent mechanisms, is expanding in the real-world management of these patients. Moreover, we provide evidence that combining conventional with novel LLTs LDL-C can be reduced to unprecedented values even in patients with the more severe phenotype. Finally, we were able to detect signals supporting the potential benefit on cardiovascular risk deriving from the combination of novel therapies in HoFH.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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Author contribution

L.D., manuscript conceptualization, data collection and analysis, and manuscript writing and revision; Si.B., data analysis and manuscript writing; Mar.A., data analysis and manuscript conceptualization, writing, and revision; M.C. and M.G., database curation and data verification and manuscript revision; St.B., manuscript conceptualization, revision of genotyping, and patient enrolment; St.B., S.C., P.T., Mau.A., and A.L.C., manuscript conceptualization, writing, and revision; G.I., patient enrolment, data verification, and manuscript writing and revision; G.F., patient enrolment, revision of genotyping, and manuscript writing and revision; and the LIPIGEN HoFH group, patient enrolment and manuscript revision.

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Data availability

The data underlying this article cannot be shared publicly for the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

Appendix

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