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A cross-national investigation of cardiovascular survival in homozygous familial hypercholesterolaemia: the Sino-Roman Study

Running title - Survival in hoFH: the Sino-Roman Study

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ABSTRACT

Background: Homozygous familial hypercholesterolaemia (hoFH) is a rare inherited disorder characterised by extreme elevation of low-density lipoprotein (LDL)-cholesterol, accelerated coronary artery disease (CAD) and premature death. Aggressive LDL-cholesterol lowering therapies are important for survival but these are not available worldwide.

Objective: To compare and contrast cardiovascular outcomes and mortality of hoFH patients in two countries with disparate use of lipoprotein apheresis (LA) and modern therapies for lowering LDL-cholesterol.

Methods: A retrospective study was undertaken comparing cardiovascular disease (CVD)-free survival and mortality in 44 hoFH patients who were treated statins but not LA, from a centre in Beijing, China, and 18 hoFH patients who were treated with LA and novel therapies from an early age, from a centre in Rome, Italy.

Results: CVD-free survival and survival was significantly reduced in the Chinese patients compared with the Italian patients after 30 years of follow-up (log-rank $p<0.01$). In a pooled analysis, cardiovascular survival was significantly increased with earlier age at treatment, longer duration of treatment and lower on-treatment LDL-cholesterol concentrations ($p<0.05$). Additionally, the probability of a CVD event and death were increased in patients that carried a null mutation in the *LDLR* or had elevated lipoprotein(a).

Conclusions: We show that CAD outcomes in patients with hoFH can be significantly improved with earlier and potent LDL-cholesterol lowering with

pharmacotherapies and LA. This has major implications for countries, such as China, where the models of care for hoFH remains underdeveloped.

Keywords: familial hypercholesterolaemia; homozygous; China; Italy; lipoprotein apheresis; novel therapies

HIGHLIGHTS

- We identified important gaps in the care of hoFH patients in China and Italy
- Chinese patients had significantly reduced survival compared with Italian patients
- Efficacious therapies (eg. drugs and LA) are crucial to improve outcomes in hoFH

INTRODUCTION

Homozygous familial hypercholesterolaemia (hoFH) is a rare disorder caused by bi-allelic mutations of genes affecting the low-density lipoprotein (LDL) receptor pathway, which result in reduced apolipoprotein B clearance and extremely elevated plasma levels of LDL-cholesterol. If untreated, hoFH accelerates the development of coronary artery disease (CAD) and results in premature death. Recent studies have demonstrated that survival in hoFH patients is improved in proportion to the extent of reduction in LDL-cholesterol, achievable using different treatments[1]. Plasma LDL-cholesterol concentration in hoFH may be lowered by diverse, conventional and affordable therapies, including statins, ezetimibe and resins, but these are ineffective in achieving recommended treatment targets[2]. Additional therapies are therefore required, such as lomitapide, PCSK9 inhibitors and non-pharmacological approaches, particularly extracorporeal removal of LDL-cholesterol by lipoprotein apheresis (LA)[3].

In Europe[3, 4], USA[5], Japan[6] and Australia[7], LA is the recommended treatment for hoFH, especially for those who do not respond sufficiently to high-dose statins and ezetimibe[8]. LA is an important treatment in hoFH patients allowing the attainment of plasma LDL-cholesterol targets and increase life expectancy, but involves continued indefinite treatment schedules and a cost burden to the health system[9, 10]. Guidelines on the value of LA[10] and disparities in FH care including LA[11] have recently been published. Services and facilities for LA are not available in many countries, such as the People's Republic of China and other countries in the Asia-Pacific region[11]. By contrast, Italy has employed LA for treating hoFH for several years and has a centre of national excellence in Rome[12]. Hence, in the context of our recent study showing the shortfall in the availability of LA in the Asia-Pacific region[11], the Sino-Roman

(Study of IncideNt Outcomes and MoRtality in HOMozygous FH Across CouNtries) study aimed to compare and contrast cardiovascular outcomes in hoFH in two national specialist centres in Beijing and Rome with disparate approaches to treating hoFH. A secondary aim was to investigate the impact of cholesterol exposure, mutation types and lipoprotein(a) [Lp(a)] on cardiovascular outcomes in hoFH.

METHODS

The Sino-Roman study was undertaken as part of the "Ten Countries Study" to compare worldwide disparities in the care of familial hypercholesterolaemia (FH), particularly in the Asia-Pacific region, and employing a European centre as benchmark[13].

Patients and study design

We employed a retrospective cohort design that reviewed data from hoFH patients attending two specialist centres in university hospitals between 1984 and 2018: the Institute of Heart, Lung and Blood Vessel Diseases, Anzhen Hospital in Beijing, China and the Extracorporeal Therapeutic Techniques Unit, Umberto I Hospital, in Rome, Italy. The centre in Rome is a specialised LA centre[12] and hoFH patients treated with weekly or biweekly LA were selected for inclusion. LA is not available in China and all hoFH patients with follow-up information were included. Criteria for the diagnosis of hoFH was confirmed by the presence of two pathogenic mutant alleles at the *LDLR*, *APOB* or *PCSK9* loci[3] and further defined as true hoFH (identical mutation in each allele of the same gene), compound heterozygous FH (non-identical mutations in each allele of the same gene) and double heterozygous FH (mutations in two different genes affecting LDL receptor function)[3]. Receptor

negative mutations were defined as nonsense, splice-site and indel frameshift mutations[14].

Medical records were reviewed to obtain genetic information and clinical data on plasma lipid profiles, lipid-lowering therapy, physical signs (tendon xanthomata and arcus cornealis), cardiovascular risk factors (hypertension, diabetes and smoking), CVD events (defined as unstable angina, myocardial infarction, coronary revascularisation, endoarteriectomy and/or aortic valves replacement), coronary atherosclerosis (defined as any plaque detected on computer tomography coronary angiography), aortic stenosis (defined as an area of aortic valves <3cm^2, transmitral pressure gradient ≥50 mmHg combined with stenosis of aortic sinus on cardiovascular imaging), carotid atherosclerosis (defined as intima-media thickness ≥1.5mm, stenosis >50% or any plaques on carotid ultrasonography), and death (any cause).

The Italian hoFH patients received weekly or biweekly LA treatment using a range of techniques, primarily the Liposorber system MA-03 (Kaneka Corp, Osaka, Japan), with adsorption columns containing negatively charged dextran sulfate (polyanion) bound on cellulose beads, as previously described[15, 16]. The treatment effects of apheresis were expressed as time-average LDL-cholesterol using Kroon's equation [Cmean=Cmin+K(Cmax-Cmin) where K is the rebound coefficient 0.65 for hoFH][17, 18].

LDL-cholesterol life-years was calculated by multiplying the untreated LDL-cholesterol by the age at treatment, and adding this to each treated LDL cholesterol multiplied by the number of years on the treatment regimen. The mean LDL-cholesterol exposure per year was calculated by dividing the LDL-cholesterol life-

years by the current age or age at death (censored at age 30 years, the common period of observation in both patient groups). Informed consent was obtained from the patients in the respective clinics for use of their de-identified clinical information for research purposes.

Laboratory analyses

Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured with standard enzymatic assays employed by the clinical service laboratories at both centres. LDL-cholesterol concentrations were calculated with the Friedewald formula[19]. Lp(a) was measured by immunoturbidimetric (SHIMA Laboratories, Tokyo, Japan) and immunonephelometric (Behring Nephelometer II, Dade Behring Inc.) assays, respectively in Beijing and Rome. Elevated Lp(a) was defined as greater than 0.5 g/L. The diagnosis of FH was confirmed by genetic analysis as previously described[20].

Statistical analyses

Data were collected using Microsoft Excel in the respective centres. All data were aligned, amalgamated and analysed using STATA (Version 13.1, StataCorp, College Station, Texas). Continuous data were expressed as mean ± standard deviation (SD) and categorical data were expressed as proportions (%). Differences in characteristics between hoFH patients from China and Italy were investigated using t-tests, Fisher's exact test and chi-square statistics. Differences in pre- and post-treatment lipid concentrations were examined using paired t-tests. Skewed variables, including triglyceride and Lp(a) were log-transformed for statistical analysis. Statistical significance was defined at the 5% level.

Kaplan-Meier survival curves with age as the time scale and survival analyses (log-rank tests) were employed to investigate CVD event-free survival and mortality according to country group, mutation status (receptor negative vs. receptor non-negative) and Lp(a) (elevated vs. non-elevated). A parametric hazard model with Weibull distribution was used to estimate the hazard ratios and 95% confidence intervals. When comparing the two country groups, the model was also adjusted for a propensity score, computed using logistic regression with the dependent variable being recipients of apheresis and the independent variables (covariates) being age at treatment, gender and the untreated LDL-cholesterol. Tertiles of variables relating to cholesterol exposure were also explored: age started treatment, duration of treatment and on-treatment LDL-cholesterol. Endpoints of CVD events and death were censored at 30 years of age.

RESULTS

The study cohort included 62 hoFH patients from China (n=44) and from Italy (n=18); 30 were male, 32 were female, with mean age at diagnosis being 8.0 ± 5.2 years. Clinical and biochemical characteristics are shown in **Table 1**. Compared with the Italian cohort, the Chinese patients were diagnosed at a significantly older age (9.1 ± 5.1 vs 5.2 ± 5.0 years, p=0.008), with no significant differences in proportion of males and females. None of the patients were diabetic or smokers. However, a significantly higher proportion of hoFH patients from China had hypertension (on antihypertensive drugs) and clinical stigmata of FH (tendon xanthomata and arcus cornealis).

A significantly higher proportion of Chinese hoFH patients had aortic stenosis and carotid atherosclerosis compared with the Italian patients, but no significant differences in clinical coronary atherosclerosis. Plasma total cholesterol and LDL-

cholesterol were significantly higher in the Italian patients compared with the Chinese patients (p=0.023 and 0.003, respectively). There were no significant differences in genotype and mutation types between the two groups.

The overall proportion of those on drug treatment were not different between the two groups, in particular there were no significant differences in those treated with statins and ezetimibe (**Table 2**); there were also no significant country differences in proportion of those treated with aspirin, other anti-platelets and anti-coagulants (**Table 1**). However, a higher proportion of hoFH patients from Italy were on resins, fibrates and lomitapide; they also commenced on treatment at an earlier age (5.6 ± 3.4 vs 10.7 ± 4.6, p<0.001) compared with the Chinese hoFH patients. A higher proportion of Chinese were treated with probucol compared with the Italian patients.

In the Italian patients, the mean age of starting LA was 8.9 ± 5.5 years. The time-average LDL-cholesterol level in the group on LA was 6.6 ± 2.7 mmol/L, a significant 65% reduction from 19.1 ± 4.8 mmol/L (p<0.001); eleven (61.1%) patients were also on lomitapide. In the Chinese hoFH patients on conventional therapy, LDL-cholesterol fell to a mean of 13.1 ± 2.7 mmol/L from 15.5 ± 3.8 mmol/L (14% reduction, p<0.001).

The Kaplan-Meier plots comparing CVD event-free survival and mortality, censored at 30 years (median 17.5 years) in the hoFH patients from the two centres are shown in **Figures 1A and 1B**, respectively. Survival analyses demonstrated significant differences in CVD and death in the Italian patients, compared with the Chinese patients (log-rank p<0.001 for both). The hazard ratio for CVD-free survival was 5.8 (95% 1.9-17.5, p=0.002) and remained significant after adjusting for the propensity

score. The hazard ratio for death was also 4.2 (95% CI 1.2-15.2, p=0.027) but did not remain significant after adjusting for the propensity score.

In the pooled analysis, Kaplan-Meier plots comparing CVD event-free survival and survival according to tertiles of: (1) age at treatment commencement, (2) treatment duration and (3) the current on-treatment LDL-cholesterol, are shown in **Figure 2**. The hazard ratio for time to first CVD event and time-to-death endpoints were significantly lower with earlier age at treatment, longer duration of treatment and lower on-treatment LDL-cholesterol concentrations (**Table 3**). **Figure 3** show the Kaplan-Meier plots comparing CVD event-free survival and survival in the hoFH patients according presence or absence of an LDL receptor negative (null) mutation, as well as in patients in relation to elevated Lp(a) above and below the cut-off of 0.5g/L. It can be seen that the probabilities of CVD event-free survival was significantly greater among patients with LDL receptor non-negative than negative mutations and amongst those with non-elevated compared with elevated Lp(a); the probability of death was also increased in those who are LDL receptor negative and had elevated Lp(a) compared with the corresponding comparator group.

DISCUSSION

The benefits of LA in patients with hoFH have been demonstrated in observational studies from the UK[21, 22], Germany[23] and South Africa[24]. However, this is the first study to compare the long-term outcomes from childhood of patients with hoFH in two countries with advanced and less advanced models of care for patients with this condition. Acknowledging environment and genetic differences in the susceptibility to CAD and the bundle of treatments included in the respective models of care, we demonstrated significant differences in CVD-free survival and survival in hoFH

patients from China not on LA compared with hoFH patients from Italy on LA. Both patient groups were also on the best and most contemporary pharmacological therapy available in their respective countries.

With a mortality rate of 32% and an average age of death at 17.9 ± 6.2 years, hoFH patients in China need to be offered treatment with LA and new therapies, such as lomitapide and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Lomitapide has been approved by both the FDA and European Medicines Agency as an orphan drug for the treatment of patients with hoFH. Although long-term use of lomitapide has been associated with an increased risk of progressing to steatohepatitis and fibrosis[25], our clinical experience has demonstrated that lomitapide is an effective adjunct to LA in hoFH patients on a low-fat diet[15, 26]. PCSK9 inhibitors can lower LDL-cholesterol levels in hoFH patients not on LA[27], but are not be efficacious in hoFH patients with two null receptor mutations[28]. HoFH patients in the present study were not treated with PCSK9 inhibitors. It must be conceded that the favourable responses of LDL-cholesterol with evolocumab in the TESLA B[27] and TAUSSIG[29] studies are significant, implying that in the future use of this treatment could achieve a greater reduction in LDL-cholesterol and improve outcomes in patients with hoFH who have residual *LDLR* function. PCSK9 inhibitors may not only reduce the frequency of LA but also enhance the effectiveness of this form of therapy[10].

Probucol, in combination with statins and ezetimibe[30] and/or LA[31, 32] can achieve regression of tendon xanthomas and atherosclerosis in a hoFH [30 33]. However, long-term randomised studies are required to confirm these reports in hoFH patients on concomitant optimal background lipid-lowering treatment. Fibrates and ezetimibe[34]

alone have limited effects on LDL-cholesterol, but may be useful adjunctive therapies to statins and LA in hoFH patients.

Other lipid-lowering treatment options for hoFH include partial ileal bypass surgery, liver transplantation and gene therapy. Partial ileal bypass surgery requires residual functional LDL receptors, is associated with gastrointestinal side effects and is of limited value[35]. Liver transplantation is restricted by availability of suitable organ donors and carries significant operative risk and risk of long-term immunosuppression[36]. Liver-directed gene therapy for hoFH is currently being trialled[37]. Although LA is considered the standard care for patients with hoFH, its limited availability, high cost, duration of the procedure and maintenance of vascular access are significant drawbacks[38]. LA also requires commitment from the patient. Nevertheless, the benefits of LA may outweigh the risks and burdens for hoFH patients, and the cost-effectiveness of treating hoFH with LA is estimated to be greater than for heterozygous FH[10, 38]. Moreover, the effectiveness of LA in our study is well emphasized by an incidence of CVD (22.2%) and death (16.7%) in the Roman cohort, which is more favourable than other published studies from Europe and South Africa[1, 14, 22-24] (**Supplementary Table 1**).

Clinical trials of new drugs in hoFH children are required, since therapeutic interventions should be initiated as early as possible. The first ever paediatric randomised trial with hoFH patients was recently published[39], demonstrating safe and effective use of Rosuvastatin 20mg (6 weeks crossover), alone or in conjunction with ezetimibe and/or LA. However, longer-term efficacy and safety data are required, particularly in a condition like hoFH where lifetime therapy is necessary.

Clinical trials of statins, ezetimibe and PCSK9 inhibitors and Mendelian randomisation studies have consistently demonstrated that the duration of treatment and the absolute reduction of LDL-cholesterol is proportional to the reduction of the risk of cardiovascular events[40]. A recent study by Thompson et al[1] emphasised this notion by demonstrating that survival in hoFH patients depends upon the extent of reduction in cholesterol, and this is consistent with our present study (**Table 3** and **Figure 2**).

Our data demonstrate that early efficacious treatment remains the key to prevent CVD and death in hoFH patients. Despite starting apheresis at a mean of 9 years and lomitapide at a mean of 24 years, the 17% mortality rate of hoFH on LA in the present study, consistent with previous observational studies[41, 42], shows that LA delays the development of atherosclerosis but does not completely arrest progression[14, 43]. Ultimately, with the advent of new therapies such as PCSK9 inhibitors and angiopoietin-like protein 3 (ANGPTL3) inhibitors [44], there is hope on the horizon for individuals with hoFH if these treatments, combined with LA[29], are instituted early enough. However, the cost of these drugs and their affordability by healthcare systems around the world are the principal barrier to access; long-term safety and tolerability in children are also important to verify.

The higher proportion of Chinese hoFH patients with tendon xanthomata compared with Italian hoFH patients also indicated greater lifetime exposure to raised plasma cholesterol. Tendon xanthomata reflects the cholesterol life-years in FH and are predictive of CAD[45]. It is difficult to precisely compare the impact of LDL-cholesterol life-years in this study because of the earlier mortality in the Chinese patients. However, after accounting for the shorter life-time exposure in the Chinese group owing to death, as well as the higher untreated LDL-cholesterol level in the Italian

group, the mean LDL-cholesterol exposure per year was significantly less in the Italian compared with the Chinese patients. The lower untreated LDL-cholesterol in the Chinese patients could be explained by the lower population mean cholesterol levels in most Asian countries[46]. The Chinese and Italians are also culturally (diet and lifestyle) and genetically different, however, it was not possible to statistically control for these differences.

Limitations of the present study was that it was not prospective or randomised for LA treatment, and that data were from a single centre in Beijing and Rome. Patients were treated with the current best available regimens in their respective countries, including full accessibility to pharmacotherapies. Previous studies of the impact of radical therapy such as LA on outcomes in hoFH patients have adopted similar retrospective case-control designs. We attempted to compare country outcomes according to the availability of LA and drug therapies. The differences in country-specific health services and government health expenditure (ie. heatlh expenditure as a share of GDP is 8.9% in Italy compared with 5.5% in China[47]) could account for differences in the availability of LA as well as diversity in other medical care offered to patients[11]. Our study has implications for other countries, particularly in the Asia-Pacific region as recently described[11]. While reimbursement is critical, the effective use of LA requires its introduction at an early age in order to be effective in preventing the development of aggressive atherosclerosis. This has been well demonstrated by our study, as well as experience from centres in Turkey[48].

We classified genetic mutations according to methodology employed in previous research[14, 49]. Studies in hoFH from the USA[50], Spain[51] and France[14] have demonstrated that patients with receptor negative mutations exhibit earlier onset of CVD and reduced survival compared with those with receptor defective mutations;

our results accords with this (**Figure 3**). Independent of mutation type and treated LDL-cholesterol, elevated Lp(a) in hoFH[52] may also bear on the already increased CVD risk. Whether this risk can be reduced by therapies that lower both LDL-cholesterol and Lp(a) concentrations, will require further research. Our present study was restricted by the availability of Lp(a) data in 70% of the cohort and we note this as a limitation. Additionally, measurement of Lp(a) employed polyclonal antibodies against apo(a) and were not strictly isoform independent nor employed the same standards. Further research, employing isoform independent assays, to ascertain the role of Lp(a) in cardiovascular outcomes in hoFH is warranted.

Another limitation was that we were not able to confirm all deaths as cardiac deaths. The deaths reported were sudden deaths at home and were assumed to be most likely due to fatal arrhythmia and cardiac arrest from an acute coronary syndrome. Post-mortem data were not available. Our hazard ratios for CVD event-free survival and mortality had wide confidence intervals, consistent with our small sample size. However, hoFH of the type selected for this study is an exceptionally rare disorder, particularly in countries without a founder-effect. Also, we did not explore adherence[53] and quality-of-life (QOL)[54] measures in relation to the use of LA in the present study. Although the impact of LA on QOL does not outweigh the CVD benefits, it is important to address QOL issues when assessing the effectiveness of a radical intervention[55].

CONCLUSION

LA in combination with pharmacological treatments, as employed in the model of care in the Rome centre, is effective in improving CAD outcomes in hoFH[56]. Early diagnosis and access to affordable and efficacious therapies are clearly fundamental

in improving the care of hoFH patients worldwide, which is particularly relevant to a populous country like China[11]. Based on population prevalence estimates[57, 58], China should have the largest number of unrelated hoFH patients in the world (approximately 4500 hoFH). To close gaps in care, the MIGHTY MEDIC (Multidisciplinary International Group for Hemapheresis TherapY and MEtabolic DIsturbances Contrast) Multinational Society has been assembled to consolidate the value of integrated therapies, including LA, in making the treatment of hoFH more equitable and effective worldwide[10]. The availability and early use of new adjunctive and pragmatic therapies, such as PCKS9 and ANGPTL3 inhibitors[29, 44], are also likely to have a major impact on CVD outcomes in this high risk group of patients.

Table 1 Clinical, biochemical and genetic characteristics of the homozygous FH patients from the Italian and Chinese centres.

	Italy (n=18)	China (n=44)	P-value
Age at diagnosis (years)	5.2 ± 5.0	9.1 ± 5.1	0.008
Gender (% male)	33.3	54.6	0.129
Hypertension (%)	0	27.4	0.001
Diabetes (%)	0	0	-
Smoking (%)	0	0	-
Tendon xanthoma (%)	72.2	100	<0.001
Arcus cornealis (%)	50.0	88.6	0.001
Cardiovascular drugs:			
Anti-hypertensives (%)	0.0	31.8	0.007
Aspirin (%)	27.8	47.7	0.148
Other anti-platelets (%)	0.0	15.9	0.072
Anti-coagulants (%)	5.6	0.0	0.115
Age at imaging (years)	8.6 ± 6.4	10.0 ± 3.9	0.401
Coronary atherosclerosis[a] (%)	38.9	54.6	0.263
Aortic stenosis[b] (%)	22.2	77.3	<0.001
Carotid atherosclerosis[c] (%)	22.2	93.2	<0.001
Untreated Total cholesterol (mmol/L)	21.2 ± 4.6	18.3 ± 4.2	0.023
Untreated LDL-cholesterol (mmol/L)	19.1 ± 4.8	15.5 ± 3.8	0.003
Untreated Triglyceride (mmol/L)	1.18 ± 0.55	1.33 ± 0.73	0.469
Untreated HDL-cholesterol (mmol/L)	0.93 ± 0.22	1.22 ± 0.71	0.093
Lipoprotein(a)[†] (g/L)	0.38 ± 0.38 (median 0.20)	0.56 ± 0.46 (median 0.47)	0.034
Elevated Lipoprotein(a)[†] (%)	26.7	45.2	0.228
Genetic diagnosis:			
True hoFH, n(%)	12 (67)	18 (41)	0.153
Compound heFH, n(%)	6 (33)	24 (55)	
Double heFH, n(%)	0 (0)	2 (5)	
Mutation type:			
Receptor negative[‡], n(%)	7 (39)	18 (41)	0.883

Continuous variables are expressed as mean±standard deviation and categorical variables are expressed as proportions.

Abbreviations: LDL: low-density lipoprotein; HDL: high-density lipoprotein; hoFH: homozygous familial hypercholesterolaemia; heFH: heterozygous familial hypercholesterolaemia.

[a]defined as any plaque detected on computer tomography coronary angiography

[b]defined as an area of aortic valves <3cm^2, transmitral pressure gradient ≥50 mmHg combined with stenosis of aortic sinus on cardiovascular imaging

[c]defined as intima-media thickness ≥1.5mm, stenosis >50% or any plaques on carotid ultrasonography

[†]Data available for n=46; elevated lipoprotein(a) defined as >0.5g/L

[‡]Negative mutations included nonsense, splice-site and indel frameshift mutations.

Table 2 Lipid-lowering treatments and outcomes of the homozygous FH patients from the Italian and Chinese centres.

	Italy (n=18)	China (n=44)	P-value
Age started pharmacotherapy treatment (years)	5.6 ± 3.4	10.7 ± 4.6	<0.001
Lipid-lowering drugs (%)	94.4	95.5	0.866
Statins (%)	88.9	95.5	0.339
Ezetimibe (%)	77.8	81.8	0.715
Resins (%)	66.7	0	<0.001
Fibrates (%)	16.7	0	0.022
Probucol (%)	0	77.3	<0.001
PCSK9 inhibitors (%)	0	0	-
Lomitapide (%)	61.1	0	<0.001
Age started lomitapide (years)	23.5 ± 4.1	-	
Lipoprotein apheresis (%)	100	0	<0.001
Age started apheresis (years)	8.9 ± 5.5	-	-
Treated LDL-cholesterol (mmol/L)	6.6 ± 2.7*	13.1 ± 2.7	<0.001
Δ LDL-cholesterol (mmol/L)	12.5 ± 5.0	2.6 ± 3.6	<0.001
Treatment duration (years)	17.4 ± 8.8	5.5 ± 4.8	<0.001
LDL-cholesterol life-years[†]	258.9 ± 98.7	227.3 ± 112.8	0.305
			0.792[‡]
Mean LDL-cholesterol exposure/year (mmol/L)[†]	12.4 ± 3.0	14.6 ± 3.0	0.011
			<0.001[‡]
CVD event, n(%)	4 (22.2)	20 (45.5)	0.088
Age at CVD event (years)	19.0 ± 9.6	16.1 ± 5.8	0.421
Death, n(%)	3 (16.7)	14 (31.8)	0.225
Age at death (years)	20.3 ± 10.7	17.9 ± 6.2	0.586

Continuous variables are expressed as mean±standard deviation and categorical variables are expressed as proportions.

Abbreviations: PCSK9: proprotein convertase subtilisin/kexin type 9; LDL: low-density lipoprotein; CAD: coronary artery disease.

*Time-average LDL-cholesterol calculated using Kroon's equation[17, 18]

[†]Censored at age 30

[‡]Adjusted for (baseline) untreated LDL-cholesterol

Table 3 Hazard ratios and 95% confidence intervals for cardiovascular event and death according to tertiles of age at treatment, treatment duration and on-treatment LDL-cholesterol (tertile 1 as reference group).

Outcome	**Tertile 2 vs Tertile 1 of age at treatment[a] [HR (95% CI]**	**Tertile 3 vs Tertile 1 of age at treatment[a] [HR (95% CI]**	**P-value[‡]**
CVD event	6.39 (1.39-29.30)	5.39 (1.20-24.15)	0.010[†]
Death	3.20 (0.66-15.44)	3.00 (0.62-14.46)	0.232
Outcome	**Tertile 2 vs Tertile 1 of treatment duration[b] [HR (95% CI]**	**Tertile 3 vs Tertile 1 of treatment duration[b] [HR (95% CI]**	**P-value[‡]**
CVD event	0.38 (0.14-0.98)	0.08 (0.03-0.25)	<0.001[††]
Death	0.11 (0.03-0.46)	0.04 (0.01-0.16)	<0.001[††]
Outcome	**Tertile 2 vs Tertile 1 of on-treatment LDL-cholesterol[c] [HR (95% CI]**	**Tertile 3 vs Tertile 1 of on-treatment LDL-cholesterol[c] [HR (95% CI]**	**P-value[‡]**
CVD event	9.22 (2.80-30.32)	6.45 (1.95-21.37)	<0.001[††]
Death	10.54 (2.21-50.41)	7.40 (1.40-39.22)	0.002[††]

[a]Tertile 1 (range 1-6 years), tertile 2 (range 7-10 years) and tertile 3 (range 11-22 years)

[b]Tertile 1 (range 0-3 years), tertile 2 (range 3.5-10 years) and tertile 3 (range 11-30 years)

[c]Tertile 1 (range 2.87-10.06 mmol/L), tertile 2 (range 10.10-13.12 mmol/L) and tertile 3 (range 13.46-17.52 mmol/L)

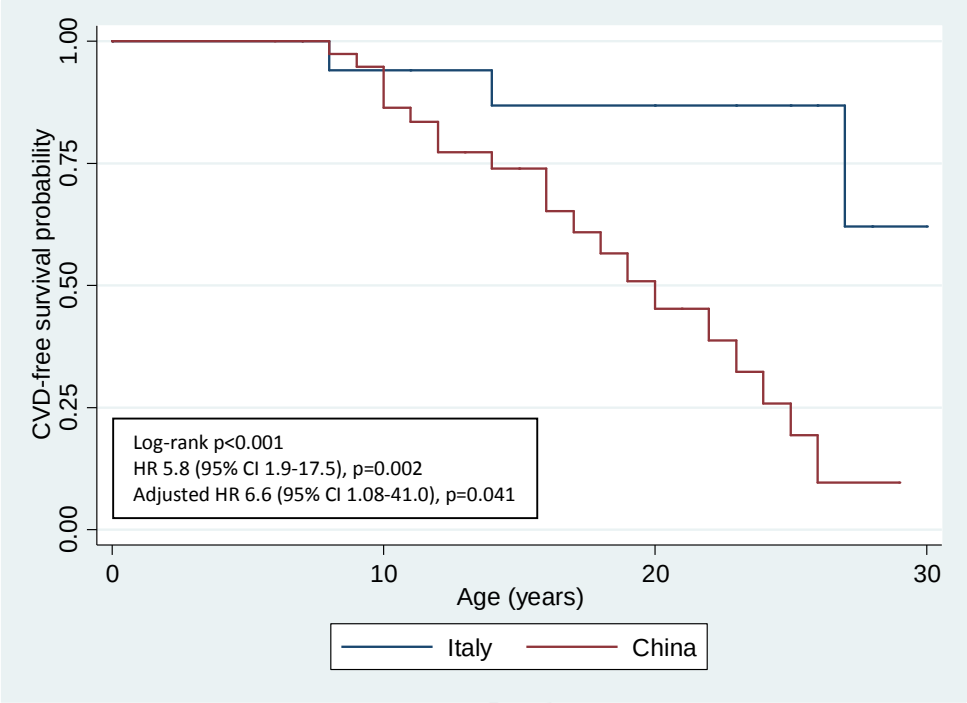
[‡]P-values indicate the level of difference in the overall model.

[†]Remains significant after adjusting for gender, pre-treatment LDL-cholesterol, apheresis and lomitapide

[††]Remains significant after adjusting for gender, age at treatment, pre-treatment LDL-cholesterol, apheresis and lomitapide

Figure 1 Kaplan-Meier plot comparing (**A**) cardiovascular disease-free survival and (**B**) survival in homozygous FH patients from the Italian centre on drug therapy and lipoprotein apheresis and patients from the Chinese centre on drug therapy alone.

A



B

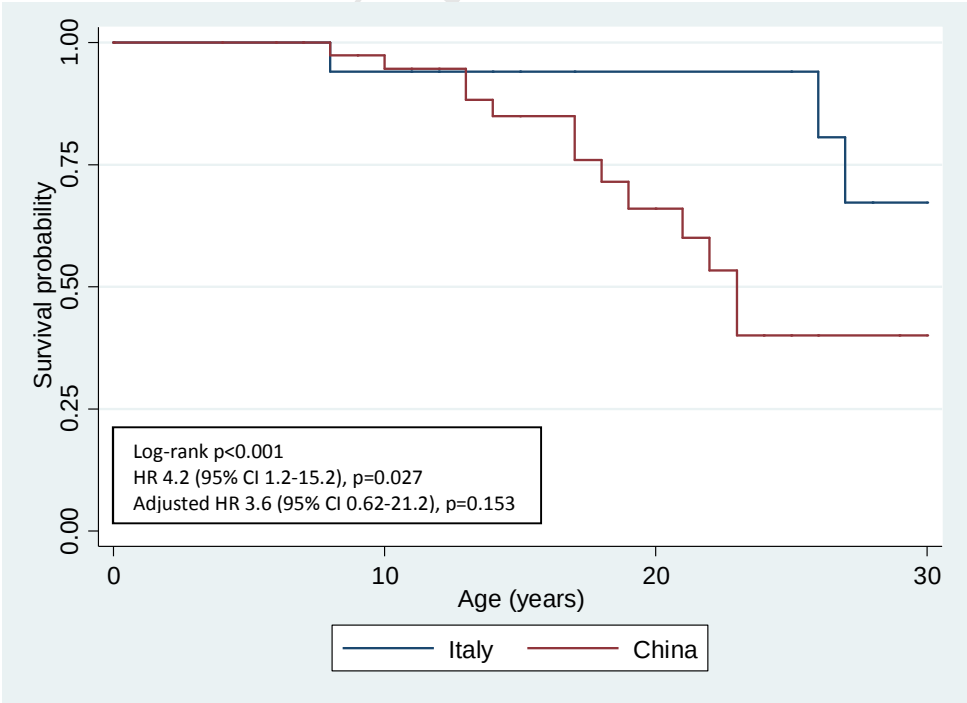


Figure 2 Kaplan-Meier plot comparing (**A**) cardiovascular disease-free survival and (**B**) survival in tertiles of (**I**) age started treatment, (**II**) duration of treatment and (**III**) on-treatment LDL-cholesterol.

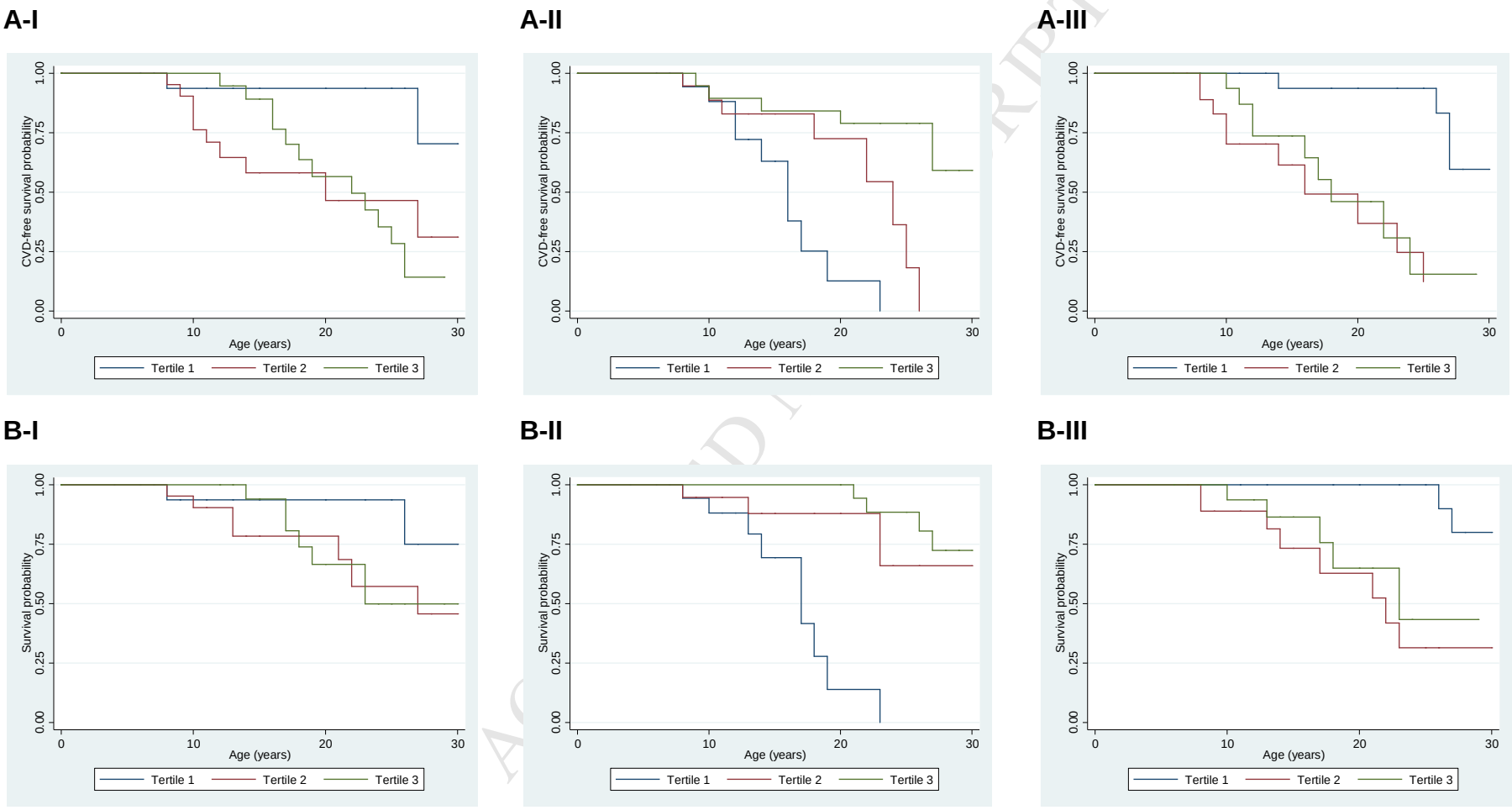
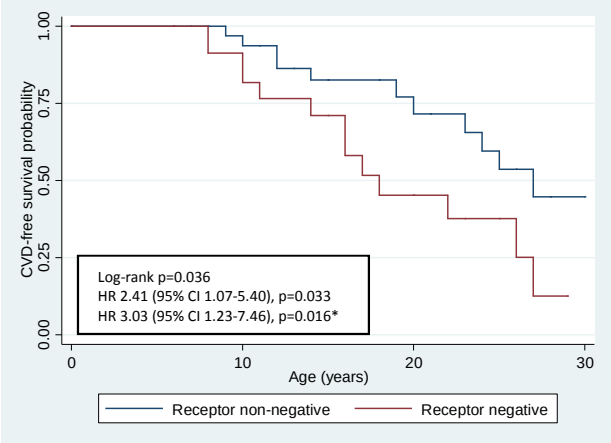
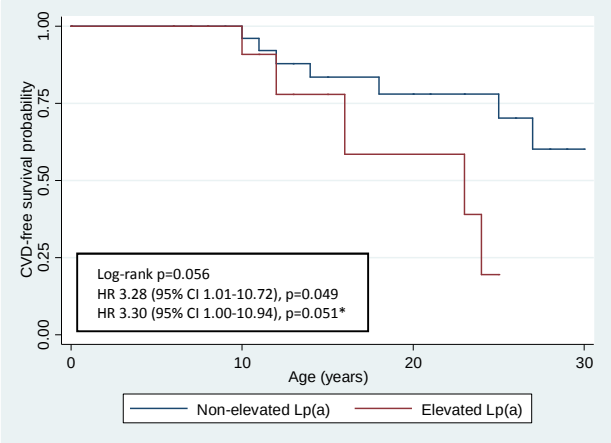


Figure 3 Kaplan-Meier plot comparing (**A**) cardiovascular disease-free survival and (**B**) survival in homozygous FH patients (**I**) with and without a receptor negative *LDLR* mutation and (**II**) with and without elevated lipoprotein(a).

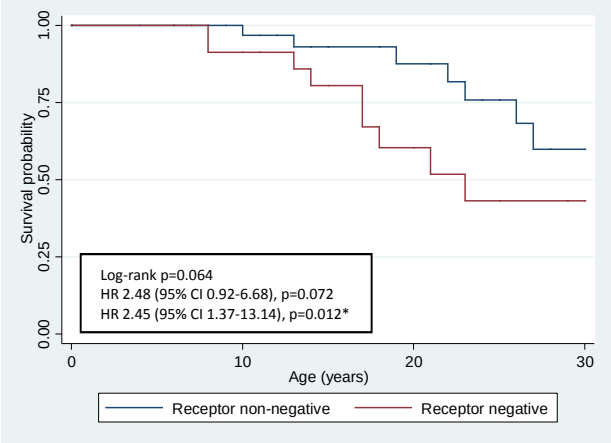
A-I



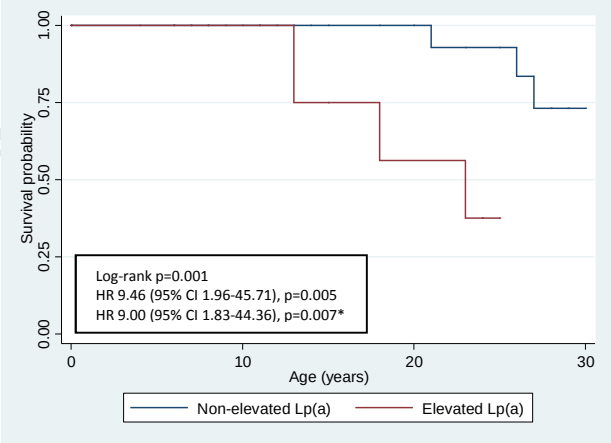
A-II



B-I



B-II



*Adjusted for pre-treatment LDL-cholesterol concentration

Supplementary Table 1 Summary of studies in homozygous familial hypercholesterolaemia where lipoprotein apheresis had been instituted at an early age with survival data with a mean follow-up to at least 25 years of age.

Reference	Country	n	CVD (%)	Death (%)	Apheresis (%)
Present study	Italy	18	22.2	16.7	100
Bruckert 2017[14]	France	53*	77.3	15.4	75
Thompson 2017[1]	United Kingdom and South Africa	133	27.1^	33.8	19
Thompson 2015[22]	United Kingdom	44†	79.5	29.5	61-62
Raal 2011[24]	South Africa	149	67.1	43.6	15.4
Keller 2009[23]	Germany	23‡	65.2	34.8	100

*16 out of 53 (30.2%) patients had ARH, heterozygous *LDLR* or undetermined/unidentified mutations

^Only fatal major adverse cardiovascular events were reported

†7 out of 44 (15.9%) patients had ARH

‡All patients had *LDLR*-defective hoFH

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Contribution Statement

CS, SDG, CM, XW, XMW and JL designed the data collection tools, monitored data collection, cleaned the data and revised the draft paper. GFW initiated the collaborative project with CS and JL. GFW also advised the statistical analysis plan. JP performed the statistical analyses and wrote the first draft of the manuscript with support from GFW. All authors discussed the results and contributed to the final manuscript.

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