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Hypercholestérolémie familiale hétérozygote : efficacité en pratique réelle des inhibiteurs de la PCSK9 et effets de la mutation génétique causale sur l'athérosclérose

Anthony Matta

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Doctorat de l'Université de Toulouse

préparé à l'Université Toulouse III - Paul Sabatier

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Thèse présentée et soutenue, le 7 mars 2024 par

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Jean FERRIERES

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2. Matta A, Rabès JP, Taraszkievicz D, Carrié D, Roncalli J, Ferrières J. Effect of causative genetic variants on atherosclerotic cardiovascular disease in heterozygous familial hypercholesterolemia patients. *Front Cardiovasc Med* 2023;10:1182554.

Case Report :

1. Matta A, Taraszkievicz D, Bongard V, Ferrières J. Ineffective subtilisin/kexin type 9(PCSK9) inhibitors monotherapy in dyslipidemia with low-density lipoprotein cholesterol (LDL-c) receptor abnormalities: a report of 2 cases. *Am J Case Rep* 2020;21:e923722.

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2. Matta A, Levai L, Roncalli J, Elbaz M, Bouisset F, Nader V, et al. Comparison of in-hospital outcomes and long-term survival for valve-in-valve transcatheter aortic valve replacement versus the benchmark native valve transcatheter aortic valve replacement procedure. *Front Cardiovasc Med* 2023;10:1113012.
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Résumé :

L'Hypercholestérolémie familiale hétérozygote (HFH) est une maladie génétique autosomique dominante caractérisée par un taux élevé de LDL-c circulant et expose à un risque accru à des accidents cardiovasculaires précoces, en particulier l'infarctus du myocarde, les accidents vasculaires cérébraux et les artériopathies périphériques. Plusieurs mutations causales ont été identifiées au niveau des gènes codant pour LDL-récepteur, apolipoprotéine B (Apo B), protéine convertase subtilisin/kexin type 9 (PCSK9) et apolipoprotéine E (Apo E). Le score de Dutch est un outil communément utilisé par les médecins pour évaluer la probabilité du diagnostic d'HFH. Selon les recommandations de la société européenne de cardiologie, les sujets avec HFH sont systématiquement classifiés à haut risque pour maladie cardiovasculaire avec un objectif LDL-c ≤ 0.7 g/dl avec une baisse initiale \geq à 50% du niveau basal de LDL-c. En pratique, la proportion des individus avec HFH contrôlés qui atteint l'objectif thérapeutique est relativement faible. Les essais cliniques ont introduit les inhibiteurs de la PCSK9 (PCSK9i) comme une nouvelle classe thérapeutique prometteuse. La valeur pronostique de l'analyse génétique par la détection d'une mutation causale génétique et son rôle dans la prédiction du développement prématuré des accidents cardiovasculaires est un sujet d'actualité en discussion. L'objectif de cette étude est d'observer tout d'abord en pratique réelle la variation de niveau basal de LDL-c ainsi que la proportion des répondeurs au PCSK9i dans une population avec HFH et d'évaluer en second temps si les porteurs d'une mutation génétique causale sont à plus haut risque de développer un événement cardiovasculaire.

Cette thèse a démontré une différence significative de la moyenne de variation de niveau de LDL-c entre le groupe des patients traités par PCSK9i et statine versus le groupe des patients traités uniquement par PCSK9i (175 ± 72 vs 125 ± 60 mg/dl, $p=0.002$). De même, le taux des répondeurs définis par une réduction $\geq 50\%$ de niveau basal de LDL-c a été significativement

plus élevé chez les patients sous PCSK9i et statine (78.6% vs. 38.6%, $p=0.001$). D'autre part, le résultat a été comparable entre le groupe de monothérapie avec PCSK9i et le groupe recevant PCSK9i avec ézetimibe au niveau de la moyenne de variation de LDL-c (125 ± 60 vs 103 ± 89 mg/dl, $p=0.86$) et le taux des répondeurs (38.6% vs 58.3%, $p=0.24$). L'incidence des évènements cardiovasculaires a été plus élevée chez les patients avec HFH et porteurs d'une mutation génétique causale (27% vs 17.4%, $p=0.048$). Cependant, la courbe de Kaplan Meier montre une survie globale sans évènements cardiovasculaires comparable entre les deux groupes des patients avec et sans mutation causale ($p=0.523$). Les analyses multivariées ajustées montrent une association positive entre la survenue d'un évènement cardiovasculaire et le niveau de LDL-c indépendamment de la présence d'une mutation génétique causale (OR=2.47 ; 95% [1.3025-4.221], $p=0.004$). En revanche, l'association entre la présence d'une mutation génétique causale et le développement d'un évènement cardiovasculaire n'est valable qu'après avoir exclu l'effet de LDL-c.

Pour conclure, uniquement l'association de PCSK9i avec statine résulte en une baisse importante du niveau de LDL-c et un taux élevé significatif des répondeurs dans une population avec HFH. Le niveau de LDL-c est le prédicteur principal de la survenue des évènements cardiovasculaires chez les patients traités pour HFH porteurs ou non porteurs d'une mutation génétique causale.

Abstract:

Heterozygous familial hypercholesterolemia (HFH) is an autosomal dominant genetic disorder characterized by a chronic exposure to a high level of circulating LDL-c and increased risk of premature cardiovascular events like myocardial infarction, stroke and peripheral arteriopathy. Numerous causal mutations have been identified in the genes encoding the LDL receptor (LDLR), apolipoprotein B (Apo B), proprotein convertase subtilisin/kexin type 9 (PSCK9) and apolipoprotein E (Apo E). Dutch score is commonly used by health care practitioners to evaluate the probability of HFH diagnosis. Based on the recommendations of the European society of cardiology, HFH- patients are classified at high risk for cardiovascular disease with a goal of LDL-c level below 70mg/dl and more than 50% reduction in baseline LDL-c level. In clinical practice, the proportion of controlled HFH-patients reaching the LDL-c target is relatively small. Recent randomized clinical trials have introduced the PCSK9 inhibitors as a novel promising therapeutic approach. In the setting of HFH, the prognostic value of a causative genetic mutation and its role to predict a premature onset of cardiovascular event is debatable. This study aims to observe in a real life experience the variation in LDL-c level and the responder's rate after initiation of PCSK9i therapy in HFH-population, then to evaluate if HFH-patients' carriers of a causative mutation are at high risk to develop cardiovascular events.

The present study showed a significant difference in the mean variation of LDL-c between the groups of HFH-patients treated with PCSK9 inhibitors and statins versus PSCK9 inhibitors alone (175 ± 72 vs 125 ± 60 mg/dl, $p=0.002$). Also, the proportion of responders defined by more than 50% reduction in baseline LDL-c level was significantly higher in HFH-patients receiving PSCK9 inhibitors and statins (78.6% vs 38.6%, $p=0.001$). Otherwise, no significant differences were observed between HFH-patients treated with PSCK9 inhibitors alone or with ezetimibe

concerning the mean variation in LDL-c level (125 ± 60 vs 103 ± 89 mg/dl, $p=0.86$) and responder's rate (38.6% vs 58.3%, $p=0.24$). The incidence of cardiovascular events was higher in HFH-patients' carriers of a causative mutation (27% vs 17.4%, $p=0.048$). However, the Kaplan Meier curve showed a comparable event free survival between HFH-patients' carriers or not of a causative mutation ($p=0.523$). The adjusted multivariate analysis showed a positive association between the LDL-c level and the occurrence of a cardiovascular event independently of the presence of a causative mutation (OR=2.47, 95%CI [1.3025-4.221], $p=0.004$). In opposition, the association of cardiovascular event and causative mutation was only valid after excluding the effect of LDL-c.

To conclude, in a context of HFH, the remarkable reduction in baseline LDL-c level and the great proportion of responders were only observed when PCSK9 inhibitors were associated with statins. The LDL-c level is the main independent predictor of the occurrence of cardiovascular events in HFH-patients' carriers or not of a causative genetic mutation.

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1. Introduction

L'hypercholestérolémie familiale est une maladie génétique de transmission autosomique dominante sous deux formes : hétérozygote (la plus fréquente) et homozygote (la plus grave). L'hypercholestérolémie familiale hétérozygote (HFH) touche 1 sur 313 à 120 individus [1-3]. Les sujets avec HFH sont exposés à un niveau élevé de LDL-c circulant plasmatique pour donner suite à un risque accru des évènements cardiovasculaires prématurés. Un infarctus de myocarde ou un accident vasculaire cérébral ou une artériopathie peuvent être la présentation initiale d'une HFH [4]. Le Dutch Lipid Clinic Network Score (DLCNS) est un outil diagnostique utilisé par les spécialistes pour évaluer la probabilité d'HFH. Il comporte les paramètres suivants : histoire familiale de maladie cardiovasculaire prématurée chez les parents de premier degré, histoire personnelle de maladie cardiovasculaire prématurée ou des signes cliniques d'hypercholestérolémie (xanthomes tendineux ou arc cornéen précoce avant 45 ans), le niveau circulant de LDL-c plasmatique avant traitement et l'analyse de génétique moléculaire [5]. Ce score classe la probabilité d'hypercholestérolémie familiale en quatre catégories : peu probable (0-2 points), possible (3-5 points), probable (6-8 points) et définie (>8 points). Il permet aussi le dépistage des sujets asymptomatiques parmi les membres de famille des patients avec HFH permettant une initiation précoce d'un traitement médical et une stratification du pronostic [6-7]. Les mutations génétiques traditionnelles décrites dans l'HFH affectent les gènes codant pour les récepteurs de LDL (LDLR), l'apolipoprotéine B (Apo B) et la pro protéine convertase subtilisine/kexin type 9 (PCSK9) [8]. Récemment, la mutation au niveau du gène codant l'apolipoprotéine E (Apo E) est considérée comme une mutation causale ou facteur d'exacerbation du phénotype d'HFH [7,9-10]. La société Européenne de cardiologie considère les patients avec HFH à haut risque pour maladie cardiovasculaire avec objectif de LDL-c ≤ 70 mg/dl et une réduction initiale $\geq 50\%$ de taux basal de LDL-c [11]. Cet objectif constitue un grand défi pour les spécialistes en pratique clinique car une faible proportion des

patients avec HFH est contrôlée. Les statines sont recommandées en première ligne thérapeutique et ont démontré la preuve de leur niveau d'efficacité. Les arthralgies et la myalgie sont des effets secondaires associés à la prise des statines et provoquant à un certain point une interruption de la prise médicamenteuse. L'arrêt d'un traitement par statine est recommandé en cas d'une augmentation des CPK dépassant dix fois la limite supérieure de la normale, augmentation des enzymes hépatiques dépassant trois fois la limite supérieure de la normale et apparition des douleurs ou faiblesse musculaires. Les inhibiteurs de la PCSK9 représentent une nouvelle classe thérapeutique chez les patients qui ne tolèrent pas les statines et/ou avec un niveau LDL-c non contrôlé malgré un traitement médical optimal. Les essais cliniques ont montré une moyenne de réduction de taux de LDL-c de 60% avec les inhibiteurs de la PCSK9 [11-12]. Certaines études cliniques ont aussi évalué l'effet synergique des inhibiteurs de la PCSK9 avec la lipoprotéine aphasée [13]. Avec l'augmentation de l'utilisation des inhibiteurs de la PCSK9, nombreuses constatations et préoccupations ont été soulevés tel que la variabilité interindividuelle, l'impact de la présence des mutations génétiques et les anomalies des récepteurs de LDL et l'importance d'un pré-traitement avec les statines. Une résistance aux inhibiteurs de la PCSK9 avec une diminution de leur efficacité a été rapportée chez les patients avec des anomalies des récepteurs de LDL ou d'autres anomalies génétiques [14-15]. D'autre part, le risque de coronaropathie est plus élevé chez les patients avec HFH. On note une multiplication du risque par six en comparaison avec les autres patients ayant une dyslipidémie et par 22 chez les patients avec HFH porteurs d'une mutation génétique causale et un taux de LDL-c ≥ 190 mg/dl [16]. Dans le contexte d'une HFH, les données en pratique réelle sur l'efficacité des inhibiteurs de la PCSK9 et en particulier si prescrit en monothérapie ou en association avec les statines sont très limitées. En plus, l'association entre la détection d'une mutation causale et l'augmentation du risque de développement d'un évènement cardiovasculaire en cas d'HFH sous traitement est rarement évalué. La plupart des

études publiées comparent la différence du risque entre les patients avec une HFH et la population générale.

2. Hypothèse et objectif

Cette thèse vise en premier à évaluer en vie réelle la moyenne de variation de LDL-c et le taux des répondeurs définies par une baisse $\geq 50\%$ de LDL-c basal chez une population avec HFH après l'introduction des inhibiteurs de PCSK9 et comparer la différence entre une introduction en monothérapie versus en association avec les statines \pm ézetimibe. Ensuite, on évalue l'association entre la détection d'une mutation génétique causale et le développement d'un évènement cardiovasculaire chez une population avec HFH sous traitement médical en comparant la différence des incidences des évènements cardiovasculaires entre HFH-porteurs d'une mutation causale versus HFH-sans mutation causale.

3. Résultats Majeurs

Après six mois d'introduction des inhibiteurs de PCSK9, la moyenne de réduction de LDL-c est de 125 ± 60 mg/dl (en monothérapie), 103 ± 77 mg/dl (en association avec ézetimibe) et 175 ± 70 mg/dl (en association avec statine et ézetimibe). Ceci correspond à une baisse de LDL-c de 44.2%, 41.7% et 66.3% respectivement. Le taux de répondeurs a été 38.6%, 58.3% et 78.6% respectivement. La moyenne de variation de LDL-c et le taux des répondeurs observés ont été significativement plus important en cas d'administration simultanée des inhibiteurs de PCSK9 avec les statines.

Une mutation génétique causale a été retrouvée chez 42.2% de la population étudiée. L'incidence des évènements cardiovasculaires est de 27% chez les patients avec HFH porteurs d'une mutation causale versus 17.4% chez les patients avec HFH sans mutation causale. La courbe de Kaplan-Meier montre l'absence de différence de survie sans évènement cardiovasculaire entre les 2 groupes ($p=0.523$). La moyenne de LDL-c est le prédicteur

principal de la survenue des évènements cardiovasculaire chez une population avec HFH sous traitement [OR=2.347, 95%CI (1.305-4.221), p=0.004].

4. Présentation des travaux de recherche

IV.1 Article : Efficacité en vie réelle des inhibiteurs de la PCSK9 chez une population avec HFH référée pour lipoprotéine aphérèse

IV.1.1 Méthodologie

IV.1.1.1 Objectif

Cette cohorte vise à évaluer l'efficacité des inhibiteurs de la PCSK9 (PCK9i) chez une population avec HFH et comparer la différence entre une monothérapie versus une association avec les autres hypolipémians.

IV.1.1.2 Schéma de l'étude

Nous avons conduit une étude observationnelle monocentrique sur les patients référés pour lipoprotéine aphérèse au département de cardiologie de CHU-Toulouse entre Mars 2018 et septembre 2019. Les patients avec un diagnostic d'HFH selon le Dutch Lipid Clinic Network Score (DLNS) [5] et ayant les critères de remboursement d'alirocumab en France ont été inclus dans cette cohorte. Par suite, les critères d'inclusion nécessitent un niveau de LDL-c ≥ 300 mg/dl si prévention primaire et ≥ 200 mg/dl si prévention secondaire. Un traitement par alirocumab a été introduit pendant 6 mois sous forme d'une injection sous cutanée de 150mg toutes les deux semaines en association au traitement médical maximal initialement toléré par les patients. Par conséquent, les participants dans cette étude ont été réparti en trois groupes

: inhibiteur de la PCSK9 (PSCK9i) en monothérapie, PSCK9i avec ézetimibe et PCSK9i avec statine et ézetimibe. Les patients des deux premiers groupes sont considérés intolérants aux statines. L'intolérance aux statines est définie par l'incapacité à tolérer au moins deux molécules de statine différentes dont une prescrite à la plus faible dose.

IV.1.1.3 Collection des données et analyses statistiques

Durant la première visite, les patients ont été examinés par un cardiologue avec collection des antécédents personnels et familiaux. Les participants dans l'étude ont été éduqués pour l'auto-réalisation de l'injection sous cutané d'alirocumab. Le bilan lipidique a été collecté avant et après 6 mois d'initiation de PCSK9i. L'objectif primaire est d'évaluer la moyenne de variation en LDL-c et de comparer la différence entre les trois groupes de l'étude. Les variables quantitatives sont représentées par moyenne et déviation standard tandis que les variables qualitatives par effectif et pourcentage. L'analyse des variances (ANOVA) a été utilisée pour comparer la moyenne des variables quantitatives et la moyenne de variation de LDL-c entre les trois groupes. Le test de Chi² ou Fischer ont été utilisés pour comparer la distribution des variables qualitatives entre les groupes. Une analyse multivariée linéaire a été conduite pour identifier les facteurs associés avec le pourcentage de réduction de LDL-c et une analyse multivariée de régression logistique a été réalisée pour identifier les prédictors des répondeurs au PCSK9i. Les analyses multivariées ont été ajustées sur l'âge, le sexe, le type de prévention (primaire ou secondaire) et le taux basal de bilan lipidique. Une valeur $p < 0.05$ est considérée statistiquement significatif.

IV.1.2. Résultats

Au total, 123 patients avec HFH ont participé dans cette étude et réparti dans trois groupes (Table 1). La table 1 montre les caractéristiques des participants. La moyenne d'âge est de 59 ± 11 ans et 66 patients (53.7%) sont des hommes. Concernant les facteurs de risque cardiovasculaire, 4 (3.3%) sont des fumeurs, 30 (24.4%) hypertendus et 9 (7.3%) diabétiques. Pour le traitement médical, 95 (77.2%) patients sont considérés intolérants à la statine et parmi eux 12 (9.8%) sont traités par ézetimibe. Les autres 28 (22.8%) participants dans l'étude sont traités par statine et ézetimibe avant l'initiation de PSCK9i. 29 (23.6%) des participants ont été traité en prévention primaire tandis que 94 (76.4%) ont été traité en prévention secondaire avec un antécédent de maladie coronarienne (87.2%), accident vasculaire ischémique (3.2%) et artériopathie périphérique (12.8%). Les moyennes basales de cholestérol total, LDL-c, HDL-c et triglycéride sont 361 ± 81 mg/dl, 277 ± 78 mg/dl, 52 ± 13 mg/dl et 168 ± 69 mg/dl respectivement. Après initiation des PSCK9i, la baisse observée de la moyenne de LDL-c est de 49.1% dans la population totale avec un passage de 277 ± 78 mg/dl à 141 ± 84 mg/dl, de 44.2% dans le groupe sous PSCK9i en monothérapie (n=83) avec un passage de 283 ± 81 mg/dl à 158 ± 83 mg/dl, de 41.7% dans le groupe sous PSCK9i et ézetimibe (n=12) avec un passage de 247 ± 68 mg/dl à 144 ± 100 mg/dl et de 66.3% dans le groupe sous PSCK9i, statine et ézetimibe (n=28) avec un passage de 264 ± 78 mg/dl à 89 ± 57 mg/dl (Figures 1, 2).

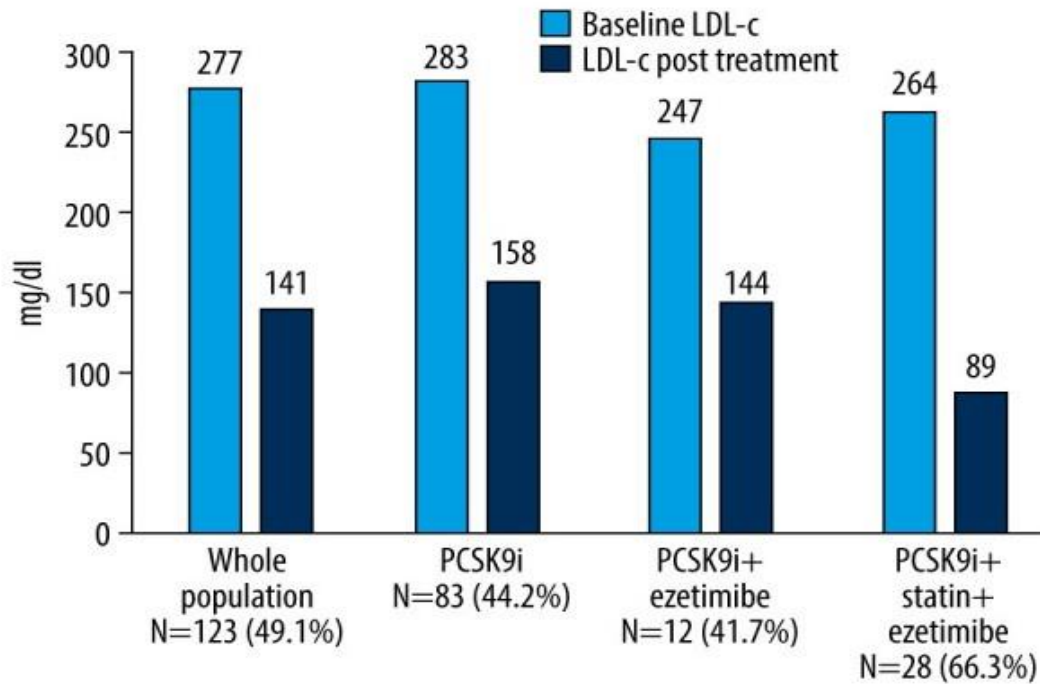


Figure 1. Le pourcentage de réduction de LDL-c (mg/dl) après l'introduction des inhibiteurs de la PCSK9 (PCSK9i).

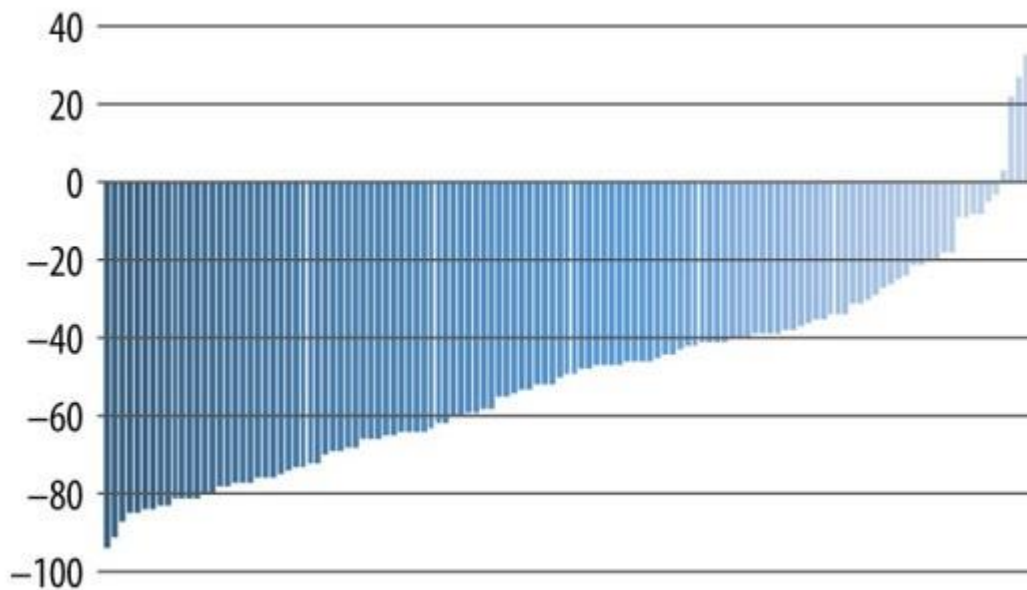


Figure 2. La cascade de variation (%) de LDL-c chez 123 participants avec hypercholestérolémie familiale hétérozygote après initiation des inhibiteurs de la PCSK9.

Les analyses statistiques (ANOVA) montrent une différence significative de la moyenne de réduction de LDL-c entre le groupe sous PCSK9i, statine et ézetimibe et les deux autres groupes

sous PCSK9i seul ($p=0.002$) et sous PCSK9i et ézetimibe ($p=0.006$). En revanche, on note une absence de différence significative entre le groupe sous PCSK9i et le groupe sous PCSK9i et ézetimibe (Table 2). En plus, l'analyse multivariée linéaire montre une association entre le groupe sous PCSK9i + statine + ézetimibe et le pourcentage de réduction de LDL-c ($\beta=-20.21$, 95% CI [-30.46 ; -9.95], $p < 0.0001$). D'autre part, le taux de répondeurs observé avec une baisse $\geq 50\%$ de taux de LDL-c est de 49.6% (61/123) dans la population étudiée, 38.6% (32/83) dans le groupe sous PCSK9i seul, 58.3% (7/12) dans le groupe sous PCSK9i + ézetimibe, et 78.6% (22/28) dans le groupe sous PCSK9i+statine+ezetimibe. Les analyses statistiques montrent une association positive entre le traitement par PCSK9i+statine+ezetimibe et le taux de répondeurs [OR=6, 95% CI (2-17.6), $p=0.001$] et une association négative entre la moyenne basale de LDL-c et le taux de répondeurs [OR=0.46, 95% CI (0.26 -0.82), $p=0.009$] (Table 3). Le tableau 4 montre la répartition individuelle des mutations génétiques chez les 123 participants.

VI.1.3 Discussion

Cette étude reflète en vie réelle et en pratique clinique l'effet de l'introduction de PCSK9i chez une population avec HFH systématiquement classifiée à haut risque cardiovasculaire avec un objectif de baisse initiale de LDL-c $\geq 50\%$ et un taux final de LDL-c $\leq 70\text{mg/dl}$ [11]. Dans cette étude, l'utilisation de PCSK9i résulte en une moyenne de réduction de 49.1% de LDL-c. Ce résultat est comparable à celui-ci rapporté dans la littérature avec une baisse de LDL-c allant de 43% jusqu'à 69% [17-25].

Les recommandations de la société Européenne de cardiologie en 2019 notent une efficacité constante des PCSK9i indépendamment de la thérapie en arrière-plan [11]. Le résultat publié par Ridker et al est en ligne avec cette recommandation [26]. Nous avons retrouvé un effet synergique entre la statine et le PCSK9i et non plus avec l'ézetimibe. En ligne avec notre

résultat, Desmukh et al rapportent l'absence des bénéfices additionnels liés à l'association de PCSK9i et l'ézetimibe d'une part [27] et Rallidis et al ont démontré une potentialisation de l'efficacité des PCSK9i avec la prise concomitante d'une statine d'autre part [28]. Les statines activent le « sterol regulatory element binding protein transcription factor » en réduisant le cholestérol hépatique intracellulaire ce qui augmente l'expression des récepteurs de LDL-c et le taux circulant de la protéine PCSK9 [12]. Une augmentation de 7.4% à 35% de taux de PCSK9 circulante est observée après l'administration des statines [29]. Cette augmentation de l'expression de la protéine PCSK9 induite par les statines expliquent la majoration de l'effet des PCSK9i en cas d'une prise concomitante avec une statine.

Dans notre étude, la proportion des répondeurs avec une baisse $\geq 50\%$ de LDL-c est de 49.6% et elle est plus faible que celle décrite dans la littérature (56% à 85.4%) [17-25] et les essais cliniques (65% à 85%) [30-32]. Ceci peut être expliqué par la proportion élevée des participants intolérants à la statine et par le niveau basal très élevé de LDL-c (277mg/dl) comparé aux autres études (de 155mg/dl à 219mg/dl). Les analyses statistiques ont démontré une association inversement proportionnelle entre le taux basal de LDL-c et le taux des répondeurs. Plus le taux de LDL-c est bas, plus la baisse de LDL-c est importante.

En résumé, cette étude conclue à l'efficacité des PCSK9i dans une population avec HFH avec une moyenne de variation de LDL-c observée comparable aux essais cliniques et compatible avec les recommandations ($\geq 50\%$). Nous avons observé une efficacité supérieure en cas d'administration avec une statine sans aucune bénéfice additionnelle attribuée à l'ézetimibe et en cas où le niveau basal de LDL-c est plus bas. Il est important à signaler que malgré la baisse significative de la moyenne de LDL-c, la proportion des participants atteignant l'objectif recommandé de LDL-c $\leq 70\text{mg/dl}$ reste relativement faible.

IV.2 Effet de la Mutation Génétique Causale sur le Risque d'Athérosclérose Chez les patients avec Hypercholestérolémie Familiale Hétérozygote

IV.2.1 Méthodologie

IV.2.1.1 Objectif

Cette étude vise à comparer si les patients avec hypercholestérolémie familiale hétérozygote (HFH) porteurs d'une mutation génétique causale sous traitement médical sont à plus haut risque de développer un évènement cardiovasculaire.

IV.2.1.2 Schéma de l'étude

Nous avons conduit une cohorte rétrospective sur 854 patients référés au département de cardiologie préventive au CHU-Toulouse et ayant le résultat d'analyse génétique de l'ADN disponible. Les patients ont été suivi de la date du premier bilan lipidique disponible jusqu'à la date de survenue d'un évènement cardiovasculaire ou la date du dernier suivi médical. Tous les bilans lipidiques réalisés durant la période de suivi ont été collectés. Nous avons calculé le DLCNS et la moyenne de LDL-c pour chaque participant dans l'étude. Les patients âgés de plus que 18 ans et qui remplissent les critères diagnostiques possible, probable et défini d'HFH selon le DLCNS ont été inclus dans l'étude (289 patients). Les patients avec des données manquantes (absence de suivi), de moins de 18 ans, avec une moyenne de LDL-c < 155mg/dl et avec un DLCNS < 3 (diagnostique peu probable pour HFH) ont été exclus de l'étude (558 patients). Un patient avec hypercholestérolémie familiale homozygote et 6 patients porteurs d'une mutation génétique d'une signification inconnue ont été aussi exclus. En premier, la population étudiée a été divisé en deux groupes en fonction de la survenue ou pas d'un

évènement cardiovasculaire et ensuite en fonction de la présence ou pas d'une mutation génétique causale.

IV.2.1.3 Collection des données et analyses statistiques

Les caractéristiques des participants et les résultats de tous les bilans lipidiques (cholestérol total, LDL-c, HDL-c, triglycéride, Lp (a), Apo A1 et Apo B) réalisés durant la période de suivi ont été collectés. Les évènements cardiovasculaires sont définis par une réduction $\geq 50\%$ des artères des membres inférieurs ou des carotides, un accident vasculaire cérébral ischémique et une sténose $\geq 50\%$ d'une artère coronaire. Le séquençage d'ADN des gènes codant pour LDL-R, PCSK9, APOB et APOE a été réalisé. Les variantes génétiques ainsi que leurs natures causales ont été vérifiées par « RJP » et consécutivement classifiées comme pathogène, probablement pathogène, variante de signification inconnue, probablement bénigne et bénigne. Ensuite, les variantes génétiques considérées comme pathogènes ou probablement pathogènes ont été divisées en fonction de leurs conséquences en deux sous-groupes : modérées ou sévères. Les formes sévères incluent les larges réarrangements, mutation « non-sens », mutations décalantes (décalage de cadre du lecture) et mutations des sites d'épissage. Les formes modérées incluent la mutation faux-sens, de délétion et de duplication. Le groupe des participants avec HFH porteurs d'une mutation causale incluent les patients avec des mutations considérées pathogènes ou probablement pathogènes. Les variables quantitatives sont représentées par moyenne et déviation standard tandis que les variables qualitatives par effectif et pourcentage. Le test de Chi² ou Fischer ont été utilisés pour comparer la distribution des variables qualitatives entre les groupes tandis que le test t de student a été utilisé pour comparer la moyenne des variables quantitatives entre les groupes. La normalité de la distribution et l'égalité des variances (homoscédasticité) pour les variables continues ont été vérifiées. La courbe de Kaplan Meier et le test de Log Rank ont été utilisés pour l'analyse de survie.

L'analyse multivariée de régression logistique a été réalisée pour évaluer l'association entre le développement d'un évènement cardiovasculaire et la présence d'une mutation génétique causale. Une valeur $p < 0.05$ est considérée statistiquement significatif.

IV.2.2 Résultat

Parmi les 854 patients filtrés, 289 ont été inclus dans l'étude. La moyenne d'âge est de 49 ± 13 ans et 37% des participants sont des hommes. Selon le DLCNS, le diagnostic d'HFH est défini (>8 points) dans 48.8%, probable (6-8 points) dans 4.2%, et possible (3-5 points) dans 47.1% des cas. L'analyse génétique détecte une mutation causale chez 42.2% des participants. La mutation causale implique le gène codant pour LDLR dans 36.6%, APOB dans 7.6%, PCSK9 dans 0.3% et APOE dans 0.7% (Figure 3).

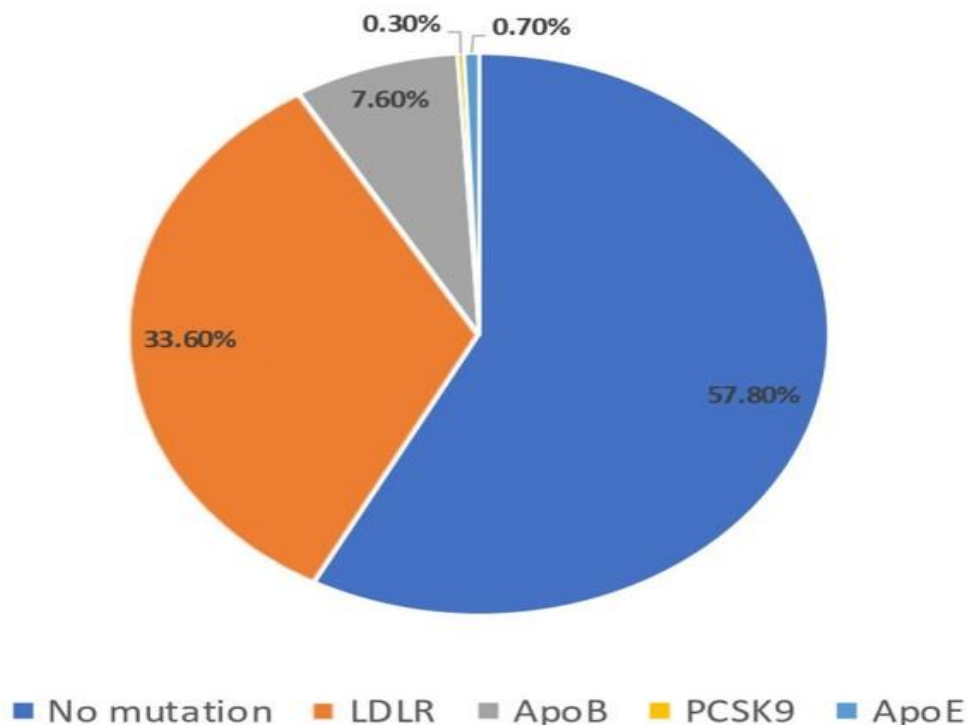


Figure 3. Diagramme représentant la prévalence des mutations parmi les participants dans l'étude.

Pendant une moyenne de suivi de 5.97 ± 5.97 années, 21.5% des participants développent un évènement cardiovasculaire : sténose coronaire (18.2%), accident vasculaire ischémique (1.4%) et artériopathie périphérique (1.4%). Comparé aux autres, les patients avec HFH qui développent un évènement cardiovasculaire sont plus des hommes (48.4% vs. 33.9%, $p=0.037$), plus âgés (52 ± 12 vs. 48 ± 13 , $p=0.027$), présentent des moyennes plus élevées de cholestérol total (310 ± 74 vs. 288 ± 46 , $p=0.029$) et de LDL-c (233 ± 66 vs. 207 ± 41 , $p=0.004$) et plus porteurs d'une mutation causale (53.2% vs. 39.2%, $p=0.048$) (Table 5). D'autre part, les patients porteurs d'une mutation causale sont plus jeunes (45 ± 14 vs 52 ± 11 , $p=0.001$) avec une moyenne de LDL-c plus élevée (226 ± 59 vs. 203 ± 37 , $p=0.001$) et une prévalence des évènements cardiovasculaires plus importante (27% vs. 17.4%, $p=0.048$) (Table 6). Après ajustement sur les facteurs de confusion (âge, sexe, HDL-c et LDL-c), l'analyse multivariée montre une association positive entre le niveau de LDL-c [OR=2.658 ; 95% CI (1.495-4.729), $p=0.001$] et l'âge [OR=1.034 ; 95% CI (1.009-1.060), $p=0.008$] et le développement d'évènement cardiovasculaire. Cependant, le niveau de HDL-c montre une tendance vers une association négative [OR=0.132, 95% CI (0.016-1.073), $p=0.058$] (Table 7- Modèle 1). En opposition de l'analyse bivariée, la détection d'une mutation causative n'est pas significativement associée avec le développement d'un évènement cardiovasculaire [OR=1.713 ; 95% CI (0.902-3.256), $p=0.100$] (Table 7- modèle 2). Seulement après l'exclusion de niveau de LDL-c du modèle statistique, l'association entre la mutation causale et l'évènement cardiovasculaire devient statistiquement significative [OR=2.149, 95% CI (1.170-3.948), $p=0.014$] (Table 7- modèle 3). Les résultats ont été comparables après stratification de la population étudiée en trois groupes : mutation sévère, modérée et absence de mutation causale (Tables 8-9). On note que la moyenne de LDL-c diffère significativement entre les sous-groupes de l'étude (Table 10) et en particulier entre les porteurs d'une mutation au niveau

du gène codant pour LDLR et les non porteurs d'une mutation causale ($p=0.003$) (Table 11).
Finalement, la courbe de Kaplan-Meier et le test de Log Rank ne montre pas une différence de survie sans évènement cardiovasculaire chez les patients avec HFH porteurs versus non porteurs d'une mutation génétique causale ($p=0.547$) (Figure 4).

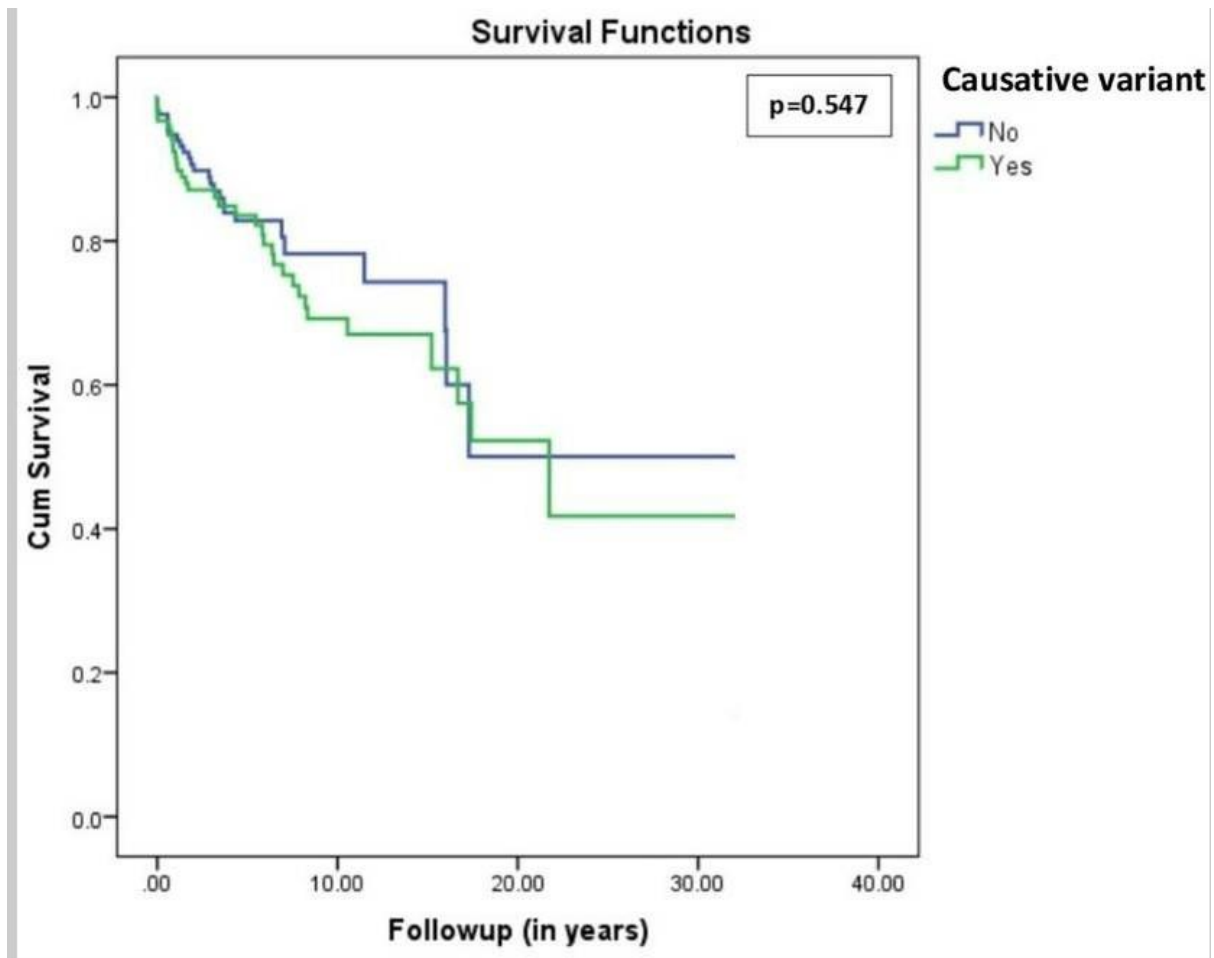


Figure 4. Courbe Kaplan Meier pour analyse de survie sans évènement cardiovasculaire chez les patients avec hypercholestérolémie familiale porteurs versus non porteurs d'une mutation causale.

IV.2.3 Discussion

Cette étude a montré que les patients avec HFH porteurs d'une mutation génétique représentent une fréquence plus élevée des évènements cardiovasculaires et une moyenne plus élevée de LDL-c, en particulier en cas d'une mutation du gène codant pour LDLR. Cependant, la détection d'une mutation génétique causale n'est pas associée en elle-même au développement d'un évènement cardiovasculaire au cours de temps. Le niveau élevé de LDL-c représente le puissant prédicteur de la maladie d'athérosclérose

Jusqu'à nos jours, les preuves disponibles de l'effet des variants génétiques sur le risque cardiovasculaire dans un contexte d'HFH sont controversées. Par exemple, les modèles statistiques ont montré un effet du variant génétique de LDLR sur la survie sans évènement cardiovasculaire chez une population néerlandaise avec HFH uniquement après l'exclusion de LDL-c du modèle [33]. Comme nous, les auteurs concluent au rôle plus important de LDL-c que la mutation en soi même sur la prédiction du risque cardiovasculaire. Cette conclusion commune résulte des données similaires entre les deux études, en particulier les résultats des analyses multivariées. Une large cohorte française récemment publiée a illustré indirectement le même résultat en montrant un taux de mortalité de toute cause comparable à la suite d'un diagnostic clinique versus génétique d'HFH (5.54 vs. 4.66 per 1000 personnes). En revanche, les taux des évènements coronaires (24.66 vs 15.89 per 1000) et cérébraux (3.44 vs 2.47 per 1000) et d'artériopathie périphérique (3.63 vs 2.66 per 1000) ont été légèrement plus élevés dans le groupe de diagnostic clinique [34]. Dans une population danoise de Copenhague, l'observation a montré l'absence de différence de risque de maladie coronaire entre les patients porteurs versus non porteurs d'une mutation du gène codant l'APOB [35]. Pareil, le niveau de LDL-c diffère d'une façon significative entre les porteurs d'une variante génétique de LDLR et les autres [35-36]. Au contraire, d'autres études ont montré que les patients avec HFH et porteurs d'une variante génétique causale sont à plus haut risque de développement d'un évènement cardiovasculaire [37-40]. Benn M, et al rapportent un risque de maladie coronaire

3.3 fois plus élevé chez les porteurs d'une mutation de gène codant LDLR [35]. Khera et al suggèrent que la connaissance du statut génétique fournit de bénéfices additionnels sur la prédiction du risque cardiovasculaire que le niveau de LDL-c isolé [39]. Les données japonaises rapportent que le diagnostic génétique reflète une exposition à long cours depuis la naissance à un niveau élevé de LDL-c [40]. En France, 1/3 des patients porteurs d'une mutation sévère développent un évènement cardiovasculaire avec une moyenne de 2.5 évènements par patient et 1/4 des patients porteurs d'une mutation modérée développent un évènement cardiovasculaire avec une moyenne de 2 évènements par patient [41]. Le risque d'une maladie coronaire est multiplié x2 et x3 chez les patients avec HFH et porteurs des variants génétiques [38]. Les études ultérieures ont été seulement intéressées par le niveau initial de LDL-c avant le début de traitement médical alors que la présente a évalué l'évolution de LDL-c sous traitement médical au cours de temps ce qui permet d'évaluer au mieux l'impact de la mutation causale sur le risque cardiovasculaire en vie réelle. Certaines études ont montré le lien entre la variante génétique de LDLR et l'athérosclérose par l'intermédiaire de la formation d'une plaque au niveau des carotides [36] et l'obstruction des artères coronaires [35-36,39,42]. Cette augmentation du risque cardiovasculaire n'a été pas observé avec les variants génétiques de PCSK9 et APOB. En ligne avec les autres études, nous n'avons pas retrouvé une différence de risque liée au sexe des patients avec HFH. En plus, il faut mentionner la responsabilité des facteurs génétiques non-LDL d'une partie des évènements cardiovasculaires indépendamment de niveau de LDL-c dans un contexte d'HFH en favorisant un état d'hyper coagulation et d'hypo fibrinolyse [43-44]. Par exemple, l'équipe Kastelein a montré une association entre le polymorphisme génétique G20210A et l'athérosclérose chez les patients avec HFH [45]. Ces investigateurs ont montré une augmentation de facteur VII dans l'HFH [46]. Ceci peut partiellement expliquer les bénéfices des statines liées à leurs effets pléiotropes et anticoagulant [47-49]. La différence entre les résultats des études sur la valeur prédictive des variantes

généétiques causales de l'athérosclérose peut dériver de la différence d'expression des facteurs génétiques non-LDL.

En résumé, il semble que l'effet de la variante génétique causale sur le développement des évènements cardiovasculaires passe uniquement par l'intermédiaire de LDL-c. L'analyse d'ADN joue un rôle essentiel dans l'établissement d'un diagnostic et le dépistage familiale permettant de réduire la durée d'exposition à un niveau élevé de LDL-c mais sa valeur de stratification du risque cardiovasculaire reste incertaine [50-51]. Une étude récemment publiée montre que l'incidence des évènements cardiovasculaires dépend de l'exposition cumulée au LDL-c [52]. L'analyse couts-avantages de test génétique reste une préoccupation même dans les pays développés tels que l'Europe et l'Australie [53-55]. Parmi les limitations de notre étude, on note sa nature rétrospective qui prédispose au biais de sélection et le critère monocentrique. En plus, on cite le nombre limité des participants porteurs des mutations causales au niveau du gène PCSK9 et APOE et la diversité thérapeutique. 60.9% des participants sont traités par PCSK9i seule, 26.6% avec PCSK9+ et statine, 9.7% avec PCSK9i, statine et ézetimibe et 2.8% avec PCSK9i et ézetimibe. Le score du risque polygénique dans le groupe des participants non porteurs d'une mutation causale n'a été pas évalué vue l'absence de preuve de son utilité [56].

5. Conclusion

Cette thèse a montré en vie réelle une efficacité des inhibiteurs de PCSK9 comparable aux essais cliniques surtout si associés à une statine dans le contexte d'HFH. Nous avons observé une différence significative dans la réduction de LDL-c en fonction de régime thérapeutique (avec ou sans statine). La mutation génétique causale ne présente pas en elle-même un prédicteur de l'évènement cardiovasculaire et le LDL-c joue le rôle clé dans la survenue des

événements ischémiques indifféremment de la présence ou non d'une variante causale. Des essais cliniques randomisés évaluant la valeur pronostic de l'analyse génétique et d'une meilleure réponse thérapeutique sont nécessaires pour améliorer la prise en charge des patients avec HFH.

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7. Listes des tableaux

Tableau 1. Caractéristiques de base de la population étudiée pour observation de l'efficacité pratique des inhibiteurs de la PCSK9.

	Whole population (n=123)	PCSK9i (n=83)	PCSK9i plus ezetimibe (n=12)	PCSK9I plus statin and ezetimibe (n=28)
Age (years±SD)	59±11	58±12	62±11	57±10
Sex				
Male, n (%)	66 (53.7%)	41 (49.4%)	5 (41.7%)	20 (71.4%)
Female, n (%)	57 (46.3%)	42 (50.6%)	7 (58.3%)	8 (28.6%)
Familial hypercholesterolemia, n (%)	123 (100%)			
Without known ASCVD, n (%)	29 (23.6%)	25 (30.1%)	3 (25%)	1 (3.6%)
With ASCVD, n (%)	94 (76.4%)	58 (69.9%)	9 (75%)	27 (96.4%)
Atherosclerotic disease				
Coronary artery disease, n (%)	82 (66.67%)	50 (60.2%)	9 (75%)	23 (82.1%)
Ischemic stroke, n (%)	3 (2.4%)	3 (3.6%)	0 (0%)	0 (0%)
Peripheral artery disease, n (%)	12 (9.8%)	10 (12%)	0 (0%)	2 (7.1%)
Cardiovascular risk factors				

Hypertension, n (%)	30 (24.4%)	21 (25.3%)	4 (33.3%)	5 (17.8%)
Diabetes mellitus, n (%)	9 (7.3%)	7 (8.4%)	1 (8.3%)	1 (3.6%)
Current smokers, n (%)	4 (3.3%)	4 (4.8%)	0 (0%)	0 (0%)
Mean total cholesterol level (mg/dl±SD)	361±83	373±83	332±66	337±82
Mean triglycerides (mg/dl±SD)	168±69	175±73	167±59	143±52
Mean HDL-c (mg/dl±SD)	52±13	53±14	50±10	48±13
Mean LDL-c level (mg/dl±SD)	277±78	283±81	247±68	264±78

ASCVD, atherosclerotic cardiovascular disease; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; n, number of patients; PCSK9i, proprotein convertase subtilisin/kexin type 9 inhibitors; SD, standard deviation.

Tableau 2. Analyse des variances (ANOVA) comparant la moyenne de réduction de LDL-c entre les 3 groupes.

	Mean variation in LDL-c (mg/dl±SD)	Compared to	95% CI	P value
PCSK9i	125±60	PCSK9i+statin	(-0.85; -0.15)	0.002
		PCSK9i+ezetimibe	(-0.28; 0.72)	0.86

		PCSK9i	(-0.72; 0.28)	0.86
PCSK9i+ezetimibe	103±89			
		PCSK9i+statin	(-1.28; -0.17)	0.006
		PCSK9i	(0.15; 0.85)	0.002
PCSK9i+statin and ezetimibe	175±72			
		PCSK9i+ezetimibe	(0.17; 1.28)	0.006

CI, confidence interval; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; PCSK9i, proprotein convertase subtilisin/kexin type 9 inhibitors.

Tableau 3. Comparaison de taux de répondeurs entre les 3 groupes

	Whole population (n=123)	PCSK9i (n=83)	PCSK9i + ezetimibe (n=12)	PCSK9i + statin and ezetimibe (n=28)
Responders, n (%)	61 (49.6%)	32 (38.6%)	7 (58.3%)	22 (78.6%)
Odds ratio			2.20	6
95% Confidence interval			(0.6-8.1)	(2-17.6)
P value			0.24	0.001

PCSK9i : Proprotein convertase subtilisin / kexin type 9 inhibitors.

Tableau 4. Répartition des mutations génétiques chez les 123 participants dans l'étude observationnelle d'efficacité des inhibiteurs de la PCSK9.

Patients number	PCSK9 gene	LDLR gene	APOB gene	APOE gene
1		X		
2		X		
3		X		
4	X			
5	X			
6		X		
7			X	
8			X	
9			X	
10		X		
11		X		
12		X		
13		X		
14		X		
15		X		
16		X		
17		X		
18		X		
19		X		
20		X		
21			X	
22		X		
23		X		

24		X
25		X
26		X
27		X
28		X
29		X
30		X
31		X
32		X
33		X
34		X
35		X
36	X	
37		X
38		X
39		X
40		X
41		X
42		X
43		X
44	X	
45	X	
46		
47	X	
48	X	
49		X
50	X	
51		X
52		X
53		X

54	X			
55				
56				
57		X		
58	X			
59		X		
60		X		
61			X	
62	X			
63		X		
64			X	
65		X		
66			X	
67		X		
68		X		
69				
70				
71		X		
72		X		
73		X		
74		X		
75	X			
76				X
77		X		
78		X		
79		X		
80	X			
81		X		
82		X		
83				X

84		X	
85	X		
86	X		
87	X		
88		X	
89		X	
90			X
91		X	
92	X		
93	X		
94		X	
95		X	
96		X	
97		X	
98	X		
99		X	
100		X	
101			X
102		X	
103		X	
104		X	
105		X	
106		X	
107		X	
108	X		
109	X		
110		X	
111		X	
112		X	
113			X

114		X
115	X	
116		X
117	X	
118		X
119		X
120		X
121		X
122		X
123		X

Tableau 5. Caractéristiques des participants avec versus sans événement cardiovasculaire.

	Study population	ASCVD-group	No ASCVD-Group	p-value
	(N=289)	(N=62)	(N=227)	
Age (year)	49±13	52±12	48±13	0.027
Males (N,%)	107 (37%)	30 (48.4%)	77 (33.9%)	0.037
BMI (kg/m²)	24.3±4.5	24.6±3.9	24.2±4.6	0.554
Smoker (N,%)	53 (18.3%)	11 (17.7%)	42 (18.5%)	0.891
Systemic hypertension (N,%)	44 (15.2%)	11 (17.7%)	33 (14.5%)	0.534
Diabetes mellitus (N,%)	8(2.8%)	4(6.5%)	4(1.8%)	0.068
Causative mutation (N,%)	122 (42.2%)	33 (53.2%)	89 (39.2%)	0.048
Mutated gene (N,%)				0.163
1. LDL-R	97(33.6%)	25(40.3%)	72(31.7%)	
2. Apo B	22(7.6%)	7(11.3%)	15(6.6%)	
3. PCSK9	1(0.3%)	0	1(0.4%)	
4. Apo E	2(0.7%)	1(1.6%)	1(0.4%)	

DLNCS (N,%)				0.001
3-5 points (possible)	136(47.1%)	13(21.0%)	123(54.2%)	
6-8 points (probable)	12(4.2%)	11(17.7%)	1(0.4%)	
>8 points (definite)	141(48.8%)	38(61.3%)	103 (45.4%)	
Mean Total cholesterol (mg/dl)	292±54	310±74	288 ±46	0.029
Mean LDL-c (mg/dl)	213±49	233±66	207±41	0.004
Mean HDL-c (mg/dl)	60±25	56±16	62±27	0.162
Mean triglyceride (mg/dl)	125±55	133±58	123±54	0.146
Mean Lp(a) (mg/dl)	44±49	49±43	43±48	0.446
<10 mg/dl (%)	33%	26.3%	34.4%	0.589
10-50 mg/dl (%)	33%	34.2%	32.8%	
>50 mg/dl (%)	34%	39.5%	32.8%	
Mean Apo A1 (mg/dl)	156±25	151±22	157±25	0.168
Mean Apo B (mg/dl)	150±33	152±39	149±34	0.667
Follow-up (year)	5.97±5.97	5.10±6.36	6.20±5.85	0.197

*BMI=body mass index; LDL-R= low density lipoprotein cholesterol receptor; PCSK9= proprotein convertase subtilisin/kexin type-9; Apo=apolipoprotein; DLNCS= Dutch lipid network clinic score; LDL-c= low density lipoprotein cholesterol; HDL-c= high density lipoprotein cholesterol; Lp(a) =lipoprotein (

Tableau 6. Caractéristiques des participants porteurs versus non porteurs d'une mutation causale.

	Study population (N=289)	Causative mutation- group (N=122)	No causative mutation-group (N=167)	p-value
Age (Year)	49±13	45±14	52±11	0.001
Males (N,%)	107(37%)	46(37.7%)	61(36.5%)	0.838
BMI (kg/m²)	24.32±4.46	24.09±5.09	24.48±3.97	0.470
Smoker (N,%)	53(18.3%)	24(19.7%)	29(17.4%)	0.617

Systemic hypertension (N,%)	44(15.2%)	14(11.5%)	30(18%)	0.129
Diabetes mellitus (N,%)	8(2.8%)	4(3.3%)	4(2.4%)	0.725
Mean Total cholesterol (mg/dl)	292±54	299±66	288±42	0.087
Mean LDL-c (mg/dl)	213±49	226±59	203±37	0.001
Mean HDL-c (mg/dl)	60±25	57±15	63±30	0.032
Mean triglyceride (mg/dl)	125±55	110±41	137±61	0.001
Mean Lpa (mg/dl)	44±49	41±44	46±52	0.405
<10mg/dl (%)	33%	32.6%	33.3%	0.532
10-50mg/dl (%)	33%	36.8%	30.4%	
>50mg/dl (%)	34%	30.5%	36.3%	
Mean Apo A1 (mg/dl)	156±25	152±27	159±23	0.033
Mean Apo B (mg/dl)	150±35	156±41	145±29	0.030
ASCVD (N,%)	62(21.5%)	33(27%)	29(17.4%)	0.048
Follow-up (year)	5.97±5.97	7.31±6.54	4.98±5.32	0.001

**BMI=body mass index; LDL-c=low density lipoprotein cholesterol; HDL-c=high density lipoprotein cholesterol; Lp(a) =lipoprotein (a); Apo=apolipoprotein; ASCVD=atherosclerotic cardiovascular disease*

Tableau 7. Modèles des analyses multivariées évaluant l’association entre la présence d’une mutation causale et la survenue d’un événement cardiovasculaire.

***Model 1**

	OR	95%CI	p-value
Sex	1.823	[1.974-3.413]	0.061
Age	1.034	[1.009-1.060]	0.008
Mean LDL-c	2.658	[1.495-4.729]	0.001
Mean HDL-c	0.132	[0.016-1.073]	0.058

***Model 2**

	OR	95%CI	p-value
Sex	1.880	[1.000-3.532]	0.050
Age	1.040	[1.014-1.067]	0.003
Causative mutation	1.713	[0.902-3.256]	0.100
Mean LDL-c	2.336	[1.298-4.205]	0.005
Mean HDL-c	0.148	[0.018-1.223]	0.076

***Model 3**

	OR	95%CI	p-value
Sex	2.003	[1.076-3.728]	0.028
Age	1.044	[1.018-1.070]	0.001
Causative mutation	2.149	[1.170-3.948]	0.014
Mean HDL-c	0.236	[0.033-1.697]	0.151

**OR=odds ratio; CI=confidence interval; LDL-c=low density lipoprotein cholesterol; HDL-c=high density lipoprotein cholesterol*

Tableau 8. Caractéristiques des participants en fonction de la sévérité de la mutation.

	Study population	Causative mutation-group (N=122)		No causative mutation-group	p-value
	(N=289)	Severe(N=36)	Moderate(N=86)	(N=167)	
Age (Year)	49±13	43±13	45±15	52±11	0.001
Males (N,%)	107(37%)	14(38.9%)	32(37.2%)	61(36.5%)	0.961
BMI (kg/m ²)	24.32±4.46	24.67±4.62	23.86±5.26	24.48±3.97	0.398
Smoker (N,%)	53(18.3%)	6(16.7%)	19(20.9%)	29(17.4%)	0.756
Systemic hypertension	44(15.2%)	1(2.8%)	13(15.1%)	30(18%)	0.071
Diabetes mellitus	8(2.8%)	0(0%)	4(4.7%)	4(2.4%)	0.442
Mean Total cholesterol (mg/dl)	292±54	284±45	306±72	288±42	0.022
Mean LDL-c (mg/dl)	213±49	217±44	229±64	203±37	0.001
Mean HDL-c (mg/dl)	60±25	53±11	58±16	63±30	0.026
Mean triglyceride (mg/dl)	125±55	102±38	113±42	137±61	0.001
Mean Lpa (mg/dl)	44±49	52±57	36±36	46±52	0.256
<10mg/dl (%)	33%	29.6%	33.8%	33.3%	0.532
10-50mg/dl (%)	33%	33.3%	38.2%	30.4%	
>50mg/dl (%)	34%	37%	27.9%	36.3%	
Mean Apo A1 (mg/dl)	156±25	144±27	155±25	159±23	0.023
Mean Apo B (mg/dl)	150±35	147±38	160±42	146±29	0.017
ASCVD (N,%)	62(21.5%)	10(27.8%)	23(27.1%)	29(17.4%)	0.130
Follow-up (year)	5.97±5.97	8±5.6	6.96±6.89	4.98±5.28	0.003

Tableau 9. Modèles de l'analyse multivariée de régression logistique évaluant l'association entre la mutation causale et le développement des évènements cardiovasculaire après stratification sur la sévérité de la mutation.

*Model 1			
	OR	95%CI	p-value
Age	1.030	[1.005-1.056]	0.020
Sex	1.813	[0.972-3.383]	0.061
Mean HDL	0.167	[0.021-1.346]	0.093
Mean LDL	2.399	[1.342-4.288]	0.003
Mutation			0.314
Moderate	1.624	[0.806-3.273]	0.175
Severe	1.698	[0.674-4.276]	0.261
*Model 2			
	OR	95%CI	p-value
Age	1.033	[1.008-1.058]	0.009
Sex	1.924	[1.041-3.555]	0.037
Mean HDL	0.256	[0.036-1.825]	0.174
Mutation			
Moderate	2.134	[1.105-4.121]	0.024
Severe	1.975	[0.798-4.890]	0.141

Tableau 10. Test de Kruskal-Wallis comparant la moyenne des composantes du bilan lipidique entre les différentes mutations.

	No causative mutation (N=167)	LDL-R (N=97)	Apo B (N=22)	PCSK9/Apo E (N=3)	p-value
Total cholesterol (mg/dl)	287±42	297±68	309±55	289±77	0.551
LDL-c (mg/dl)	203±37	225±61	231±52	218±51	0.014
HDL-c (mg/dl)	63±30	55±14	63±15	64±14	0.017
Lpa (mg/dl)	46±52	42±46	37±38	36±34	0.986
Apo A1 (mg/dl)	159±23	149±26	159±26	174±41	0.033
Apo B (mg/dl)	146±29	153±42	162±30	194±81	0.150

* *LDL-R= low density lipoprotein cholesterol receptor; PCSK9= proprotein convertase subtilisin/kexin type-9; Apo=apolipoprotein; LDL-c= low density lipoprotein cholesterol; HDL-c= high density lipoprotein cholesterol; Lpa=lipoprotein a*

Tableau 11. Test de Bonferroni comparant la moyenne de différence de LDL-c entre les sous-groupes.

		95%CI	p-value
	LDL-R	[-0.3733; -0.0506]	0.003
No mutation	PCSK9/Apo E	[-0.8827;0.5895]	1
	Apo B	[-0.5645;0.0086]	0.063
	No mutation	[0.0506;0.3733]	0.003
LDL-R	PCSK9/Apo E	[-0.6753;0.8062]	1
	Apo B	[-0.3643;0.2324]	1
	No mutation	[-0.5895;0.8827]	1
PCSK9/Apo E	LDL-R	[-0.8062;0.6753]	1
	Apo B	[-0.9091;0.6463]	1
	No mutation	[-0.0086;0.5645]	0.063

Apo B	LDL-R	[-0.2324;0.3643]	1
	PCSK9/Apo E	[-0.6463;0.9091]	1

* *LDL-R= low density lipoprotein cholesterol receptor; PCSK9= proprotein convertase subtilisin/kexin type-9;*
Apo=apolipoprotein

5. Annexes

Real-World Efficacy of Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors (PCSK9i) in Heterozygous Familial Hypercholesterolemia Patients Referred for Lipoprotein Apheresis

Authors' Contribution:
Study Design: A
Data Collection: B
Statistical Analysis: C
Data Interpretation: D
Manuscript Preparation: E
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Background: A small proportion of familial hypercholesterolemia (FH) patients can adequately control this condition, although achieving the recommended targets for low-density lipoprotein cholesterol (LDL-c) levels remains a challenge. Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) are new and potent lipid-lowering drugs. However, there is scarce literature on real-world data about their use in patients with FH.

Material/Methods: We examined the reduction in LDL-c levels from the baseline, after PCSK9i initiation in heterozygous familial hypercholesterolemia patients referred for lipoprotein apheresis in our regional lipid clinic. The study was conducted from March 2018 to September 2019, the period immediately after PCSK9i reimbursement was available in France. PCSK9i was added on top of the patients' maximal tolerated lipid-lowering regimens.

Results: The study had 123 patients with heterozygous FH. The mean age of the patients was 59±11 years. The mean baseline LDL-c for all the participants was 277±78 mg/dl. It was 283±81 mg/dl in the PCSK9i monotherapy group (n=83), 247±68 mg/dl in the PCSK9i plus ezetimibe group (n=12), and 264±78 mg/dl in the PCSK9i plus statin and ezetimibe group (n=28). The mean decrease observed in the LDL-c level from baseline was 136±70 mg/dl (n=123), 125±60 mg/dl (n=83), 103±77 mg/dl (n=12), and 175±70 mg/dl (n=28), respectively.

Conclusions: An overall reduction of 49.1% from the baseline LDL-c was observed in the heterozygous FH population after PCSK9i initiation in a real-world experience. The group treated with PCSK9i ezetimibe plus statin showed further reduction of their LDL-c levels with a better responder rate, achieving the target 50% reduction in LDL-c from the baseline.

Keywords: Cholesterol, LDL • Dyslipidemias • Proprotein Convertases

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/928784>

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Background

Familial hypercholesterolemia (FH) is a common, autosomal dominant hereditary disease caused by mutations in the genes encoding key proteins involved in the low-density lipoprotein (LDL) receptor's (LDLR) endocytic and recycling pathways. The LDLR gene encodes the LDLR, the apolipoprotein B gene encodes apolipoprotein B (the ligand of the LDL receptor), and the PCSK9 gene encodes a serine protease (proprotein convertase subtilisin/kexin type 9 or PCSK9), which plays a role in the degradation of the LDLR [1]. Mutations in the LDLR adaptor protein 1 gene are responsible for the recessive form of the disease [2]. However, FH is an underdiagnosed and undertreated genetic disorder that results in premature mortality and morbidity due to atherosclerotic disease. Myocardial infarction, stroke, or other cardiovascular events could be the first clinical presentation of FH [3]. European guidelines classify FH patients at high cardiovascular risk and the optimal target for low-density lipoprotein cholesterol (LDL-c) is ≤ 70 mg/dl with $\geq 50\%$ reduction from the level at baseline [4]. Reaching these LDL-c goals in clinical practice remains a challenge for physicians.

All the statins have proven their effectiveness in significantly lowering LDL-c levels (upregulating the LDL receptor in the liver and peripheral tissues) and are recommended as first-line therapy. Nevertheless, muscle and joint pain are common adverse effects associated with statin therapy, which lead to an interruption of medical treatment in real-life practice. PCSK9 inhibitors (PCSK9i) have recently emerged as novel medications indicated for patients who cannot tolerate statins, and cannot achieve the required optimal LDL-c levels [5].

Clinical trials have shown that PCSK9i can effectively reduce the baseline LDL-c levels by 60% on average, when used alone or combined with other lipid-lowering agents [4]. Current trials are investigating PCSK9i as an alternative therapeutic option for patients undergoing lipoprotein apheresis, as it shows a synergistic effect leading to a drop in extracorporeal apheresis sessions and lipoprotein apheresis withdrawal (in some cases) [6]. Concerns regarding the inter-individual variability, FH mutations, LDLR abnormalities, and the importance of pre-treatment with statins in patients on PCSK9i have been raised, as all these factors are well-known predictors of the efficacy of lipid-lowering drugs. In the Trial Evaluating PCSK9 Antibody in Subjects With LDL Receptor Abnormalities (TESLA) [7], neither a single autosomal recessive hypercholesterolemia patient nor a single null/null LDLR homozygote patient responded to evolocumab. Similar results were observed in larger numbers of patients for both these patient categories in the Trial Assessing Long-Term Use of PCSK9 Inhibition in Subjects With Genetic LDL Disorders (TAUSSIG) study [8]. Robinson et al reported that PCSK9i is more effective in patients with higher levels of circulating PCSK9 stimulated by previous statin therapy [9].

In addition, the failure to respond to PCSK9i occurs more frequently, as PCSK9i is more commonly prescribed [10].

The real-world efficacy of PCSK9i regarding patients' characteristics and the severity of LDL-c levels are not known since the vast majority of randomized controlled trials have been conducted on patients receiving combination therapy (PCSK9i and statin), and patients with low LDL-c before treatment [11-14]. Therefore, we aimed to investigate the variation in LDL-c levels after initiation of PCSK9i, either as monotherapy, or in combination with previously prescribed ezetimibe or statin plus ezetimibe in a real-life cohort study of severely dyslipidemic heterozygous FH patients referred for lipoprotein apheresis. In this study population, the baseline LDL-c level was one of the highest reported in literature.

Material and Methods

Study Design and Population

An observational single-center cohort study was conducted on patients referred for lipoprotein apheresis at our Lipid, Atherosclerosis, and Preventive Cardiology Clinic at Rangueil University Hospital, Toulouse, France between March 2018 and September 2019. Patients were eligible for participating in the study if they were diagnosed with FH according to the Dutch Lipid Clinic Network score [15]. In France, alirocumab is reimbursed only for patients treated for primary prevention (LDL-c ≥ 300 mg/dl) or secondary prevention (LDL-c ≥ 200 mg/dl) in the setting of an experienced lipoprotein apheresis center. Therefore, patients with these baseline LDL-c levels were requested to participate in the study. The patients were divided into 3 groups based on their real-world prescriptions in this observational study: those treated with PCSK9i only, those treated with PCSK9i in addition to the preexisting ezetimibe therapy, and those treated with PCSK9i in addition to the preexisting statin plus ezetimibe therapy. Alirocumab monotherapy was initiated in the statin-intolerant patients. Statin intolerance was defined as an inability in the past to tolerate 2 or more statins (1 statin at the lowest daily starting dose). Alirocumab was added on top of a maximal tolerated lipid-lowering regimen in patients taking either ezetimibe or a statin plus ezetimibe. The alirocumab was prescribed as a 150 mg subcutaneous injection administered every 2 weeks.

Data Collection and End Points

During the initial visit, a physician performed a detailed physical examination, and recorded family and personal medical histories of the participants. The issues of incorrect injection techniques and non-adherence to treatment were eliminated, as the medical team supervised the injections. Full lipid

Table 1. Baseline characteristics of the study population.

	Whole population (n=123)	PCSK9i (n=83)	PCSK9i plus ezetimibe (n=12)	PCSK9i plus statin and ezetimibe (n=28)
Age (years±SD)	59±11	58±12	62±11	57±10
Sex				
Male, n (%)	66 (53.7%)	41 (49.4%)	5 (41.7%)	20 (71.4%)
Female, n (%)	57 (46.3%)	42 (50.6%)	7 (58.3%)	8 (28.6%)
Familial hypercholesterolemia, n (%)	123 (100.0%)			
Without known ASCVD, n (%)	29 (23.6%)	25 (30.1%)	3 (25.0%)	1 (3.6%)
With ASCVD, n (%)	94 (76.4%)	58 (69.9%)	9 (75.0%)	27 (96.4%)
Atherosclerotic disease				
Coronary artery disease, n (%)	82 (66.7%)	50 (60.2%)	9 (75.0%)	23 (82.1%)
Ischemic stroke, n (%)	3 (2.4%)	3 (3.6%)	0 (0.0%)	0 (0.0%)
Peripheral artery disease, n (%)	12 (9.8%)	10 (12.0%)	0 (0.0%)	2 (7.1%)
Cardiovascular risk factors				
Hypertension, n (%)	30 (24.4%)	21 (25.3%)	4 (33.3%)	5 (17.8%)
Diabetes mellitus, n (%)	9 (7.3%)	7 (8.4%)	1 (8.3%)	1 (3.6%)
Current smokers, n (%)	4 (3.3%)	4 (4.8%)	0 (0.0%)	0 (0.0%)
Mean total cholesterol level (mg/dl±SD)	361±83	373±83	332±66	337±82
Mean triglycerides (mg/dl±SD)	168±69	175±73	167±59	143±52
Mean HDL-c (mg/dl±SD)	52±13	53±14	50±10	48±13
Mean LDL-c level (mg/dl±SD)	277±78	283±81	247±68	264±78

ASCVD – atherosclerotic cardiovascular disease; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; n – number of patients; PCSK9i – proprotein convertase subtilisin/kexin type 9 inhibitors; SD – standard deviation.

panels at the initiation of the PCSK9i therapy (baseline LDL-c) and 6 months after the initiation of the PCSK9i treatment were collected. Based on the 2019 European Society of Cardiology (ESC) guidelines for FH management, ≥50% reduction from the LDL-c baseline was considered a satisfactory response to PCSK9i in this study. The main outcome was the variation in LDL-c levels, 6 months from the baseline levels after initiation of the PCSK9i treatment in the 3 groups of patients. None of the patients had received a statin without ezetimibe before inclusion in the study. The secondary outcome was the percentage of responders at 6 months (ie, ≥50% drop in LDL-c).

Statistical Analysis

Continuous data were summarized as means and standard deviations, and categorical variables were summarized as counts and percentages. The continuous variables were compared between the 3 groups through an analysis of variance (ANOVA). The bivariate associations with the categorical variables were studied with the chi-square test or Fisher's exact test as appropriate.

ANOVA was used to compare the mean change in LDL-c levels between the groups. A multivariate linear regression analysis was conducted for studying percentage variations in the

LDL-c levels from baseline in the 3 groups. The patients treated with PCSK9i alone were considered the reference group. A multivariate logistic regression analysis was conducted to study the satisfactory response to treatment as a dependent variable. The multivariate analyses were adjusted for age, sex, type of prevention (primary or secondary), and the baseline lipid panel. A P value <0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 20, (SPSS Inc., IBM, Chicago, IL, USA).

The patients were seen at our clinic within a regular follow-up; therefore, approval from the Ethics Committee was not needed. The patients gave oral consent for participation in the study, which followed the institution's ethical rules.

Results

A total of 123 patients with heterozygous FH were included in the study (**Supplementary Online Table 1**). They were divided into 3 groups. The baseline characteristics of these participants are shown in **Table 1**. Their mean age was 59±11 years; there were 66 (53.7%) men and 57 (46.3%) women. Four (3.3%) patients were smokers, 30 (24.4%) patients had systemic hypertension, 9 (7.3%) patients had diabetes mellitus,

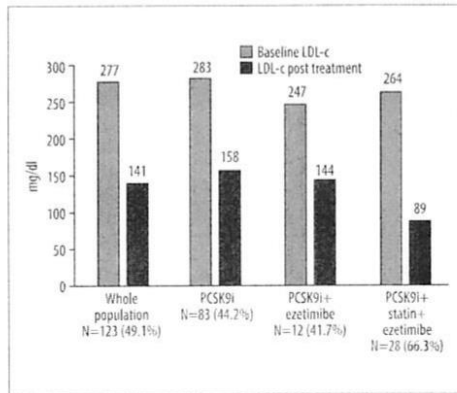


Figure 1. Low-density lipoprotein cholesterol reduction (%) from the baseline after PCSK9i initiation. PCSK9i – proprotein convertase subtilisin/kexin type 9 inhibitor.

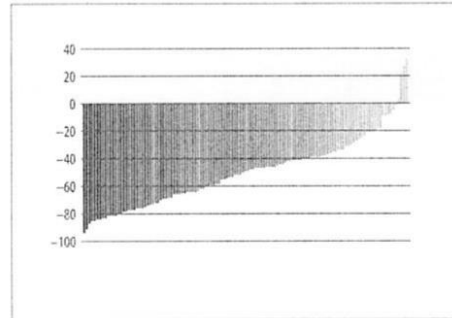


Figure 2. Waterfall plot of the variation (%) in low-density lipoprotein cholesterol of 123 heterozygous familial hypercholesterolemia patients after initiation of PCSK9i. PCSK9i – proprotein convertase subtilisin/kexin type 9 inhibitor.

Table 2. Analysis of variance (ANOVA) comparing the reduction in mean low-density lipoprotein cholesterol between the 3 groups after adding PCSK9i.

	Mean variation in LDL-c (mg/dl±SD)	Compared to	95% CI	P value
PCSK9i	125±60	PCSK9i+statin	(-0.85; -0.15)	0.002
		PCSK9i+ezetimibe	(-0.28; 0.72)	0.86
PCSK9i+ezetimibe	103±89	PCSK9i	(-0.72; 0.28)	0.86
		PCSK9i+statin	(-1.28; -0.17)	0.006
PCSK9i+statin and ezetimibe	175±72	PCSK9i	(0.15; 0.85)	0.002
		PCSK9i+ezetimibe	(0.17; 1.28)	0.006

CI – confidence interval; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; PCSK9i – proprotein convertase subtilisin/kexin type 9 inhibitors.

28 (22.8%) patients were taking statin and ezetimibe prior to the PCSK9i initiation, and 95 (77.2%) patients were statin-intolerant. Of the statin-intolerant patients, 12 (9.8%) patients were taking ezetimibe prior to the PCSK9i initiation and 83 (67.5%) patients did not receive any lipid-lowering agents before the PCSK9i initiation. Twenty-nine (23.6%) patients were being treated for primary prevention. Among the 94 (76.4%) patients treated for secondary prevention, 82 (87.2%) patients had coronary artery disease, 3 (3.2%) patients had a history of ischemic stroke, and 12 (12.8%) patients had peripheral artery disease. The overall mean total cholesterol, LDL-c, high-density lipoprotein cholesterol (HDL-c), and triglyceride baseline levels were 361±83 mg/dl, 277±78 mg/dl, 52±13 mg/dl, and 168±69 mg/dl, respectively.

The mean baseline LDL-c level dropped from 277±78 mg/dl to 141±84 mg/dl in the entire study population, accounting for a 49.1% reduction. The mean baseline LDL-c level showed

a 44.2% reduction (from 283±81 mg/dl to 158±83 mg/dl) in the PCSK9i monotherapy group (n=83), a 41.7% reduction in LDL-c (from 247±68 mg/dl to 144±100 mg/dl) in the PCSK9i plus ezetimibe group (n=12), and a 66.3% reduction in LDL-c (from 264±78 mg/dl to 89±57 mg/dl) in the PCSK9i plus statin and ezetimibe group (n=28) (Figures 1, 2). Statistical analysis (ANOVA) showed a statistically significant difference in the mean LDL-c reduction between the PCSK9i plus statin and ezetimibe group compared to the other 2 groups (PCSK9i only, P=0.002, and PCSK9i plus ezetimibe, P=0.006), while no significant difference was noted between the PCSK9i only and PCSK9i plus ezetimibe groups (Table 2). A secondary multivariate analysis using linear regression (% of LDL-c reduction=dependent variable) showed a significant correlation between the statin group (PCSK9i plus statin and ezetimibe) and the percentage of LDL-c reduction (β =-20.21, confidence interval [CI] [-30.46 to -9.95], P<0.0001).

Table 3. A comparison of the responder rate between the 3 groups.

	Whole population (n=123)	PCSK9i (n=83)	PCSK9i+ezetimibe (n=12)	PCSK9i+statin and ezetimibe (n=28)
Responders, n (%)	61 (49.6%)	32 (38.6%)	7 (58.3%)	22 (78.6%)
Odds ratio			2.20	6
95% Confidence interval			(0.6-8.1)	(2-17.6)
P value			0.24	0.001

PCSK9i – proprotein convertase subtilisin/kexin type 9 inhibitors.

There were 61/123 (49.6%) responders in the study population, who achieved the target of at least 50% reduction in their LDL-c level from the baseline. They were 32/83 (38.6%) patients from the PCSK9i only group, 7/12 (58.3%) patients from the PCSK9i plus ezetimibe group, and 22/28 (78.6%) patients from the PCSK9i plus statin and ezetimibe group. Statistical analysis showed a positive statistically significant relationship between the PCSK9i plus statin and ezetimibe group and the responder rate (OR=6, CI [2-17.6], $P=0.001$) (Table 3), and a negative statistically significant relationship between the baseline LDL-c level and the responder rate (Odds ratio=0.46, CI [0.26-0.82], $P=0.009$).

Discussion

All FH patients are systematically classified as high cardiovascular risk patients according to the ESC guidelines for cardiovascular prevention [4]. A target of 70 mg/dl for LDL-c with at least 50% reduction from the baseline is recommended [4]. Recently, patients who could not achieve the target LDL-c value on statin and ezetimibe received the PCSK9i as triple combination therapy [4,16,17]. However, few reports reflected the application of PCSK9i in clinical practice, especially in FH patients. Here, we present a real-world, single-center experience of PCSK9i use in the FH population referred for lipoprotein apheresis.

In our study population, the use of PCSK9i was associated with a 49.1% reduction in LDL-c from the baseline. This result is similar to the 43% to 69% drop in LDL-c levels reported in previously published studies [18-26]. We found a potential synergic effect between the statin and PCSK9i, revealed through a statistically significant ($P<0.01$) reduction in the LDL-c levels observed in the patients taking PCSK9i on top of the statin, compared to the patients treated only with PCSK9i or with PCSK9i plus ezetimibe. A similar result was reported by Deshmukh et al, which showed no significant difference with the addition of ezetimibe [23]. Currently, there is no clear indication that the statin type or intensity relevantly affects the LDL-c lowering by PCSK9i. The 2019 ESC guidelines [4] and Ridker et al [17]

have endorsed a constant PCSK9i efficacy, regardless of the background therapy. Rallidis et al [27] reported a potent effect of PCSK9i on top of the statin in LDL-c reduction compared to those receiving PCSK9i monotherapy. By reducing the intracellular hepatic cholesterol, statin subsequently activates the sterol regulatory element binding protein transcription factor, increases not only the LDLR expression but also the PCSK9 expression, and consequently the circulating PCSK9 protein [5]. A 7.4% to 35% increase in the PCSK9 protein level was shown with the use of statins [28]. This boosts the efficacy of PCSK9i and facilitates the expected reduction in the LDL-c level. It explains the smaller reduction in LDL-c that is observed in patients treated with PCSK9i alone or combined with ezetimibe, making it more difficult to achieve the target goals in statin-intolerant patients. For this reason, the proportion of patients achieving the target 50% reduction in LDL-c from the baseline is 49.6% and it is lower than found in the literature (56% to 85.4%) [18-26]. Clinical trials have reported that 65% to 85% of the studied populations reach their treatment targets [29-31]. This discrepancy can be linked to the higher baseline LDL-c levels of our study population (277 mg/dl) compared to the previously described LDL-c levels in the literature (155 mg/dl to 219 mg/dl). The multivariate statistical analysis showed a statistically significant negative relationship between the baseline LDL-c levels and the potential satisfactory reduction in LDL-c levels ($P=0.009$); starting from a lower baseline, LDL-c is predicted to show a greater drop in LDL-c, and a significantly ($P=0.001$) higher proportion of responders are shown among those treated with statin and PCSK9i.

The recommended satisfactory reduction in the LDL-c levels in the present study's FH population were observed, while PCSK9i was associated with statin. In parallel, a low baseline LDL-c level is a predictor for a greater reduction in LDL-c, and no additional benefits were attributed to ezetimibe. Regardless of an overall observed alirocumab efficacy similar to the results of large randomized clinical trials, we highlight a statistically significant difference between the 2 prescribed regimens (with and without statin). It is important to consider that in our study, the treatment regimen excluding statin was prescribed in totally statin-intolerant patients.

To summarize, we emphasize the value of PCSK9i use in the FH population referred for lipoprotein apheresis in real-life practice, especially when combined with statin. Consequently, in order to obtain the best efficacy, PCSK9i is the appropriate therapy only in FH patients already on statin therapy and with moderate LDL-c levels before PCSK9i initiation.

Limitations

This study was a single-center experience reflecting the findings for PCSK9i use in a regional lipid clinic. Only alirocumab was used and its effects are reported in this study. The restricted inclusion criteria were defined according to the conditions for PCSK9i-reimbursement approval by the French health authorities. The 6-month follow-up is a short period to monitor the long-term LDL-c efficacy in FH patients.

Supplementary Data

Supplementary Online Table 1. Heterozygous mutated genes of the study participants.

Supplementary/raw data available from the corresponding author on request.

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Conclusions

We presented a single-center study showing an overall efficacy of PCSK9i in real life for the heterozygous FH population referred for lipoprotein apheresis. In these patients, the association of statin is essential for a greater reduction in LDL-c and an improved chance to achieve the target goals as a significant difference has been shown between PCSK9i monotherapy and the statin-associated regimen. Further research is needed to disentangle the best predictive parameters of PCSK9i efficacy in this severe FH population.

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Conflict of Interest

The authors declare they have no conflict of interest. Prof. Jean Ferrières has received lecture fees from Amgen, MSD, Sanofi, and Sèrvier.

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Effect of causative genetic variants on atherosclerotic cardiovascular disease in heterozygous familial hypercholesterolemia patients

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Background: Heterozygous familial hypercholesterolemia (HFH) is an autosomal dominant genetic disorder leading to a lifetime exposure to high low-density lipoprotein cholesterol (LDL-c) level and an increased risk of premature atherosclerotic cardiovascular disease (ASCVD). We evaluate the effect of a causative genetic variant to predict ASCVD in HFH patients undergoing treatment.

Materials and methods: A retrospective cohort was conducted on 289 patients with possible, probable, and definite diagnosis of HFH according to Dutch Lipid Clinic Network Score and in whom DNA analyses were performed and mean LDL-c level was above 155 mg/dl. The study population was divided into groups based on the presence or not of a causative variant (pathogenic or likely pathogenic). We observed each of the study's participants for the occurrence of ASCVD.

Results: A causative variant was detected in 42.2% of study participants, and ASCVD has occurred in 21.5% of HFH patients. The incidence of ASCVD (27% vs. 17.4%, $p = 0.048$) and the mean of LDL-c under an optimal medical treatment (226 ± 59 mg/dl vs. 203 ± 37 mg/dl, $p = 0.001$) were higher in HFH-causative variant carriers than others. After adjusting on confounders, ASCVD was positively associated with LDL-c level [OR = 2.347; 95% (1.305–4.221), $p = 0.004$] and tends toward a negative association with HDL-c level [OR = 0.140; 95% (0.017–1.166), $p = 0.059$]. There is no more association between the detection of a causative variant and the occurrence of ASCVD [OR = 1.708; 95% (0.899–3.242), $p = 0.102$]. Kaplan Meier and log rank test showed no significant differences in event-free survival analysis between study groups ($p = 0.523$).

Conclusion: In this study population under medical care, it seems that the presence of a causative variant did not represent an independent predictor of adverse cardiovascular outcomes in HFH patients, and LDL-c level played an undisputable causal role.

KEYWORDS

heterozygous familial hypercholesterolemia, genetic variant, cardiovascular disease, LDL-c, atherosclerosis

Introduction

Heterozygous familial hypercholesterolemia (HFH) is an autosomal dominant genetic disorder leading to a lifetime exposure to high low-density lipoprotein cholesterol (LDL-c) level and an increased risk of premature atherosclerotic cardiovascular disease (ASCVD). In most countries, the heterozygous form of familial hypercholesterolemia (FH) usually affects 1 in 313 to 120 individuals (1–3). The Dutch Lipid Clinic Network Score (DLCNS) is a valid diagnostic score for FH. It includes a set of criteria: patient's family history of early-onset cardiovascular disease in his first-degree relatives, personal history of cardiovascular disease, physical signs of hypercholesterolemia (tendinous xanthoma and/or arcus cornealis prior to age 45 years), circulating level of LDL-c, and positive DNA analysis for a genetic variant in *LDLR* (low-density lipoprotein cholesterol receptor), *APOB* (apolipoprotein B), or *PCSK9* (proprotein convertase subtilisin/kexin type-9) gene (4). DLCNS stratifies the diagnosis of FH into four categories: unlikely (<3 points), possible (3–5 points), probable (6–8 points), and definite (>8 points). Thus, DNA testing is recommended in FH patients by several international and scientific societies. It ensures a precise molecular diagnosis, a screening cascade identifying unknown and asymptomatic FH patients among closed family members, an early initiation of optimal medical therapy, and a prognostic stratification (5, 6). Apart from the traditional HFH-causing variants in *LDLR*, *APOB*, and *PCSK9* genes, the detection of mutant *APOE* gene in HFH patients is recently considered as a cause or an exacerbating factor of HFH phenotype (6–8). It seems that patients with digenic causality, combined *LDLR* and *PCSK9* gene variants, experienced poor cardiovascular outcomes marked by a high frequency of non-fatal myocardial infarction (9). It is noteworthy that the risk of obstructive coronary artery disease in HFH patients with pathogenic variant and LDL-c level of ≥ 190 mg/dl was 22 times higher than that of general population with LDL-c level of ≤ 130 mg/dl (10). It was also six times higher in HFH patients without pathogenic variant compared with the reference group (10). In the setting of HFH, clinical trials evaluating the risk of atherosclerosis depending on DNA analysis are scarce in literature. Most published ones assess the difference in risk between FH patients and the general population. The present study compares cardiovascular outcomes in HFH population under medical care with versus without a causative variant and evaluates the association between different gene variants and ASCVD.

Materials and methods

Study design and population

A retrospective cohort was conducted on 854 patients who were referred to the Department of Preventive Cardiology at Toulouse University Hospital, Rangueil, France, and for whom the results of DNA analysis test are available. We collected the available controls of lipid panel during the follow-up period while receiving the maximum tolerated medical therapy. The follow-up period

extended from the date of the first lipid panel till the occurrence of ASCVD or the last available follow-up. The DLCNS and means of collected LDL-c levels in the course of time were calculated for each of the study's participants. Patients aged above 18 years old and who fulfilled the diagnostic criteria of possible, probable, and definite HFH according to DLCNS were included in this study (289 patients). Patients with incomplete data (lack of follow-up information), younger than 18 years old, with mean LDL-c of <155 mg/dl, and unlikely for HFH diagnosis (DLCNS of <3) were excluded from this study (558 patients). One patient with homozygous FH was also excluded. Six patients with genetic variant of unknown significance were excluded. Then, we observed study participants till the occurrence of a significant atherosclerotic cardiovascular event or the last available follow-up. The study population was divided into two groups: first, according to the development or not of ASCVD and, second, according to the detection or not of a causative variant. We evaluate the differences in the incidence of ASCVD and means of total cholesterol, LDL-c, HDL-c, triglycerides, lipoprotein(a) [Lp(a)], apolipoprotein A1 (Apo A1), and apolipoprotein B (Apo B) levels among the study groups.

Data collection and end point

Baseline characteristics of study population, results of DNA analysis, and full lipid panel tests [total cholesterol, LDL-c, HDL-c, triglycerides, Lp(a), Apo A1, and Apo B] under an optimal tolerated lipid-lowering therapy were collected throughout the follow-up period. The DLCNS and means of cholesterol, LDL-c, HDL-c, triglycerides, Lp(a), Apo A1, and Apo B levels were calculated for each of study's participants, respectively. ASCVD was defined by a more than 50% reduction in the diameter of peripheral arteries or carotids on Doppler ultrasound, an ischemic stroke was revealed on cerebral imaging, and more than 50% reduction in the coronary artery lumen was detected on a coronary scanner or coronary angiography. DNA sequencing of the *LDLR*, *PCSK9*, *APOB*, and *APOE* genes were performed. All genetic variants and their causal effects were verified by "RJP" and subsequently classified as pathogenic, likely pathogenic, variant of unknown significance, likely benign, or benign. Considering their consequences, pathogenic and likely pathogenic causative genetic variants were segregated into two subtypes: moderate or severe. Severe variants encompass large rearrangements and point mutations accounting for non-sense, frameshifts, splicing, and initiation codon loss mutations. Moderate variants include missense, in-frame deletion, or duplication and 5' regulatory mutations. The HFH-causative variant carriers group includes pathogenic and likely pathogenic variants, whereas HFH-no causative variant group includes variants of unknown significance, benign and likely benign variants, and patients with undetected genetic variant. We aim to evaluate if HFH with a causative variant patient undergoing medical care was more associated with ASCVD compared with HFH-no causative variant carrier. Patients were informed at hospital admissions that their clinical data could be used for research purposes in anonymous form, and non-opposition consent forms were obtained. The cohort was

registered by the Ministry of Research and the Regional Health Agency Occitanie (no. DC-2017-298).

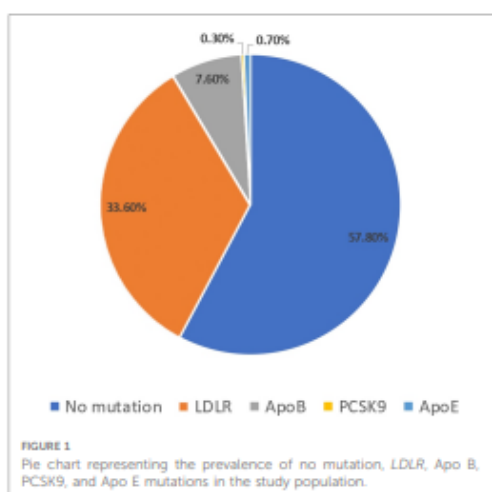
Statistical analysis

Statistical analyses were performed using SPSS version 20.0. Qualitative variables were expressed by frequency and percentages, while quantitative variables were summarized as means and standard deviations. Categorical variables were compared with the use of χ^2 test or Fisher's exact test as appropriate, while continuous variables were studied with the use of *t*-test. Normality and variance homogeneity for continuous variables were checked. Kaplan-Meier curve and log rank test were used for survival analysis. Multivariable logistic regression analysis was used to test the association of ASCVD with HFH-causative variants. A two-sided *p*-value of ≤ 0.05 was considered to be of statistical significance.

Results

Out of 854 screened patients, a total of 289 patients were included in this study. The mean age of study population was 49 ± 13 years old, and 37% of study participants were males. Based on DLCNS, the diagnosis of HFH was definite (>8 points) in 48.8%, probable (6–8) in 4.2%, and possible (3–5) in 47.1%. The DNA analysis detected a genetic causative variant in 42.2% of study participants. The causative variants were found on *LDLR* gene in 33.6%, *APOB* gene in 7.6%, *PCSK9* in 0.3%, and *APOE* in 0.7% (Figure 1). Over a mean follow-up period of 5.97 ± 5.97 years, ASCVD has occurred in 21.5% of study participants. The observed cardiovascular events were coronary artery disease (18.2%), ischemic stroke (1.4%), and peripheral

artery disease (1.4%). Compared with no ASCVD group, HFH patients who developed ASCVD were commonly males (48.4% vs. 33.9%, $p = 0.037$), were older (52 ± 12 vs. 48 ± 13 , $p = 0.027$), and had higher means of total cholesterol (310 ± 74 vs. 288 ± 46 , $p = 0.029$) and LDL-c (233 ± 66 vs. 207 ± 41 , $p = 0.004$) levels. In addition, causative variants were significantly more expressed in study participants with versus without ASCVD (53.2% vs. 39.2%, $p = 0.048$) (Table 1). On the other hand, HFH-causative variant carriers were younger (45 ± 14 vs. 52 ± 11 , $p = 0.001$) with higher mean LDL-c level (226 ± 59 vs. 203 ± 37 , $p = 0.001$). Also, HFH patients with pathogenic and likely pathogenic variant were at greater risk of ASCVD (27% vs. 17.4%, $p = 0.048$) (Table 2). After adjusting on confounders (age, sex, HDL-c and LDL-c levels), the multivariable logistic regression showed a positive association of LDL-c level [OR = 2.658; 95% (1.495–4.729), $p = 0.001$] and age [OR = 1.034; 95% (1.009–1.060), $p = 0.008$] with ASCVD, respectively. The HDL-c level tends toward a negative association with ASCVD [OR = 0.132; 95% (0.016–1.073), $p = 0.058$], whereas male sex tends toward a positive association [OR = 1.823; 95% (1.974–3.413), $p = 0.061$] (Table 3—model 1). Unlike the results of bivariate analyses, the detection of a causative variant becomes no more significantly associated with ASCVD [OR = 1.713; 95% (0.902–3.256), $p = 0.100$] (Table 3—model 2). Only after excluding LDL-c from the statistical model, the association between the presence of causative genetic variant and ASCVD was statistically significant [OR = 2.149; 95% (1.170–3.948), $p = 0.014$] (Table 3—model 3). Similar results were found after a second revision of genetic analyses stratifying study participants into three categories: severe, moderate, and no causative genetic variant (Supplementary Tables S1 and S2). Note that the means of LDL-c differed significantly among study sub-groups (Table 4) and this difference was mainly observed between *LDLR* variant carriers and those with no causative variant ($p = 0.003$) (Table 5). Lastly, the Kaplan-Meier curve and log rank test failed to detect a significant difference in survival analysis for freedom of ASCVD between study groups (no causative variant vs. causative variant carriers, $p = 0.547$) (Figure 2).



Discussion

The present study is one of the few available studies to report on the prediction of HFH-causative variants including *APOE* variant type. It showed that HFH-causative variant carriers are more likely exposed to cardiovascular events and expressed a higher level of LDL-c, especially those with *LDLR* variant type. However, the detection of HFH-causative variant *per se* was not significantly associated with the occurrence of ASCVD in the course of time. Thus, the increased level of LDL-c remains the strongest independent predictor of ASCVD.

To date, available evidence on the effect of genetic variants on cardiovascular risk in HFH patients is controversial. For example, in Dutch HFH patients, the effect of *LDLR* variant type on survival analysis for freedom of cardiovascular event was only observed in the statistical models after excluding LDL-c level (11). Like us, authors conclude to a greater role of LDL-c level

TABLE 1 Characteristics of study population with versus without atherosclerotic cardiovascular disease (ASCVD).

	Study population (N = 289)	ASCVD group (N = 62)	No ASCVD group (N = 227)	p-Value
Age (year)	49 ± 13	52 ± 12	48 ± 13	0.027
Males (N, %)	107 (37%)	30 (48.4%)	77 (33.9%)	0.037
BMI (kg/m ²)	24.3 ± 4.5	24.6 ± 3.9	24.2 ± 4.6	0.554
Smoker (N, %)	53 (18.3%)	11 (17.7%)	42 (18.5%)	0.891
Systemic hypertension (N, %)	44 (15.2%)	11 (17.7%)	33 (14.5%)	0.534
Diabetes mellitus (N, %)	8 (2.8%)	4 (6.5%)	4 (1.8%)	0.068
Causative mutation (N, %)	122 (42.2%)	33 (53.2%)	89 (39.2%)	0.048
Mutated gene (N, %)				0.163
LDLR	97 (33.6%)	25 (40.3%)	72 (31.7%)	
Apo B	22 (7.6%)	7 (11.3%)	15 (6.6%)	
PCSK9	1 (0.3%)	0	1 (0.4%)	
Apo E	2 (0.7%)	1 (1.6%)	1 (0.4%)	
DLNCS (N, %)				0.001
3–5 points (possible)	136 (47.1%)	13 (21.0%)	123 (54.2%)	
6–8 points (probable)	12 (4.2%)	11 (17.7%)	1 (0.4%)	
>8 points (definite)	141 (48.8%)	38 (61.3%)	103 (45.4%)	
Mean total cholesterol (mg/dl)	292 ± 54	310 ± 74	288 ± 46	0.029
Mean LDL-c (mg/dl)	213 ± 49	233 ± 66	207 ± 41	0.004
Mean HDL-c (mg/dl)	60 ± 25	56 ± 16	62 ± 27	0.162
Mean triglyceride (mg/dl)	125 ± 55	133 ± 58	123 ± 54	0.146
Mean Lp(a) (mg/dl)	44 ± 49	49 ± 43	43 ± 48	0.446
<10 mg/dl (%)	33%	26.3%	34.4%	0.589
10–50 mg/dl (%)	33%	34.2%	32.8%	
>50 mg/dl (%)	34%	39.5%	32.8%	
Mean Apo A1 (mg/dl)	156 ± 25	151 ± 22	157 ± 25	0.168
Mean Apo B (mg/dl)	150 ± 33	152 ± 39	149 ± 34	0.667
Follow-up (year)	5.97 ± 5.97	5.10 ± 6.36	6.20 ± 5.85	0.197

BMI, body mass index; LDLR, low-density lipoprotein cholesterol receptor; PCSK9, proprotein convertase subtilisin/kexin type-9; Apo, apolipoprotein; DLNCS, Dutch Lipid Network Clinic Score; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; Lp(a), lipoprotein (a).

TABLE 2 Characteristics of study population with versus without causative mutation.

	Study population (N = 289)	Causative mutation group (N = 122)	No causative mutation group (N = 167)	p-Value
Age (year)	49 ± 13	45 ± 14	52 ± 11	0.001
Males (N, %)	107 (37%)	46 (37.7%)	61 (36.5%)	0.838
BMI (kg/m ²)	24.32 ± 4.46	24.09 ± 5.09	24.48 ± 3.97	0.470
Smoker (N, %)	53 (18.3%)	24 (19.7%)	29 (17.4%)	0.617
Systemic hypertension (N, %)	44 (15.2%)	14 (11.5%)	30 (18%)	0.129
Diabetes mellitus (N, %)	8 (2.8%)	4 (3.3%)	4 (2.4%)	0.725
Mean total cholesterol (mg/dl)	292 ± 54	299 ± 66	288 ± 42	0.087
Mean LDL-c (mg/dl)	213 ± 49	226 ± 59	203 ± 37	0.001
Mean HDL-c (mg/dl)	60 ± 25	57 ± 15	63 ± 30	0.032
Mean triglyceride (mg/dl)	125 ± 55	110 ± 41	137 ± 61	0.001
Mean Lp(a) (mg/dl)	44 ± 49	41 ± 44	46 ± 52	0.405
<10 mg/dl (%)	33%	32.6%	33.3%	0.532
10–50 mg/dl (%)	33%	36.8%	30.4%	
>50 mg/dl (%)	34%	30.5%	36.3%	
Mean Apo A1 (mg/dl)	156 ± 25	152 ± 27	159 ± 23	0.033
Mean Apo B (mg/dl)	150 ± 35	156 ± 41	145 ± 29	0.030
ASCVD (N, %)	62 (21.5%)	33 (27%)	29 (17.4%)	0.048
Follow-up (year)	5.97 ± 5.97	7.31 ± 6.54	4.98 ± 5.32	0.001

BMI, body mass index; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; Lp(a), lipoprotein (a); Apo, apolipoprotein; ASCVD, atherosclerotic cardiovascular disease.

than causative variant *per se* on predicting cardiovascular risk. This shared conclusion ensues from the similarity between both study's findings, in particular multivariable analysis results. Also, a recently

published large French cohort has indirectly illustrated the same finding. This cohort has showed almost similar all-cause mortality rate in HFH patients with clinical versus genetic

TABLE 3 Statistical models of multivariable logistic regression investigating the association between the presence of causative mutation and development of atherosclerotic cardiovascular disease adjusted on confounders.

Model 1			
	OR	95% CI	p-Value
Sex	1.823	(1.974–3.413)	0.061
Age	1.034	(1.009–1.060)	0.008
Mean LDL-c	2.658	(1.495–4.729)	0.001
Mean HDL-c	0.132	(0.016–1.073)	0.058
Model 2			
	OR	95% CI	p-Value
Sex	1.880	(1.000–3.532)	0.050
Age	1.040	(1.014–1.067)	0.003
Causative mutation	1.713	(0.902–3.256)	0.100
Mean LDL-c	2.336	(1.298–4.205)	0.005
Mean HDL-c	0.148	(0.018–1.223)	0.076
Model 3			
	OR	95% CI	p-Value
Sex	2.003	(1.076–3.728)	0.028
Age	1.044	(1.018–1.070)	0.001
Causative mutation	2.149	(1.170–3.948)	0.014
Mean HDL-c	0.236	(0.033–1.697)	0.151

OR, odds ratio; CI, confidence interval; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

TABLE 4 Kruskal-Wallis test comparing the means of lipid panel components between study population sub-groups.

	No causative mutation (N = 167)	LDLR (N = 97)	Apo B (N = 22)	PCSK9/Apo E (N = 3)	p-Value
Total cholesterol (mg/dl)	287 ± 42	297 ± 68	309 ± 55	289 ± 77	0.551
LDL-c (mg/dl)	203 ± 37	225 ± 61	231 ± 52	218 ± 51	0.014
HDL-c (mg/dl)	63 ± 30	55 ± 14	63 ± 15	64 ± 14	0.017
Lpa (mg/dl)	46 ± 52	42 ± 46	37 ± 38	36 ± 34	0.986
Apo A1 (mg/dl)	159 ± 23	149 ± 26	159 ± 26	174 ± 41	0.033
Apo B (mg/dl)	146 ± 29	153 ± 42	162 ± 30	194 ± 81	0.150

LDL-R, low-density lipoprotein cholesterol receptor; PCSK9, proprotein convertase subtilisin/kexin type-9; Apo, apolipoprotein; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; Lpa, lipoprotein a.

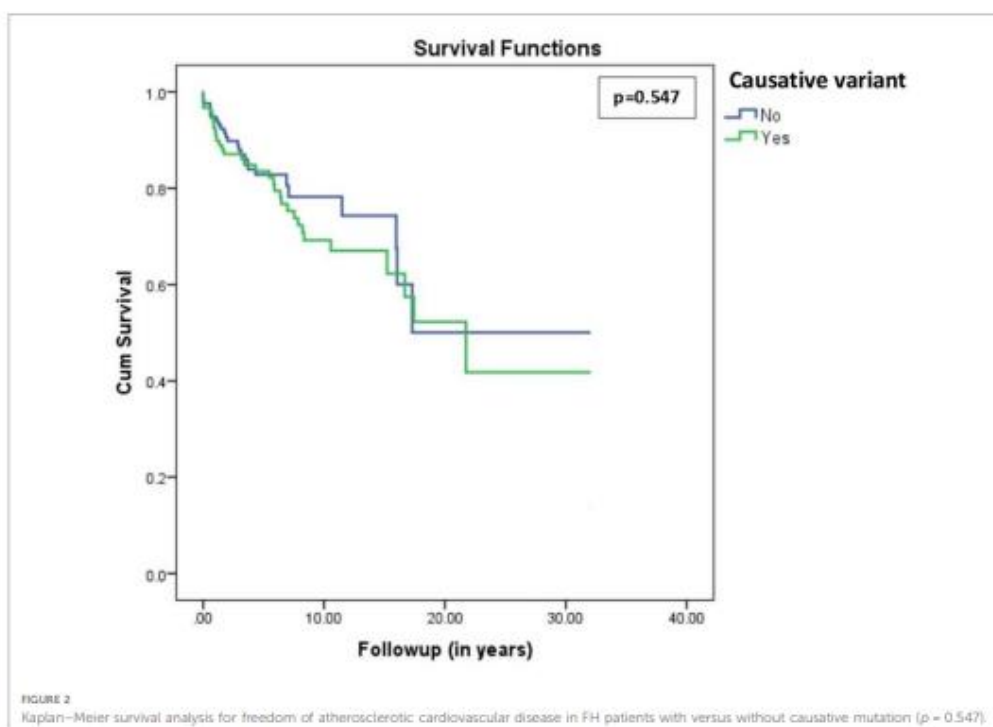
diagnosis (5.54 vs. 4.66 per 1,000 persons). Therefore, the rates of coronary events (24.66 vs. 15.89 per 1,000), cerebral events (3.44 vs. 2.47 per 1,000), and peripheral artery disease (3.63 vs. 2.66 per 1,000) were slightly higher in those with clinical diagnosis (12). In Copenhagen general population, no significant differences in coronary artery disease and myocardial infarction-free survival were observed between *APOB* variant carriers versus non-carriers (13). A significant difference in LDL-c level was mainly observed in patients expressing *LDLR* variant type (13,14). In opposition, some studies found that HFH patients with genetic variant are at elevated risk for adverse cardiovascular outcomes compared with no-variant group (15–18). Benn M. et al. reported a risk of coronary artery disease in LDL-c receptor gene mutation carriers 3.3 times higher than that in non-carriers (13). Khera and co-

TABLE 5 Bonferroni test comparing the mean difference of low-density lipoprotein cholesterol between study sub-groups.

		95% CI	p-Value
No mutation	<i>LDLR</i>	(-0.3733; -0.0506)	0.003
	PCSK9/Apo E	(-0.8827; 0.5895)	1
	Apo B	(-0.5645; 0.0086)	0.063
<i>LDLR</i>	No mutation	(0.0506; 0.3733)	0.003
	PCSK9/Apo E	(-0.6753; 0.8062)	1
	Apo B	(-0.3643; 0.2324)	1
PCSK9/Apo E	No mutation	(-0.5895; 0.8827)	1
	<i>LDLR</i>	(-0.8062; 0.6753)	1
	Apo B	(-0.9091; 0.6463)	1
Apo B	No mutation	(-0.0086; 0.5645)	0.063
	<i>LDLR</i>	(-0.2324; 0.3643)	1
	PCSK9/Apo E	(-0.6463; 0.9091)	1

LDLR, low-density lipoprotein cholesterol receptor; PCSK9, proprotein convertase subtilisin/kexin type-9; Apo, apolipoprotein.

workers reported that being aware of FH mutation provides additional benefits on cardiovascular risk prediction than LDL-c level alone (17). Data from Japan suggest that genetic diagnosis may identify individuals at high risk by reflecting a lifetime exposure to the increased level of LDL-c (18). In France, we observed that one-third of patients carrying a severe mutation experienced a cardiovascular event with an average of 2.5 events per patient, while one-fourth of patients carrying a moderate mutation experienced a cardiovascular event with an average of two events per patient (19). A 2- to 3-fold increase in the risk of coronary artery disease has also been reported in HFH variant carriers (16). Indeed, the baseline LDL-c before initiating a medical treatment has been only used (16). In the present study, we were interested in LDL-c profile under optimal medical therapy as it may reflect more precisely the atherosclerotic impact of a causative variant in the real-world practice. Other studies have identified an association between *LDLR* variant type and ASCVD by revealing a link with carotid plaque formation (14) and obstructive coronary artery disease (13, 14, 17, 20). This augmentation in cardiovascular risk was not observed with the remaining genetic variants, *PCSK9* and *APOB*. In line with previously published studies, we do not reveal a significant difference in cardiovascular risk related to gender in HFH patients. However, it seems likely that males could be at higher risk and HDL-c level could be inversely associated with ASCVD in such HFH-treated population (21–23). Also, it is worth highlighting the potential role of non-LDL genetic factors that result in hypercoagulation and hypofibrinolysis as causal components of ASCVD in HFH patients, independent of elevated LDL-c (24, 25). For example, Kastelein's group has shown an association between coagulation gene polymorphisms, e.g., G20210A, and ASCVD in FH patients (26). These investigators showed that FH individuals had increased factor VIII compared with non-FH (27). A literature review of these findings has been provided by Ravnkov et al. (28, 29). These findings may partially explain the beneficial effects of statins in FH due to their pleiotropic and anticoagulant effects (30–32). Thus, the discrepancy between the study's results on



atherosclerosis risk prediction of causative variant in HFH patients may be related to differences in non-LDL genetic factors' expression among the study's populations.

To summarize, it seems that the effect of a causative variant on atherosclerosis in HFH patients solely passes via the LDL-c level. Then, DNA analysis mainly plays a key role in the diagnosis and screening cascade. It provides an early diagnosis among family members and may reduce the lifelong exposure to high LDL-c level, whereas its usefulness for risk stratification remains uncertain (33, 34). A recently published paper highlights that the risk of incident cardiovascular disease event depends on a cumulative exposure to LDL-c (35). Otherwise, the cost-benefit analysis of genetic analysis tests is another concern even in the developed countries like Europe and Australia (36–38). Lastly, non-LDL genetic factors that result in hypercoagulation and hypofibrinolysis play a potential role as causal components of ASCVD in HFH patients, independent of elevated LDL-c.

Limitations

The study design may predispose to selection bias. This study was carried out in a single large tertiary center, but this also promotes the homogeneity of the patient's management and

follow-up approach. The differences in lipid-lowering therapy and dose changes over time were not discussed. A large proportion of study participants were statin-intolerant patients. Statin intolerance is defined as the inability to tolerate at least two statins, one at the lowest starting dose. However, we assessed the last medical treatment of each of the study's participants. We observed that 60.9% of study participants were treated with PCSK9i alone; 26.6% with PCSK9i and statins; 9.7% with PCSK9i, statins, and ezetimibe; and 2.8% with PCSK9i and ezetimibe. The number of collected lipid panel tests varies between study participants. In addition, we mention the small sample size and limited number of study participants in PCSK9 and APOE sub-groups reducing the ability to make conclusion about differences among study sub-groups. The polygenic risk score in HFH-no causative variant carriers was not evaluated noticing that it is not yet widely performed due to a less robust evidence base for utility (39).

Conclusion

The present study emphasizes the undisputable causal role of LDL-c for the occurrence of ischemic cardiovascular events in HFH patients with and without the causative genetic variant. While the incidence of ASCVD and level of LDL-c were higher

in HFH pathogenic or likely pathogenic variant carriers, the detection of a causative variant did not represent *per se* an independent predictor of adverse cardiovascular outcomes. Thus, the usefulness of DNA analysis on top of LDL-c level for prognostic classification is uncertain. Additional larger prospective studies are warranted to examine this question.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients'/participants' legal guardian/next of kin was not required for participation in this study in accordance with the national legislation and the institutional requirements.

Author contributions

All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2023.1182554/full#supplementary-material>

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