lek. Joanna Katarzyna Rogozik

Ocena wpływu mutacji genetycznych w hipercholesterolemii rodzinnej na efekty nowoczesnej terapii hipolipemizującej

Assessment of the impact of genetic mutations in familial hypercholesterolemia on the efficacy of contemporary lipid-lowering therapy

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

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Spis treści

1. WYKAZ ZASTOSOWANYCH SKRÓTÓW

APOB	apolipoproteina B (ang. *apolipoprotein B*)
ARH	autosomalna recesywna hipercholesterolemia (ang. *autosomal recessive hypercholesterolemia)*
ACGPR	receptor asialoglikoproteinowy (ang. *asialoglycoprotein receptor)*
eGFR	wskaźnik przesączania kłębuszkowego (ang. *estimated glomerular filtration rate*)
FH	hipercholesterolemia rodzinna (ang. *familial hypercholesterolemia*)
HeFH	heterozygotyczna postaci hipercholesterolemii rodzinnej (ang. *heterozygous familial hypercholesterolemia*)
LDL	lipoproteina o małej gęstości (ang. *low-density lipoprotein*)
LDLR	receptor dla lipoproteiny o małej gęstości (ang. *low-density lipoprotein receptor*)
LDLRAP1	białko adaptorowe receptora LDL1 (ang. *LDL-receptor adaptor protein 1*)
MACE	poważne zdarzenia sercowo-naczyniowe (ang. *major adverse cardiovascular events*)
GalNAc	N-acetylogalaktozamina (ang. *N-acetylgalactosamine*)
PCSK9	konwertaza proproteinowa subtylizyny/keksyny 9 (ang. *proprotein convertase subtilisin/kexin type 9*)
PCSK9-I	inhibitory konwertazy proproteinowej subtylizyny-keksyny 9 (ang. *proprotein convertase subtilisin/kexin type 9 inhibitors*)
RISC	indukowany RNA kompleks wyciszający (ang. *RNAinduced silencing complex*)
SCORE 2	skala oceny ryzyka sercowo-naczyniowego (ang. *SystematicCOronaryRisk Evaluation*)

siRNA	małe interferujące RNA (ang. *small interfering RNA*)
VUS	wariant o nieznanym znaczeniu (ang. *variant of uncertain significance*)

2. SPIS RYCIN I TABEL

Ryciny

Tabele

3. STRESZCZENIE W JĘZYKU POLSKIM

Niniejszą rozprawę doktorską tworzy cykl trzech spójnych tematycznie publikacji, poruszających tematykę hipercholesterolemii rodzinnej (FH ang. *familial hypercholesterolemia*), a w szczególności determinantę genetyczną, możliwości diagnostyki oraz leczenia. Cykl publikacji składa się z pracy przeglądowej, pracy oryginalnej oraz publikacji kazuistycznej.

Publikacja przeglądowa zatytułowana „Genetic backgrounds and diagnosis of familial hypercholesterolemia" zawiera systematyczny przegląd literatury podsumowujący aktualny stan wiedzy dotyczący częstości występowania i obrazu klinicznego hipercholesterolemii rodzinnej. W pracy omówiono szczegółowo rodzaje mutacji genetycznych leżących u podłoża FH, rolę badań genetycznych oraz skale służące do diagnostyki. Zwrócono również uwagę na istotną rolę prewencji chorób sercowo-naczyniowych i wczesnego rozpoznawania choroby u pacjentów z podejrzeniem FH. Publikacja stanowi wprowadzenie do dalszych prac w cyklu, gdyż zaburzenia lipidowe stanowią jeden z głównych czynników ryzyka miażdżycy, a FH często pozostaje nierozpoznana. Wczesne potwierdzenie choroby oraz wdrożenie leczenia pozostają podstawą zmniejszenia ryzyka przedwczesnych zdarzeń sercowo-naczyniowych i poprawy oczekiwanej długości życia.

W publikacji oryginalnej zatytułowanej „Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment—A Single-Centre Analysis" przedstawiono wpływ mutacji genetycznych na odpowiedź podczas leczenia alirokumabem. W jako jednej z nielicznych prac wykazano, że heterozygoty z podwójną mutacją wykazują słabszą odpowiedź na zastosowaną terapię alirokumabem. Pokazano jak ważne jest przeprowadzanie badań genetycznych w celu doboru odpowiedniej terapii hipolipemizującej.

Cykl zamyka publikacja kazuistyczna zatytułowana "Challenges in the management of familial hypercholesterolemia: a case report" przedstawiająca przypadek pacjentki z heterozygotyczną postacią FH, całkowitą nietolerancją statyn, u której nie uzyskano odpowiedzi na leczenie alirokumabem. Włączenie inklisiranu pozwoliło na uzyskanie spadku frakcji LDL cholesterolu. Praca ta pokazuje jak ważny jest wybór odpowiedniej terapii oraz jak wiele trudności można napotkać na drodze do osiągnięcia celu terapeutycznego.

Wszystkie trzy prace stanowią spójny cykl tematyczny prezentujący podłoże genetyczne, obraz kliniczny hipercholesterolemii rodzinnej oraz rodzaje i odpowiedni dobór dostępnej nowoczesnej terapii hipolipemizującej.

4. STRESZCZENIE W JĘZYKU ANGIELSKIM (SUMMARY)

Title: Assessment of the impact of genetic mutations in familial hypercholesterolemia on the efficacy of contemporary lipid-lowering therapy

This doctoral dissertation comprises three thematically coherent publications focused on familial hypercholesterolemia (FH), specifically emphasizing genetic determinants, diagnostic approaches, and treatment strategies. The series includes a review paper, an original paper, and a case report. The review paper, titled "Genetic Backgrounds and Diagnosis of Familial Hypercholesterolemia," presents a comprehensive analysis of existing literature, summarizing the current understanding of FH's prevalence and clinical manifestations. It delves into the various genetic mutations associated with FH, the significance of genetic testing, and diagnostic scales. Furthermore, it highlights the crucial role of early disease diagnosis and cardiovascular disease prevention in suspected FH cases. This paper sets the stage for subsequent publications in the series, as lipid disorders significantly contribute to atherosclerosis risk, and FH often goes undetected. Early disease confirmation and timely treatment are pivotal in mitigating the risk of premature cardiovascular events and improving overall life expectancy.

The original paper "Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment—A Single-Centre Analysis" explores the impact of genetic mutations on the efficacy of alirocumab treatment. It is one of the few studies demonstrating that individuals with a double mutation exhibit a diminished response to alirocumab therapy. This underscores the significance of genetic testing to tailor the most suitable lipid-lowering therapy.

The series concludes with a case report titled "Challenges in the management of familial hypercholesterolemia: a case report," which details the management of a patient with heterozygous FH and complete statin intolerance. Despite not responding to alirocumab treatment, the patient experienced a decreased LDL cholesterol fraction only after the introduction of inclisiran. This case highlights the criticality of selecting the appropriate therapy and the challenges that may arise in achieving therapeutic goals.

These three publications form a cohesive thematic series that explores the genetic basis and clinical manifestations of familial hypercholesterolemia and the various types of modern lipid-lowering therapy and their appropriate selection.

5. WSTĘP

Zaburzenia lipidowe są głównym czynnikiem ryzyka sercowo-naczyniowego będącym przyczyną rozwoju miażdżycy i poważnych konsekwencji zawału serca czy udaru mózgu. Stanowią one istotny problem w populacji polskiej. W badaniach NATPOL oraz WOBASZ hipercholesterolemię stwierdzono u 55-60% Polaków [1-2]. Wyniki badań LIPIDOGRAM2015 oraz LIPIDOGEN2015 przeprowadzonych w latach 2015‑2016, pokazały nadal utrzymujący się odsetek pacjentów z zaburzeniami lipidowymi, oszacowanymi na 58% u pacjentów w wieku > 18. roku życia [3-4].

Hipercholesterolemia może być sklasyfikowana na dwie grupy: pierwotną i wtórną. Pierwotna hipercholesterolemia, również znana jako hipercholesterolemia rodzinna (*familial hypercholesterolemia, FH*), jest spowodowana mutacjami genetycznymi, które wpływają na zdolność organizmu do przetwarzania i eliminacji cholesterolu. Natomiast wtórna hipercholesterolemia jest często wynikiem chorób przewlekłych: zaburzeń endokrynologicznych, przewlekłej choroby nerek, zespołu nerczycowego, zaburzeń czynności wątroby lub działań niepożądanych niektórych leków.

Hipercholesterolemia rodzinna jest dziedziczona autosomalnie dominująco i zalicza się ją do najczęściej występujących chorób genetycznych monogenowych. Charakteryzuje się znacznie podwyższonymi stężeniami cholesterolu we krwi. Najnowsze dane częstość występowania heterozygotycznej postaci FH *(heterozygous familial hypercholesterolemia, HeFH.*) szacują na 1:311 populacji ogólnej [5-6]. W Polsce może ona wynosić nawet jedna na 250 osób, a chorobą być dotkniętych nawet 120 000‑140 000 dorosłych Polaków [7-9]. Dziedziczenie autosomalne recesywne jest rzadkie, oceniane na 1:1 000 000 [10]. Ostatnio opublikowane prace sugerują wyższą częstość występowania w zakresie 1:160 000 do 1:300 000 [11-15].

FH pozostaje istotnym problemem w praktyce klinicznej. Nadal zbyt mała liczba pacjentów ma odpowiednio szybko postawioną diagnozę. Dane europejskie oraz z Polskiego Rejestru Hipercholesterolemii Rodzinnej wskazują, że < 1 % dotkniętych chorobą ma postawione rozpoznanie [7,10], natomiast średni wiek rozpoznania FH to 45 lat [16].

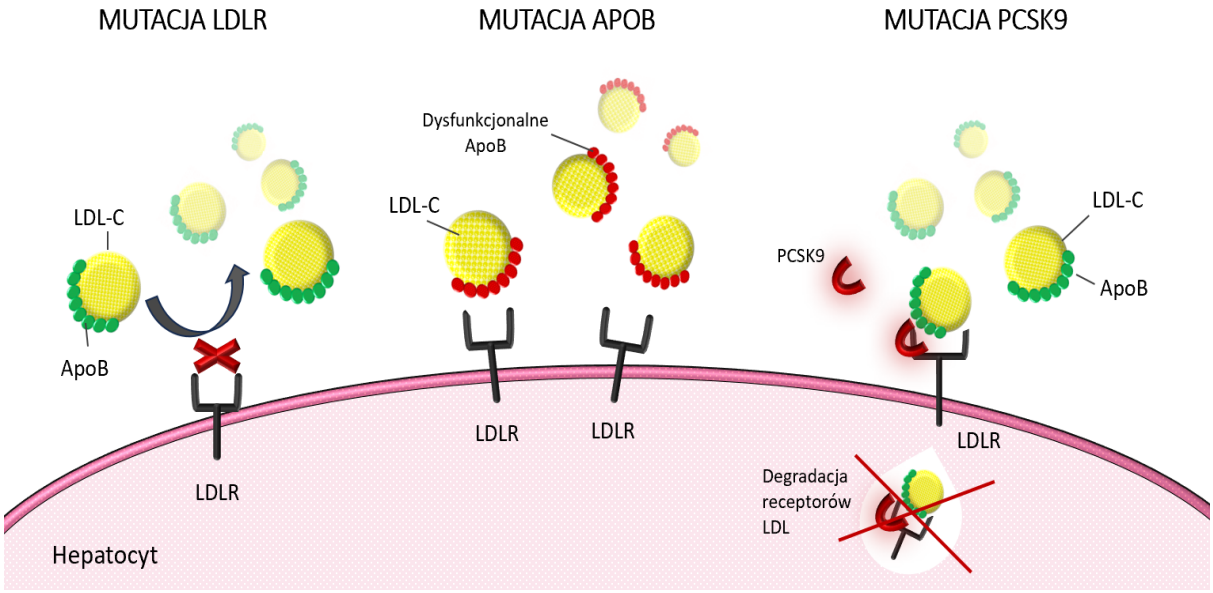
Podłoże genetyczne FH stanowią przede wszystkim mutacje monogenowe prowadzące do upośledzenia funkcji genu kodującego receptor lipoprotein o niskiej gęstości *(low-density lipoprotein receptor, LDLR*), genu apolipoproteiny B (*apolipoprotein B, APOB*) lub nabycia funkcji genu konwertazy proproteinowej subtylizyny/keksyny typu 9 (*proprotein convertase*

subtilisin/kexin type 9, PCSK9) [10,17-19]. Główne mutacje i ich skutki zostały ujęte w tabeli 1 oraz na rycinie 1. W przypadkach autosomalnej recesywnej hipercholesterolemii (*autosomal recessive hypercholesterolemia, ARH*) stwierdzano zmienność bialleliczną w białku adaptorowym receptora LDL 1 (*LDL-receptor adaptor protein 1, LDLRAP1*) [19-21].

Tabela 1. Główne mutacje genowe determinujące FH.

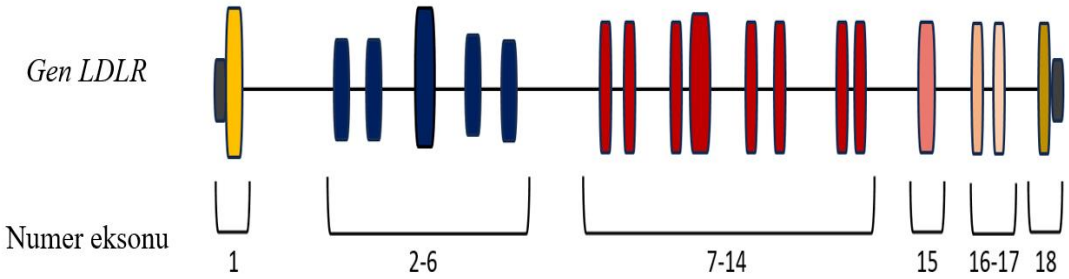
Gen	**Funkcja**	**Częstość występowania mutacji**	**Mutacja**
LDLR (Receptor lipoproteiny o niskiej gęstości)	Wychwyt lipoprotein o małej gęstości (LDL-C), co skutkuje obniżeniem ich poziomu	60-90%	Utrata funkcji
APOB (Apolipoproteina B-100)	Budowa lipoprotein zawierających LDL i ich transport do receptora LDL	5-10%	Utrata funkcji
PCSK9 (Konwertaza proproteinowa subtylizyny/keksyny 9)	Hamowanie recyklingu receptorów LDL poprzez promowanie ich degradacji w lizosomach	1-3%	Nabycie funkcji

Rycina 1. Schemat efektów trzech najczęstszych mutacji w kontekście metabolizmu cholesterolu (rysunek własny).



Gen *LDLR* znajduje się na chromosomie 19 i jest zbudowany z 18 eksonów. Na rycinie 1 przedstawiono schematyczną budowę genu *LDLR*. Istnieje ponad 3842 wariantów mutacji, które zostały zgłoszone do bazy danych wariantów LDLR University College London (UCL) w bazie danych Leiden Open Variation Database (LOVD 3) (http://www.lovd.nl/LDLR) [22]. The American College of Medical Genetics and Genomics (ACMG) wprowadziło w 2015 r. algorytm, który klasyfikuje wszystkie warianty mutacji genu *LDLR* do pięciu grup: patogenne, prawdopodobnie patogenne, wariant o nieznanym znaczeniu (VUS), prawdopodobnie łagodne i łagodne [23-26].

Rycina 2. Schematyczna budowa genu *LDLR*.



Wczesna diagnoza pacjentów z podejrzeniem FH jest kluczowa, żeby odpowiednio szybko wdrożyć terapię hipolipemizującą oraz zapobiec powikłaniom ze strony układu sercowo naczyniowego. W Polsce jedną z najczęściej wybieranych skal do klinicznego rozpoznania FH jest skala Dutch Lipid Clinic Network przedstawiona w Tabeli 2. Jej kryteria stanowi między innymi wywiad kliniczny oraz badanie fizykalne z uwzględnieniem charakterystycznych dla hipercholesterolemii cech fenotypowych takich jak rąbek rogówkowy, czy żółtaki ścięgien. Przy jej pomocy możemy określić rozpoznanie FH jako pewne, możliwe i prawdopodobne. Alternatywnie wykorzystuje się również pochodzące z Wielkiej Brytanii kryteria z rejestru Simone Broome (The Simon Broome Register Group) [11,26-27] zwłaszcza w populacji pediatrycznej. Wspomniany schemat diagnostyczny przedstawiono w Tabeli 3.

Tabela 2. Skala Dutch Lipid Clinic Network do oceny prawdopodobieństwa FH.

Dutch Lipid Clinic Network kryteria	Punkty
Wywiad rodzinny	
Krewni I stopnia z przedwczesną chorobą wieńcową lub naczyniową lub krewni I stopnia ze stężeniem cholesterolu LDL > 190 mg/dl	1
Krewni I stopnia z Żółtakami ścięgien i/lub rąbkiem rogówkowym lub dzieci i młodzież < 18 rż. ze stężeniem cholesterolu LDL > 155 mg/dl	2
Wywiad kliniczny	
Przedwczesna choroba wieńcowa (M< 55rż., K< 60 rż.)	2
Przedwczesna choroba naczyń mózgowych lub obwodowych	1
Badanie przedmiotowe	
Żółtaki ścięgien	6
Rąbek rogówkowy < 45 roku życia	4
Stężenie LDL-C (bez leczenia)	
LDL-C ≥ 8.5 mmol/L (≥325 mg/dL)	8
LDL-C 6.5-8.4 mmol/L (251-325 mg/dL)	5
LDL-C 5.0-6.4 mmol/L (191-250 mg/dL)	3
LDL-C 4.0-4.9 mmol/L (155-190 mg/dL)	1
Badanie genetyczne	
Mutacja genu *LDLR, APOB* lub *PCSK9*	8
‘pewna’ diagnoza hipercholesterolemii rodzinnej	**> 8 punktów**
‘prawdopodobna’ diagnoza hipercholesterolemii rodzinnej	**6- 8 punktów**
‘możliwa’ diagnoza hipercholesterolemii rodzinnej	**<6 punktów**

Tabela 3. Kryteria z rejestru Simone Broome (The Simon Broome Register Group).

Kryteria	Opis
A	Dorośli: stężenie cholesterolu całkowitego > 290 mg/dl (7,50 mmol/L) lub stężenie LDL> 190 mg/dl (4,91 mmol/L) U dzieci i młodzieży < 16 rż.: stężenie cholesterolu całkowitego. > 260 mg/dL

	(6,72 mmol/L) lub lub stężenie LDL > 155 mg/dL (4,01 mmol/L)
B	Obecność żółtaków ścięgien u pacjenta lub krewnego I stopnia
C	Potwierdzona w badaniach genetycznych mutacja genu LDLR, APOB lub PCSK9
D	Zawał serca u krewnego I stopnia <60. rż. lub krewnego II stopnia <50. rż
E	Stężenie cholesterolu całkowitego > 290 mg/dL (7,5 mmol/L) u krewnego I lub II stopnia
PEWNA FH: spełnienie kryteriów A oraz B lub C **PRAWDOPODOBNA FH:** spełnienie kryteriów A oraz D lub E	

Złotym standardem w rozpoznawaniu FH pozostają badania genetyczne. Z uwagi jednak na ich ograniczoną dostępność oraz koszt nie są powszechnie wykonywane. Przedstawione powyżej skale używane do diagnozowania pacjentów z FH mogą być użyteczne ze względu na swoją prostotę i ogólną powszechność, jednakże nie są tak dokładne i nie dają szczegółowych informacji jak badania genotypu. Badania genetyczne potrafią wykazać zmiany w sekwencjonowaniu, delecji lub duplikacji u około 60–80% osób [27-28]. Warto jednak zauważyć, że u około 20–40% pacjentów z fenotypem FH mutacje mogą nie zostać wykryte za pomocą badań genetycznych [29-30]. Testy genetyczne ułatwiają przeprowadzenie kaskadowych badań przesiewowych u członków rodziny i wcześniejsze rozpoznanie FH, niż na podstawie klinicznej fenotypu. Dodatkowym argumentem przemawiającym za zwiększeniem wykorzystania testów genetycznych w codziennej praktyce jest zidentyfikowanie mutacji, a następnie dostosowanie terapii do stwierdzonego genotypu.

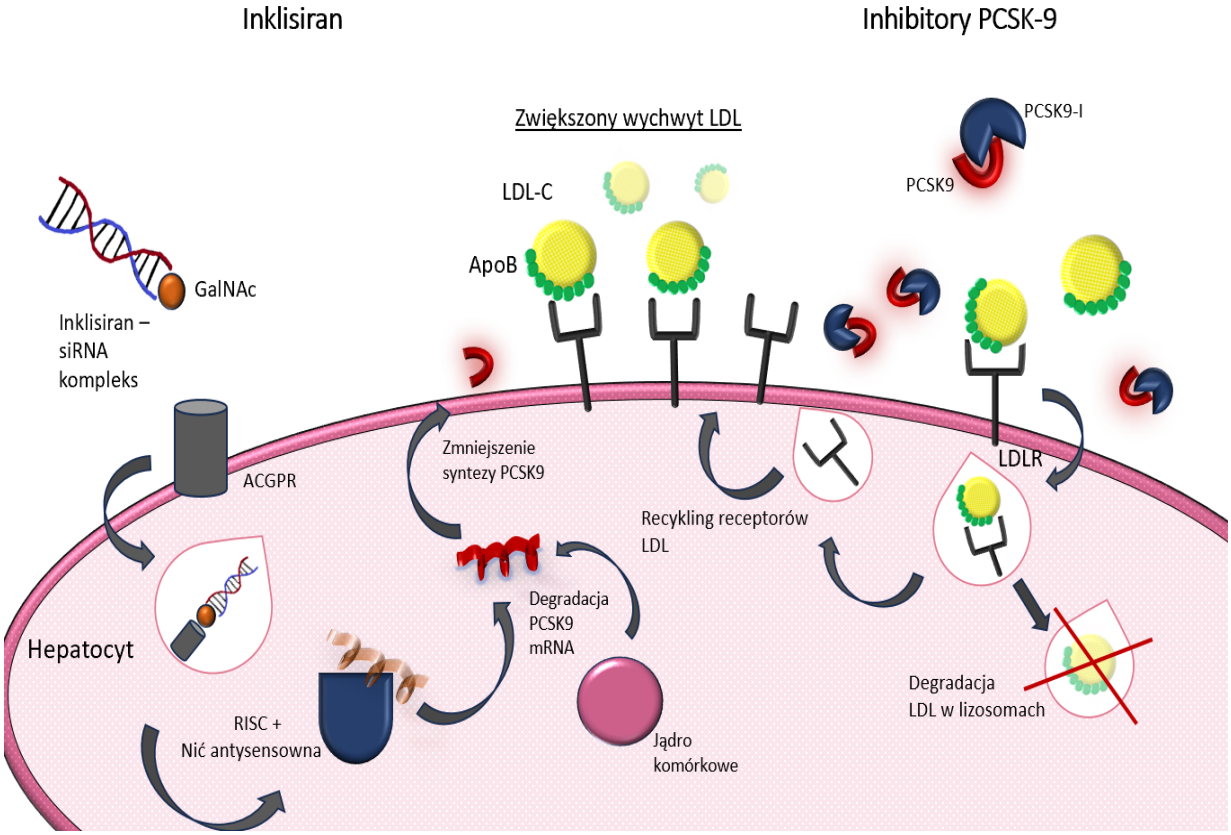
Pacjenci z FH należą do grupy wysokiego i bardzo wysokiego ryzyka sercowo naczyniowego wg skali SCORE 2 (SystematicCOronaryRisk Evaluation) [31]. Wytyczne Europejskiego Towarzystwa Kardiologicznego z 2019 roku zalecają u tych pacjentów jak najszybsze osiągnięcie celu terapeutycznego wynoszącego LDL< 70mg/dL (<1.8 mmol/L) odpowiednio dla grupy dużego ryzyka i LDL <55mg/dL (<1.4 mmol/L) dla grupy bardzo dużego ryzyka sercowo-naczyniowego [11].

Aktualnie dostępny jest szeroki wachlarz leków hipolipemizujących. Podstawę terapii stanowią statyny, leki o udowodnionym działaniu redukującym stężenie LDL, jak również plejotropowym, zmniejszającym ryzyko rozwoju miażdżycy i powikłań sercowo-naczyniowych. W przypadku nieosiągnięcia celu terapeutycznego, bądź nietolerancji statyn

można zastosować ezetymib, który wybiórczo hamuje wchłanianie cholesterolu i pochodnych steroli roślinnych w jelicie cienkim.

Nowoczesna terapia hipolipemizująca obejmująca inhibitory konwertazy proproteinowej subtylizyny-keksyny 9 (PCSK9-I) oraz inklisiran stanowi ogromne wsparcie dla pacjentów z hipercholesterolemią. Do grupy PCSK9-I zalicza się przeciwciała monoklonalne alirokumab oraz ewolokumab. Leki te wykazują wysoką skuteczność w redukcji stężenia LDL. Poprzez wysokie powinowactwo oraz swoistość hamują proces łączenia się PCSK9 z receptorami LDLR, co prowadzi do wzrostu liczby receptorów LDLR na powierzchni hepatocytów, a tym samym nasila wychwyt LDL. Skutkuje to znaczącym obniżeniem stężenia LDL w osoczu. Na rycinie 3 przedstawiono schemat działania inhibitorów PCSK9 oraz inklisiranu.

Rycina 3. Schemat działania inklisiranu i inhibitorów PCSK9 (rysunek własny). Wyjaśnienia skrótów: ACGPR- receptor asialoglikoproteinowy; APOB- apolipoproteina B; GalNAc- N-acetylogalaktozamina, LDLR- receptor dla lipoproteiny o małej gęstości, PCSK9- konwertaza proproteinowa subtylizyny/keksyny 9 RISC- indukowany RNA kompleks wyciszający; siRNA-małe interferujące RNA.



W badaniach ODYSSEY FH I oraz FH II oceniano wpływ alirokumabu na leczenie pacjentów z FH. W obu badanych grupach, otrzymujących maksymalną tolerowaną terapię hipolipemizującą, po 24 tygodniach leczenia uzyskano znaczący spadek LDL w porównaniu z placebo wynoszący odpowiednio −57.9% w FH I oraz −51.4% w FH II [32]. U pacjentów z HeFH i poziomem LDL wynoszącym 160 mg/dL lub wyższym pomimo maksymalnej tolerowanej terapii statynami i/lub dodatkowej terapii hipolipemizującej w badaniu ODYSSEY HIGH FH, stwierdzono 45,7% redukcję poziomu LDL w grupie otrzymującej alirokumab [33-34].

Brak odpowiedzi na leczenie PCSK9-I występuje bardzo rzadko. Niemniej jednak w takich przypadkach należy wziąć pod uwagę możliwość obecności przeciwciał przeciwlekowych, które mogą mieć wpływ na skuteczność leczenia. Podczas badania ODYSSEY FH zaobserwowano, że u 3 z 735 pacjentów, w 12 tygodniu leczenia stwierdzono obecność przeciwciał neutralizujących alirokumab [32, 34]. W badaniach fazy 3 ODYSSEY zjawisko hiporeaktywności na alirokumab, zdefiniowanej jako zmniejszenie poziomu LDL < 15%, obserwowano u 1% populacji pacjentów objętych badaniem [35]. Możliwymi przyczynami hiporeaktywności mogą być: brak przestrzegania terapii, konieczność oceny stężenia alirokumabu w trakcie terapii, teoretyczna i rzadka wspomniana możliwość biologicznego braku reakcji spowodowanego przez utrzymujące się przeciwciała przeciwlekowe lub inne niezidentyfikowane przyczyny [35].

Szacuje się, że prawdopodobieństwo znalezienia podwójnych heterozygot z dwoma różnymi genami wynosi 1 do 1,4 miliona [36-37]. Połączenie rzadkich mutacji w genach LDLR i APOB wykazuje fenotyp zbliżony do postaci homozygotycznej i wiąże się z wyższym ryzykiem rozwoju miażdżycy w porównaniu z występowaniem każdej z tych mutacji osobno [38]. Wynika to z kumulatywnego efektu tych mutacji, który prowadzi do znacznie podwyższonego poziomu lipidów we krwi [39]. Kilka przeprowadzonych badań wykazało, że podwójne heterozygoty (mutacje w genach LDLR i APOB) mogą słabiej reagować na terapię PCSK9-I [40,41]. Przeprowadzonych sześć badań wykazało, że osoby z pierwotnymi mutacjami monogenowymi związanymi z FH wykazywały istotną odpowiedź na terapię alirokumabem [38]. Dostępne dowody wykazują, że efekt redukcji poziomu LDL przez PCSK9-I jest spójny we wszystkich genotypach [42]. W opublikowanych badaniach odpowiedź na PCSK9-I była zauważalnie zmniejszona u osób ze złożonymi mutacjami heterozygotycznymi oraz u homozygot [43,44]. W badaniu GENRE-FH z ewolokumabem, wykazano spadek stężenia LDL porównywalny z alirokumabem, podczas gdy u pacjentów, u

których nie stwierdzono patogennych wariantów mutacji lub mieli tylko defekt w obrębie patogennej mutacji, odnotowano wyższy odsetek osiągniętej redukcji LDL niż u pacjentów z całkowitym brakiem ekspresji genu (ang. null pathogenic variant) [45].

Cząsteczkę inklisiranu stanowi małe interferujące RNA (ang. *siRNA- small interfering RNA*), który działając na komórki hepatocytów, hamuje translację PCSK9. Prowadzi to do zwiększonego recyklingu receptorów LDLR, co z kolei zwiększa wychwyt LDL i zmniejsza jego poziom we krwi. W badaniach klinicznych ORION-9, inklisiran wykazał znaczną skuteczność w obniżaniu poziomu LDL w porównaniu z placebo, u pacjentów z HeFH leczonymi maksymalnymi tolerowanymi dawkami statyn (46). W 510. dniu badania obserwowano, że 99% wszystkich pacjentów otrzymujących inklisiran, osiągnęło znaczącą 39,7% redukcję poziomu LDL (46).

Od 2018 roku jest dostępny w Polsce program B101 dedykowany dla pacjentów z zaburzeniami lipidowymi. Po spełnieniu wymaganych kryteriów, między innymi pewnej diagnozy FH, utrzymującego się stężenia LDL > 100mg/dL pomimo stosowania maksymalnej tolerowanej dawki statyn i terapii skojarzonej z ezetymibem, możliwe jest leczenie nowymi lekami hipolipemizującymi tj. PCSK9- I oraz inklisiranem. Pełne kryteria kwalifikacji oraz wyłączenia udziału w programie dla pacjentów z FH przedstawiono w Tabeli 3.

Tabela 3. Kryteria kwalifikacji oraz wyłączenia udziału w programie B101 dla pacjentów z FH.

Kryteria kwalifikacji dla pacjentów z hipercholesterolemią rodzinną	Kryteria wyłączenia
1) wiek 18 lat i powyżej; 2) pewna diagnoza rodzinnej heterozygotycznej hipercholesterolemii; tj. > 8 punktów w skali Dutch Lipid Clinic Network; 3) LDL-C > 100 mg/dL (2,5 mmol/dL) pomimo stosowania diety i:	1) wystąpienie ciężkich reakcji alergicznych po podaniu leku; 2) brak skuteczności terapii: 3) wystąpienie objawów nadwrażliwości na którykolwiek ze stosowanych leków lub na którąkolwiek substancję pomocniczą leku, uniemożliwiających kontynuację leczenia;

a) intensywnego leczenia statynami w maksymalnych dawkach, tj. atorwastatyna 80 mg lub rosuwastatyna 40 mg, a następnie atorwastatyna 40–80 mg lub rosuwastatyna 20–40 mg w skojarzeniu z ezetymibem 10 mg, stosowanego łącznie przez 3 miesiące, w tym leczenie skojarzone przez minimum miesiąc lub b) pacjenci z całkowitą nietolerancją statyn, definiowaną według obowiązujących wytycznych towarzystw naukowych w zakresie diagnostyki i leczenia zaburzeń lipidowych (PTL/KLRWP/PTK/PTDL/PTD/PTNT), jako udokumentowany brak tolerancji co najmniej 2 statyn – jednej w najmniejszej początkowej dawce na dobę i drugiej w dowolnej dostępnej dawce (okres leczenia statynami ustalony przez lekarza prowadzącego, ale nie krótszy niż przez 3 miesiące).	4) okres ciąży lub karmienia piersią; 5) wystąpienie chorób lub stanów, które według oceny lekarza prowadzącego uniemożliwiają dalsze prowadzenie leczenia; 6) wystąpienie nieakceptowalnej lub zagrażającej życiu toksyczności, pomimo zastosowania adekwatnego postępowania; 7) brak współpracy lub nieprzestrzeganie zaleceń lekarskich, w tym dotyczących okresowych badań kontrolnych oceniających skuteczność i bezpieczeństwo leczenia, ze strony świadczeniobiorcy lub jego opiekuna prawnego.

W badaniach FOURIER oceniającym skuteczność ewolokumabu [47] oraz ODYSSEY OUTCOMES z alirokumabem [48] wykazały redukcję o 15% głównego punktu końcowego, definiowanego jako zgon z przyczyn sercowo-naczyniowych, zawał mięśnia sercowego, udar, hospitalizacja z powodu niestabilnej dławicy piersiowej lub rewaskularyzacja wieńcowa. Dodatkowo wykazano, że stosowanie alirokumabu wpływa również na 15% redukcję zgonów bez względu na przyczynę [48].

6. ZAŁOŻENIA I CEL PRACY

Hipercholesterolemia rodzinna pozostaje istotnym problemem w praktyce klinicznej. Nadal zbyt mała liczba pacjentów ma odpowiednio szybko postawioną diagnozę. Celem pracy było zwrócenie uwagi na możliwości diagnostyki i leczenia pacjentów z FH, żeby zapobiegać rozwojowi miażdżycy i wystąpieniu przedwczesnych zdarzeń sercowo-naczyniowych.

Główny cel pracy

Ocena jak mutacje genetyczne determinujące hipercholesterolemię rodzinną wpływają na efekty zastosowanej nowoczesnej terapii hipolipemizującej.

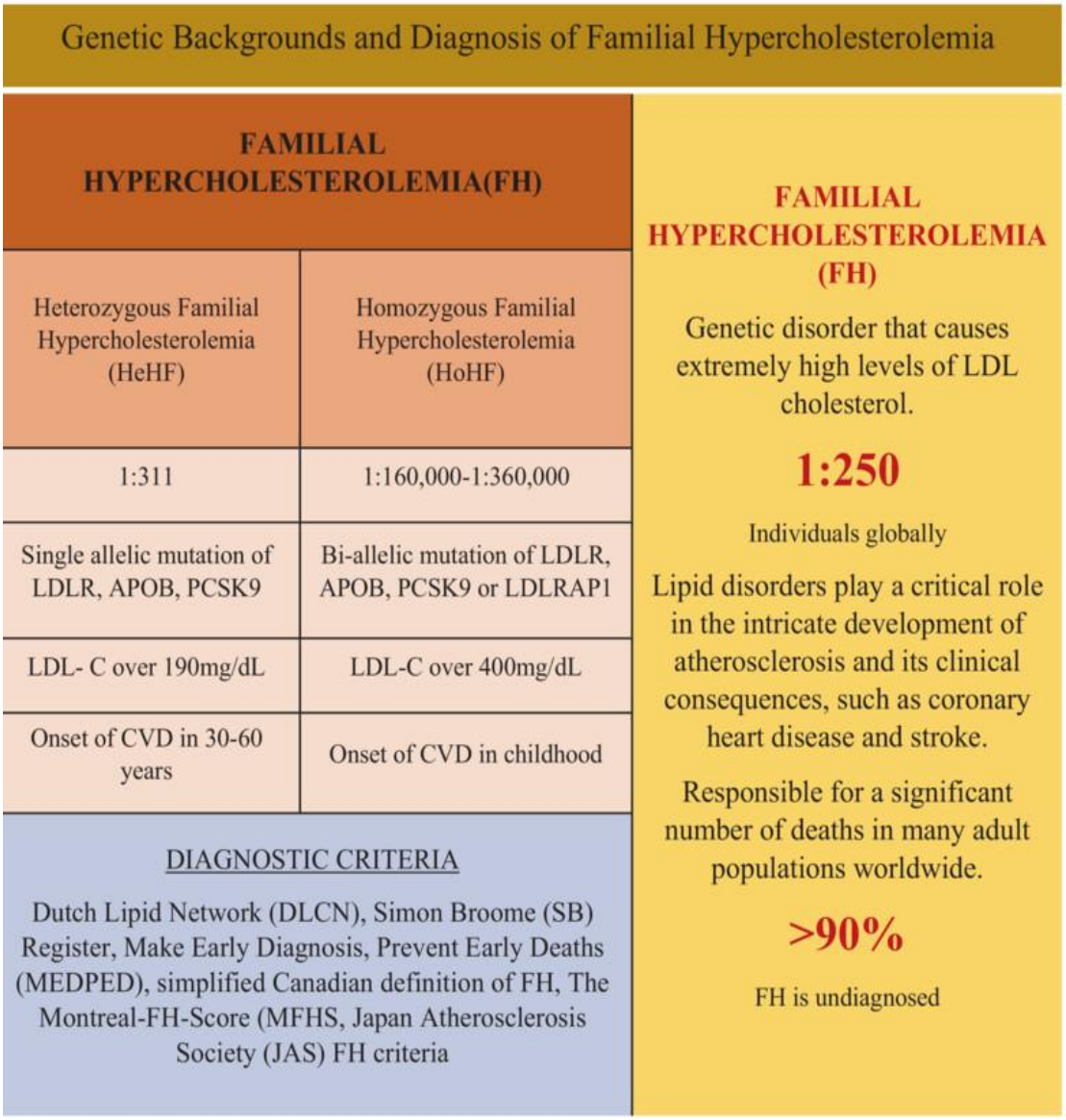
Cele szczegółowe pracy:

1. Omówienie aktualnego stanu wiedzy dotyczącego podłoża genetycznego hipercholesterolemii rodzinnej (FH).
2. Ocena skuteczności alirokumabu w leczeniu osób z heterozygotyczną FH, w zależności od genotypu.
3. Ocena związku pomiędzy genotypem pacjentów z FH, a stwierdzanym fenotypem**.**
4. Ocena skuteczności, bezpieczeństwa w czasie trwania rocznej terapii alirokumabem.
5. Ocena wystąpienia u pacjentów leczonych alirokumabem poważnych zdarzeń sercowo-naczyniowych (MACE) tj. zawał serca, udar mózgu, zgon z przyczyn sercowo-naczyniowych.
6. Analiza doboru leczenia u pacjentów z całkowitą nietolerancją statyn

7. KOPIE OPUBLIKOWANYCH PRAC

7.1 Genetic backgrounds and diagnosis of familial hypercholesterolemia

Graphical abstract



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REVIEW

CLINICAL GENETICS WILEY

Genetic backgrounds and diagnosis of familial hypercholesterolemia

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Abstract

Lipid disorders play a critical role in the intricate development of atherosclerosis and its clinical consequences, such as coronary heart disease and stroke. These disorders are responsible for a significant number of deaths in many adult populations worldwide. Familial hypercholesterolemia (FH) is a genetic disorder that causes extremely high levels of LDL cholesterol. The most common mutations occur in genes responsible for low-density lipoprotein receptor (LDLR), apolipoprotein B (APOB), or proprotein convertase subtilisin/kexin type 9 (PCSK9). While genetic testing is a dependable method for diagnosing the disease, it may not detect primary mutations in 20%–40% of FH cases.

KEYWORDS
familial combined hyperlipidemia, familial hypercholesterolemia, low-density lipoprotein receptor, low-density lipoprotein cholesterol, proprotein convertase subtilisin/kexin type 9

1 | INTRODUCTION

Abnormalities in lipid levels in the blood, known as lipid disorders, are a significant threat to cardiovascular health. Atherosclerosis, a condition characterized by plaque buildup in the arteries, is a common consequence of lipid disorders and can lead to serious cardiovascular diseases such as myocardial infarction (heart attack) and stroke. However, early detection and proper treatment can greatly reduce the risk of premature cardiovascular events and improve life expectancy.

Hypercholesterolemia, a type of lipid disorder characterized by the presence of high cholesterol levels in the blood, can be classified into two groups: primary and secondary.[1] Primary hypercholesterolemia, which is also known as familial hypercholesterolemia (FH), is caused by genetic mutations that affect the body's ability to process and eliminate cholesterol. On the other hand, secondary hypercholesterolemia is often a result of underlying chronic diseases, unhealthy lifestyle choices, or certain medications. It is important to detect and diagnose hypercholesterolemia early and choose appropriate treatment options to prevent the development of cardiovascular diseases. Chronic conditions like endocrine disorders, chronic kidney disease, nephrotic syndrome, primary biliary cholangitis, and liver function disorders are chronic conditions that can contribute to lipid disorders. Additionally, certain lifestyle choices, such as obesity, excessive alcohol consumption, smoking, improper diet, and insufficient physical activity, can increase the risk of hypercholesterolemia and hypertriglyceridemia. It is important to note that certain medications used to treat hypertension or other conditions, such as diuretics, beta-blockers, and steroids, estrogens, may also raise lipid levels. Therefore, regular monitoring of lipid levels is necessary when taking such drugs.[2] Maintaining a healthy lifestyle is essential for preventing and managing lipid disorders. This includes adhering to a balanced diet, engaging in regular physical activity, and avoiding excessive alcohol consumption and smoking. These lifestyle choices have been scientifically proven to positively impact lipid levels and reduce the likelihood of cardiovascular events.

This review focuses on two primary types of genetic mutations that cause a significant increase in blood lipid levels: FH and familial combined hyperlipidemia (FCHL). FH is an inherited condition that impedes the body's ability to remove low-density lipoprotein cholesterol (LDL-C) from the blood, leading to a buildup of LDL-C in the

arteries. FCHL, on the other hand, is a more complex genetic disorder that results in elevated levels of both LDL-C and triglycerides (another type of fat in the blood). The information contained in this review was collected from several reputable sources, including the EMBASE, MEDLINE, and PubMed databases.

2 | FAMILIAL HYPERCHOLESTEROLEMIA

2.1 | Definition and epidemiology of FH

FH is a common inherited lipid disorder that causes high levels of LDL-C and increases the risk of premature atherosclerotic cardiovascular disease (ASCVD).[3–5] There are two types of FH: heterozygous mutation carriers (HeFH), which occur in ~1 in 311 people,[6,7] and rare homozygous carriers (HoFH), which have a prevalence of about 1 in 1 000 000.[8] Recent studies suggest the prevalence of homozygous FH ranges from 1 in 160 000 to 1 in 300 000.[9]

Table 1 shows the prevalence of HeFH and HoFH in different countries and populations.

2.2 | Diagnosis

Establishing a diagnosis of FH relies on the use of various criteria, including the Dutch Lipid Network (DLCN); criteria adopted in Geneva in 1998 by the WHO and the Simon Broome (SB) Register (Table 2), the Make Early Diagnosis, Prevent Early Deaths (MEDPED) (Table 3), a simplified Canadian definition of FH, The Montreal-FH-Score (MFHS),[27] and the 2017 Japan Atherosclerosis Society (JAS) FH criteria.[28] These criteria facilitate the diagnosis of FH as either certain, probable, or possible.[18,27,29,30] While genetic testing remains the gold standard, it may not be widely available or cost-effective. Nonetheless, clinical scores can be utilized to establish a diagnosis of FH without genetic testing, although they may not be as precise as genetic testing. It is imperative to diagnose FH promptly and to initiate appropriate therapeutic strategies to normalize life expectancy.

The Make Early Diagnosis Prevent Early Deaths (MEDPED) program is a global non-profit organization aimed at providing humanitarian aid. It operates in various countries across the globe, including the United States, Asia, Australia, and many European nations. The primary objective of the MEDPED program is to identify, diagnose, and

TABLE 1 Prevalence of HeFH and HoFH.

Prevalence rate	Country/population	Author; references	Year of publication
Heterozygous familial hypercholesterolemia (HeFH)			
1:500	United States	Goldstein et al[9]	1973
1:900	Japan	Mabuchi et al.[10]	1977
1:260	Québécois French Canadians	Moorjani et al.[11]	1989
1:67	Ashkenazi Jews	Seftel et al.[12]	1989
1:165	Tunisia	Slimane et al.[13]	1993
1:72	South Africa/Afrikaners	Steyn et al.[14]	1996
1:441	Finnish North Karelia	Vuorio et al.[15]	1997
1:538	Hungary	Kalina et al.[16]	2001
1:623	United Kingdom	Austin et al.[17]	2004
1:85	Lebanon	Austin et al.[18]	2004
1:137	Denmark	Benn et al.[19]	2012
1:267	Australia	Pang et al.[19]	2016
1:212–1:357	China	Zhou and Zhao[20]	2016
1:310	United States	SEARCH Study (Safarova et al.)[21]	2016
1:250	United States	NHANES Study (de Ferranti et al.)[22]	2016
1:192	Catalan	Zamora et al.[23]	2017
1:108	West Siberian (Russian Federation)	Ershova et al.[24]	2017
1:311	General population	Beheshti et al.[6] Hu P et al.[7]	2020
Homozygous familial hypercholesterolemia (HoFH)			
1:275 000	Québécois French Canadians	Moorjani et al.[12]	1989
1:300 000	Netherlands (Dutch)	Sjouke et al.[25]	2015
1:450 000	Spain	Sanchez-Hernandez et al.[26]	2016
1:325 774	Catalan	Zamora et al.[23]	2017
1:160 000–1:360 000	General population	Hu P et al.[7]	2020

TABLE 2 Diagnosis of familial hypercholesterolemia based on Dutch Lipid Clinic Network and Simon Broome Criteria (SB; United Kingdom) diagnostic criteria for familial hypercholesterolemia.

Dutch lipid clinic network criteria	Points
(1) Family history	
First-degree relative with known premature (men aged <55 years; women <60 years) coronary or vascular disease, or a first-degree relative with known LDL-C above the 95th percentile	1
First-degree relative with tendinous xanthomata and/or arcus corneal, or children aged <18 years with LDL-C above the 95th percentile	2
(2) Clinical history	
Patient with premature (men aged <55 years; women <60 years) CAD	2
Patient with premature (men aged <55 years; women <60 years) cerebral or peripheral vascular disease	1
(3) Physical examination A	
Tendinous xanthomata	6
Arcus corneal before age 45 years	4
(4) LDL-C levels (without treatment)	
LDL-C ≥ 8.5 mmol/L (≥325 mg/dL)	8
LDL-C 6.5–8.4 mmol/L (251–325 mg/dL)	5
LDL-C 5.0–6.4 mmol/L (191–250 mg/dL)	3
LDL-C 4.0–4.9 mmol/L (155–190 mg/dL)	1
(5) DNA analysis	
Functional mutation in the LDLR, apoB, or PCSK9 genes	8
Choose only one score per group, the highest applicable, diagnosis is based on the total number of points obtained	
A "definite" FH diagnosis requires >8 points	
A "probable" FH diagnosis requires 6–8 points	
A "possible" FH diagnosis requires 3–5 points	
Simon Broome Criteria (SB; United Kingdom)	
Total cholesterol (LDL-C) of 290 mg/dL (190 mg/dL) in adults or 260 mg/dL (155 mg/dL) in pediatrics (age < 16 years) AND	
1. DNA mutation	Definite FH
2. Tendon xanthomas in the patient or a first- or second-degree relative	Probable FH
3. Family history of MI at age < 50 years in second-degree relative or at age < 60 years in first-degree relative OR Family history of total cholesterol >290 mg/dL in first- or second-degree relative	Possible FH

Abbreviations: CAD, coronary artery disease; FH, familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PCSK9, proprotein convertase subtilisin/kexin type 9.

promote the treatment of patients suffering from FH. To estimate the risk of FH, age-specific parameters and total cholesterol concentration are taken into consideration, along with a family history of FH. It is important to note that the serum cholesterol cut-off points used in general populations are higher than those applied when patients have first-, second-, or third-degree relatives with FH.[31]

In Canada, a simplified definition is utilized to diagnose FH that is comparable to pre-existing criteria. The initial step involves identifying individuals whose LDL-C concentrations are ≥4 or 5 mmol/L based on their age. A definite diagnosis of FH can be made if the person exhibits elevated LDL-C (≥8.50 mmol/L), tendon xanthomas, or a causal DNA mutation in the LDLR, APOB, or PCSK9 genes in themselves or a first-degree relative. Probable FH is diagnosed if a first-degree relative has elevated LDL-C not caused by secondary factors or if the person or a first-degree relative exhibits elevated LDL-C (≥5.0 mmol/L) and early-onset atherosclerotic cardiovascular disease (ASCVD) (men under 55 years old, women under 60 years old). If none of these criteria are met, severe hypercholesterolemia is diagnosed.[27]

The Multivariate Familial Hypercholesterolemia Score (MFHS) is a valuable tool for evaluating the risk of cardiovascular disease in individuals with familial hypercholesterolemia. This score takes into consideration multiple independent risk factors, such as age, gender, high-density lipoprotein cholesterol, smoking, and hypertension. Its predictive accuracy is notably high, with an area under the curve (AUC) of 0.84.[32] A score of 20 or more is indicative of a significantly elevated risk of cardiovascular disease, with 10 times higher risk than those with lower scores. Furthermore, the MFHS can effectively identify patients who are currently receiving statin therapy but may require additional treatment to manage their cardiovascular risk. This can assist clinicians in identifying patients who require more intensive treatment.[32,33] The latest Canadian Cardiovascular Society Position Statement recommends the use of MFHS in the FH population.[34]

Japan Atherosclerosis Society (JAS) FH criteria are focused on only three essential clinical manifestations: (1) LDL cholesterol ≥4.65 mmol/L; (2) tendon xanthomas (thickening of tendons on dorsal side of the hands, knees, elbows, or Achilles tendon hypertrophy or xanthoma tuberosum; and (3) a family history of FH or premature CAD within patient's second-degree relatives. FH diagnosis is if ≥2 of the above-mentioned criteria are fulfilled.[35] The diagnostic criteria for heterozygous FH in individuals aged 15 years or older are well established and include the following parameters. According to X-ray measurements, the minimum thickness for Achilles tendon hypertrophy is defined as ≥9.0 mm.[36] If there is a suspicion of heterozygous FH, it is recommended to use genetic testing for diagnosis. Excluding secondary dyslipidemia is a crucial step in making an accurate diagnosis. Premature coronary artery disease (CAD) is defined by the development of CAD in males under the age of 55 or females under the age of 65. When FH is detected, it is advised to investigate the relatives of the patient. The same standards for diagnosis are applicable to HoFH as well. Pediatric FH can be diagnosed using two primary criteria. The first is hypercholesterolemia, which is indicated by an untreated LDL-C level of ≥140 mg/dL (if the total cholesterol level is ≥220 mg/dL, then the LDL-C level should be measured). The second criterion is a family history of FH or premature CAD, specifically in a blood relative closer than the two parents.[35] The simplicity and ease

of use of these criteria make them a practical tool for healthcare professionals.

The European Atherosclerosis Society (EAS) has recommended guidelines for the diagnosis of Homozygous FH (HoFH), a rare and life-threatening condition. These guidelines should be followed when there is suspicion of the disease to ensure accurate diagnosis and appropriate treatment. HoFH is a condition that is marked by high levels of LDL-C in the blood from birth. It is also associated with the early onset of atherosclerotic cardiovascular disease (ASCVD). The clinical and genetic criteria for HoFH have been updated in 2023. The new criteria now include two main aspects: (1) LDL criteria, which is untreated LDL-C > 10 mmol/L (> ~ 400 mg/dL) that requires further investigation to confirm the diagnosis, and (2) additional criteria, which is cutaneous or tendon xanthomas appearing before the age of 10 years and/or untreated elevated LDL-C levels that are consistent with heterozygous FH in both parents. It is worth noting that in digenic form, one parent may have normal LDL-C levels while the other may have LDL-C levels consistent with HoFH. To meet the genetic criteria, there must be confirmation of pathogenic or likely pathogenic variants on different chromosomes in the LDLR, APOB, PCSK9, or LDLRAP1 genes, or at least two such variants at different loci.[37]

3 | FH-CAUSING GENES

The predominant causes of FH that are observed in over 90% of patients are attributed to loss-of-function mutations in the gene responsible for encoding the low-density lipoprotein receptor (LDLR), apolipoprotein B (APOB) genes, or gain-of-function mutations in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene (Table 4).[38–42]

HeFH is typically caused by a single pathogenic variant in one of three main genes: LDLR, APOB, and PCSK9. Table 5 includes the most common mutations. HoFH is usually associated with biallelic mutations, primarily in LDLR.[44,45] In autosomal recessive hypercholesterolemia (ARH) cases are reported mutations in LDL-receptor adaptor protein 1 (LDLRAP1).[46–48] Mutations in LDL-receptor adaptor protein 1 (LDLRAP1) are reported in cases of autosomal recessive hypercholesterolemia (ARH). It is feasible to identify alterations, such as modifications in sequencing, deletion, or duplication, in at least one CanGen in about 60%–80% of cases. However, genetic testing is inconclusive in detecting these mutations in 20%–40% of patients with the FH phenotype.[49] Additionally, FH may also result from pathogenic mutations in unidentified genes or multiple genes, referred to as polygenic FH.[7,49]

The expression of different phenotypes, such as autosomal dominant (AD) or autosomal recessive (AR), may be linked to various mutations of genes in probands. Table 5 below illustrates the association between genes and phenotypes in different types of FH.

An algorithm for detecting phenotypic HoFH in suspected cases without genetic data is presented in Figure 1. It is important to note that phytosterolemia and Lysosomal Acid Lipase Deficiency are disorders with comparable phenotypes that should be considered in the differential diagnosis of affected individuals.

The LIPA gene, which encodes lysosomal acid lipase (LAL), is located in the chromosomal region 10q23.31.[50] When this enzyme malfunctions, it leads to the accumulation of cholesteryl esters and

TABLE 3 Make Early Diagnosis to Prevent Early Deaths (MEDPED) diagnostic criteria for Heterozygous Familial Hypercholesterolemia (FH)*

FH is diagnosed if total cholesterol exceeds these cutpoints in mg/dL (mmol/L)				
Age (years)	**First-degree relative with FH**	**Second-degree relative with FH**	**Third-degree relative with FH**	**General population**
<20	220 (5.7)	230 (5.9)	240 (6.2)	270 (7.0)
20–29	240 (6.2)	250 (6.5)	260 (6.7)	290 (7.5)
30–39	270 (7.0)	280 (7.2)	290 (7.5)	340 (8.8)
> = 40	290 (7.5)	300 (7.8)	310 (8.0)	360 (9.3)

*The total cholesterol cutpoints for FH depends upon the confirmed FH cases in the family. If FH is not diagnosed in the family, then the cutpoint for diagnosis is as per the "general population."

TABLE 4 Main gene mutations in FH.

Gene	Prevalence of mutation	Functions	Mutation
LDLR *Low-density lipoprotein receptor*	60%–90% of monogenic FH	Uptake of low-density lipoprotein cholesterol (LDL-c), thus decreasing systemic LDL-c levels	Loss-of-function
APOB *Apolipoprotein B-100*	5%–10%	Building of LDL-containing lipoproteins and transporting to the LDL receptor	Loss-of-function
PCSK9 *Proprotein convertase subtilisin/kexin 9*	1%–3%	Inhibiting LDL receptors recircularization by promoting its degradation in the lysosomes	Gain-of-function

Abbreviations: APOB, apolipoprotein B-100; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin 9.

TABLE 5 Phenotype-gene relationships.

Phenotype	Inheritance	Gene/locus	Location	Phenotype MIM number
Familial hypercholesterolemia 1	AR, AD	LDLR	19p13.2	143 890
Familial hypercholesterolemia 2	AD	APOB	2p24.1	144 010
Familial hypercholesterolemia 3	AD	PCSK9	1p32.3	603 776
Familial hypercholesterolemia 4	AR	LDLRAP1	1p36.11	603 813

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.
Source: OMIM-Online Mendelian Inheritance in Man, An Online Catalog of Human Genes and Genetic Disorders (https://omim.org/).[43]

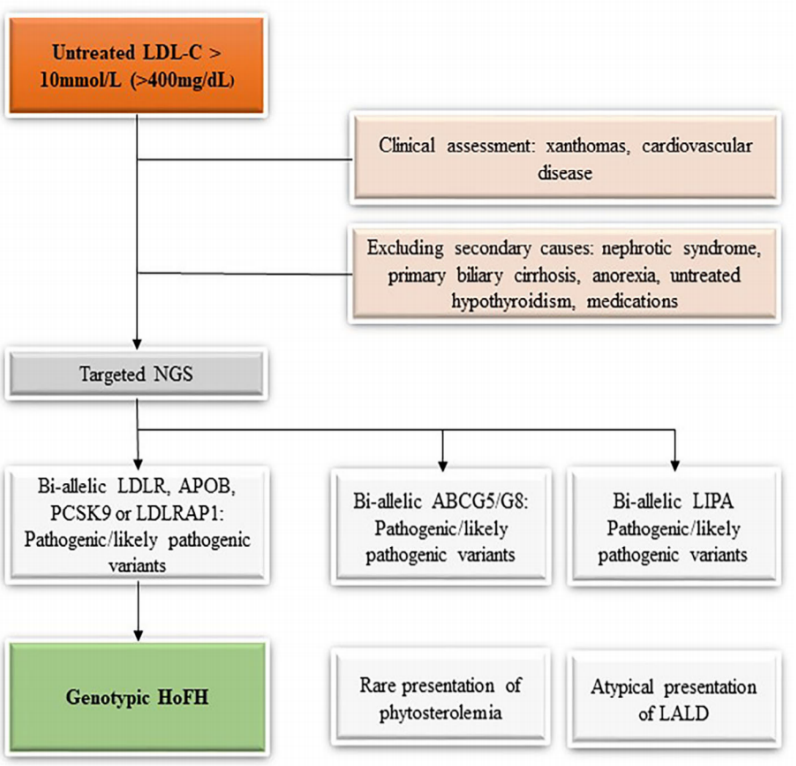

FIGURE 1 The algorithm for identifying phenotypic homozygous familial hypercholesterolemia (HoFH) without genetic data (based on the EAS 2023 Guidelines).

triglycerides in the liver, intestines, adrenal glands, and hepatocytes.[51] This deficiency can cause the lethal Lysosomal Acid Lipase Deficiency-LALD (previously known as Wolman disease) or the late-onset lysosomal acid lipase (CESD), which is characterized by hyperlipidemia and hepatosplenomegaly).[52] Some studies have identified patients with a clinical diagnosis of FH and mutation of the LIPA gene.[53]

Mutations in ATP-binding cassette subfamily G members 5 (ABCG5) and ATP-binding cassette subfamily G members 8 (ABCG8) can cause the accumulation of phytosterol in the body, leading to a condition known as phytosterolemia.[54] These genes are located on chromosome 2p21 and encode subunits of a membrane transporter of sterols.[55] ABCG5/ABCG8 regulate and eliminate sterols via the biliary tree and the intestinal tract.[56] Patients with phytosterolemia exhibit a wide range of symptoms, from almost asymptomatic to early CV death, premature atherosclerosis, hypercholesterolemia, splenomegaly, and arthritis.[57,58] Recent studies show that the prevalence of mutations in ABCG5 and/or ABCG8 genes is ~1 in 200 000 individuals among the general population.[59] Genetic mutations of ABCG and ABCG 8 can cause a recessive form of FH and increase the risk of developing atherosclerosis.[57,60] Therefore, it is important to conduct genetic testing, measure phytosterol levels, and administer treatment with a sterol absorption inhibitor in patients with lipid disorders to prevent CV incidents.[60]

3.1 | The low-density lipoprotein receptor

The LDL receptor gene is positioned at 19p13.2, the short arm on chromosome 19, and is made up of 18 coding regions (exons). The type and location of LDLR gene mutations are linked to receptor activity[61] and may be classified into five classes[62]: Class 1 mutations

prevent the synthesis of detectable LDLR, Class 2 mutations result in defective LDLR transport, and are either completely (Class 2a) or partially (Class 2b) retained in the endoplasmic reticulum (ER). Class 3 mutations lead to reduced LDLR binding at the cell surface, while Class 4 mutations result in mutant LDLRs that cannot concentrate in clathrin-coated pits. Class 5 mutations disrupt the release of LDL in the endosome, and are subsequently degraded intracellularly.[62] A sixth class of mutations has recently been suggested, where mutations are incorrectly inserted in the cell membrane.[63,64]

The American College of Medical Genetics and Genomics (ACMG) introduced an algorithm in 2015 that categorizes all variants of LDLR gene mutations into five groups: pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign, and benign.[64-66]

There are 3842 public variants, comprising 2095 unique events that have been reported to the University College London (UCL) LDLR variant database on the Leiden Open Variation Database (LOVD 3) (http://www.lovd.nl/LDLR).[67] Out of these variants, ~72% were classified as pathogenic or likely pathogenic, 21% as benign/likely benign, and 7% were identified as variants of unknown significance (VUS). Regarding Classification of unique public LDLR gene mutation variants (LOVD3), pathogenic/likely pathogenic variants, benign/likely benign variants, and variants of unknown significance were found in 72%, 21%, and 7%, respectively.

Numerous studies have explored the correlation between genetic variants and their impact on biological and clinical severity. The variants such as nonsense, splicing alterations, frameshift variants, large deletions or altered initiation codons, can cause a complete absence or abnormality of the LDL receptor protein and are known as "null" or "negative" alleles, resulting in a total loss of function (LOF) of the LDL receptor. Other nucleotide changes, typically missense changes, result in defective alleles that lead to partial LOF or altered receptor function. In individuals with HeFH cells carrying a null allele a residual LDL receptor activity of about 50%, while individuals with defective alleles present greater residual activities.[68,69] The carriers of a receptor-negative/null allele with total loss of LDL receptor function, have a more severe phenotype than those with defective receptor mutations. It is crucial to distinction because this severe phenotype includes increased LDL cholesterol levels and a higher prevalence of tendon xanthomas, early onset of atherosclerosis and CVD.[70-72]

3.2 | Apolipoprotein B

ApoB is the primary apolipoprotein found on lipoprotein molecules, and it facilitates the attachment of LDLs to specific receptors on cell surfaces, particularly in the liver.[29] A less common form of FH is a point mutation in the apolipoprotein B-100 gene, located on the short arm of chromosome 2, leading to a change in the amino acid—arginine to glutamine at position 3500 in the apolipoprotein B molecule (ApoB p.Arg3500Gln).[73,74] It was the first mutation in APOB to be identified and characterized and has also been referred to as familial defective apoB (FDB). The other point mutations p.Arg3500Trp (substitution of Arginine by Tryptophan at codon 3500) and p.Arg3531Cys (substitution of Arginine by Cysteine at codon 3531) are less common causes of FDB.[75,76] FDB is transmitted by autosomal codominant inheritance and the prevalence of this disorder is estimated at ~1:1000 Caucasians and Europeans.[73] Among the most commonly reported in studies mutations of apoB are: p.Arg3527Gln,[77-80] p.Ala3527Ala, p.Leu3517Leu,[81] p.Glu3405Gln, p.Leu3350Leu,[82] p.Thr3540Thr, p.Thr3552Thr,[83] p.Ala3426Val[84] and p.Arg50Trp.[85]

3.3 | Proprotein convertase subtilisin/kexin type 9

The PCSK9 gene can be found on chromosome 1p32.3 and was first characterized in 2003 by Ambifadel et al. This gene consists of 12 exons and produces a human subtilase-neutral apoptosis-regulated convertase (NARC-1) that is primarily expressed in the liver and plays a key role in maintaining cholesterol homeostasis.[86,87] When PCSK9 binds to LDLR, it leads to the internalization and degradation of LDLR in lysosomes. Autosomal codominant inheritance is observed with mutations in the PCSK9 gene.[88] Gain-of-function mutations result in increased LDL-receptor degradation and reduced receptor numbers on the cell surface, ultimately leading to significantly elevated blood cholesterol levels.

There are currently over 20 known PCSK 9 mutation variants worldwide. The most common of these variants is p.Asp374Tyr, with an estimated 2% prevalence rate in UK FH patients. Two rare mutations, p.Ser127Arg and p.Phe216Leu, have been reported in France's population as essential for the development of hypercholesterolemia.[86,89] The p.Asp374Tyr mutation was discovered in Norwegian patients by Leren and simultaneously by Timmsy Timms et al. in a Utah pedigree.[90,91] The p.Arg496Trp mutation was discovered in a 35-year-old Sicilian woman[92] and is thought to result in a variant of FH with a better prognosis than that associated with the p.Asp374Tyr mutation.[93] Kaya et al. evaluated PCSK9 mutations in FH patients from Turkey and found only p.Asp374Tyr and p. Arg496Trp mutations in the Turkish population.[94]

It should be noted that when PCSK 9 mutations cause loss of function, there is a decrease in the degradation of the LDL receptor, resulting in lower levels of cholesterol in the plasma. This reduction in LDL cholesterol is linked to a significant decrease in the risk of cardiovascular disease.[88]

4 | RESIDUAL GENETIC CAUSES IN FH

It is worth mentioning the original research by Zorzo et al in 2023[95] regarding LDLR gene's promoter region hypermethylation in patients with FH. A research study examined samples from patients diagnosed with FH according to DCLN criteria, who previously tested negative for structural changes in known genes, as well as samples from a control group of patients with normal blood lipids. The research team tested all DNA samples for

methylation of three genes' CpG islands and determined FH prevalence for each gene in both groups, calculating respective prevalence ratios (PRs). Methylation analysis of APOB and PCSK9 showed no relationship between methylation in those genes and FH phenotype in either group. Analysis of LDLR-island1 indicated that methylation had no relationship with the FH phenotype but showed a possible association between methylation on this LDLR-island2 and the FH phenotype, with a PR of 4.12 and a significant p-value. The authors have identified a connection between DNA methylation and the expression of FH phenotype. They have conducted tests on the four CpG islands on the CanGens, which suggests the possibility of explaining the origin of the FH phenotype in patients where variants in the CanGens were not found. Considering the role of the LDLR gene in cholesterol metabolism, it is possible that its methylated status may have an epigenetic impact on the FH phenotype. This finding could be groundbreaking as it would explain the FH+ phenotype in patients who have no changes in the DNA structure of CanGen.[95]

5 | CONCLUSIONS

FH is a hereditary condition that impedes the body's capacity to eliminate LDL cholesterol from the bloodstream, posing a significant risk for cardiovascular disease. Despite established clinical assessment guidelines, FH frequently remains undiagnosed. Genetic testing is a crucial tool for identifying patients with FH, as it can uncover mutations in genes that contribute to the disorder. Ongoing research is essential for identifying new mutations linked to FH and other genetically determined lipid disorders, which can enhance diagnostic accuracy and treatment options. Nonetheless, early detection and prompt initiation of therapeutic strategies with statins and other lipid-lowering drugs have been demonstrated to be effective in delaying or preventing the development of coronary artery disease. Therefore, it is vital to increase awareness among healthcare professionals and the general public regarding the importance of timely diagnosis and treatment of FH.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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7.2 Gene mutation in patients with familial hypercholesterolemia and response to alirocumab treatment. The single-centre analysis.

Graphical abstract

FAMILIAL HYPERCHOLESTEROLEMIA (FH)

Gene mutation in patients with familial hypercholesterolemia and response to alirocumab treatment.

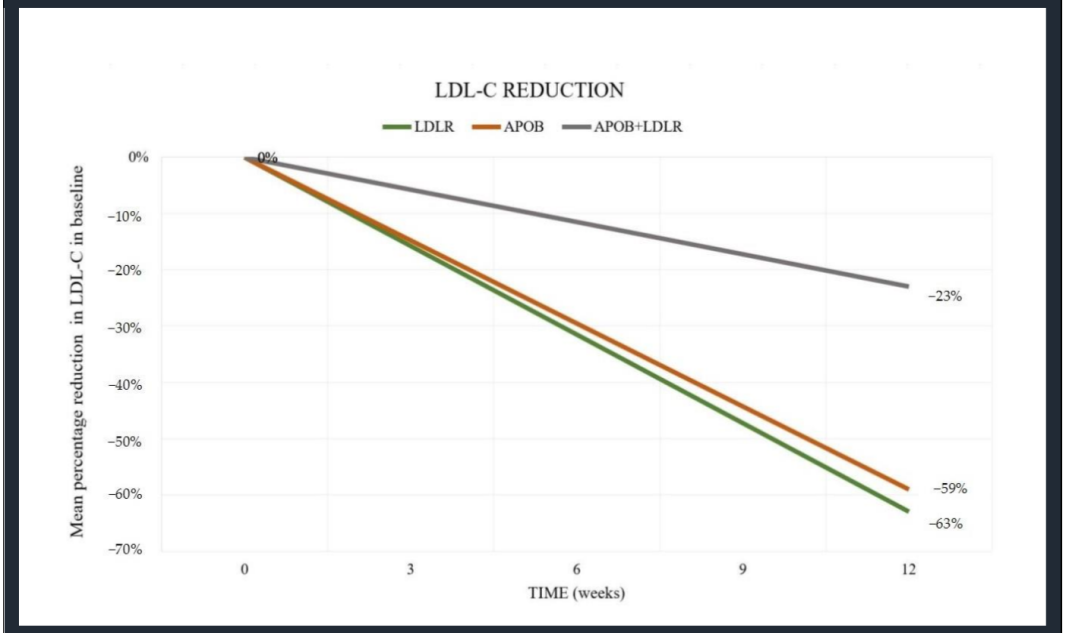
GENE MUTATION

LDLR	*APOB*	*APOB+LDLR*

INCLUSION CRITERIA

- > 18 years old
- confirmed FH mutation with genetic tests
- LDL-C above 100 mg (2.5 mmol/l) despite undergoing diet and intensive statin treatment at maximum tolerated doses for a minimum of 3 months, which also involved combined treatment with ezetimibe 10 mg for at least 1 month
- patients with documented intolerance to at least 2 statins

3-months of alirocumab 150mg sc every two weeks



MACE defined as a composite of nonfatal stroke, nonfatal myocardial infarction, and cardiovascular death was not observed during one year of treatment in patients with response to alirocumab.

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Article

Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment—A Single-Centre Analysis

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Abstract: Background: Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder characterized by significantly elevated levels of low-density lipoprotein (LDL) cholesterol, which plays a major role in the progression of atherosclerosis and leads to a heightened risk of premature atherosclerotic cardiovascular disease. **Methods**: We have carried out an observational study on a group of 17 patients treated at the Outpatient Lipid Clinic from 2019 to 2024. **Result**: The most frequent mutation observed was found in the *LDL receptor (LDLR)* gene, which was identified in ten patients (58.8%). Five patients were identified to have a mutation in the *apolipoprotein B (APOB)* gene, whereas two patients had two points mutations, one in the *LDLR*, and the other in the *APOB* gene. The average age of patients with *LDLR* mutation was 54.8 (12.3); for *APOB* mutation it was 61.4 (9.3) and for patients with two points mutation it was 61.5 (14.8). The study results showed that at Week 12, individuals with *LDLR*-defective heterozygotes who were given alirocumab 150 mg every two weeks experienced a 63.0% reduction in LDL cholesterol levels. On the other hand, individuals with *APOB* heterozygotes experienced a 59% reduction in LDL cholesterol levels. However, in patients with double heterozygous for mutations in *LDLR* and *APOB* genes, there was a hyporesponsiveness to alirocumab, and the reduction in LDL-C was only by 23% in two individuals. **Conclusions**: In patients with a single mutation, there was a greater response to treatment with alirocumab in contrast to patients with double heterozygous mutation, who did not respond to treatment with PCSK9 inhibitors.

Keywords: alirocumab; APOB; familial hypercholesterolemia; genes; hyperlipidemia; LDLR; PCSK9

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1. Introduction

Disorders involving lipids are pivotal in the progression of atherosclerosis and its associated clinical outcomes such as coronary heart disease and stroke. These disorders are a primary contributor to mortality in adult populations globally. However, timely identification and effective management can greatly diminish the likelihood of early cardiovascular events and enhance longevity. Familial hypercholesterolemia (FH), also referred to as primary hypercholesterolemia, is a genetic condition resulting from mutations that impair the ability to process and eliminate cholesterol. FH leads to extremely high levels of low-density lipoprotein (LDL) cholesterol, which is a key contributor to the development of atherosclerosis and raises the risk of an early onset of atherosclerotic cardiovascular disease (ASCVD) [1–3].

FH occurs in heterozygous mutation carriers (HeFH) and rare homozygous carriers (HoFH). HeFH in the general population has a relatively high prevalence of about 1 in 311 people [4,5], while HoFH is much rarer with a prevalence of about 1 in 1,000,000 [6]. Recent research indicates that the occurrence of HoFH is from 1 in 160,000 to 1 in 300,000 worldwide [5,7–9]. The estimated prevalence of FH in Poland is 1 in 250 individuals [10]. Genetically determined hypercholesterolemia is a medical condition that needs a proper

and accurate diagnosis. Several diagnostic criteria for facilitating the diagnosis of FH have been established. These include the Dutch Lipid Network (DLCN), chosen most often in Poland to predict FH (presented in Table 1) [11], the 2017 Japan Atherosclerosis Society (JAS) FH criteria, the Make Early Diagnosis, a simplified Canadian definition of FH, the Prevent Early Deaths (MEDPED) criteria, the Simon Broome (SB) Register, and the Montreal-FH-Score (MFHS) [12–19]. These criteria help categorize FH as definite, probable, or possible. The diagnosis is established by identifying the characteristic physical symptoms, including the presence of arcus corneal, tendinous xanthomata, a premature history of cardiovascular diseases, and elevated LDL levels. Genetic testing is the gold standard for diagnosing FH due to its high accuracy [14,20,21]. However, it may not be practical for everyone due to its limited availability and cost. Clinical scores can also be utilized to diagnose FH, but may not be as accurate as genetic testing. Establishing an accurate diagnosis of FH is essential to ensure proper treatment and management of the condition, which can help prevent severe cardiovascular complications. The other reason to obtain a molecular diagnosis is to make it easier to conduct cascade screening for family members.

Table 1. Diagnosis of familial hypercholesterolemia based on Dutch Lipid Clinic Network criteria for FH [11] *.

Dutch Lipid Clinic Network Criteria	Points
(1) Family history	
First-degree relative with premature (men < 55 years; women < 60 years) coronary or vascular disease, or a first-degree relative with LDL-C above the 95th percentile	1
First-degree relative with tendinous xanthomata and/or arcus corneal, or children < 18 years with LDL-C above the 95th percentile	2
(2) Clinical history	
Patient with premature (men aged < 55 years; women < 60 years) CAD	2
Patient with premature (men aged < 55 years; women < 60 years) cerebral or peripheral vascular disease	1
(3) Physical examination	
Tendinous xanthomata	6
Arcus corneal < 45 years	4
(4) LDL-C levels (without treatment)	
LDL-C ≥ 8.5 mmol/L (≥325 mg/dL)	8
LDL-C 6.5–8.4 mmol/L (251–325 mg/dL)	5
LDL-C 5.0–6.4 mmol/L (191–250 mg/dL)	3
LDL-C 4.0–4.9 mmol/L (155–190 mg/dL)	1
(5) DNA analysis	
Functional mutation in the *LDLR*, *APOB*, or *PCSK9* genes	8

* A 'definite' FH diagnosis requires >8 points; A 'probable' FH diagnosis requires 6–8 points; A 'possible' FH diagnosis requires 3–5 points. Abbreviations: CAD—coronary artery disease; FH—familial hypercholesterolemia; LDL-C—low-density lipoprotein cholesterol; MI—myocardial infarction, PCSK9—proprotein convertase subtilisin/kexin type 9.

FH primarily arises from genetic mutations leading to the impaired function of the gene that encodes the *low-density lipoprotein receptor (LDLR)*, *apolipoprotein B (APOB)* genes, or the increased function of the *proprotein convertase subtilisin/kexin type 9 (PCSK9)* gene [22–26]. These mutations are found in more than 90% of FH patients and are listed in Table 2. In autosomal recessive hypercholesterolemia (ARH) cases, biallelic variation in the *LDL-receptor adaptor protein 1 (LDLRAP1)* has been reported [27]. Genetic testing can detect changes in sequencing, deletion or duplication, in at least one CanGen in approximately 60–80% of

individuals [28,29]. Although, it is worth noting that in about 20–40% of patients with the FH phenotype, these mutations may not be detected through genetic testing [29,30]. Polygenic hypercholesterolemia, which is caused by pathogenic mutations in unidentified genes or multiple genes, is another factor that contributes to FH [7,30].

Table 2. The primary gene mutations found in FH.

Gene	Functions	Prevalence of Mutation	Mutation
LDLRlow-density lipoprotein receptor	Uptake of low-density lipoprotein cholesterol (LDL-C), resulting in lower levels of LDL-C	60–90% of monogenic FH	Loss-of-function
APOBapolipoprotein B-100	Building of LDL-containing lipoproteins and transporting to the LDL receptor	5–10%	Loss-of-function
PCSK9proprotein convertase subtilisin/kexin 9	Recycling of LDL receptors is inhibited by promoting their demotion in the lysosomes	1–3%	Gain-of-function

Alirocumab is a monoclonal antibody that targets PCSK9, which is a protein that joins to LDLR found on the surface of liver cells and it competes with LDL for its binding to LDL receptors. When it occurs, it leads to the degradation of these receptors and is responsible for removing LDL-C from circulation. In addition, PCSK9 facilitates the breaking down of LDLR intracellularly for recycling. Alirocumab is a fully human immunoglobulin G1 antibody that effectively treats hypercholesterolemia in high-risk patients by blocking the binding of PCSK9 to LDLR. Through this process, it upregulates the number of receptors available for LDL-C removal, leading to a reduced concentration of LDL-C in the blood. Alirocumab can be employed as a standalone therapy or in conjunction with different lipid-lowering agents for patients who are unable to tolerate statins or for whom the administration of statins is medically prohibited.

Recent updates in European guidelines have revised the recommended target purpose of low-density lipoprotein cholesterol (LDL-C) for individuals who are at a significantly higher likelihood of experiencing major adverse cardiovascular events (MACE). Individuals who have been diagnosed with HeFH are classified as being at high to very high risk for developing cardiovascular diseases. For individuals with FH and atherosclerotic cardiovascular disease (ASCVD) who are at very high risk, the 2019 European Society of Cardiology/European Atherosclerosis Society guidelines suggest therapy aimed at achieving a minimum of 50% reduction from the initial values and LDL-C target of <1.4 mmol/L (55 mg/dL) [31]. If these goals cannot be met, a combination of drugs is recommended. In very high-risk FH patients who have not attained treatment goals with maximal tolerated statin plus ezetimibe, treatment with PCSK9 inhibitors (PCSK9-I) is recommended.

This retrospective study aims to present the genotype-specific effectiveness of alirocumab in treating individuals with heterozygous FH.

2. Materials and Methods

From 1 April 2019 to 1 February 2024, a comprehensive retrospective analysis was conducted at the University Clinic Center of the Medical University of Warsaw. This analysis focused on a cohort of 17 patients with FH who underwent our outpatient lipid clinic. Our study protocol was accepted by the Bioethics Committee at the Medical University of Warsaw (decision number AKBE/68/2023) and the ethics committee desisted the exigences for informed consent. The study was performed following the ethical guidelines of the 1975 Declaration of Helsinki. Our patients were identified through genetic testing. Molecular

analysis was performed using the direct sequencing technique of the *LDLR* gene and a fragment of exon 26 of the *APOB* gene, as well as the MLPA technique. The material for the genetic test was DNA isolated from peripheral blood leukocytes. All genetic testing was completed before the patient's referral to our outpatient clinic.

Patients eligible for the study met the criteria of the Polish FH treatment program, including being over 18 years old, having a confirmed FH mutation with genetic tests, and maintaining an LDL-C above 100 mg (2.5 mmol/L) even when undergoing diet and intensive statin therapy at maximum tolerated doses for a minimum of 3 months, which also involved combined treatment with ezetimibe 10 mg for at least 1 month. Additionally, patients with a documented intolerance to at least 2 statins, as per the guidelines of scientific societies specializing in the diagnosis and treatment of lipid disorders, were also considered for the study. The exclusion criteria included individuals younger than 18 years, those who had not undergone genetic testing, pregnant and breastfeeding women, individuals with kidney impairment (eGFR < 60 mL/min/1.73 m^2), and those with severe liver dysfunction. Figure 1 summarizes the stages of participant inclusion in the study.

The Dutch Lipid Clinic Network scale was employed to evaluate the patients. Alirocumab was applied subcutaneously at a dose of 150 mg once every two weeks throughout the treatment period. After the initial administration of the first two doses by a trained nurse in a clinical setting, patients were provided with the necessary training to self-administer four out of six doses of medication at home. The study involved measuring the lipid profile of individuals, including the concentration of total cholesterol (TC), LDL-C (calculated using Friedewald formula), HDL cholesterol (HDL), non-HDL cholesterol (non-HDL), and triglycerides (TG). The measurements were taken before treatment and 12 weeks after the start of treatment. To qualify for further therapy, patients must have achieved a reduction of at least 30% in serum LDL levels from the baseline. Participants who experienced a reduction in more than 30% in their baseline LDL-C levels were evaluated for ongoing alirocumab usage. The effects of alirocumab treatment after 1 year have been summarized separately. Additionally, alanine aminotransferase (ALT) concentration was measured to exclude patients with liver disease, and serum creatinine levels were assessed to evaluate kidney function as part of the initial qualification process. To evaluate the efficacy of treatment, we analyzed the impact of the detected mutation on the concentration of LDL-C before and after the onset of therapy.

Statistical Analysis

Statistical analysis was performed using the MedCalc program. Group differences were presented as the mean value and standard deviation. Continuous variables were analyzed using nonparametric tests, specifically the Wilcoxon signed-rank test for paired samples and the Mann–Whitney U test for independent samples. The association of non-numeric variables was assessed using the chi-square test. Statistical significance was defined as a p-value < 0.05 for all tests.

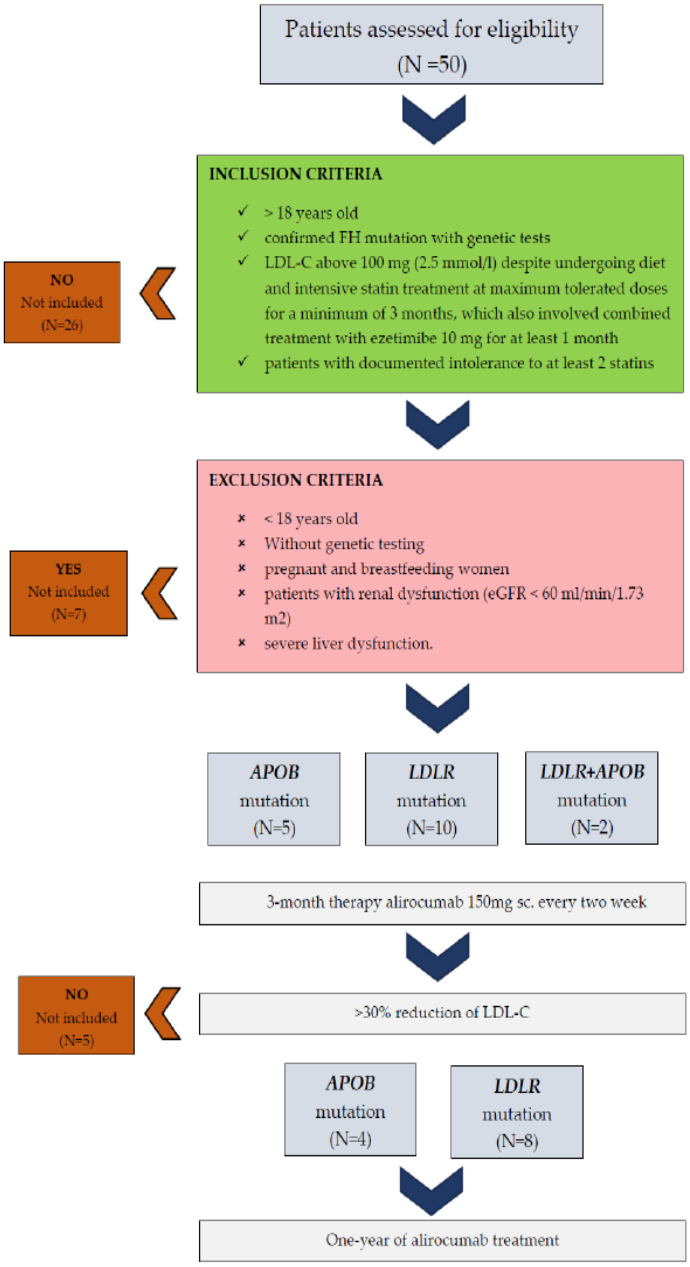

Figure 1. The stages of participant inclusion in the study.

3. Results

The study involved a sample of 17 individuals, with the majority being females (76.5%) and only 4 males. The mean age of the participants was 57.5 (11.1) years. The average age of patients with *LDLR* mutation was 54.8 (12.3); for the *APOB* mutation it was 61.4 (9.3), and for patients with two points mutation, it was 61.5 (14.8). Table 3 presents the baseline characteristics of the patients. The overall cholesterol level in the participants before initiating the therapy of alirocumab was 281.3 (85.1) mg/dL, while the LDL-C level was 206.1 (82.6) mg/dL. After 12 weeks of treatment with a dose of 150 mg of alirocumab every two weeks, the mean TC was 169.7 (98.9) mg/dL and LDL-C was 95.8 (95.7) mg/dL. In the study, 70% of patients were treated with combination therapy with the maximum

tolerated dose of steroid and ezetimibe. Additionally, statin intolerance was identified in four patients. Among the patients, 35.3% were diagnosed with coronary artery disease, and two of them had a history of myocardial infarction. Carotid artery atherosclerosis was found in 29.4% of the patients, and three patients had a history of stroke.

Table 3. Baseline characteristics of patients.

Characteristic	*LDLR* Mutation (n = 10)	*APOB* Mutation (n = 5)	*APOB* + *LDLR* Mutation (n = 2)	All Patients (n = 17)
Age, year	54.8 (12.3)	61.4 (9.3)	61.5 (14.80)	57.5 (11.1)
Female sex- no (%)	7 (70.0)	4 (80.0)	2 (100.0)	13 (76.5)
Male sex- no (%)	3 (30.0)	1 (20.0)	0	4 (23.5)
DLCN	16.5	13.6	22.5	16.5
Variables				
Family history of ASCVD, n (%)	8 (80%)	5 (100%)	2 (100%)	15 (88.2%)
Hypertension, n (%)	1 (10%)	2 (40%)	0 (0%)	3 (17.6%)
T2DM, n (%)	1 (10%)	0 (0%)	0 (0%)	1 (5.8%)
Premature CVD	2 (20%)	0 (0%)	0 (0%)	2 (11.7%)
Myocardial infarction, n (%)	2 (20%)	1 (20%)	0 (0%)	3 (17.6%)
CAD, n (%)	3 (30%)	2 (40%)	1 (50%)	6 (35.3%)
PCI, n (%)	1 (10%)	0 (0%)	0 (0%)	1 (5.8%)
CABG, n (%)	1 (10%)	0 (0%)	0 (0%)	1 (5.8%)
Stroke/TIA, n (%)	1 (10%)	2 (40%)	0 (0%)	3 (17.6%)
Carotid disease, n (%)	5 (50%)	0 (0%)	0 (0%)	5 (29.4%)
PAD, n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Physical examination				
Arcus corneal <45 y, n (%)	6 (60%)	1 (20%)	0 (0%)	7 (41.2%)
Tendinous xanthomata, n (%)	1 (10%)	1 (20%)	1 (50%)	3 (17.6%)
Lipid-lowering treatment				
Statins, n (%)	8 (80%)	5 (100%)	0 (0%)	13 (76.5%)
Ezetimibe, n (%)	9 (90%)	5 (100%)	0 (0%)	14 (82.4%)
Statin intolerance, n (%)	2 (20%)	0 (0%)	2 (100%)	4 (23.5%)
Laboratory results before treatment				
Total cholesterol (mg/dL)	272.8 (63.9)	228.4 (33.8)	456.0 (21.2)	281.3 (85.1)
LDL cholesterol (mg/dL)	198.5 (57.8)	151.4 (34.1)	381.0 (4.9)	206.1 (82.6)
HDL cholesterol (mg/dL)	50.1 (12.6)	59.4 (9.8)	56.6 (17.7)	53.6 (13.5)
non-HDL cholesterol (mg/dL)	223.6 (62.5)	169.0 (37.7)	399.5 (3.5)	228.2 (84.3)
Triglycerides (mg/dL)	130.8 (57.3)	88.8 (32.9)	95.0 (7.1)	114.2 (48.6)

Table 3. *Cont.*

Characteristic	*LDLR* Mutation (n = 10)	*APOB* Mutation (n = 5)	*APOB* + *LDLR* Mutation (n = 2)	All Patients (n = 17)
Serum creatinine (mg/dL)	0.74 (0.1)	0.76 (0.1)	0.85 (0.1)	0.8 (0.2)
Estimated glomerular filtration rate (mL/min/1.73 m^2)	97.3 (7.4)	88.0 (5.6)	76.0 (17.0)	92.1 (12.2)
ALT (U/L)	27.2 (13.5)	28.2 (12.2)	20.0 (5.7)	26.6 (12.0)
Laboratory results after 12 weeks of treatment				
Total cholesterol (mg/dL)	149.2 (81.2)	135.6 (46.4)	357.5 (89.8)	169.7 (98.9)
LDL cholesterol (mg/dL)	72.6 (73.1)	62.8 (27.9)	294.5 (81.3)	95.8 (95.7)
HDL cholesterol (mg/dL)	51.2 (16.5)	60.0 (23.1)	48.5 (9.2)	53.5 (17.1)
non-HDL cholesterol (mg/dL)	98.0 (68.4)	75.6 (29.0)	309.0 (80.6)	116.2 (93.4)
Triglycerides (mg/dL)	98.7 (39.4)	63.6 (28.1)	74.0 (4.2)	85.5 (36.4)

All data are presented as mean and standard deviation or as n (%). Abbreviations: ALT: alanine aminotransferase; ASCVD: atherosclerotic cardiovascular disease; *APOB*: apolipoprotein B gene; CABG: coronary artery bypass grafting; CAD: coronary artery disease; CVD: cardiovascular disease; DLCN: Dutch Lipid Clinic Network; HDL: high-density lipoprotein; LDL: lower-density lipoprotein; LDLR: lower-density lipoprotein receptor; non-HDL: non-high-density lipoprotein;T2DM: type 2 diabetes mellitus; PAD: peripheral artery disease; PCI: percutaneous coronary intervention; TIA: transient ischemic attack.

The patients were evaluated concerning a confirmed mutation in the genetic testing of underlying FH. Among the enrolled population, the most frequent mutation observed was found in the *LDLR* gene, identified in ten patients (58.8%). Five patients had a mutation in the *APOB* gene while two patients were identified with mutations in both the *LDLR* and *APOB* genes. There were no patients with the *PCSK9* mutation in the study group. The genotype and correlation with phenotype are shown in Table 4. The most prevalent mutation observed among patients with *APOB* gene defects was mutation *p. R3527Q*. In the study, it was found that 60% of patients with the *LDLR* mutation displayed arcus cornealis before the age of 45, while two patients with a *p. R3527Q* mutation in the *APOB* gene exhibited tendinous xanthomata. The chi-square test did not show a significant association between the mutation and the occurrence of one of these phenotypes. No significant association was found between the concentration of either cholesterol fractions and the clinical presentation (tendinous xanthomata, arcus cornealis) typically associated with FH.

Patients demonstrated compliance with the prescribed treatment regimen and did not experience any adverse effects. The patients did not make any modifications to their diet or their previous lipid-lowering therapy. The study results showed that at Week 12, individuals with *LDLR*-defective heterozygotes who were given alirocumab 150 mg sc. every two weeks experienced a 63.0% reduction in LDL-C levels. On the other hand, individuals with *APOB* heterozygotes experienced a 59.0% reduction in LDL-C levels. Patients carrying single gene mutations, as previously mentioned, have been identified as responders to alirocumab therapy. However, in patients with complex *APOB* + *LDLR* mutations, there were non-responders to alirocumab, and the reduction in LDL-C was only 23% in two individuals (Figure 2).

Table 4. Genotype and phenotype of each patient.

Patient (No.)	Age	Sex (F—Female M—Male)	Gene Mutation	Genotype	DLCN Points	Phenotype 1—Tendinous Xanthomata, 2—Arcus Corneal < 45y 0—None	CAD History (1—Yes, 0—No)
1	58	F	*LDLR*	*p.C95R, exon 3*	20	2	1
2	71	F	*LDLR*	*c.2311+56G>C/ p.intron 15 c.941-2140del.*	21	0	0
3	40	F	*LDLR*	*c.314-1186dup, exons 4-8 duplication*	25	2	0
4	68	F	*LDLR*	*exons 4-7 duplication*	10	2	0
5	69	F	*LDLR*	*c.940+2T>C, exon 6*	15	2	1
6	55	F	*LDLR*	*p. G592E, exon 12*	12	0	0
7	57	M	*LDLR*	*c.1187-10G>A*	11	0	1
8	42	F	*LDLR*	*c.1775G>A p.R3527Q*	14	2	0
9	41	M	*LDLR*	*No data*	15	2	0
10	47	M	*LDLR*	*c.1871_1873del (p.1624del) exon 13*	22	1	0
11	51	F	*APOB*	*c.10580G>A/p.R3527Q exon 26*	12	0	0
12	60	F	*APOB*	*p.R3527Q exon 26*	10	0	0
13	66	F	*APOB*	*p.R3527Q exon 26*	15	1	1
14	73	F	*APOB*	*p.R3527Q exon 26*	19	2	1
15	57	M	*APOB*	*p.R3527Q exon 26*	12	0	1
16	51	F	*LDLR + APOB*	*c.1117G>T exon 8, c.10580G>A/p. R3527Q exon 26,*	25	1	0
17	72	F	*LDLR + APOB*	*p.G373C, exon 8, p.R3527Q, exon 26*	20	0	1

Abbreviations: *APOB*: apolipoprotein B-100 gene; CAD: coronary artery disease; DLCN: Dutch Lipid Clinic Network; *LDLR*: Low-density lipoprotein receptor gene.

Figure 3 shows the LDL-C level fluctuations before and after a 12-week therapy of alirocumab for each patient. A single patient with a mutation in the *LDLR* gene showed a 95% reduction in LDL-C levels. This patient experienced a remarkable drop in LDL-C from a starting point of 306 mg/dL to 14 mg/dL after 12 weeks of treatment. However, one of the patients with combined *APOB+LDLR* mutation showed a poor response to treatment, with only an 8.3% decrease recorded from 384 mg/dL to 352 mg/dL.

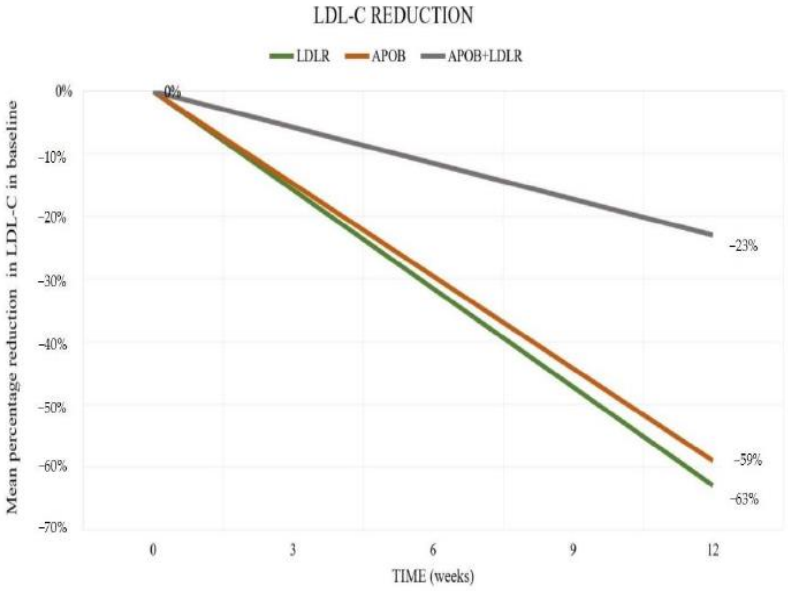

Figure 2. Mean percentage reduction from baseline in LDL-C level depending on the mutation after 12 weeks of treatment alirocumab in dose 150 mg sc. Abbreviations: *APOB*: apolipoprotein B gene; LDL-C: low-density lipoprotein cholesterol; *LDLR:* low-density lipoprotein receptor gene.

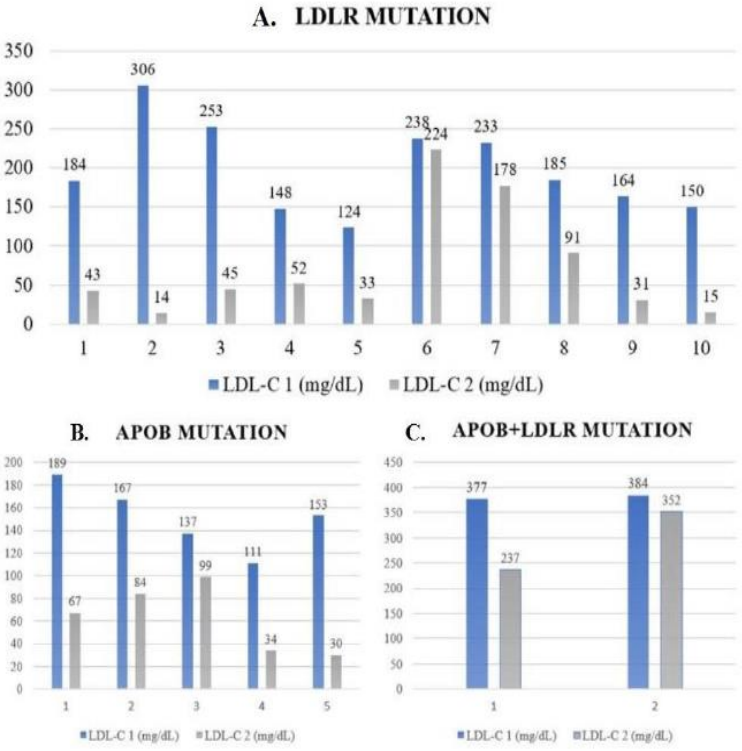

Figure 3. The LDL-C level fluctuations before and after a 12-week therapy of alirocumab for each patient. (**A**). Patients with *LDLR* mutation (n = 10). (**B**). Patients with *APOB* mutation (n = 5). (**C**). Patients with two genes mutation: *APOB* + *LDLR* (n = 2). Abbreviations *APOB:* Apolipoprotein B-100 gene; *LDLR:* Low-density lipoprotein receptor gene, LDL-C: lower-density lipoprotein concentration.

One-Year Follow-Up

Twelve patients who experienced a reduction in more than 30% in LDL-C levels from their initial values were evaluated for ongoing treatment with alirocumab. After 1 year of therapy, the mean LDL-C levels were 49.4 mg/dL (33.8 mg/dL) for the *LDLR* mutation group and 59.8 mg/dL (29.7 mg/dL) for the *APOB* mutation group. For the non-responder group, which comprised the remaining five patients, the average LDL-C level was 258.7 mg/dL (100.1 mg/dL). In patients with mutations in the *LDLR* gene, there was a notable decrease in LDL, non-HDL, and TC levels, indicating a positive response to treatment. However, this effect was not observed in HDL and TG levels. Conversely, no significant differences were found in the treatment effect among the group with *APOB* mutation, potentially due to the small sample size. In a 12-month observation, no significant variations in cholesterol fraction concentrations were noted between responders classified by identified gene mutations and non-responders grouped by gene mutations. One-year follow-up laboratory results are shown in Table 5.

Table 5. Laboratory results of patients after one year of treatment with alirocumab and patients showing no response to the treatment.

Patients with Response to Alirocumab Treatment				
Characteristic	*LDLR* **Mutation (n = 8)**	*APOB* **Mutation (n = 4)**	*APOB* + *LDLR* **Mutation (n = 0)**	**All Patients (n = 12)**
Laboratory results after one-year treatment				
Total cholesterol (mg/dL)	126.5 (36.5)	133.3 (30.3)	0	128.8 (33.3)
LDL cholesterol (mg/dL)	49.4 (33.8)	59.8 (29.7)	0	52.8 (31.5)
HDL cholesterol (mg/dL)	51.8 (17.8)	57.3 (8.8)	0	53.6 (15.2)
non-HDL cholesterol (mg/dL)	74.8 (33.8)	76.0 (34.1)	0	75.2 (32.2)
Triglycerides (mg/dL)	111.1 (53.5)	76.3 (38.0)	0	99.5 (50.1)
Patients with no Response to Alirocumab Treatment				
Characteristic	*LDLR* **Mutation (n = 2)**	*APOB* **Mutation (n = 1)**	*APOB* + *LDLR* **Mutation (n = 2)**	**All Patients (n = 5)**
Laboratory results after one-year treatment				
Total cholesterol (mg/dL)	378	209	407	344 (105.2)
LDL cholesterol (mg/dL)	288.5	127	331	258.7 (100.1)
HDL cholesterol (mg/dL)	69.5	72	51	65.5 (10.1)
non-HDL cholesterol (mg/dL)	308.5	137	356	277.5 (108.9)
Triglycerides (mg/dL)	99.0	52	125	93.7 (47.0)

Abbreviation: apolipoprotein B-100 gene; *LDLR*: low-density lipoprotein receptor gene, LDL: lower-density lipoprotein, HDL: high-density lipoprotein; non-HDL: non-high-density lipoprotein.

MACE defined as a composite of nonfatal stroke, nonfatal myocardial infarction, and cardiovascular death was not observed during treatment with alirocumab.

4. Discussion

FH is a genetic condition caused by the *LDLR*, *APOB*, *PCSK9*, and *LDLRAP1* genes mutations. Recent studies suggest that the loss of function of the *LDLR* is responsible for a majority (60–90%) of all detected mutations [32]. Our research supports this finding, with patients possessing a confirmed *LDLR* mutation comprising 58.8% of our study population.

The most prevalent mutation observed among patients with *APOB* gene defects was mutation *p. R3527Q*. This single variant is found in >95% of patients population with FH cases caused by mutations in the *APOB* gene [33–35].

Alirocumab, a monoclonal antibody that targets protease PCSK9, has proven to be highly effective in patients with HeFH who require further reduction in LDL-C levels. The ALTERNATIVE study correlated alirocumab and ezetimibe in individuals at moderate to high cardiovascular risk with statin intolerance. The 12-week dosing of alirocumab resulted in a significant reduction in LDL-C by 47.0% (1.9) [36]. In a Phase 2 trial, treatment with alirocumab of 150 mg every 2 weeks resulted in an average LDL-C reduction of 67.90% (4.85) from baseline to week 12 [37]. The phase 3 clinical trials evaluated the percentage decrease in LDL-C levels after 24 weeks of drug administration. The latest research revealed that the mean LDL-C reductions from the baseline were as follows. In the FH I and FH II trials, individuals with HeFH and inadequate LDL-C control on maximally tolerated lipid-lowering therapy (LLT) were treated with alirocumab for 78 weeks. The LDL-C reductions observed were 48.8% and 48.7% in FH I and FH II, respectively [38]. In a LONG TERM study randomized controlled trial with high-risk patients with LDL-C > 70 mg/dL observed a significant reduction in LDL-C levels of 61.0% when alirocumab was added to statin therapy at the maximum tolerated dose. This treatment effect remained consistent over a 78-week period [39]. According to the ODYSSEY HIGH FH study, there was a 45.7% reduction in LDL-C levels among patients with HeFH and LDL-C levels of 160 mg/dL or higher, even though they were subject to the maximum tolerated statin therapy and/or additional lipid-lowering treatments [40,41]. Our research findings revealed that the mean percentage reduction in LDL-C among our study participants was consistent with the data reported for the population that received alirocumab treatment, as mentioned in the studies we referred to earlier.

The identification of mutation in genetic testing is important for understanding the efficacy of alirocumab treatment. Patients with a single mutation (*LDLR* or *APOB*) showed a more positive reaction to alirocumab treatment compared to those with double heterozygous mutation (*LDLR* and *APOB*), who did not have a response to PCSK9-I treatment.

The incidence of non-responsiveness to human PCSK9 monoclonal antibodies is very rare. Nevertheless, in such cases, clinicians need to consider the possibility of the presence of anti-drug antibodies, which can impact the efficacy of the treatment. During the ODDYSEY FH clinical trial, it was observed that 3 of the included 735 patients experienced positive alirocumab-neutralizing antibody status at one time, all at Week 12 [42]. The Phase 3 ODYSSEY studies investigated the phenomenon of apparent hyporesponsiveness to alirocumab, defined as less than a 15% reduction in LDL-C levels. This was observed in only 1% of the patient study population [42]. The possible reasons for this hyporesponsiveness could be a lack of adherence to therapy, a theoretical and infrequent opportunity of biological non-responsiveness caused by persistent antidrug antibodies, or other unidentified reasons.

Our patients showed a high level of tolerance towards alirocumab, and there were no reported adverse effects. According to the patient's self-report, all prescribed doses were administered as scheduled following prior training. Nonadherence to the prescribed dosage regimen may have contributed to the observed lack of treatment response. However, the treatment was not effective, and we are still uncertain about the reason for this lack of effectiveness.

In the observation, during alirocumab treatment, there were no instances of MACE, which were defined as a combination of nonfatal stroke, nonfatal myocardial infarction, and cardiovascular death. In the ODDYSEY study alirocumab significantly reduced the risk of cardiovascular events [43,44].

The probability of finding double heterozygotes with two distinct genes is estimated to be approximately 1 in 1.4 million [45,46]. The combination of rare mutations in *LDLR* and *APOB* genes results in a more severe phenotype of atherosclerotic vascular diseases compared to either mutation alone. This is due to the cumulative effect of these mutations, which leads to elevated blood lipid levels [47]. Several studies have reported that individuals with double heterozygosity (mutations in both *LDLR* and *APOB* genes) may respond lower to PCSK9-I therapy [48,49]. Six studies found that individuals with primary mutations in genes linked to FH showed consistently positive responses to alirocumab therapy, with no significant differences observed [50]. The available evidence consistently showed that the LDL-C-lowering effect of PCSK9-I is consistent across genotypes [51]. The response to PCSK9-I was notably reduced in individuals carrying compound heterozygous and homozygous mutations across the published studies [51,52]. In the GENRE- FH study with evolocumab, human monoclonal immunoglobulin G2 that joins specifically to human PCSK9 had a similar lowering LDL-C effect comparable to alirocumab, whereas patients who did not have pathogenic variants or had only defective pathogenic variants experienced a higher percentage of achieved LDL-C reduction than those with at least one null pathogenic variant [53].

One of the primary limitations that ought to be considered when interpreting the results of the presented study is the small number of patients included in the analysis. Additional research is required using a more extensive sample of patients; we are planning on undertaking a cascade screening of patients' families to investigate the underlying causes for the absence of noteworthy LDL cholesterol reduction in patients receiving alirocumab treatment.

5. Conclusions

Patients with single mutation (*LDLR* or *APOB*) responded more to treatment with alirocumab than patients with double heterozygous mutation (*LDLR* and *APOB*), who did not respond to treatment with PCSK9-I. The administration of PCSK9-I was well-tolerated and did not lead to any severe side effects. No major adverse cardiovascular events occurred during the alirocumab treatment observation. Identifying double heterozygous through genetic testing is crucial for determining the suitability of alirocumab as a treatment for FH. This finding highlights the importance of genetic diagnosis in establishing a theoretical framework for personalized patient treatment.

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7.3 Challenges in the management of familial hypercholesterolemia: a case report

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Challenges in the management of familial hypercholesterolemia: a case report

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Background: Familial hypercholesterolemia (FH) is a serious genetic condition that results in abnormally high levels of low-density lipoprotein cholesterol (LDL-C) in the bloodstream, significantly increasing the risk of early onset of cardiovascular disease. The heterozygous form of FH (HeFH) is widespread, affecting around 1 in 500 people worldwide.
Case report: In this clinical report, we present the case of a patient who suffers from HeFH due to a mutation in the LDL receptor (LDLR) gene. A woman exhibited intolerance to statin therapy and did not attain adequate reduction in low-density lipoprotein cholesterol (LDL-C) levels on ezetimibe monotherapy. Genetic testing confirmed the presence of a pathogenic variant for FH with the deletion of exons 7–14. The administration of alirocumab (a dose of 150 mg sc) as the primary therapy did not exhibit the desired therapeutic outcome. Consequently, the patient was given inclisiran therapy (a dose of 284 mg sc), which significantly reduced LDL cholesterol levels after 3 months of treatment and during the 1-year follow-up.
Conclusion: Inclisiran therapy has shown promising results for individuals with HeFH who experience statin intolerance. This therapy works by using a small interfering RNA (siRNA) to target the mRNA of proprotein convertase subtilisin/kexin type 9 (PCSK9), which leads to a significant reduction of LDL-C levels. This approach can be an alternative for patients without significant reductions in LDL-C levels with PCSK9 inhibitor therapy. For HeFH patients with limited treatment options due to statin intolerance and genetic mutations, inclisiran can represent a promising therapeutic option.

KEYWORDS
case report, familial hypercholesterolemia, inclisiran, LDLR gene, PCSK9

Introduction

Familial hypercholesterolemia (FH) is a severe genetic disorder that leads to high levels of low-density lipoprotein cholesterol (LDL-C) in the blood and increases the risk of early onset of cardiovascular diseases (CVD) (1). It is an autosomal codominant disease and the most common form of monogenic hypercholesterolemia (2, 3). Cholesterol-lowering therapies have been found to reduce the risk of mortality and major cardiovascular events in individuals with FH. The heterozygous FH (HeFH) form is quite prevalent, with 1 in 500 individuals affected worldwide (4, 5). The estimated prevalence of FH in Poland is 1 in 250 individuals (6, 7).

According to the latest guidelines on lipid-lowering treatment of the European Society of Cardiology (8), patients with FH who have not achieved target LDL-C levels should be treated with proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. This group includes human monoclonal antibodies, i.e., alirocumab and evolocumab, by inhibiting the binding

of PCSK9 to low-density lipoprotein receptor (LDLR), increases the number of available LDL receptors thereby reducing LDL-C levels from the circulation (9–14). Inclisiran is a novel small interfering RNA (siRNA) that is administered subcutaneously and works by inhibiting the synthesis of PCSK9 in the liver. This mechanism leads to a significant reduction in circulating LDL-C levels. Inclisiran is a first-in-class drug that has shown high effectiveness as a treatment for patients with hypercholesterolemia (15).

This study presents a case report of a woman with HeFH triggered by a mutation in the LDLR gene. The patient had a complete intolerance to statins, which led to the decision to initiate alirocumab therapy. Unfortunately, the therapy turned out to be ineffective. However, the latest treatment method with inclisiran was implemented which resulted in a significant reduction of LDL levels and the risk of adverse cardiovascular events.

Case report

Patient information and clinical findings

In December 2021, a 42-year-old woman was referred to our Outpatient Lipid Clinic (University Clinic Center of the Medical University of Warsaw) for the management of FH. The individual had a genetically confirmed mutation in the LDLR gene and had a family history with documented premature CVD. Carotid ultrasound revealed the presence of atherosclerosis plaques. On physical examination, the patient had corneal arcus, a typical finding in severe hypercholesterolemia. The patient's hypercholesterolemia was found to have no underlying secondary causes such as diabetes mellitus, thyroid gland disorders, renal or hepatic dysfunctions, or hypertension. Additionally, the patient was not taking any systemic corticosteroids or estrogens. In Table 1 we presented patient characteristics based on Dutch Lipid Clinic Network.

Previously, the patient was administered atorvastatin and rosuvastatin, leading to myalgia and elevated creatine kinase (CK) levels. The patient's intolerance to statins was confirmed, and during ezetimibe monotherapy, the patient did not achieve the desired therapeutic outcome. In the absence of any lipid-lowering treatment, the patient had the following lipid values: total cholesterol (TC) 315 mg/dL, non-HDL cholesterol (n-HDL) 250 mg/dL, high-density lipoprotein cholesterol (HDL-C) 65 mg/dL, triglycerides (TG) 58 mg/dL and LDL-C 238 mg/dL (calculated using Friedewald formula).

Genetic analysis

The Dutch Clinic Lipid Network (DLCN score)—17 points indicated that the patient had a definite clinical diagnosis of HeFH. The genetic using direct sequencing and MLPA techniques test confirmed the presence of a pathogenic variant for FH, classified as c.2311 + 36G > C/p. (deletion of exons 7–14 of the LDLR gene in heterozygosis). This particular variant has a 50% chance of being passed down to first-degree relatives.

Therapeutic intervention

The patient had met the necessary criteria in the Polish FH treatment program to receive alirocumab, which was administered subcutaneously at a dose of 150 mg every 2 weeks. The woman underwent clinical evaluation and lab test follow-up after 3 months of treatment. Alirocumab was well-tolerated. No adverse effects were reported. The results of the laboratory tests showed that there was no significant decrease in LDL cholesterol. After unsuccessful treatment with a PCSK-9 inhibitor, the decision was made to begin administering inclisiran therapy. The patient was given 284 mg of inclisiran subcutaneously and follow-up tests were conducted after 3 months. The results showed a significant decrease in LDL cholesterol along with detailed lipid profile parameters: TC 114 mg/dL, non-HDL at 45 mg/dL, HDL-C at 69 mg/dL, TG at 53 mg/dL and LDL-C at 34 mg/dL. The next drug administration was scheduled in 6 months. Following a year of administering inslisiran, a noticeable positive therapeutic effect with LDL-C 54 mg/dL has been consistently observed and maintained. Individual LDL-C values over time are illustrated in Figure 1.

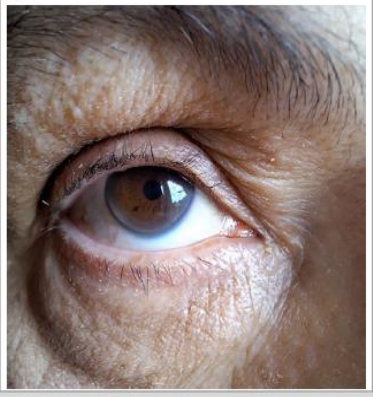
Discussion

FH is primarily caused by loss-of-function mutations in the gene responsible for encoding LDLR, apolipoprotein B (APOB) genes, or gain-of-function mutations in the PCSK9 gene. These mutations are observed in over 90% of patients (16). Typically, HeFH is caused by a single pathogenic variant in one of three main genes: LDLR, APOB, and PCSK9 (17). Although genetic testing is the most reliable way to diagnose FH, it is not always available or affordable. However, clinical scores based on criteria such as the DCLN score, WHO, Simon Broome Register, MEDPED, Montreal-FH-Score (18), and JAS FH criteria (19) can be used to diagnose FH without genetic testing. These criteria help classify FH as certain, probable, or possible (18, 20, 21). Early diagnosis and treatment of FH is crucial to normalize life expectancy.

In this research, we present a case study of a patient diagnosed with HeFH resulting from a mutation in the LDLR gene. In monogenic FH, the LDLR gene is found to be dysfunctional in 60%–90% of cases, leading to impaired LDL clearance from the bloodstream (17, 22). The LDL receptor gene is located on the short arm of chromosome 19 (19p13.2) and consists of 18 coding regions (exons).

The American College of Medical Genetics and Genomics (ACMG) introduced an algorithm in 2015 that categorizes all variants of LDLR gene mutations into five groups: pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign, and benign (23, 24). Deletions encompassing exon 7–14 within the LDLR gene identified in the patient based on the algorithm mentioned above are classified as pathogenic.

TABLE 1 Patient characteristics based on Dutch Lipid Clinic Network.

Dutch lipid clinic network criteria	Patient's personal history	Points
Family history		
First-degree relative with premature coronary or vascular disease, or a first-degree relative with LDL-C >the 95th percentile	Yes Father had MI at 40 years of age Grandmother had a stroke at 60 years old	1
First-degree relative with tendinous xanthomata and/or arcus corneal, or children aged <18 years with LDL-C >95th percentile	Yes Son with confirmed FH	**2**
Clinical history		
Patient with premature CAD	No	2
Patient with premature vascular disease	No	1
Physical examination		
Tendinous xanthomata	No	6
Arcus corneal before age 45 years	Yes 	**4**
LDL-C levels (without treatment)		
LDL-C ≥8.5 mmol/L (≥325 mg/dL)		8
LDL-C 6.5–8.4 mmol/L (251–325 mg/dL)		5
LDL-C 5.0–6.4 mmol/L (191–250 mg/dL)	LDL-C 250 mg/dL	**3**
LDL-C 4.0–4.9 mmol/L (155–190 mg/dL)		1
DNA analysis		
Functional mutation in the LDLR, apoB, or PCSK9 genes	Yes LDLR 7–14 exon deletion	**8**
A "definite" FH diagnosis requires >8 points	Definitive diagnosis	**17**

Points where the patient met the DCLN criteria and the summary score are highlighted in bold.
APOB, apolipoprotein B; CAD, coronary artery disease; FH, familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol concentration; LDLR, low-density lipoprotein receptor; MI, myocardial infarction.

The patient under consideration has a confirmed mutation in the LDLR gene, with a heterozygous variant. In addition, she has been diagnosed with complete statin intolerance (SI). Discontinuation of statin therapy is most frequently caused by statin-associated muscle symptoms (SAMS), which are also the most common adverse effects of statins (25, 26). Other potential statin-related adverse effects include neurocognitive disorders, hepatotoxicity, haemorrhagic stroke, and renal toxicity (27, 28). The issue of statin intolerance and the resulting discontinuation of statin therapy is a persistent clinical challenge that is prevalent on a global scale (29, 30). According to the subgroup analysis of a 2022 meta-analysis titled "Prevalence of statin intolerance", the prevalence of statin intolerance (SI) was found to be 9.0% in primary prevention patients with FH (31).

Alirocumab, a monoclonal antibody that targets protease PCSK9, has proven to be highly effective in patients with atherosclerotic CVD and/or HeFH who require further reduction in LDL-C levels. Studies have shown that alirocumab can achieve reductions of between 55% and 60% in LDL-C levels in such patients (32). In a recent clinical trial, ODYSSEY OUTCOMES, it was found that the use of alirocumab, a PCSK9 inhibitor, significantly reduced the risk of major adverse cardiovascular events (MACE) when compared to a placebo (33). Non-responsiveness to human PCSK9 monoclonal antibodies is exceedingly low. However, in the rare cases where non-responsiveness does occur, clinicians are concerned about the potential presence of anti-drug antibodies. Phase 3 ODYSSEY studies examined apparent

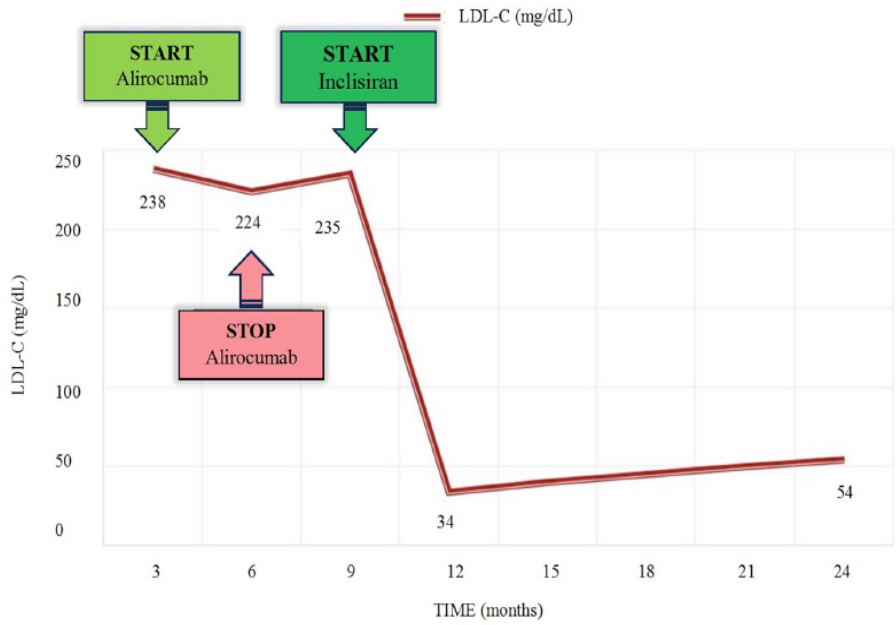


FIGURE 1
Timeline of LDL-C level variations according to lipid-lowering therapy. LDL-C, low-density lipoprotein cholesterol.

hyporesponsiveness to alirocumab, defined as <15% LDL-C reduction from and was reported in 1% of the patient study population appeared to be due to lack of adherence to therapy, a theoretical and rare possibility of biological non-responsiveness due to persistent antidrug antibodies, or other causes, as yet unidentified (34).

Alirocumab was found to be highly tolerable in our patient. According to the patient's self-report, all prescribed doses were administered as scheduled following prior training, and no reported adverse effects. In the study, inclisiran was administered by healthcare professionals on site, while patients self-administered four out of six doses of alirocumab at home. Nonadherence to the prescribed dosage regimen may have contributed to the observed lack of treatment response.

Inclisiran is a novel small interfering RNA (siRNA) that selectively targets the liver and suppresses the translation of PCSK9, a protein that regulates cholesterol metabolism. This leads to increased recycling of LDL receptors, which in turn increases the uptake of LDL-C and reduces its levels in the bloodstream. In clinical trials known as ORION, inclisiran has shown significant efficacy in reducing LDL-C levels when used as an adjunct to maximally tolerated statin therapy, specifically in patients with HeFH (15, 35, 36). On the 510th day of the study, it was observed that 99% of all patients who were enrolled and administered inclisiran achieved a significant 39.7% reduction in LDL-C levels (15). In the presented case the attainment of the therapeutic goal became possible solely due to the application of insclisiran. The present state of the patient is stable, and she gave her consent to proceed with the inclisiran therapy.

Conclusions

In the case of a patient with HeFH, the process of selecting a suitable treatment option posed a considerable challenge due to their inability to tolerate statins and inadequate response to alirocumab. This rare condition is not commonly encountered in clinical practice, making it a complex case to manage effectively. However, with the use of inclisiran, a modern therapy, it was possible to effectively lower LDL-C levels and significantly decrease the patient's cardiovascular risk.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Bioethics Committee at the Medical University of Warsaw. The studies were conducted in accordance with the local legislation and

institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JR: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. MG: Conceptualization, Formal Analysis, Supervision, Validation, Writing – original draft, Writing – review & editing. RG: Formal Analysis, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

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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.

Publisher's note

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8. WNIOSKI

8.1 Publikacja przeglądowa

W publikacji przeglądowej zatytułowanej „Genetic backgrounds and diagnosis of familial hypercholesterolemia", która stanowi wprowadzenie do dalszych prac w cyklu, zawarto systematyczny przegląd literatury podsumowujący aktualny stan wiedzy dotyczący częstości występowania i obrazu klinicznego hipercholesterolemii rodzinnej. W pracy omówiono szczegółowo rodzaje mutacji genetycznych leżących u podłoża FH, rolę badań genetycznych oraz skale służące do diagnostyki. Zwrócono również uwagę na istotną rolę prewencji chorób sercowo-naczyniowych i wczesnego rozpoznawania choroby u pacjentów z podejrzeniem FH. Nadal prowadzonych jest wiele badań dążących do identyfikacji nowych mutacji związanych z FH, co może pozwolić na zwiększenie precyzji diagnostycznej. Głównym wnioskiem z publikacji jest podkreślenie istotności wczesnego wykrycia i szybkiego rozpoczęcia strategii terapeutycznych z zastosowaniem statyn i innych leków hipolipemizujących, by skutecznie opóźniać lub zapobiegać rozwojowi choroby wieńcowej. Dlatego też kluczowe jest zwiększenie świadomości wśród pracowników służby zdrowia i społeczeństwa w zakresie znaczenia terminowej diagnozy i leczenia FH.

8.2 Publikacja oryginalna

W oryginalnej publikacji zatytułowanej „Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment—A Single-Centre Analysis" przedstawiono wpływ mutacji genetycznych na odpowiedź podczas leczenia alirokumabem. Do badania włączono 17 pacjentów z potwierdzoną genetycznie hipercholesterolemią rodzinną, z czego większość stanowiły kobiety (76.5%). Pacjenci spełniali kryteria polskiego programu leczenia FH, w tym: skończyli 18 lat, mieli potwierdzoną mutację FH w testach genetycznych oraz stężenie LDL >100 mg/dL (2,5 mmol/L) podczas stosowania diety i intensywnej terapii statynami w maksymalnych tolerowanych dawkach przez co najmniej 3 miesiące (obejmującej również skojarzone leczenie ezetymibem 10 mg przez co najmniej 1 miesiąc). Pacjenci z udokumentowaną nietolerancją co najmniej 2 statyn, zgodnie z wytycznymi towarzystw naukowych specjalizujących się w diagnostyce i leczeniu zaburzeń lipidowych, stanowili 23,5%. Kryteria wykluczenia obejmowały osoby poniżej 18 roku życia, bez wykonanych testów genetycznych, kobiety w ciąży i karmiące piersią, pacjentów z niewydolnością nerek (eGFR < 60 ml/min/1,73 m2) oraz z ciężką dysfunkcją wątroby.

Przed rozpoczęciem terapii alirokumabem poziom cholesterolu całkowitego u uczestników wynosił 281,3 (85,1) mg/dL, podczas gdy poziom LDL wynosił 206,1 (82,6) mg/dL. Po 12 tygodniach leczenia dawką 150 mg alirokumabu podawaną co dwa tygodnie podskórnie, średnie stężenie cholesterolu całkowitego wynosiło 169,7 (98,9) mg/dL, a stężenie LDL 95,8 (95,7) mg/dL.

U dziesięciu pacjentów (58,8%) potwierdzono mutację w genie *LDLR*. Pięciu pacjentów miało mutację w genie *APOB*, podczas gdy u dwóch pacjentów zidentyfikowano mutacje zarówno w genach *LDLR*, jak i *APOB*.

Najczęstszą obserwowaną mutacją u pacjentów z defektami genu *APOB* była mutacja p. R3527Q. Rąbek rogówkowy przed ukończeniem 45 roku życia stwierdzono u 60% pacjentów z mutacją *LDLR*, podczas gdy u dwóch pacjentów z mutacją p. R3527Q w genie *APOB*, występowały żółtaki ścięgien. Ponadto u badanej grupy nie stwierdzono zależności między fenotypem (obecność rąbka rogówkowego i/lub żółtaków ścięgien), a zidentyfikowaną patogenną mutacją. Nie znaleziono również powiązania pomiędzy stężeniem którejkolwiek z frakcji cholesterolu, a objawem klinicznym (żółtaki ścięgien, rąbek rogówkowy) jako cech typowo związanych z FH.

W badaniu wykazano, że u heterozygot z podwójną mutacją w genach *LDLR* i *APOB* nie uzyskano istotnej redukcji LDL w trakcie otrzymywania alirokumabu. Wśród pacjentów z mutacją monogenową (*LDLR* lub *APOB*) stwierdzono wysoką skuteczność terapii i redukcję LDL na poziomie −63% dla grupy z mutacją genu *LDLR* oraz −59% u chorych z mutacją w genie *APOB*.

Dwunastu pacjentów, u których nastąpił spadek poziomu LDL o ponad 30% w stosunku do wartości wyjściowych, kontynuowali leczenie alirokumabem. Po roku terapii średni poziom LDL wynosił 49,4 mg/dL (33,8 mg/dL) wśród pacjentów z mutacją *LDLR* i 59,8 mg/dL (29,7 mg/dL) u osób z mutacją *APOB*. W grupie, która nie zareagowała na leczenie, obejmującą pozostałych pięciu pacjentów, średni poziom LDL wynosił 258,7 mg/dL (100,1 mg/dL).

W czasie rocznej obserwacji utrzymywał się wysoki poziom skuteczności stosowanej terapii. Badani pacjenci bardzo dobrze tolerowali leczenie alirokumabem, nie obserwowano działań niepożądanych. Warto również zauważyć, że nie odnotowano żadnych przypadków MACE, które zdefiniowano jako wystąpienie udaru mózgu, zawału serca i zgonu z przyczyn sercowo-naczyniowych.

Wyniki publikacji wskazują, że identyfikacja podwójnej heterozygotyczności poprzez testy genetyczne jest kluczowa dla określenia skuteczności alirokumabu jako wyboru leczenia u pacjentów z FH. Rezultaty podkreślają znaczenie diagnostyki genetycznej w ustalaniu spersonalizowanej terapii dla pacjentów.

8.3 Publikacja kazuistyczna

Zamykająca cykl publikacja kazuistyczna zatytułowana “Challenges in the management of familial hypercholesterolemia: a case report” przedstawia przypadek pacjentki z heterozygotyczną postacią FH, spowodowaną mutacją w obrębie genu LDLR oraz całkowitą nietolerancją statyn. W trakcie terapii alirokumabem nie uzyskano odpowiedzi na leczenie. Włączenie inklisiranu doprowadziło do znacznej, kilkukrotnej redukcji LDL z wyjściowych wartości wynoszących 235mg/dL do 34mg/dL. Podczas rocznej terapii utrzymywał się skuteczny efekt leczenia hipolipemizującego.

Terapia inklisiranem wykazuje obiecujące wyniki u osób z HeFH, u których rozpoznano nietolerancję statyn. Poprzez wykorzystanie małego, interferującego RNA (siRNA) i ingerowanie w mRNA proproteinowej konwertazy subtilizyny/keksyny typu 9 (PCSK9) skutkującym ograniczaniem wytwarzania białka PCSK9, ostatecznie prowadzi do znacznego obniżenia poziomu LDL. Takie podejście może być alternatywą dla pacjentów, którzy nie osiągnęli znacznego obniżenia poziomu LDL podczas terapii inhibitorami PCSK9. W przypadku pacjentów z HeFH z ograniczonymi możliwościami leczenia z powodu nietolerancji statyn, inklisiran może stanowić obiecującą opcję terapeutyczną.

Publikacja pokazuje jak istotny jest wybór odpowiedniej terapii oraz ile trudności można napotkać na drodze do osiągnięcia celu terapeutycznego.

8.4 Wnioski szczegółowe

Identyfikacja mutacji genetycznych determinujących hipercholesterolemię rodzinną ma istotne znaczenie przy wyborze nowoczesnych leków hipolipemizujących. Diagnostyka genetyczna stanowi kluczową rolę w ustalaniu spersonalizowanej terapii dla pacjentów.

1. Hipercholesterolemia rodzinna pozostaje istotnym problemem w praktyce klinicznej. Zbyt mała liczba pacjentów ma odpowiednio szybko postawioną diagnozę. Podłoże genetyczne FH stanowią przede wszystkim mutacje monogenowe w obrębie genu kodującego receptor lipoprotein o niskiej gęstości *(LDLR),* genu apolipoproteiny B (*APOB*) oraz genu konwertazy proproteinowej subtylizyny/keksyny typu 9 (*PCSK9*).

2. U heterozygot z podwójną mutacją w genach *LDLR* i *APOB* nie uzyskano istotnej redukcji LDL w trakcie otrzymywania alirokumabu. Wśród pacjentów z mutacją monogenową (*LDLR* lub *APOB*) stwierdzono wysoką skuteczność terapii i redukcję LDL.

3. Nie stwierdzono zależności między fenotypem (obecność rąbka rogówkowego i/lub żółtaków ścięgien), a zidentyfikowaną patogenną mutacją.

4. W czasie rocznej obserwacji utrzymywał się wysoki poziom skuteczności stosowanej terapii. Leczenie było dobrze tolerowane, nie obserwowano działań niepożądanych.

5. W trakcie rocznej obserwacji nie odnotowano żadnych przypadków MACE, które zdefiniowano jako wystąpienie udaru mózgu, zawału serca i zgonu z przyczyn sercowo-naczyniowych.

6. U pacjentów z heterozygotyczną postacią hipercholesterolemii rodzinnej (HeFH), nietolerancją statyn oraz niewystarczającym spadkiem LDL w trakcie terapii inhibitorami PCSK9, inklisiran może stanowić obiecującą opcję terapeutyczną.

9. PODSUMOWANIE

Zaburzenia lipidowe stanowią jeden z głównych czynników ryzyka rozwoju miażdżycy, a hipercholesterolemia rodzinna często pozostaje nierozpoznana. Wczesne potwierdzenie choroby jest kluczowe, aby odpowiednio szybko wdrożyć skuteczną terapię hipolipemizującą i zapobiec przedwczesnym powikłaniom ze strony układu sercowo naczyniowego.

Wszystkie trzy publikacje stanowią spójny cykl tematyczny prezentujący podłoże genetyczne, obraz kliniczny hipercholesterolemii rodzinnej oraz rodzaje i odpowiedni dobór dostępnej nowoczesnej terapii hipolipemizującej.

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11. OPINIA KOMISJI BIOETYCZNEJ

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303
Fax: 022/ 57 - 20 -165

ul. Żwirki i Wigury nr 61
02-091 Warszawa

e-mail: komisja.bioetyczna@wum.edu.pl
www.komisja-bioetyczna.wum.edu.pl

Warszawa, dnia 06.02 2023

AKBE/ 68 / 2023

Dr hab. n. med. Renata Główczyńska
Katedra i Klinika Kardiologii
ul. Banacha 1a,
02-097 Warszawa

OŚWIADCZENIE

Niniejszym oświadczam, że Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym w dniu 06 lutego 2023 r. przyjęła do wiadomości informację na temat badania pt. "Pierwotne i wtórne przyczyny hiperlipidemii."
Przedstawione badanie nie stanowi eksperymentu medycznego w rozumieniu art. 21ust.1 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (Dz.U. z 2018 r poz. 617) i nie wymaga uzyskania opinii Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym, o której mowa w art. 29 ust.1 ww. ustawy.

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma –Kozakiewicz

12. OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI

WARSZAWSKI
UNIWERSYTET
MEDYCZNY

I KATEDRA I KLINIKA I KARDIOLOGII

Warszawa, 10.10.2024

Prof. dr hab. n. med. Renata Główczyńska

OŚWIADCZENIE

1. Jako współautor pracy pt. *Genetic backgrounds and diagnosis of familial hypercholesterolemia* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: **opracowanie koncepcji pracy, przegląd literatury, krytyczna analiza i korekta artykułu, ostateczna akceptacja artykułu.**

   Mój udział procentowy w przygotowaniu publikacji określam jako **10 %.**

   Wkład lek. Joanny Rogozik w powstawanie publikacji określam jako **85%** obejmował on: udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników konceptualizację i identyfikację luk w wiedzy, organizację pracy, przegląd literatury, tworzenie elementów graficznych, napisanie artykułu oraz jego korekta w trakcie recenzji.

2. Jako współautor pracy pt. *Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment—A Single-Centre Analysis* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: **konceptualizacja i projekt badania, krytyczna analiza i korekta artykułu, ostateczna akceptacja artykułu.**

   Mój udział procentowy w przygotowaniu publikacji określam jako **10 %.**

   Wkład lek. Joanny Rogozik w powstawanie publikacji określam jako **80 %** obejmował on: udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników konceptualizację i projekt badania, organizację pracy, zbieranie i opracowywanie danych, analiza statystyczna, krytyczna analiza wyników, przegląd literatury, tworzenie elementów graficznych, napisanie manuskryptu oraz jego korekta w trakcie recenzji.

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3. Jako współautor pracy pt. *Challenges in the management of familial hypercholesterolemia: a case report* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: **opracowanie koncepcji pracy, krytyczna analiza i korekta artykułu, ostateczna akceptacja artykułu.**

Mój udział procentowy w przygotowaniu publikacji określam jako **10%.**

Wkład lek. Joanny Rogozik w powstawanie publikacji określam jako **85%**

obejmował on: konceptualizację i projekt badania, organizację pracy, zbieranie i opracowywanie danych, przegląd literatury, tworzenie elementów graficznych, napisanie manuskryptu oraz jego korekta w trakcie recenzji.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Joanny Rogozik.

(podpis oświadczającego)

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WARSZAWSKI
UNIWERSYTET
MEDYCZNY

I KATEDRA I KLINIKA I KARDIOLOGII

Warszawa, 10.10.2024

Prof. dr hab. n. med. Marcin Grabowski

OŚWIADCZENIE

1. Jako współautor pracy pt. *Genetic backgrounds and diagnosis of familial hypercholesterolemia* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: **krytyczna analiza i korekta artykułu, ostateczna akceptacja artykułu.**

   Mój udział procentowy w przygotowaniu publikacji określam jako **5 %.**

   Wkład lek. Joanny Rogozik w powstawanie publikacji określam jako **85%** obejmował on: udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników konceptualizację i identyfikację luk w wiedzy, organizację pracy, przegląd literatury, tworzenie elementów graficznych, napisanie artykułu oraz jego korekta w trakcie recenzji.

2. Jako współautor pracy pt. *Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment—A Single-Centre Analysis* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: **krytyczna analiza i korekta artykułu, ostateczna akceptacja artykułu.**

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Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Joanny Rogozik.

KIEROWNIK
I Katedra i Klinika Kardiologii WUM

prof. dr hab. n. med. Marcin Grabowski

.....

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WARSZAWSKI
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I KATEDRA I KLINIKA I KARDIOLOGII

Warszawa, 10.10.2024

Lek. Jakub Kosma Rokicki

OŚWIADCZENIE

1. Jako współautor pracy pt. *Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment—A Single-Centre Analysis* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: **opracowywanie danych, analiza statystyczna, ostateczna akceptacja artykułu.**

   Mój udział procentowy w przygotowaniu publikacji określam jako **5 %.**

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Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Joanny Rogozik.

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13. ANALIZA BIBLIOMETRYCZNA

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Nr referencyjny
BIBG/Punktacja/ 572/2024/KK

Warszawa, 15.10.2024

Sz. Pani
Joanna Rogozik

ANALIZA BIBLIOMETRYCZNA PUBLIKACJI
PANI JOANNY ROGOZIK,
WCHODZĄCYCH W SKŁAD CYKLU PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

Lp.	Opis bibliograficzny	Impact Factor	MNiSW
Artykuły			
1.	**Rogozik J** [aut. koresp.], Główczyńska R, Grabowski M. Genetic backgrounds and diagnosis of familial hypercholesterolemia. Clinical Genetics. 2024;105(1):3-12 [Rodzaj publikacji: praca poglądowa]	2,900	100
2.	**Rogozik J** [aut. koresp.], Rokicki J, Grabowski M, Główczyńska R. Gene Mutation in Patients with Familial Hypercholesterolemia and Response to Alirocumab Treatment - A Single-Centre Analysis. Journal of Clinical Medicine. 2024;13(18):5615 [Rodzaj publikacji: praca oryginalna]	3,000	140
3.	**Rogozik J** [aut. koresp.], Grabowski M, Główczyńska R. Challenges in the management of familial hypercholesterolemia: a case report. Frontiers in Cardiovascular Medicine. 2024;11:1417432 [Rodzaj publikacji: opis przypadku]	2,800	40
	Łącznie:	**8,700**	**280**
Książki			
1.	-		
Rozdziały w książkach			
1.	-		

DYREKTOR
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