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Prevalence of pathogenic genetic variants associated with familial hypercholesterolemia in Ghanaian children

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ABSTRACT

Familial hypercholesterolemia (FH) is an important contributor to atherosclerotic cardiovascular disease (ASCVD) burden globally. FH disrupts cholesterol metabolism and causes lifelong elevation in low-density lipoprotein cholesterol (LDL-C). In sub-Saharan Africa (SSA), the increasing burden of ASCVD may be partly driven by genetic dyslipidemias of which FH is the commonest. However, there is absence of data on FH prevalence in SSA which delineates an important gap in the management of ASCVD. This study is the first to investigate the prevalence of pathogenic variants associated with FH in Ghana. We used 96 deidentified archived dried blood spot samples collected from a Ghanaian cohort, to determine the prevalence of pathogenic genetic variants associated with FH. These samples were collected from children under 9 years old as part of surveillance for antimalarial drug resistance in 2021. We searched the NCBI's ClinVar database and used *in silico* tools to identify 500–800 nucleotide base pair regions of interest in 3 genes known to harbor the commonest genetic variants associated with FH. We selected these regions of interest from the *LDLR* gene exons 4, 9 and 10, *APOB* exon 26 and *PCSK9* exon 2 loci. Next, we amplified these regions of interest using conventional polymerase chain reaction. Finally, we sequenced the amplicons using paired-end Sanger sequencing and called variants from the chromatogram files using an in-house custom built bash script utilizing the open access program Tracy. Subsequently, we did quality checks on all reported pathogenic variant calls manually using Benchling's sequence alignment tool. We identified one pathogenic variant V523 M in the *LDLR* exon 10 region and report an FH prevalence of 1% (1/96), 95% CI: [0%,3.07%], in our Ghanaian cohort. Our finding underscores the importance of FH in driving ASCVD burden in Ghana and advocates the need for implementation science-driven programs to manage this genetic dyslipidemia in Ghana and SSA.

1. Introduction

Familial hypercholesterolemia (FH) is an inherited monogenic disorder of cholesterol metabolism that results in life-long exposure to low-density lipoprotein cholesterol (LDL-C) from birth and a resulting higher risk of premature cardiovascular disease (from age 30 in men and age 40 in women) and premature deaths (Roth et al., 2020). Heterozygous FH (HeFH) describes an individual with one inherited pathogenic genetic variant from a parent. The global prevalence of HeFH is estimated at 1 in 311 (Hu et al., 2020). It is also estimated that 35 million people are affected globally with FH and only 10% diagnosed (Watts et al., 2020; Ray et al., 2022). The most common genes associated with FH are the LDL receptor (*LDLR*), apolipoprotein B (*APOB*), proprotein convertase subtilisin/kexin type 9 (*PCSK9*) and *LDLR* adaptor protein 1 (*LDLRAP1*) genes accounting for about 80–85%, 10–15%, 2% and 1% of all FH cases respectively (Toft-Nielsen et al., 2022). FH is an autosomal genetic disease with dominant heritability of most pathogenic variants (Suryawanshi and Warbhe, 2023; Watts et al., 2015; Srivastava, 2023).

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The *LDLR* gene is located on chromosome 19, spans 44358 base pairs (bp) and has 18 exons. It codes for the LDL receptor responsible for removing LDL-C from the bloodstream. Pathogenic variants of *LDLR* gene result in a partial or complete malfunction of LDLR and a corollary impairment in LDL-C removal from the blood (Srivastava, 2023). Over 1500 pathogenic variants of this gene have thus far been reported including D154 N, D206E, V408 M which were reported in South Africa (Raal et al., 2020). The *APOB* gene is located on chromosome 2, spans 42645 bp and has 29 exons. Familial defective APOB is the second commonest cause of FH with pathogenic variants in exons 26 and 29 of the *APOB* gene leading to deficient binding of LDL-C to LDLR (Vrablik et al., 2020). A common pathogenic variant of *APOB* affecting the binding capacity of LDL receptors is R3527Q (Rodríguez-Jiménez et al., 2023). The *PCSK9* gene is located on chromosome 1, has a size of 25305 bp and 12 exons. PCSK9 binds to the internalized LDL-LDLR complex and targets the LDLR in the cytosol for cellular degradation. Gain-of-function (GOF) pathogenic variants in the *PCSK9* gene lead to increased LDLR degradation and cause increased LDL-C levels in the bloodstream (Guo et al., 2020). Loss-of-function (LOF) mutations typically result in lower levels of LDL-C (Grejtakova et al., 2024). Some reported LOF variants that can lower LDL cholesterol include R46L, E144K and C378W (Grejtakova et al., 2024; Meng et al., 2023) whereas some common GOF variants that have been well-documented in various populations include R357H, D374Y and E670G (Guo et al., 2020). *LDLRAP1* is located on chromosome 1 and spans 46795 bp with 10 exons. The gene aids in the internalization of the LDL-LDLR complex. Pathogenic variants of this gene hinder the internalization of the LDL-LDLR complex by preventing the proper formation of clathrin-coated endosomes thus inhibiting LDL uptake (Benito-Vicente et al., 2018).

FH is known to have a high prevalence in human populations of 1 in 200 to 1 in 500 (Hu et al., 2020; Benn et al., 2012; Goldberg et al., 2011; Singh and Bittner, 2015). Despite this, most cases of FH are underdiagnosed and poorly managed (Mainieri et al., 2022; Society, 2024). The clinical suspicion of an FH diagnosis typically relies on a combination of factors including levels of LDL-C in the blood, the presence of subcutaneous cholesterol deposits like tendon xanthomas, and a family history of early-onset heart disease or elevated cholesterol among close relatives (Watts et al., 2023). The common clinical diagnosis criteria used are the Dutch Lipid Clinic Network criteria (DLCN), Simon Broome criteria and Make Early Diagnosis to Prevent Early Deaths (MEDPED) (McGowan et al., 2019), however, a genetic test is needed to conclusively confirm the diagnosis.

While FH is commonly reported in Western countries and South Africa, its unknown prevalence in SSA might be a critical factor underlying the region's burden of non-communicable diseases (Hu et al., 2020; Beheshti et al., 2020; Gratton et al., 2023a). However, there is little to no data on the prevalence of FH in sub-Saharan African countries like Ghana, even in important risk groups such as persons with premature myocardial infarction. This is a critically important gap as it has been shown that early identification of FH, family cascade genetic testing, and administration of lipid-lowering medication is integral to its management (Ray et al., 2022). Therefore, the aim of this study was to determine the prevalence of FH causally associated genetic variants in children aged less than 9 years old in the Ghanaian populace. Reliable determination of the true prevalence of FH and its geographic distribution will help inform regional policies and adoption of evidence-based interventions aimed at early detection and prevention of ASCVD.

2. Method

2.1. Literature search for FH data on sub-Saharan African countries

To determine the geographical distribution of studies conducted on FH in the last five years, our search strategy utilized the PubMed database. We queried in "[All Fields] using the search terms "familial hypercholesterolaemia", "hypercholesterolaemia", "hyperlipoproteinemia type ii", "hyperlipoproteinemia", "familial hypercholesterolemia" and "hypercholesterolemia" on PubMed (https://pubmed.ncbi.nlm.nih.gov/). Boolean operators including "AND" and "OR" were used in combination with the above-mentioned search terms to identify relevant papers published in the last five years (Supplementary Table 1). The screening strategy included independently reviewing titles and abstracts of studies retrieved from the databases to identify information relevant to the study. The full texts were retrieved and scrutinized to extract data of interest which includes the study type and the country it was conducted. All papers published in other languages aside English were translated and included. Publications were considered of interest if they addressed any aspect of FH including prevalence, clinical presentation, diagnosis, prevention and treatment. All review papers, commentaries, books and viewpoints were excluded from the analysis. For papers where the country of study was not mentioned, the country of the authors was used if all authors were from the same country. Instances where authors were from different locations, the country of the corresponding author was used. All articles that had multiple study locations were unnested. The distribution of study types by continent was visualized on a bar chart with the study location on the x-axis and number of studies stacked by different study types on the y-axis. Africa was highlighted and further visualized on a pie chart to identify the distribution of studies by country within that location.

2.2. In silico analysis to identify gene regions of interest

Current Sanger sequencing supports the generation of nucleotide sequences within the ranges 800–1000 base pairs (bp) of which the first 15–40 bp are low-quality sequences due to primer binding. In addition to this, the quality of the sequences becomes poor after 750 bases (Sanger et al., 1977; Stranneheim and Lundeberg, 2012). Taking note of these limitations, about 800 bp region that had pathogenic variants for each gene (*LDLR, PCSK9* and *APOB*) causally associated with FH were selected *in silico* for amplicon generation and sequencing. The ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) aggregates information about genomic variants and its relationship to human health and was used to search for genetic variants associated with FH (*LDLR, PCSK9 and APOB*). Subsequently, this set of genetic variants were filtered for highly pathogenic variants as classified by the American College of Medical Genetics and the Association for Molecular Pathology (ACMG – AMP) (Richards et al., 2015). Next, the distribution of pathogenic variants data for each gene was analysed by plotting a stacked bar chart with nucleotide positions binned into 800bp windows on the y-axis and total variant counts on the x-axis. Highly pathogenic regions for *LDLR* (exon 4, 9, and 10) and *PCSK9* (exon 2) were amplified using primers previously published by (Ho et al., 2012; Leren, 2004) respectively. From the ClinVar data, *APOB* gene had most pathogenic variants in exon 26 thus primers targeting that region as described by (Vaseghi et al., 2021) were used. Quality check on the primers were done *in silico* using Benchling (https://www.benchling.com) and AmplifX 2.1.1 (https://inp.univ-amu.fr/en/amplifx-manage-test-and-design-your-primers-for-pcr).

2.3. Study sites and samples

The study used 96 archived dried blood spot samples from children aged less than 9 years old with uncomplicated malaria recruited from designated health facilities at ten sentinel sites under the National Malaria Elimination Program (NMEP) and the Noguchi Memorial Institute for Medical Research (NMIMR) for Therapeutic Efficacy Studies (TES) in 2021. The sites were Ada (5.7882° N, 0.6337° E) and Cape Coast (5.1315°N, 1.2795°W) located in the Coastal Savanna zone with perennial malaria transmission, Begoro (6.3916°N, 0.3795°W), Bekwai (6.4532°N, 1.5838°W), Hohoe (7.1519°N, 0.4738°E), Sunyani (7.3349°N, 2.3123°W) and Tarkwa (5.3018°N, 1.9930°W) in the Forest

zone with perennial malaria transmission, Navrongo (10.8940°N, 1.0921°W), Wa (10.0601°N, 2.5099°W) and Yendi (9.4450°N, 0.0093°W) in the Guinea savanna zone with seasonal malaria transmission. These sites are inhabited by different ethnic/tribal groups (Ada-Adangmes, Cape Coast-Fantis, Begoro- Akans, Bekwai-Akans, Hohoe-Ewes, Sunyani-(admixture of Akans, Northerners, Ewes, and Ga-Adangbes, Tarkwa-Fantis, Wa-Walaas, Navrongo-Kassenas, Yendi-Dagombas). The samples were then stored in plastic bags containing silica gels and kept at room temperature until use.

2.4. Ethics statement

Written informed consent was obtained from all parents and guardians of the study participants approved by the Noguchi Memorial Institute for Medical Research Institutional Review Board (IRB CPN 032/05-06a amed.2021). The consent also covered the future use of the archived samples for molecular analysis.

2.5. Sample size calculation

The sample size for this study was calculated using a single proportion population formula by Cochran (1997), $n = \frac{z^2\, p(1-p)}{d^2}$ where n = Sample size to be determined, z = Z-score (2.58 at 99 % confidence interval), P= Prevalence of FH in human population 1 in 200 (0.5%) to 1 to 500 (0.2%) (Singh et al., 2015), d = Precision or margin of error (0.02). The reliability coefficient to be used is 2.58 since the confidence interval is 99%. Using a prevalence of 0.5% and 2% allowable margin error, the minimum sample size required was 83 for which we rounded up to 96 to fit a sequencing plate.

2.6. DNA extraction and PCR amplification of genes

Genomic DNA was extracted from dried blood blots using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) per the manufacturer's protocol. Conventional PCR was performed to amplify the regions of interest using published protocols (Ho et al., 2012; Leren, 2004; Vaseghi et al., 2021) with minimal modifications. Successfully amplified samples were Sanger sequenced by Macrogen, Europe (Netherlands) in both the forward and reverse directions. All primers used in this study are presented in Table 1. Conventional PCR for all genes was performed in a total volume of 35 µl consisting of 1X One Taq Quick-load master mix (New England Biolabs, Massachusetts, USA), 0.2 µM of each primer (Eurogentec, Liege, Belgium), nuclease-free water and 5 µl of extracted DNA.

2.7. Computational data analysis

Sequences obtained for all the genes were verified using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/) upon receipt from Macrogen Europe. All chromatograph files (.ab1) for each gene were converted to Fastq to access the sequence qualities using fastqc (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Analysis of the Sanger sequencing data (.ab1 files), including base calling, alignment to reference sequence, variant identification, and deconvolution of heterozygous mutations, were performed with an in-house custom bash script using Tracy 0.7.6 (Rausch et al., 2020). Sequences of *LDLR* (exon 4 & 9/10), *PCSK9* (exon 2) and *APOB* (exon 26) were aligned to the human reference sequence obtained from the NCBI database with accession numbers NG_009060.1, NG_009061.1 and NG_011793.2 respectively. The output binary variant call format (bcf) files for each sample were converted to human-readable variant call format (vcf) files with custom bash scripts using BCFtools (version 1.8). Low-quality nucleotide variants (Phred score <40) were filtered out from the vcf. Variant annotation and effect prediction, including the variant impact on protein was done using SnpEff v 5.2a (Cingolani et al., 2012). All annotated variants that have been previously reported were germline classified by sorting these variants from ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) using custom R scripts. Pathogenic variants observed were manually confirmed using Benchling.com (California, CA, USA). All variant calls that were not in concordance with Benchling were removed. The prevalence of FH was calculated as: $\frac{No\ of\ samples\ with\ pathogenic\ variants}{Total\ no\ of\ samples\ analysed} x\ 100$

Finally, exploratory data analysis and graphical summaries were done using custom R scripts in R version 4.0 (Core TeamR., 2021).

3. Results

3.1. There is very little FH data from sub-Saharan Africa

To assess the contribution of Ghana and other African countries to the body of knowledge on FH, we retrieved a total of 801 studies conducted in the last five years. After full-text review and data extraction, 710 studies were classified as original research articles, while the others comprised of 48 review papers, 14 letters, 17 commentaries, 11 books and documents, and 1 article correction. By unnesting original research articles with multiple study countries, the final dataset included 1164 studies for further analysis, excluding all non-original research papers. Among these, Europe accounted for the highest number of published studies (608), followed by the Americas (243) and Asia (257). In contrast, Africa and Oceania contributed the fewest publications, with only 28 original articles each (Fig. 1). A closer examination of Africa revealed that only three of its five sub-regions were represented in FH research. Southern Africa had the highest output (82%; 23/28), followed by Eastern Africa (11%; 3/28), and Northern Africa (7%; 2/28), while Western and Central Africa had no published studies during the period analysed (Fig. 2).

3.2. Pathogenic variants associated with FH are mostly found between regions 23500 - 24300bp in exons 9 and 10 for LDLR, 17300 - 18100bp in exon 2 for PCSK9, and exon 26 of APOB

In silico analysis of FH pathogenic variants utilizing the ClinVar database identified key regions within the *LDLR, PCSK9, and APOB* genes with the highest concentration of pathogenic variants in certain exons. The *LDLR* gene (NG_009060.1), located on chromosome 19, exhibited the highest number of reported pathogenic variants in exons 9

Table 1
Primer sequences used and amplicon sizes.

Gene	Exon Region	Primer	Primer Sequence (5′-3′)	Product size (bp)
APOB100	Exon 26	Forward	ATGGAAGTGTCAGTGGCAAC	842
		Reverse	TGCTGTCTCCTACCAATGCT	
LDL-R	Exon 9/10	Forward	CTGCAGGATGACACAAGGGG	648
		Reverse	CACTAACCAGTTCCTGAAGCTC	
	Exon 4	Forward	GGTGTTGGGAGACTTCACACG	542
		Reverse	GTTGTTGGAAATCCACTTCGG	
PCSK9	Exon 2	Forward	ATCATCTACTATACTCTGTTGTGT CTCT	477
		Reverse	ACAGAAAGCCAGTAGTTACTGTGCT	

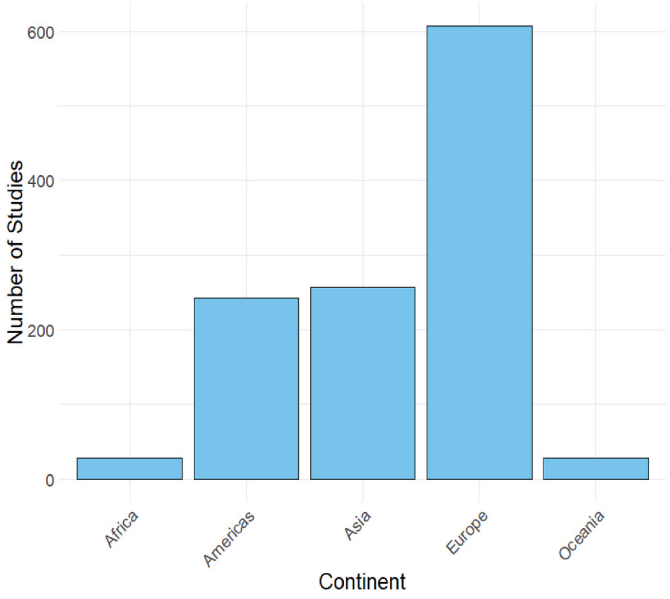

Fig. 1. A bar chart showing counts of the distribution of published original research articles on familial hypercholesterolemia in the last 5 years. The x-axis shows the provenance of the articles by continents. The y-axis shows the number of studies published from each continent.

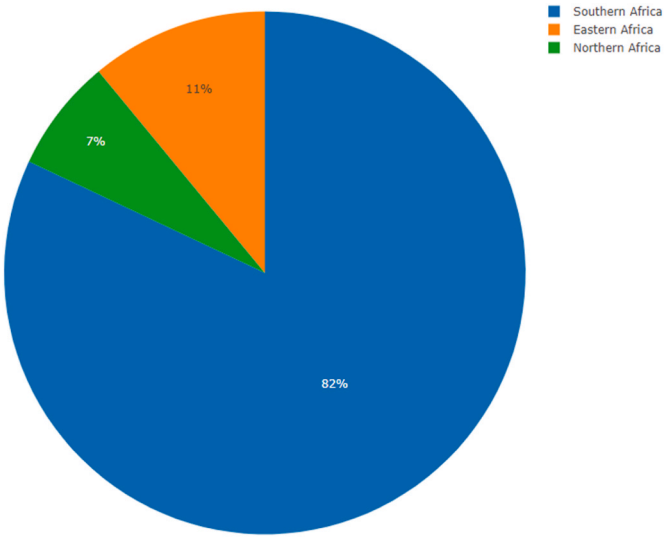

Fig. 2. A pie chart showing the distribution of the reviewed studies by the sub-regions in Africa. Out of 28 studies reviewed from Africa, the highest number were reported in Southern African region with the least studies done in the Northern African region. There were no studies done on FH in West Africa.

and 10 with a total of 179 pathogenic variants identified between nucleotide positions 23,500 and 24,300. Additionally, 163 pathogenic variants were reported between nucleotide positions 15,900 and 16,700 in exon 4 of the *LDLR* gene, with most of these variants classified as single nucleotide variants (SNVs) (Fig. 3). In the *PCSK9* gene (NG_009061.1) located on chromosome 1, the most reported pathogenic variants were located between nucleotide positions 4070 and 4800 in exon 2, followed by positions 17,300 to 18,100 in exon 7 (Fig. 4). For the *APOB* gene (NG_011793.2), located on chromosome 2, the analysis revealed that most reported pathogenic variants in ClinVar clustered in exon 26.

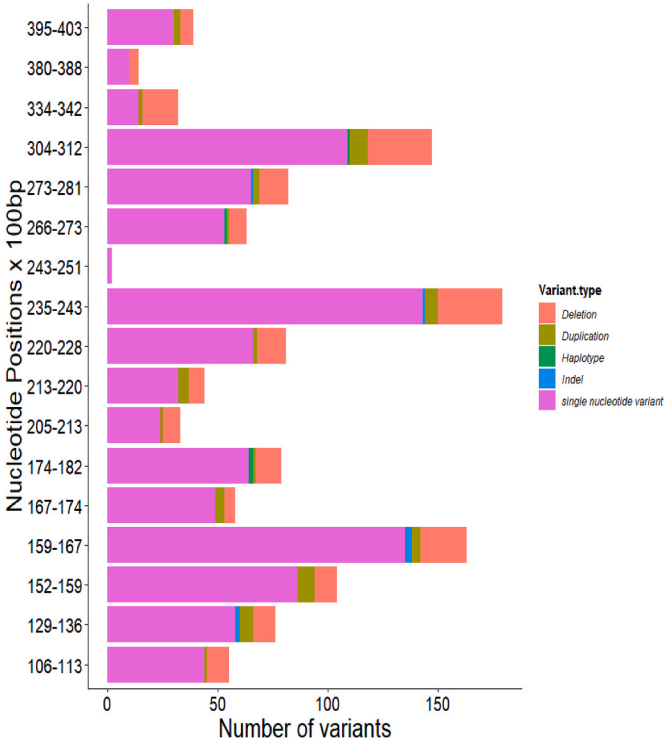

Fig. 3. A stacked bar chart showing the distribution of pathogenic variants found in the *LDLR* gene with nucleotide positions (23500–24300) and (15900–16700) as the highest. The y-axis represents the nucleotide positions binned into about 800bp window. The x-axis represents the number of pathogenic variants stacked by variant types for each binned window.

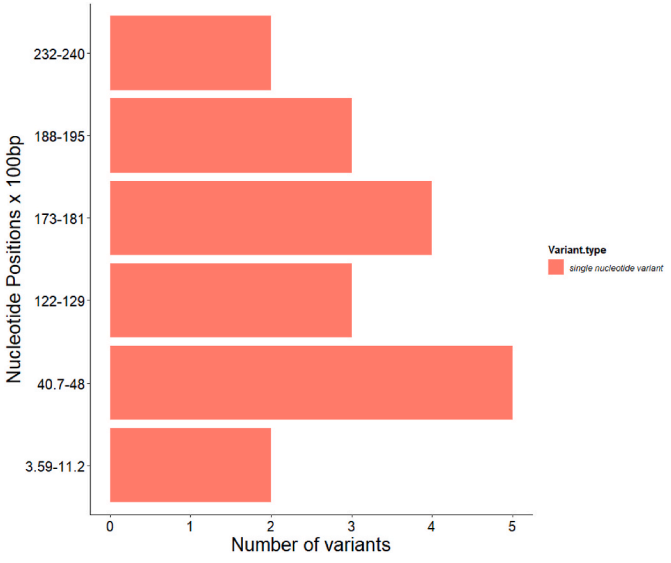

Fig. 4. A stacked bar chart showing the distribution of pathogenic single nucleotide variants found in the *PCSK9* gene with nucleotide positions (4070–4800) as the highest. The x-axis represents the number of pathogenic variants stacked by variant types for each binned window. The y-axis represents the nucleotide positions binned into about 800bp window.

3.3. The V523 M pathogenic variant was observed at a prevalence of 1%

A total of 96 samples were successfully sequenced for all three genes. No pathogenic variants as classified by the ACMG – AMP were observed in all samples analysed for *LDLR* exons 4 and 9, *APOB* exon 26, and

PCSK9 exon 2 regions. However, the previously reported pathogenic variant V523 M in the *LDLR* exon 10 region was observed in a study sample from Bekwai in the forest ecological zone in Ghana. This finding translates the prevalence of pathogenic variants associated with FH in Ghana as 1 in 96 (1%) [95% CI: [0%,3.07%]].

The molecular characteristics of the V523 M variant are highlighted below.

- Pathogenicity: Classified as pathogenic according to ACMG – AMP
- HGVS Nomenclature: NM_000527.5 (LDLR): c.1567G > A (p. Val523Met)
- rs Number: rs28942080

4. Discussion

Atherosclerotic cardiovascular diseases (ASCVD) such as infarctive strokes and myocardial infarctions are the leading causes of death globally (Roth et al., 2020; WHO, 2024). In low and middle-income countries like Ghana, the contribution of ASCVD has been on the rise and is currently listed in the top 10 causes of mortality by the World Health Organization (WHO) (Ray et al., 2022; WHO, 2024; Boakye et al., 2023; Owusu et al., 2021). The burden of ASCVD increases in proportion to the duration of exposure to risk factors such as hypertension, diabetes mellitus and hypercholesterolemia in individuals. Risk factors, like the genetic dyslipidaemia FH, increase the likelihood of life-long exposure to elevated levels of LDL-C from birth and the resulting higher risk for ASCVD events. FH is classified as a tier 1 genetic disease by the National Institutes of Health as there is ample evidence to show that its contribution to ASCVD/NCD burden can be significantly managed by early detection, family cascade screening, and evidence-based reduction in LDL-C (Pang et al., 2020).

The findings of this study are in concordance with the reports that FH is the most common genetic cholesterol metabolic disorder in the world and is a major risk factor for premature ASCVD events (Ray et al., 2022; Benito-Vicente et al., 2018). Compared to the global average of FH prevalence of 1 in 311 (0.3%) (Hu et al., 2020), our finding of a prevalence of 1 in 96 (1%) shows a higher-than-average prevalence of FH in Ghana. Our study provides knowledge to fill in a critically important gap on the molecular epidemiology of FH in Ghana. As FH clinical or genetic diagnosis is largely neglected in SSA countries like Ghana, our findings suggest that FH might be a significant contributor to the disease burden of ASCVD. Therefore, the need for SSA countries like Ghana to adopt evidence-based guidelines for FH management erected on the pillars of FH genetic testing, family cascade testing and intensive use of lipid lowering medication naturally comes to the fore.

Ghana's national policy on Non-Communicable Diseases (NCDs) has highlighted the need for interventions to reduce exposures to risk factors and increase their early detection and management using a plethora of strategies that include the promotion of prevention and routine screening for diagnosis of NCDs (MOH, 2022). However, risk mitigation strategies for genetic risk factors were not addressed. The World Heart Federation guidelines on genetic dyslipidemias recommend the need for country-tailored implementation of FH screening and diagnosis programs driven by public education and clinician engagement campaigns (Ray et al., 2022). In this regard how acceptable, feasible, accessible, sustainable and cost-effective an FH management program, in SSA countries like Ghana, is the next important question to be answered. Both facts highlight the gap and justification for an FH management program in Ghana driven by well-established implementation science strategies. A well-grounded approach to explore this question will be to employ the methods and conceptual frameworks of implementation science (Jones et al., 2024).

We further contend that to facilitate FH detection and genetic testing, tools like the DLCN and SBC ought to be validated in uncharted populations like that of Ghana and new tools developed to capture the idiosyncrasies of the Ghanaian population. This position is even more important when juxtaposed to the fact that these non-genetic screening tools have not been optimised for screening in the general population (McGowan et al., 2019). For resource constrained settings like SSA, a two-tier FH genetic testing program comprising of an initial non-expensive genetic tool to identify individuals most likely to carry FH pathogenic variants for confirmatory genetic testing will be a feasible and sustainable strategy for the SSA context. Inserting this two-tier FH testing program into existing wellness clinics that offer routine health checks could provide an important framework to systematically identify adult carriers of pathogenic FH variants. This practical approach and its sustainability have been tested in the UK by (Gratton et al., 2023b). Their findings highlight the use of a population-bespoke models to identify people with suspected FH for genetic testing. Therefore, future studies evaluating the performance of non-genetic screening tools for FH and modifying them accordingly for the SSA population are needed.

The V523 M pathogenic variant identified by this study was reported earlier in Greek children (Mollaki et al., 2014). Other studies done in Argentina in FH clinically diagnosed individuals that explored next-generation sequencing techniques also reported this variant (Corral et al., 2018, 2020). Again, in a study done on clinically diagnosed FH individuals from Turkey (Turkyilmaz et al., 2021), the variant was reported after genetic testing, validating its role in elevating LDL-C levels.

Furthermore, *in vitro* studies have provided evidence to buttress the finding that this variant affects LDL receptor function, resulting in impaired cellular uptake of LDL-C resulting in elevated plasma LDL-C levels characteristic of FH (Bertolini et al., 2013). The V523 M mutation occurs within the epidermal growth factor (EGF) precursor homology domain of the LDLR protein (Galicia-Garcia et al., 2020; Goldstein and Brown, 2009). This EGF-like domain plays an important role in LDL release and receptor recycling processes (Galicia-Garcia et al., 2020; Zhao and Michaely, 2008). Thus, it is possible this variant can offset the activity of the EGF-like domain resulting in impaired LDL uptake and subsequently the development of FH.

This finding of our study must be interpreted in the light of the following factors. First, we sampled deidentified archived dried blood spots without corresponding clinical data on LDL-C and non-HDL-C levels. Therefore, the reported pathogenic variants are still yet to be phenotypically validated in the Ghanaian population. Secondly, we assumed as similar and representative and used the global prevalence of heterozygous FH for our sample size calculation. This may have resulted in a sample size underpowered to determine the true prevalence of FH in Ghana. Future studies should be mindful of this. Thirdly, we used paired-end Sanger sequencing which is inherently limited by the inability to properly resolve polymorphic single nucleotide positions resulting in us discarding a good proportion of these ambiguous base calls. Future studies would benefit from leveraging the higher sequencing depth of next-generation sequencing techniques in determining the genetic epidemiology of FH. Finally, the Sanger sequencing platform used was also limited by its inability to determine phasing and delineate individuals with homozygous from heterozygous FH.

In conclusion, our finding of a prevalence of 1% of pathogenic variants associated with FH highlights the possibility that FH might be a major contributor to ASCVD burden in Ghana. The implication of this finding is the need for the implementation of evidence based guidelines for FH management as proscribed by the World Heart Federation (FH genetic testing, family cascade testing and aggressive lipid lowering medication for life) (Ray et al., 2022). In this regard, future clinical epidemiology studies will need to determine the national prevalence of pathogenic variants associated with FH in Ghana using larger sample sizes, identify the current state of FH management and the potential barriers and facilitators to FH management. Overall, our work sounds the opening salvos for future efforts to implement an FH management program in Ghana and the SSA region.

CRediT authorship contribution statement

Philip Opoku-Agyeman: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Prince Ameyaw:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Selassie Bruku:** Writing – review & editing, Methodology. **Gideon Ofori Addo:** Writing – review & editing, Methodology. **Gideon Tetteh:** Writing – review & editing, Methodology. **Esther Baafi:** Writing – review & editing, Writing – original draft, Methodology. **Sandra Korkor Asare:** Writing – original draft, Methodology. **Abigail Foriwaah Frimpong:** Writing – review & editing, Writing – original draft, Methodology. **Samuel Adu-Poku:** Writing – review & editing, Writing – original draft, Methodology. **Sena Adzoa Matrevi:** Writing – review & editing, Writing – original draft, Methodology. **Nancy Odurowah Duah-Quashie:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Kwesi Z. Tandoh:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Data availability statement

Raw sequence data on some of the sequences have been deposited at the GenBank (NCBI) database with accession numbers **PQ635077-PQ635111**.

Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amolm.2025.100067.

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