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The APOB R3500Q Mutation in Libya: Universal Heterozygosity and the Statin Masking Effect

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ABSTRACT

Familial defective apolipoprotein B-100 (FDB) is primarily driven by the APOB R3500Q (c.10580G>A) mutation. While extensively characterized in European cohorts, its prevalence and phenotypic expression in the Middle East and North Africa (MENA) region remain contested, and the diagnostic utility of standard lipid profiles in mutation carriers is increasingly compromised by aggressive lipid-lowering therapy. This study investigated the genetic prevalence, phenotypic expression, and cardiovascular implications of the APOB R3500Q mutation in a Libyan cohort. A retrospective case-control design with concurrent biomarker profiling was employed, comprising 59 participants (48 cases with severe coronary artery disease [CAD] and 11 healthy controls). Genotyping was performed using multiplex PCR and reverse-hybridization (CVD StripAssay®). Data were analyzed by multivariable logistic regression, Principal Component Analysis (PCA), and hierarchical clustering. All 59 participants (100%) carried the heterozygous (G/A) R3500Q genotype, a distribution that deviated significantly from Hardy-Weinberg equilibrium in the control group (χ^2=11.0, df=1, P<0.001), suggesting a localized founder effect or a previously unmapped regional polymorphism. Cases unexpectedly showed lower LDL-C (P=0.034) and total cholesterol (P=0.044) than controls, a "pharmacological masking" effect attributable to high-intensity statin therapy in 87.5% of cases. The residual atherogenic risk was instead reflected in significant HDL-C depletion (P=0.047) and elevated TG/HDL ratios. Logistic regression identified age (OR=4.91, P=0.002) and hypertension (OR=3.75, P=0.008) as the principal independent predictors of CAD, consistent with a "triple hit" model in which genetic susceptibility requires cumulative temporal exposure and hemodynamic stress to precipitate clinical atherosclerosis. Cluster analysis further resolved this genetically uniform cohort into three distinct metabolic phenotypes. These findings indicate that the APOB R3500Q mutation is highly prevalent among Libyan patients but exhibits incomplete early-life penetrance, and that diagnostic and therapeutic strategies for FDB in heavily medicated populations should pivot from absolute LDL-C targets toward composite residual-risk markers such as HDL-C and the TG/HDL ratio. KEYWORDS: Apolipoprotein B; R3500Q mutation; familial defective apolipoprotein B-100; HDL-C depletion; cardiovascular disease; Libya

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, with global mortality exceeding 20.5 million per year and a trajectory that continues to rise, a burden that is particularly pronounced in the Middle East and North Africa (MENA) region owing to rapid epidemiological transition (World Heart Federation, 2025; GBD 2021 Causes of Death Collaborators, 2024; Ghadamosi et al., 2025). Atherosclerotic CVD is fundamentally driven by prolonged elevation of low-density lipoprotein cholesterol (LDL-C), and a sustained 1 mmol/L reduction in LDL-C is associated with a proportionate reduction in major adverse cardiovascular events (Mach et al., 2020). Among monogenic causes of severe hypercholesterolemia, familial defective apolipoprotein B-100 (FDB) arises from mutations in the APOB gene (chromosome 2p24-p23), which encodes the 4,536-amino-acid ligand for the LDL receptor (LDLR) (Abifadel and Boileau, 2023).

The R3500Q (c.10580G>A) missense mutation substitutes glutamine for arginine at residue 3500 within the LDLR-binding domain of apolipoprotein B-100, reducing receptor affinity to below 10% of normal and causing sustained plasma retention of LDL particles (Benn et al., 2009). This variant is the classical cause of FDB in European populations, where it has been linked to founder effects in cohorts such as the Copenhagen General Population Study (Benn et al., 2016) and the Old Order Amish community (Andersen, 2016), but is reported at near-zero frequency in most MENA screening studies (Durmus et al., 2020; Younis et al., 2023). In Libya, molecular screening for APOB mutations remains essentially absent despite a high clinical burden of coronary artery disease (CAD). The CVD StripAssay® platform, which combines multiplex PCR with reverse hybridization to allele-specific probes, offers a standardized and sensitive method for R3500Q genotyping (ViennaLab Diagnostics GmbH, 2024) and was used here to characterize the APOB genetic landscape of a Libyan cardiovascular cohort for the first time.

A further diagnostic challenge is that standard lipid panels may be poorly informative in mutation carriers who receive intensive statin therapy, since aggressive up-regulation of hepatic LDL receptors can drive LDL-C into the normal range independently of the underlying genetic defect - a phenomenon we term "Pharmacological masking." Whether this obscures the R3500Q phenotype in treated Libyan patients, and whether age or metabolic comorbidities (hypertension, diabetes) are required to convert genetic susceptibility into overt CAD, had not previously been investigated. The present study therefore aimed to (i) establish the carrier frequency of the APOB R3500Q mutation among Libyan CAD patients and healthy controls; (ii) test whether genotype interacts with age and metabolic comorbidity to precipitate CAD; (iii) characterize the distortion of standard lipid parameters by statin therapy and identify residual atherogenic markers; and (iv) resolve phenotypic heterogeneity among mutation carriers using unsupervised multivariate methods.

MATERIALS AND METHODS

Study design, setting, and participants

This retrospective case-control study with concurrent biomarker profiling was conducted at the Alfouad Cardiac Center, Tripoli, Libya, between March and August 2025. Of 82 adults (>25 years) screened, 59 met all eligibility criteria and were enrolled: 48 cases with confirmed CAD, hypertension and/or hyperlipidemia (age >40 years), and 11 age- and sex-matched healthy controls with no personal or family history of CVD. Exclusion criteria included severe unrelated comorbidities, prior lipid-lowering therapy at the time of initial lipid assessment, incomplete records, and poor-quality blood samples. Sample size was determined a priori using G*Power (version 3.1.8) for a case-control chi-square design (α=0.05, power=0.80). The study was approved by the National Committee for Biosafety and Bioethics in Libya and conducted in accordance with the Declaration of Helsinki; written informed consent was obtained from all participants.

Sample collection and DNA extraction

Venous blood (5 mL) was collected into EDTA tubes. Genomic DNA was extracted from whole blood using the extraction reagents supplied with the CVD StripAssay® kit (ViennaLab Diagnostics GmbH, Vienna, Austria, Ref. 4-240), and DNA concentration and purity were assessed spectrophotometrically (A260/A280 and A260/A230 ratios).

APOB R3500Q genotyping

Genotyping for the APOB R3500Q variant (c.10580G>A; rs5742904) was performed using the CVD StripAssay® kit, which employs multiplex

PCR followed by reverse hybridization of biotinylated amplicons to allele-specific oligonucleotide probes immobilized on nitrocellulose strips. PCR products were denatured, hybridized to the strips at 45°C for 30 min, washed stringently, and detected colorimetrically via a streptavidin-alkaline phosphatase conjugate and NBT/BCIP substrate. Genotypes (G/G, G/A, or A/A) were determined from the banding pattern by two independent, blinded readers, and 10-20% of samples were re-genotyped for quality control. A random subset of 10 samples underwent independent re-genotyping for analytical validation.

Lipid profile

Fasting total cholesterol, triglycerides, and HDL-C were measured by standard enzymatic colorimetric assays on an automated biochemistry analyzer. LDL-C was calculated using the Friedewald formula (LDL-C = TC - HDL-C - TG/5) for triglycerides <400 mg/dL, and VLDL-C was estimated as TG/5. Atherogenic ratios (TC/HDL, LDL/HDL, TG/HDL) were derived from these values.

Statistical analysis

Analyses were performed in IBM SPSS Statistics (version 26.0) and Python, with two-tailed significance set at $P<0.05$. Missing clinical data were addressed by Multiple Imputation (15 imputations). Normality was assessed using the Shapiro-Wilk test; continuous variables are reported as mean ± SD (or median and IQR where non-normally distributed) and compared using independent-samples t-tests or the Mann-Whitney U test as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact test. The control group's genotype distribution was tested for deviation from Hardy-Weinberg equilibrium using a chi-square goodness-of-fit test (df=1). Spearman and Pearson correlations assessed bivariate relationships among age, lipid parameters, electrolytes, and coagulation markers. Binary logistic regression identified independent predictors of case status, and multiple linear regression identified predictors of HDL-C and the TC/HDL ratio. Principal Component Analysis was applied to eight lipid-related variables, and hierarchical cluster analysis (Ward's method, Euclidean distance) was used to stratify metabolic phenotypes.

RESULTS

Demographic and clinical characteristics of 82 individuals initially screened, 59 participants (48 CAD cases and 11 healthy controls) met all eligibility criteria. Gender distribution was closely matched between groups (37.5% vs. 36.4% male, $\chi^2=0.000$, $P=0.952$). Cases were markedly older than controls (62.7 ± 12.3 vs. 37.5 ± 7.1 years; Mann-Whitney $U=497.0$, $P<0.001$, $r=-0.883$), and showed a significantly higher prevalence of hypertension (66.7% vs. 0%, $P<0.001$) and diabetes mellitus (35.4% vs. 0%, $P=0.024$) (Table 1). Coagulation and cardiac injury markers (APTT, PT, D-dimer, troponin) did not differ significantly between groups, consistent with pharmacological suppression of coagulation by dual antiplatelet therapy in the majority of cases (Table 2).

Table 1. Demographic and clinical characteristics of the study population.

Parameter	Cases (n=48)	Controls (n=11)	P-value
Age (years, mean ± SD)	62.73 ± 12.33	37.45 ± 7.06	<0.001*
Male gender, n (%)	18 (37.5%)	4 (36.4%)	0.952
Coronary artery disease, n (%)	35 (72.9%)	0 (0.0%)	<0.001*
Hypertension, n (%)	32 (66.7%)	0 (0.0%)	<0.001*
Diabetes mellitus, n (%)	17 (35.4%)	0 (0.0%)	0.024*
Cerebrovascular accident, n (%)	5 (10.4%)	0 (0.0%)	0.572

APOB R3500Q genotyping

Genotyping of all 59 participants revealed a uniform heterozygous (G/A) genotype in every individual (100%), with no wild-type (G/G) or homozygous mutant (A/A) genotypes detected in either group (Table 3). Allele frequencies were consequently balanced (G=50.0%, A=50.0%).

Table 2. Coagulation parameters and cardiac biomarkers.

Biomarker	Cases (n=48)	Controls (n=11)	P-value
APTT (sec)	25.39 ± 1.42	25.17 ± 1.06	0.869
PT (sec)	12.51 ± 1.02	11.95 ± 0.90	0.055
D-Dimer (µg/mL)	1.50 ± 1.73	1.17 ± 1.61	0.053
Troponin (ng/mL)	1.09 ± 2.46	0.89 ± 1.03	0.806

The control group's genotype distribution deviated significantly from Hardy-Weinberg equilibrium (observed GG=0, GA=11, AA=0 vs. expected GG=2.75, GA=5.50, AA=2.75; χ^2=11.0, df=1, P<0.001). Independent re-genotyping of a random subset of 10 samples (9 cases, 1 control) showed 100% concordance with the primary screening, confirming the reliability of the assay.

Table 3. APOB R3500Q genotype distribution.

Genotype	Cases	Control	Total	%
G/G (wild-type)	0	0	0	0.0%
G/A (heterozygous)	48	11	59	100.0 %
A/A (homozygous mutant)	0	0	0	0.0%

Lipid profile and pharmacological masking

The overall cohort showed generally favorable absolute lipid values (mean total cholesterol 151.8 ± 45.7 mg/dL; LDL-C 90.1 ± 33.9 mg/dL), but low HDL-C (<40 mg/dL) was present in 50.8% of participants. Contrary to the expected FDB phenotype, cases showed significantly lower total cholesterol (147.2 ± 48.5 vs. 171.5 ± 23.8 mg/dL, P=0.044) and LDL-C (86.1 ± 34.0 vs. 107.5 ± 28.3 mg/dL, P=0.034) than controls, while HDL-C was markedly and significantly lower in cases (41.2 ± 12.9 vs. 52.5 ± 18.3 mg/dL, P=0.047) (Table 4). Triglycerides did not differ significantly (138.2 ± 90.9 vs. 107.1 ± 48.4 mg/dL, P=0.403) but remained numerically elevated in cases. This pattern coincided with high-intensity statin use in 87.5% of cases. Atherogenic lipid ratios (TC/HDL, LDL/HDL, TG/HDL) did not differ significantly between groups, although the mean case TC/HDL ratio (3.75) exceeded the conventional high-risk threshold of 3.5, and the TG/HDL ratio showed a non-significant elevation in cases (3.74 vs. 2.45, P=0.092).

Table 4. Lipid profile comparison between cases and controls.

Parameter	Cases (n=48)	Controls (n=11)	Test	P-value
Total cholesterol (mg/dL)	147.2 ± 48.5	171.5 ± 23.8	t-test	0.044*
HDL-C (mg/dL)	41.22 ± 12.9	52.45 ± 18.33	Mann-Whitney U	0.047*
LDL-C (mg/dL)	86.14 ± 34.0	107.5 ± 28.35	t-test	0.034*
Triglycerides (mg/dL)	138.23 ± 90.90	107.13 ± 48.4	Mann-Whitney U	0.403

Predictors of cardiovascular disease status

Because genotype was invariant, binary logistic regression was used to identify clinical predictors of case status. Age was the strongest independent predictor (OR=4.913, 95% CI 1.844-13.089, P=0.002), followed by hypertension (OR=3.750, 95% CI 1.407-9.991, P=0.008); overall model accuracy was 96.6% (Table 5). Lipid parameters, gender, diabetes, and clinical hyperlipidemia were not independently associated with case status, consistent with confounding by indication (statins being preferentially prescribed to cases). Age-stratified analysis of mechanical intervention showed that the proportion of participants requiring coronary stenting rose from 15.3% below 40 years to 23.7% between 40-55 years and 61.0% above 55 years, indicating a clinical "age threshold" of approximately 55 years.

Table 5. Binary logistic regression analysis for cardiovascular disease status.

Predictor	B	Odds ratio	95% CI	P-value
Age	1.592	4.913	1.844–13.089	0.002*
Hypertension	1.322	3.750	1.407–9.991	0.008*
Diabetes mellitus	0.566	1.762	0.661–4.694	0.258
Gender (male)	-0.449	0.638	0.239–1.700	0.369
Total cholesterol	-0.139	0.870	0.327–2.319	0.781
HDL-C	-0.307	0.736	0.278–1.947	0.540
LDL-C	-0.755	0.470	0.176–1.252	0.131
Triglycerides	0.223	1.250	0.463–3.370	0.655

Principal component and cluster analyses

PCA of eight lipid-related variables extracted three components explaining 85.6% of total variance. PC1 (49.5%) reflected general atherogenic burden (high loadings for TC/HDL, LDL/HDL and TG/HDL ratios, triglycerides, total cholesterol and LDL-C); PC2 (25.0%) reflected an HDL-protective profile (dominant HDL-C loading of 0.591); and PC3 (11.1%) was driven almost exclusively by age (loading 0.887) (Table 6). Cases showed higher PC1 and lower PC2 scores than controls, indicating a shift toward greater atherogenic burden and reduced HDL-mediated protection despite an identical genotype. Hierarchical clustering (Ward's method) resolved the cohort into three metabolically distinct phenotypes:

Cluster 1 (n=18, 30.5%), older and pharmacologically well-controlled but with low HDL-C (36.5 mg/dL); Cluster 2 (n=2, 3.4%), severely dyslipidemic (triglycerides 473.1 mg/dL) with poor metabolic control; and Cluster 3 (n=39, 66.1%), younger, with moderate lipid elevation and relatively preserved HDL-C (46.7 mg/dL) (Table 7).

Table 6. Principal component loadings for the first three components (lipid-related variables).

Variable	PC1	PC2	PC3
Age	-0.013	-0.346	0.887
Total cholesterol	0.325	0.488	0.165
HDL-C	-0.186	0.591	0.282
LDL-C	0.330	0.447	0.175
Triglycerides	0.408	-0.122	0.175
TC/HDL ratio	0.462	-0.062	-0.169
LDL/HDL ratio	0.436	-0.015	-0.126
TG/HDL ratio	0.425	-0.273	0.035

DISCUSSION

This study provides the first molecular characterization of the APOB R3500Q mutation in a Libyan cardiovascular cohort and yields two findings that depart markedly from expectation: a universal heterozygous carrier state across both cases and controls, and a lipid profile in which cases showed lower, rather than higher, LDL-C and total cholesterol than controls.

The 100% carrier frequency, together with the significant deviation from Hardy-Weinberg equilibrium in the control group, is most plausibly explained by a pronounced localized founder effect, a phenomenon previously documented for R3500Q in the Copenhagen General Population Study (Benn et al., 2016) and, at a much higher magnitude, in the Old Order Amish community (Andersen, 2016). Extreme, geographically restricted enrichment of APOB variants has also been reported in other Middle Eastern cohorts (Al-daraji et al., 2018), consistent with the broader principle that APOB variant frequency in the MENA region may be characterized by isolated pockets of high prevalence against a general background of near-absence (Durmus et al., 2020; Younis et al., 2023). An alternative, non-exclusive explanation is that the CVD StripAssay® probes, validated against European loci, may cross-react with a benign, population-specific North African polymorphism, as has been described for other underrepresented populations subjected to whole-exome sequencing (Abdullah-Zawawi et al., 2025; Jang et al., 2022). Definitive resolution of these possibilities will require next-generation or whole-exome sequencing of this cohort.

The unexpected finding of lower LDL-C and total cholesterol in cases is fully explained by aggressive secondary-prevention pharmacotherapy: 87.5% of cases received high-intensity statins, consistent with contemporary AHA/ACC and ESC/EAS guidelines mandating LDL-C reductions of at least 50% after coronary events (Virani et al., 2023; Mach et al., 2020). Because the primary defect in

APOB R3500Q carriers is impaired LDL particle-receptor binding rather than hepatic cholesterol overproduction, statin-driven up-regulation of LDL receptors can drive circulating LDL-C to artificially low values without correcting the underlying molecular lesion - a "pharmacological masking" effect also described in other statin-treated FDB cohorts (Lehner, 2021; Houttu et al., 2023). Critically, statin therapy did not normalize HDL-C, which remained significantly depleted in cases; together with a numerically elevated TG/HDL ratio, this indicates that HDL-C depletion, rather than absolute LDL-C, is the more informative residual-risk marker in heavily medicated APOB mutation carriers.

Logistic regression identified age and hypertension, rather than lipid parameters, as the dominant independent predictors of case status, supporting a "triple hit" model of pathogenesis in which the R3500Q genotype provides an atherogenic substrate that requires both cumulative temporal exposure (an apparent vulnerability window opening around 55 years) and hemodynamic stress to precipitate clinical atherosclerosis. This is compatible with the known synergy between hypertension and dyslipidemia in accelerating endothelial injury (Chen et al., 2019), and is further supported by a strong positive correlation between LDL-C and serum sodium, suggesting a compounded osmotic and atherogenic stress in this predominantly hypertensive cohort (Gao et al., 2017).

Despite sharing an identical genotype, PCA and hierarchical clustering revealed pronounced phenotypic heterogeneity, resolving the cohort into pharmacologically controlled, severely dyslipidemia, and standard atherogenic subgroups. This heterogeneity, together with sex-specific differences in lipid parameters likely mediated by estrogen-dependent effects on HDL metabolism, argues against a uniform treatment approach and for phenotype-driven management of APOB mutation carriers, consistent with the broader recognition that genotype alone poorly predicts phenotypic severity in familial hypercholesterolemia (Kamar et al., 2020).

Several limitations should be considered. The case and control groups were not age-matched, which, while informative for identifying the age-threshold effect, introduces age as a potential confounder. The sample size (n=59) and unbalanced case:control ratio (48:11) limit broader epidemiological extrapolation, and the absence of the wild-type genotype precluded conventional case-control genetic association testing.

The CVD StripAssay® targets European-validated loci, and cross-reactivity with an uncharacterized North African variant cannot be excluded without

sequencing confirmation. Finally, the concurrent biomarker design captured a pharmacologically treated snapshot rather than a pre-treatment genetic baseline, limiting inference about the natural history of the mutation in this population.

CONCLUSION

In conclusion, the APOB R3500Q mutation is highly prevalent in this Libyan cohort but shows incomplete penetrance before the sixth decade of life, and its lipid phenotype is substantially obscured by intensive statin therapy in treated patients. Diagnostic and management strategies for FDB in similarly medicated populations should therefore incorporate HDL-C depletion and the TG/HDL ratio as composite residual-risk markers, alongside genetic confirmation and cascade screening of first-degree relatives, and future work should prioritize sequencing-based validation of the genetic finding together with longitudinal, pre-treatment biomarker profiling.

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