*Article*

# Applicability of Diagnostic Criteria and Prevalence of Familial Dysbetalipoproteinemia in Russia

**Anastasia V. Blokhina [1,*], Alexandra I. Ershova [1], Anna V. Kiseleva [1], Evgeniia A. Sotnikova [1], Anastasia A. Zharikova [1,2], Marija Zaicenoka [3], Yuri V. Vyatkin [1,4], Vasily E. Ramensky [1,2], Vladimir A. Kutsenko [1,5], Svetlana A. Shalnova [1], Alexey N. Meshkov [1,6,7,8] and Oxana M. Drapkina [1]**

1 National Medical Research Center for Therapy and Preventive Medicine, Ministry of Healthcare of the Russian Federation, Petroverigsky per.10, Bld. 3, 101000 Moscow, Russia; blokhina0310@gmail.com (A.V.B.); alersh@mail.ru (A.I.E.); sanyutabe@gmail.com (A.V.K.); sotnikova.evgeniya@gmail.com (E.A.S.); azharikova89@gmail.com (A.A.Z.); vyatkin@gmail.com (Y.V.V.); ramensky@gmail.com (V.E.R.); vlakutsenko@yandex.ru (V.A.K.); sshalnova@gnicpm.ru (S.A.S.); meshkov@lipidclinic.ru (A.N.M.); drapkina@bk.ru (O.M.D.)

2 Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, 1-73, Leninskie Gory, 119991 Moscow, Russia

3 Moscow Institute of Physics and Technology, Dolgoprudny, Institutskiy per.9, 141701 Dolgoprudny, Russia; marija.zaicenoka@gmail.com (M.Z.)

4 Novosibirsk State University, 1, Pirogova Str., 630090 Novosibirsk, Russia

5 Faculty of Mechanics and Mathematics, Lomonosov Moscow State University, 1-73, Leninskie Gory, 119991 Moscow, Russia

6 National Medical Research Center for Cardiology, 3–ya Cherepkovskaya Street, 15A, 121552 Moscow, Russia

7 Research Centre for Medical Genetics, 1 Moskvorechye St, Moscow 115522, Russia

8 Pirogov Russian National Research Medical University, 1 Ostrovityanova st., Moscow 117997, Russia

* Correspondence: blokhina0310@gmail.com (A.V.B)

**Abstract:** Familial dysbetalipoproteinemia (FD) is a highly atherogenic genetically-based lipid disorder with the underestimated actual prevalence. In the recent years, several biochemical algorithms have been developed to diagnose FD using available laboratory tests. However, there is not enough data on their use in real-world clinical implementation. We studied the applicability of the most accessible biochemical algorithms to diagnose FD in clinical practice. We also investigated the prevalence of FD in one of the European regions of Russia based on a population sample. In this study there was detected a high prevalence of FD: 1 in 151. We demonstrated that the diagnostic algorithms of FD including a diagnostic apoB levels require correction, taking into account the characteristics of the distribution of apoB levels in the population. At the same time a triglycerides cutoff ≥1.5 mmol/L may be a useful tool in identifying subjects with FD. We also analyzed the presence and pathogenicity of *APOE* variants associated with the autosomal dominant FD in a large research sample.

**Keywords:** familial dysbetalipoproteinemia; hyperlipoproteinemia type III; *APOE*; apolipoprotein E; apolipoprotein B; autosomal dominant; remnant lipoproteins; hyperlipidemia; population; genetic

## 1. Introduction

Familial dysbetalipoproteinemia (FD), also known as hyperlipoproteinemia type III (OMIM #617347), is a genetically-based lipid disorder caused by variants in the *APOE* gene. FD is associated with elevated levels of lipoprotein remnants and an increased risk of premature atherosclerosis [1,2].

*APOE* has three main alleles that encode apolipoprotein E isoforms: ε2, ε3, ε4. As a result, there are three homozygous (ε2ε2, ε3ε3, and ε4ε4) and three heterozygous (ε2ε3, ε3ε4, and ε4ε2)

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haplotypes. The haplotype composition is determined by the combination of two variants: rs7412 (NP_000032.1:Arg176Cys or Arg158Cys according to the old nomenclature) and rs429358 (NP_000032.1:Cys130Arg or Cys112Arg). Homozygotes for the ε2 allele (T/T genotype), in the absence of changes in rs429358 (T/T genotype), will form the ε2ε2 haplotype [2–5]. In more than 90% of cases, the ε2ε2 haplotype predisposes to the development of an autosomal recessive form of FD [6]. However, for the development of FD, the ε2ε2 haplotype alone is not sufficient. Additional factors leading to increased synthesis of very low-density lipoproteins or impaired excretion of lipoprotein remnants, including impaired functioning of the heparan sulfate proteoglycan receptor, are necessary. These factors include overweight or obesity [3,7,8], insulin resistance [3,7,9], diabetes mellitus [3,10], hypothyroidism, some medications, menopause [3], and pregnancy [11,12]. In the presence of these conditions, inhibition or even degradation of the heparan sulfate proteoglycan receptor can occur, leading to the accumulation of remnants and the development of FD [3].

It was also shown that around 10% of patients could have the autosomal dominant FD associated with a single copy of the defective *APOE* allele. Approximately 30 *APOE* variants associated with the autosomal dominant FD have been reported [13–15]. However, there is insufficient data regarding the relationship between the described *APOE* variants and FD [15].

Previous epidemiological studies demonstrated a variety of prevalences of FD (0.2%–2.7%) [16,17]. Thus, it was shown that the prevalence of FD was 0.7% (1 in 143) in the population of Utah, USA, and 2.7% (1 in 37) among patients with premature coronary artery disease [16]. In a more recent study, the prevalence of FD among US adults in two population-based samples was 0.2–0.8% [17]. This estimate is based on various ultracentrifugation criteria. However, the availability of these criteria for real clinical practice is limited, and recent data suggests that the real prevalence of FD is underestimated [18,19].

In recent years, several biochemical algorithms have been developed to diagnose FD using available laboratory tests. These algorithms rely on assessing the ratios between the levels of apolipoprotein B (apoB), total cholesterol (TC), triglycerides (TG) (the apoB algorithm of Sniderman 2010 [20]), and non-high density lipoprotein cholesterol (non-HDL-C) [21,22]. However, there is not enough data on their use in real-world clinical implementation.

The aim of this study was to investigate the prevalence of FD in one of the European regions of Russia based on a population sample. Additionally, we first simultaneously assessed the applicability of previously developed biochemical FD criteria (the apoB algorithm: apoB <1.2 g/L, TG ≥1.5 mmol/L, TG/apoB <10.0 mmol/g, TC/apoB ≥6.2 mmol/g, the non-HDL-C/apoB ratio ≥3.69 mmol/g and the non-HDL-C/apoB ratio >4.91 mmol/g). We also analyzed the presence and pathogenicity of *APOE* variants associated with the autosomal dominant FD in a large research sample.

## 2. Results

### *2.1. APOE variants associated with the autosomal dominant FD*

We have identified six variants that are associated with the autosomal dominant FD. Four of these variants have been described previously [14,15]. Additionally, we have identified two rare *APOE* variants that have not been previously associated with FD. **Supplementary Table S1** presents characteristics of all detected *APOE* variants.

The median age of subjects with these variants was 48 years [40; 54], and 30.8% were men. The majority (92.3%) were at least overweight, and 15.4% of them were obese. One subject with a pathogenic (p.Glu63ArgfsTer15) and another subject with a likely pathogenic (rs121918393, *APOE2* Heidelberg) *APOE* variant had xanthomas (tendon or cutaneous eruptive, respectively). The TG level among all subjects was less than 1.7 mmol/L, but subjects with pathogenic or probably pathogenic *APOE* variants had a TG level greater than 1.5 mmol/L. **Table 1** presents the characteristics of subjects with available clinical data (n = 13).

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**Table 1.** Characteristics of subjects with variants associated with the autosomal dominant FD.

| Parameter | All subjects (n = 13) | Subjects with VUS (n = 7) | Subjects with LP or P variants (n = 6) |
|---|---|---|---|
| Men, n (%) | 4 (30.8%) | 2 (28.6%) | 2 (33.3%) |
| Age, years | 48 [40; 54] | 48 [30; 54] | 45 [40; 57] |
| BMI, kg/m2 | 26.3 [26.0; 28.9] | 26.3 [26.0; 28.9] | 27.4 [25.7; 30.0] |
| Presence of xanthoma, n (%) | 2 (15.4%) | 0 | 2 (33.3%) |
| CHD, n (%) | 0 | 0 | 0 |
| TG, mmol/L | 1.36 [1.11; 1.95] | 1.36 [1.11; 1.70] | 1.55 [0.69; 4.75] |
| LDL-C, mmol/L | 3.0 [2.32; 3.85] | 3.04 [1.66; 3.94] | 2.96 [2.32; 3.54] |
| HDL-C, mmol/L | 1.23 [1.0; 1.81] | 1.18 [0.98; 2.07] | 1.27 [1.09; 1.66] |

BMI—body mass index; CHD—coronary heart disease; HDL-C—high density lipoprotein cholesterol; LDL-C—low density lipoprotein cholesterol; LP—likely pathogenic; P—pathogenic; TG—triglycerides; VUS—variant of uncertain significance.

Given the small sample of subjects with pathogenic or likely pathogenic variants associated with the autosomal dominant FD, this group was not included in further analysis of this study.

*2.2. Applicability of biochemical FD criteria*

After analyzing the genetic data of 3408 subjects, we identified 37 carriers of the ε2ε2 haplotype. To assess the prevalence of FD, it was necessary to establish criteria for identification subjects with not only the ε2ε2 haplotype, but with FD. That is why we applied the most widely-used apoB algorithm to the ε2ε2 haplotype carriers who had both complete lipid values and clinical data, and did not have pathogenic or likely pathogenic variants in other lipid metabolism genes (n = 33).

In order to diagnose FD according to the apoB algorithm, laboratory data must meet all the following cutoffs: apoB <1.2 g/L, TG ≥1.5 mmol/L, TG/apoB <10.0 mmol/g, and TC/apoB ≥6.2 mmol/g [20]. Out of 33 ε2ε2 haplotype carriers, only 66.7% passed the apoB algorithm. Moreover, three subjects with the FD phenotype did not pass the main cutoff of the algorithm, which is apoB <1.2 g/l (**Figure 1**). This threshold represents the 75th percentile of the distribution of apoB in persons ≥20 years of age from the National Health and Nutrition Examination Survey III (NHANES III) study [23].

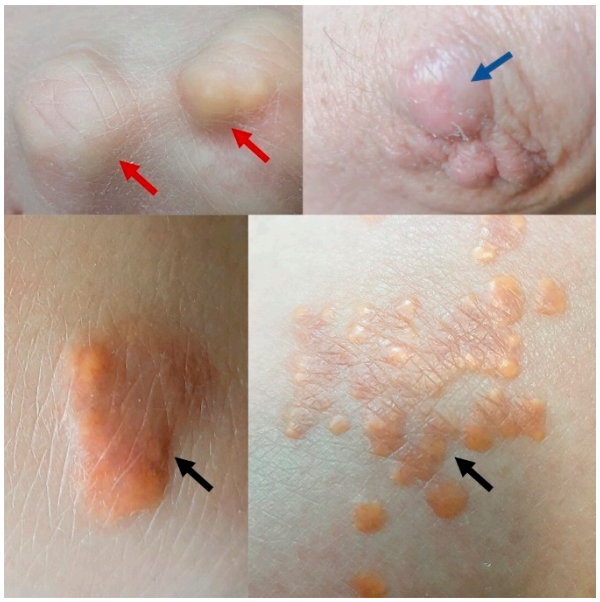

**Figure 1.** Cutaneous: eruptive (black arrows), tubero-eruptive (blue arrow), and tendon (metacarpal, red arrows) xanthomas in patients with FD who did not pass the apoB algorithm.

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Considering exclusion subjects with the ε2ε2 haplotype and FD phenotype according to the apoB level, we studied the distribution of apoB levels in the ESSE-Ivanovo sample (**Table 2**).

**Table 2.** The distribution of apoB levels in the ESSE-Ivanovo sample (n = 1550).

| Age, years | Number of subjects | apoB, g/l | | |
|---|---|---|---|---|
| | | 50th percentile | **75th percentile** | 95th percentile |
| | | **All subjects** | | |
| **25–64** | 1550 | 0.89 | **1.06** | 1.35 |
| 25–34 | 285 | 0.73 | 0.86 | 1.14 |
| 35–44 | 325 | 0.84 | 0.98 | 1.24 |
| 45–54 | 472 | 0.93 | 1.08 | 1.37 |
| 55–64 | 468 | 0.98 | 1.16 | 1.43 |
| | | **Men** | | |
| **25–64** | 574 | 0.88 | **1.05** | 1.33 |
| 25–34 | 167 | 0.78 | 0.92 | 1.18 |
| 35–44 | 142 | 0.87 | 1.02 | 1.29 |
| 45–54 | 151 | 0.96 | 1.11 | 1.43 |
| 55–64 | 114 | 0.96 | 1.13 | 1.36 |
| | | **Women** | | |
| **25–64** | 976 | 0.90 | **1.07** | 1.35 |
| 25–34 | 118 | 0.67 | 0.79 | 1.06 |
| 35–44 | 183 | 0.81 | 0.95 | 1.14 |
| 45–54 | 321 | 0.92 | 1.06 | 1.34 |
| 55–64 | 354 | 1.00 | 1.17 | 1.45 |

The results showed that the level of apoB was lower (1.06 g/L (75th percentile)) compared to the NHANES III study.

The sensitivity and specificity of biochemical FD criteria were analyzed (**Table 3**).

**Table 3.** The sensitivity and specificity of biochemical FD criteria in the ESSE-Ivanovo sample (n = 1550).

| Criterion | Passed the criterion for subjects with the ε2ε2 haplotype (n = 13) | Did not pass the criterion for subjects without the ε2ε2 haplotype (n = 1537) | Sensitivity, % | Specificity, % |
|---|---|---|---|---|
| | | **apoB algorithm of Sniderman 2010 [20]** | | |
| apoB <1.2 g/L | 13 | 185 | 100 | **12.0** |
| TG ≥1.5 mmol/L | 10 | 1001 | **76.9** | **65.1** |
| TG/apoB <10.0 mmol/g | 13 | 2 | 100 | **0.1** |
| TC/apoB ≥6.2 mmol/g | 12 | 842 | 92.3 | 54.8 |
| | | **non-HDL-C/apoB ratio** | | |
| non-HDL-C/apoB ≥3.69 mmol/g [21] | 13 | 19 | 100 | **1.2** |
| non-HDL-C/apoB >4.91 mmol/g [22] | 12 | 1364 | **92.3** | **88.7** |

non-HDL-C—non-high density lipoprotein cholesterol; TG—triglycerides.

The apoB algorithm analysis showed the low specificity of apoB level (12.0%) and TG/apoB-ratio (0.1%) for identifying subjects without the ε2ε2 haplotype. TG level ≥1.5 mmol/L had sufficient sensitivity (76.9%) and specificity (65.1%) to identify subjects with the ε2ε2 haplotype, as well as the non-HDL-C/apoB ratio >4.91 mmol/g. At the same time, the non-HDL-C/apoB ratio ≥3.69 mmol/g

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had a low specificity (1.2%) for identifying subjects without the ε2ε2 haplotype in the study population.

The low specificity of the apoB algorithm, as well as the pronounced differences in sensitivity and specificity of the non-HDL-C/apoB ratios, may be attributed to the lower levels of apoB in the ESSE-Ivanovo sample. Specifically, the non-HDL-C/apoB ratio in this sample initially exceeded the cutoff ≥3.69 mmol/g in 98.7% of subjects. Moreover, the subjects with FD from the Boot 2019 study [22] showed a similar cutoff (the means were 4.0 mmol/g) to that of all subjects in the ESSE-Ivanovo sample, regardless of their FD status (the median was 4.51 [4.27; 4.73] mmol/g) (**Table 4**).

**Table 4.** The distribution of apoB levels and the non-HDL-C/apoB ratio in the ESSE-Ivanovo sample and other studies.

| Parameter | ESSE-Ivanovo sample | non-HDL-C/apoB ratio ≥3.69 mmol/g Paquette 2020 [21] | | non-HDL-C/apoB ratio >4.91 mmol/g[1] Boot 2019 [22] | |
|---|---|---|---|---|---|
| | All subjects (n = 1550) | With FD subjects (n = 188) | Without FD subjects (n = 4703) | With FD subjects (n = 63) | Without FD subjects (n = 1397) |
| apoB, g/L | 0.89 [0.74; 1.06] | 1.30 [1.04; 1.83] | 1.81 [1.50-2.17] | 1.35 (0.4) | 1.00 (0.3) |
| non-HDL-C/apoB, mmol/g | 4.51 [4.27; 4.73] | 5.36 [4.54-6.64] | 3.38 [2.97-3.87] | 4.0 (0.5) | 7.3 (1.5) |

[1] – the means with standard deviations in parentheses (Boot 2019 [22]). FD – familial dysbetalipoproteinemia; non-HDL-C—non-high density lipoprotein cholesterol.

In this case, the most appropriate combination for identifying subjects with FD appears to be the ε2ε2 haplotype and the TG level of ≥1.5 mmol/l.

*2.3. The prevalence of FD*

The prevalence of the ε2ε2 haplotype carriers was 0.8% (one in 118) (95% CI: 0.46%-1.41%). When the TG ≥1.5 mmol/L cutoff was added to the ε2ε2 haplotype carriage, the FD was identified in 11 subjects. Thus, the prevalence of FD was 0.7% (1 in 151) (95% CI: 0.33–1.19). It is important to emphasize that none of these patients had previously been diagnosed with FD. **Table 5** presents the characteristics of subjects with FD: presence of the ε2ε2 haplotype and TG ≥1.5 mmol/L.

**Table 5.** Characteristics of subjects with FD.

| Parameter | The subjects with FD (n = 11) |
|---|---|
| Men, n (%) | 5 (45.5%) |
| Age, years | 50 [49; 58] |
| BMI, kg/m2 | 31.6 [29.0; 33.9] |
| Presence of diabetes, n (%) | 0 |
| Presence of arterial hypertension, n (%) | 10 (90.9%) |
| CHD, n (%) | 1 (9.1%) |
| Carotid atherosclerosis, n (%) | 7 (77.8%) n = 9 |
| Femoral atherosclerosis, n (%) | 3 (33.3%) n = 9 |
| apoB, g/L | 0.67 [0.54; 0.75] |
| TC, mmol/L | 5.4 [4.47; 6.28] |
| TG, mmol/L | 2.31 [1.86; 2.81] |
| LDL-C, mmol/L | 2.12 [1.92; 2.48] |

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| | |
|---|---|
| HDL-C, mmol/L | 1.19 [1.05; 1.51] |

BMI—body mass index; CHD—coronary heart disease; HDL-C—high density lipoprotein cholesterol; LDL-C—low density lipoprotein cholesterol; TG—triglycerides; TC—total cholesterol.

The median age was 50 years [49; 58], and 45.5% were men. Among those with FD, 63.6% were obese. Coronary heart disease was present in 9.1%, and the majority (77.8%) had carotid atherosclerosis. Femoral atherosclerosis affected 33.3% of subjects with FD. The apoB level was 0.67 [0.5; 0.75] g/L. **Figure 2** shows the distribution of TG levels, with the minimum value being 1.54 mmol/L and the maximum value being 6.0 mmol/L.

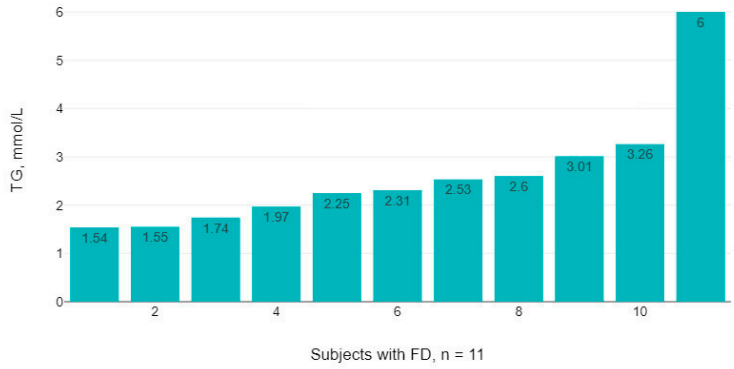


**Figure 2.** The TG levels among the subjects with FD.

## 3. Discussion

### *3.1. APOE variants associated with the autosomal dominant FD*

We have identified four *APOE* variants previously associated with the autosomal dominant FD [14,15]. However, only one of these variants, NM_000041.4:c.460C>T; NP_000032.1:p.Arg154Cys; rs121918393 – *APOE2* Heidelberg, was classified as likely pathogenic according to ACMG/AMP2015 pathogenicity criteria (PM1 [24], PM2, PP1_Moderate [24], PP3, PP4, and PP5). The pathogenicity of three other previously described *APOE* variants (rs267606664, rs199768005, and rs267606661) in the development of the autosomal dominant FD remains uncertain. These variants have a prevalence exceeding 0.01% in control samples, and there is limited data on their segregation with the FD phenotype.

In addition, we have identified two new frameshift *APOE* variants that are pathogenic (NM_000041.4:c.184_187del, NP_000032.1: p.Glu63ArgfsTer15) or likely pathogenic (NM_000041.4: c.434_461del, NP_000032.1: p.Gly145AlafsTer97) in the development of the autosomal dominant FD. It would be important to analyze their segregation in the proband`s relatives.

Therefore, further studies analyzing the phenotype-genotype relationship and conducting functional analysis can contribute to establishing the causal role of *APOE* variants in the development of the autosomal dominant FD. Determination the contribution of these variants to the development of the autosomal dominant FD will help to increase the detection of individuals with this disease.

### *3.2. Applicability of biochemical FD criteria*

We studied the applicability of the most accessible biochemical algorithms to diagnose FD in clinical practice. In the first stage, we applied the apoB algorithm of Sniderman to subjects with the ε2ε2 haplotype and found that some subjects with FD were excluded by this algorithm. Further, the distribution of apoB levels in a population sample was analyzed. It was shown that the distribution of apoB levels was lower (75th percentile - 1.06 g/L) compared to the apoB algorithm (75th percentile - 1.2 g/L according to the NHANES III study). The analysis of sensitivity and specificity revealed a low specificity of the apoB level, which may be due to the initially lower levels of apoB in subjects

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from the ESSE-Ivanovo population sample. This could result in non-carriers of the ε2ε2 haplotype being incorrectly identified as carriers.

The differences in sensitivity and specificity of the non-HDL-C/apoB ratio cutoffs ≥3.69 mmol/g and >4.91 mmol/g may also be associated with a lower level of apoB in the ESSE-Ivanovo sample, as described in our study results. Furthermore, the level of apoB may have interindividual variability depending on concomitant metabolic diseases and may decrease due to the intake of lipid-lowering therapy. This casts doubt on the unambiguous use of apoB level as the first or main indicator for identifying FD in clinical practice [25,26].

On the other hand, we found that the TG cutoff ≥1.5 mmol/l had sufficiently high sensitivity and specificity (76.9% and 65.1%, respectively) in identifying carriers of the ε2ε2 haplotype. This finding is consistent with the Varghese 2021 study, which showed that the TG cutoff ≥1.5 mmol/L was optimal for identifying individuals with FD. Increasing the TG cutoff in the apoB algorithm may result in missing subjects with a less pronounced phenotype of FD [27].

Thus, algorithms that include the apoB level require correction, taking into account the characteristics of the distribution of apoB levels in the population. Additionally, the TG cutoff ≥1.5 mmol/L may be a useful tool in identifying subjects with FD.

### *3.3. The prevalence of FD*

According to the European Medicines Agency (EMA), a disease with a maximum prevalence of 5 per 10,000 people is considered rare [28]. Therefore, FD is not a rare disease (with a prevalence of 66 per 10,000 people in the Ivanovo region). Moreover, the prevalence of FD in the Ivanovo region is comparable to that of familial hypercholesterolemia (FH), the prevalence of which we have previously reported (1 per 111 people [29], p = 0.453). However, while the prevalence of FH has been widely studied in certain populations, including Russia [29] and among various ethnic groups [30], and is covered at the global level [31,32], the equally common and no less atherogenic FD remains understudied.

## 4. Materials and Methods

### *4.1. Sampling*

We analyzed genetic data from three large samples which are described below.

The population sample was selected from the "Epidemiology of Cardiovascular Diseases and Risk Factors in Regions of the Russian Federation" (ESSE-RF) study which was a cross-sectional study conducted from 2012 to 2013 across 13 regions of Russia [33]. The current study included a sample from the Ivanovo region (n = 1858) [34]. After quality control and estimation of relatedness, 202 subjects were excluded so that data from 1656 subjects of ESSE-Ivanovo (median age was 49 years old [39; 57]; 37.3% were men) were finally used in the study (**Supplementary Table S2)**.

The ESSE-FH-RF sample consisted of participants from the ESSE-FH-RF study who had clinical diagnosis of definite or probable heterozygous FH (n=161) [29].

The Russian patient sample (RPS) was formed of patients with diverse medical conditions who were observed at the National Medical Research Center (NMRC) for Therapy and Preventive Medicine (Moscow, Russia) (n=1591).

Genetic data from the total of 3408 samples were used to identify the ε2ε2 haplotype and variants associated with the autosomal dominant FD.

### *4.2. Applicability of biochemical FD criteria*

The lipid concentrations (apoB, LDL-C, HDL-C, TC and TG) were measured with the Abbott Architect C-8000 system (Abbott Laboratories, Illinois, USA).

We assessed the sensitivity and specificity of biochemical FD criteria, such as the apoB algorithm [20] (combination of criteria apoB <1.2 g/L, TG ≥1.5 mmol/L, TG/apoB <10.0 mmol/g, TC/apoB ≥6.2 mmol/g), the non-HDL-C/apoB ratio ≥3.69 mmol/g [21], and the non-HDL-C/apoB ratio >4.91 mmol/g [22], using the ESSE-Ivanovo population sample (for subjects without lipid-lowering therapy and

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with complete lipid values, n = 1550). The ε2ε2 haplotype carriers were considered conditionally ill, whereas conditionally healthy are those without the ε2ε2 haplotype. The sensitivity was calculated as the ratio of true positive (the number of cases correctly identified as ε2ε2 haplotype carriers)/true positive + false negative (the number of cases incorrectly identified as healthy). The specificity was calculated as the ratio of true negative (the number of cases correctly identified as healthy)/true negative + false positive (the number of cases incorrectly identified as ε2ε2 haplotype carriers) [35].

The prevalence of FD was determined based on the ESSE-Ivanovo population sample (n = 1656) **(Figure 3).**

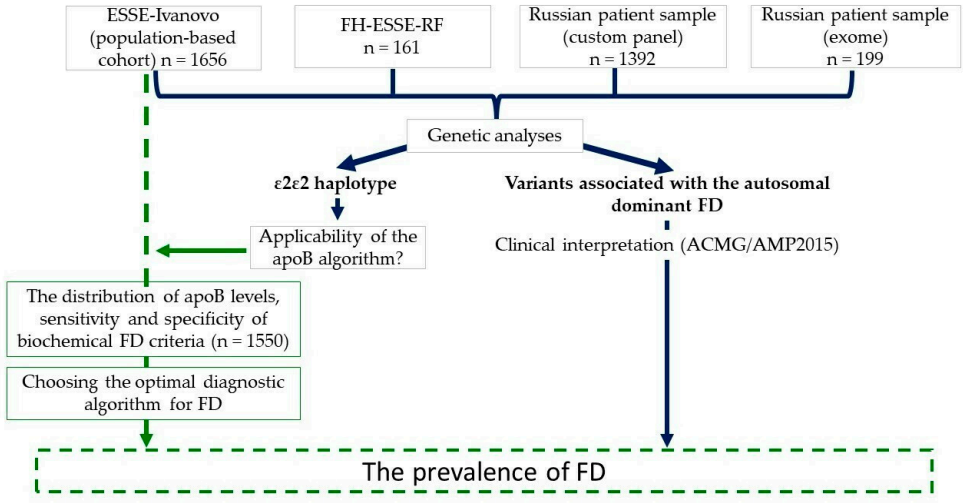


**Figure 3.** Study design.

*4.3. Next Generation Sequencing*

Genomic DNA was isolated from peripheral blood with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). DNA concentration was measured on the Qubit 4.0 fluorimeter (Thermo Fisher Scientific, Waltham, MA, USA). For next generation sequencing (NGS), the libraries were prepared using either the SeqCap EZ Prime Choice Library kit (Roche, Basel, Switzerland) for targeted sequencing or the IDT-Illumina TruSeq DNA Exome protocol (Illumina, San Diego, CA, USA) for exome libraries. Sequencing was performed on a Nextseq 550 (Illumina, San Diego, CA, USA). All stages of sequencing were conducted according to the manufacturers' protocols.

*4.4. Bioinformatic Processing of Sequencing Data*

All bioinformatic analyses were described in more detail in a previous study of the ESSE-Ivanovo sample [34].

The processing of sequencing data and quality control evaluation were done using the custom-designed pipeline [34] based on GATK 3.8 [36]. Paired-end reads were aligned to the GRCh37/hg19 reference genome. As a result, individual VCF files were obtained for each subject, containing a list of variants, their genomic coordinates, coverage data, and other characteristics. Low-quality variants, which are presumably sequencing errors, were filtered out. The coverage depth of the reference and alternative alleles, the quality of reads and mapping, and other relevant factors were reported and analyzed.

The ε2ε2 haplotype of the *APOE* gene was determined to be homozygous for the ε2 allele (rs7412) in combination with no alteration in rs429358 (**Table 6**).

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**Table 6.** *APOE* variants that define the ε2ε2 haplotype.

| Gene | Variant | Genomic Coordinates (GRCh37) | Reference allele | Alternative allele | Genotype | HGVSc, HGVSp |
|---|---|---|---|---|---|---|
| *APOE* | rs7412 | chr19: 45412079_C/T | C | T | T/T | NM_000041.4:c.526C>T NP_000032.1:p.Arg176Cys |
| *APOE* | rs429358 | chr19: 45411941_T/C | T | C | T/T | NM_000041.4:c.388T>C NP_000032.1:p.Cys130Arg |

HGVSc —Human Genome Variation Society coding sequence name; HGVSp — Human Genome Variation Society protein sequence name.

Previously reported *APOE* variants associated with the autosomal dominant FD, as well as rare *APOE* variants, were analyzed [14,15].

The annotation of rare *APOE* variants was performed with OMIM [37], gnomAD (v2.1.1) [38]), ClinVar (2021/01/10) [39], Human Gene Mutation Database (HGMD) [40], Leiden Open Variation Database (LOVD) [41], dbSNP [42] databases, and literature data. The clinical interpretation is based on the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP2015) guidelines [43].

### *4.5. Sanger Sequencing*

The validation of results by Sanger sequencing was conducted for the c.526C>T (p.Arg176Cys) variant, all known variants associated with the autosomal dominant form of FD, as well as rare *APOE* variants. The DNA sequencer Applied Biosystem 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) was used for Sanger sequencing together with the ABI PRISM BigDye Terminator v3.1 reagent kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's protocol.

### *4.6. Statistical Analyses*

Statistical analyses were done using R v. 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) [*44*]. The data is presented as a median (25th–75th percentile). We calculated the prevalence of FD by dividing the number of people with FD by the total sample size. The prevalence of FD was worked out as a percentage for all participants. The Clopper-Pearson exact method was used for the estimation of the 95% confidence interval. A p-value of less than 0.05 was considered statistically significant.

## 5. Conclusions

A high prevalence of FD (1 in 151) was detected in one of the European regions of Russia. Underdiagnosis is typical for FD. The diagnostic algorithms of FD including a diagnostic apoB levels require correction, taking into account the characteristics of the distribution of apoB levels in the population. At the same time the TG cutoff ≥1.5 mmol/L may be a useful tool in identifying subjects with FD.

**Supplementary Materials:** The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: The clinical interpretation of the detected *APOE* variants, associated with the autosomal dominant FD; Table S2: Characteristics of ESSE-Ivanovo sample

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administration, A.I.E. and A.N.M.; software, V.A.K., A.A.Z., V.E.R., Y.V.V. and M.Z.; resources, A.I.E., S.A.S., A.N.M. and O.M.D.; supervision, O.M.D.; funding acquisition, A.I.E., S.A.S., A.N.M. and O.M.D. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Written informed consent has been obtained from all the patients involved in this study.

**Data Availability Statement:** The data used and/or analyzed during the current study are available from the corresponding authors on reasonable request. Individual genotype information cannot be made available in order to protect participant privacy.

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