Polygenic risk in Type III hyperlipidaemia and risk of cardiovascular disease: An epidemiological study in UK Biobank and Oxford Biobank

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ABSTRACT

Background: Type III hyperlipidaemia (T3HL) is characterised by equimolar increases in plasma triglycerides (TG) and cholesterol in <10% of APOE22 carriers conveying high cardiovascular disease (CVD) risk. We investigate the role of a weighted triglyceride-raising polygenic score (TG.PS) precipitating T3HL.

Methods: The TG.PS (restricted to genome-wide significance and weighted by published independent effect estimates) was applied to the Oxford Biobank (OBB, n = 6952) and the UK Biobank (UKB, n = 460,037), to analyse effects on plasma lipid phenotypes. Fasting plasma lipid, lipoprotein biochemistry and NMR lipoprotein profiles were analysed in OBB. CVD prevalence/incidence was examined in UKB.

Results: One TG.PS standard-deviation (SD) was associated with 13.0% (95% confidence-interval 12.0–14.0%) greater TG in OBB and 15.2% (15.0–15.4%) in UKB. APOE22 carriers had 19.0% (1.0–39.0%) greater TG in UKB. Males were more susceptible to TG.PS effects (4.0% (2.0–6.0%) greater TG with 1 TG.PS SD in OBB, 1.6% (1.3–1.9%) in UKB) than females. There was no interaction between APOE22 and TG.PS, BMI, sex or age on TG. APOE22 carriers had lower apolipoprotein B (apoB) (OBB: −0.35 (−0.29 to −0.40)g/L, UKB: −0.41 (−0.405 to −0.42)g/L). NMR lipoprotein lipid concentrations were discordant to conventional biochemistry in APOE22 carriers.

In APOE22 compared with APOE33, CVD was no more prevalent in similarly hypertriglyceridaemic participants (OR 0.97 (95%CI 0.76–1.25)), but was less prevalent in normolipidaemia (OR 0.81, 95%CI 0.69–0.95); no differences were observed in CVD incidence.

Conclusions: TG.PS confers an additive risk for developing T3HL, that is of comparable effect size to conventional risk factors. The protective effect of APOE22 for prevalent CVD is consistent with lower apoB in APOE22 carriers.

1. Introduction

Type III hyperlipidaemia (T3HL), also known as dysbeta lipoproteinaemia or remnant removal disease, is a rare type of hyperlipidaemia, mostly seen in males. It is characterised by remarkable equimolar increases in triglycerides and cholesterol, precipitating early-onset atherosclerotic disease. T3HL is seen in individuals homozygous for APOE ε2 allele, the genetic basis of which was first described by Utermann and colleagues [1,2]. Approximately 1% of the population carry the APOE22 genotype, but <10% of them exhibit T3HL [1,3,4].

There are three common APOE alleles (ε2, ε3, ε4) determined by two single nucleotide polymorphisms (SNPs), rs7412 and rs429358, situated...
on chromosome 19 [3]. The protein encoded by APOE, apolipoprotein E (apoE), plays a role in hepatic clearance of triglyceride-rich lipoproteins (Very Low-Density Lipoprotein [VLDL]), chylomicrons and their remnants) through its ligand action to the Low-Density Lipoprotein receptor (LDLR), but also to LDL receptor related protein (LRP1) and other heparan sulphate proteoglycan receptors. Additional actions of apoE include modulating lipoprotein triglyceride lipolysis and hepatic VLDL production [1,3]. The ε2 variant of apoE, through an Arg158 > Cys substitution, causes a change in salt-bridge formation, consequently reducing ApoE2/2/ε2 binding capacity to LDLR to <1% [3,6]. Defective lipolysis of hepatic VLDL and intestinal chylomicron remnants is caused by slower lipoprotein lipase-mediated lipolysis, due to displacement/masking of Apo-CII [1].

The combined effect of these events is prolonged residence time of remnant lipoprotein particles in the circulation, allowing for cholesteryl ester transfer protein-mediated deposition of cholesteryl esters into remnant particles. The ApoE2/2/ε2 remnant lipoprotein particles are therefore characterised by unusually high cholesterol:triglyceride and cholesteryl:apolipoprotein B (apoB) ratios. The abnormal remnant particles can no longer be lipolyzed into LDL, resulting in low transfer of VLDL apoB into LDL apoB in APOE22 carriers. [7]. Therefore, APOE22 carriers exhibit low LDL cholesterol and apoB plasma concentrations, compared with non-carriers [5].

The causes of APOE22 carrier status leading to T3HL are not fully understood. Utermann suggested that it is potentially a “polygenic disorder” precipitating the APOE22 carrier status into T3HL [8]. The established acquired risk factors such as obesity, Type-2 Diabetes and alcohol excess represent a metabolic overload on the lipoprotein transport system. A recent prospective study highlighted deleterious effects of prolonged adiposity in precipitating T3HL [5].

The unusual characteristics of T3HL often lead to delayed diagnosis, although lipidologists typically recognise T3HL. Although fibrates constitute the first line pharmacological treatment, reversal of underlying precipitating factors is equally important. Polygenic lipid risk scores have not been explored as a potential T3HL risk factor, despite growing knowledge from large scale genome-wide association studies (GWAS) on lipid phenotypes [9].

We aimed to examine the effect of a polygenic lipid risk score on plasma triglyceride concentration (TG) and hyperlipidaemia expression, in the context of APOE22. We aimed to explore the effect of a weighted triglyceride-raising polygenic score (TG.PS) in the context of other established risk factors such as age and sex, and compare its effect with a modifiable risk factor, BMI. With the availability of NMR-based plasma lipidomic assessments, we aimed to investigate the performance of this platform for the unusual lipoprotein profile associated with APOE22 carrier status. Finally, we aimed to investigate the relationship of normolipidaemia and hypertriglyceridaemic APOE22 carriers with prevalent and incident cardiovascular disease (CVD) in the UK Biobank.

2. Methods

2.1. Population

Oxford Biobank (OBB) collected data on ~8000 participants living in Oxfordshire, of ages 30–50 on enrolment [10]. UK Biobank (UKB) collected data on ~500,000 participants living in the UK, of ages 40–69 on enrolment [11,12]. Past medical history, anthropometric measurements and biological samples were obtained at screening visits in both biobanks. Body mass index (BMI) was calculated as kg/m² based on direct measurements. We provide a detailed general description of the studies in supplementary 1. The methods of these studies have been described elsewhere [10–12]. OBB was granted ethical approval by the Oxfordshire Clinical Research Ethics Committee (08/H0606/107 + 5). UKB was granted ethical approval by the Northwest Centre for Research Ethics Committee (11/NW/0382) [10–12]. The population in this analysis comprised of 6952 participants from the OBB and 460,037 participants from the UKB. Participant and analysis-results flow charts are shown in supplementary 2.

2.2. Genotyping

The two APOE-determining SNPs on chromosome 19, rs7412 and rs429358, were extracted to derive the APOE genotype for each participant in OBB and UKB (see supplementary 3). OBB in-house APOE genotyping was generated using Taqman Genotyping assays (AOD ABI assay) and data were concordant with the APOE genotype of the Affymetrix chip. Genotyping in UKB was performed using the UK Biobank Axiom array for 438,427 individuals and the UK BILEVE Axiom array (Affymetrix) for 49,950 individuals, with the two arrays exhibiting 95% common marker content [11].

2.3. Lipid measurements

For standard biochemistry in OBB, fasting TG and total cholesterol were analysed with Instrumentation Laboratory II Test™ kits on ILab 600/650 clinical chemistry analysers (Werfen, Warrington, UK). High Density Lipoprotein (HDL) cholesterol and apoB were analysed with Random kits adapted for lab 600/650 analysers (Random Laboratories, Crumlin, Northern Ireland) [10].

NMR metabolomics (Nightingale Health, Helsinki, Finland) analysis was undertaken on 6573 OBB participant plasma specimens, with information available on ~230 metabolites [10]. A list of metabolites is shown in supplementary 4.

For standard biochemistry in UKB, non-fasting TG were assayed using enzymatic analysis and apoB with immuno-turbidimetric analysis. The analytical platform used for assay outputs was Beckman Coulter AU800 (Beckman Coulter, UK) [13].

2.4. Cardiovascular disease (CVD) profile

UKB linkage to Hospital Episode Statistics (HES) and death registry was utilised to define CVD status of each UKB participant, using ICD9 and ICD10 codes informed by UKB data showcase. Death registry data were available from April 2006 until 31st December 2020. HES data were available from April 1991 until 31st December 2020. Where participants had recurrent CVD events, only the first event was included. Prevalent CVD was defined as any episode of angina, acute or chronic ischaemic heart disease, ischaemic stroke, peripheral vascular disease, myocardial infarction of any type and any complications recorded in HES and/or death registry, but not self-reported. Incident CVD was defined as the first time CVD appeared in participants’ HES or death records during the follow-up period (i.e. after the baseline visit). Participants with recorded inpatient CVD; ischaemic stroke/cerebral ischaemia; peripheral vascular disease or atherothrombotic disease episodes prior to baseline visit were excluded from incident analyses (details shown in supplementary 5).

2.5. Statistical analysis

All analyses were performed using R, version 4.0.0 [14,15]. Complete case analyses were used for both OBB and UKB main analyses based on information availability for the following parameters; age, sex, BMI, TG, apoB, APOE genotype and gene dosages for the 107 TG.PS variants. Separately, for the NMR analysis in OBB, the study population was based on availability of information on APOE genotype, TG (standard biochemistry) and biochemistry and NMR results for apoB, total cholesterol and HDL cholesterol concentrations.

Summary statistics are presented as mean (standard deviation; SD), unless skewed, in which case they are presented as median (interquartile range; IQR). Hyperlipidaemia (in relation to the T3HL phenotype) was defined as TG >3.0 mmol/L and T3HL was defined as hyperlipidaemia in an APOE22 carrier. Normolipidaemia was defined as TG ≤3.0 mmol/ L.
To derive the TG.PS we used results from the GWAS conducted by the Global Lipids Genetic Consortium (GLGC), which identified 444 independent variants with effects on plasma lipid phenotypes. The OBB contributed genetic data for 4442 of about 300,000 individuals to the variants restricted to genome-wide significance. Clumping was performed at $r^2 < 0.1$ and a window of 3 megabase pairs (Mb). Four variants within 1 Mb proximity to APOE-related variants rs769455, rs769449 and rs7412 as identified by GLGC GWAS, which remained after clumping were removed (rs28399654, rs769449, rs439401, rs445925). Clumping was carried out using the MRCIEU/Two Sample MR package in R [17]. TG.PS for each individual were constructed as the sum of the number of triglyceride-associated effect alleles weighted by their published single-variant effect sizes on triglyceride. In total, 107 independent variants were included in the TG.PS. TG.PS was $Z$-transformed to present the scores in standard units for ease of comparison. Included variants were extracted from UKB genotype data. The genotype and quality control procedures for the UKB and OBB are described in detail elsewhere [10,11,18]. Additional information on the APOE and TG.PS variants in OBB and UKB are shown in Supplementary 6 (spreadsheet).

Heterogeneity of variance in TG of APOE22 compared with the rest of APOE genotypes was examined using Levene’s test. Log-transformation was used to account for skewed TG distribution. General linear regression was used for continuous outcome variables (TG, apoB) and logistic regression was used for the binary outcome variable (hyperlipidaemia) to compute odds ratios and associated 95% confidence intervals. TG and hyperlipidaemia were regressed on TG.PS and adjusted for age, sex, BMI and APOE genotype. Along with the main effects terms, interaction terms (Age $\times$ APOE22, Sex $\times$ APOE22, BMI $\times$ APOE22, TG.PS $\times$ APOE22 and in a separate model, Sex $\times$ TG.PS) were included to assess effect modification. Sensitivity analyses with additional adjustments were performed in UKB to assess the robustness of the results. These sensitivity analyses accounted for: sample dilution errors, fasting status, lipid-lowering medication (restricting analyses to participants not on lipid-lowering medication), of whom 500 (299 males) were defined as having hyperlipidaemia, of whom 7 (all males) were defined as having T3HL. In UKB, 46,082 participants were defined as hyperlipidaemic, of whom 500 (299 males) were defined as having T3HL. Baseline characteristics of incomplete case datasets in OBB and UKB are shown in supplementary 7. Participant and analysis-results flow charts are shown in supplementary 2.

### 3. Results

#### 3.1. Baseline characteristics

Table 1 shows the baseline characteristics for OBB and UKB participants included in the main analyses. In OBB, 175 individuals were defined as having hyperlipidaemia, of whom 7 (all males) were defined as having T3HL. In UKB, 46,082 participants were defined as hyperlipidaemic, of whom 500 (299 males) were defined as having T3HL. Baseline characteristics of incomplete case datasets in OBB and UKB are shown in supplementary 7. Participant and analysis-results flow charts are shown in supplementary 2.

#### 3.2. Heterogeneity of variance and regression analyses

In OBB, the variance in TG was greater in APOE22 carriers compared with other APOE genotypes (1.47 mmol/L versus 0.53 mmol/L, $p = 5.31 \times 10^{-6}$). Similar heterogeneity of variance in TG was observed in UKB, (1.31 mmol/L versus 0.99 mmol/L, $p = 2.39 \times 10^{-6}$).

The TG.PS effect on TG, in the context of other innate and modifiable risk factors, is shown in Table 2 for UKB (effect estimates and standard error shown in supplementary 8). One SD higher TG.PS was associated with 15.2% higher TG. Carrier status of the APOE22 genotype was associated with 19.0% higher TG; male sex with 21.0% higher TG; 3.4% increase in TG for 1 kg/m$^2$ increase in BMI and by $0.63\%$ for each year of age. APOE22 did not show interaction with the TG.PS, BMI, age or sex in raising TG (Table 2). In OBB, the TG.PS effect was comparable to UKB, with 1 SD associated with 13.0% higher TG (shown in supplementary 8). Sensitivity analyses assessing the impact of model composition on the TG.PS effect, showed similar effect estimates (see supplementary 9).

### Table 1

<table>
<thead>
<tr>
<th>Demographics</th>
<th>OBB</th>
<th>%</th>
<th>UKB</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>6952</td>
<td>–</td>
<td>460,037</td>
<td>–</td>
</tr>
<tr>
<td>Male (n)</td>
<td>3025</td>
<td>43.5</td>
<td>210,284</td>
<td>45.7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.6 (SD 5.9)</td>
<td>–</td>
<td>56.5 (SD 8.1)</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.8 (SD 4.6)</td>
<td>–</td>
<td>27.4 (SD 4.8)</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipid concentrations</th>
<th>Triglyceride plasma concentration (mmol/L)$^a$</th>
<th>0.94 (IQR)</th>
<th>1.48 (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.70–1.35)</td>
<td>1.05–2.14</td>
<td></td>
</tr>
<tr>
<td>apoB plasma concentration (g/L)</td>
<td>0.89 (SD 0.23)</td>
<td>1.03 (SD 0.24)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APOE genotype (n)</th>
<th>E2/E2</th>
<th>53</th>
<th>0.8</th>
<th>2564</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E1/E3</td>
<td>4205</td>
<td>60.5</td>
<td>270,425</td>
<td>58.8</td>
</tr>
<tr>
<td></td>
<td>E3/E4</td>
<td>142</td>
<td>2.0</td>
<td>10,993</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>E2/E3 (or E3/E2)$^b$</td>
<td>839</td>
<td>12.1</td>
<td>56,198</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>E2/E4 (or E4/E2)$^b$</td>
<td>156</td>
<td>2.2</td>
<td>11,526</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>E3/E4 (or E4/E3)$^b$</td>
<td>1557</td>
<td>22.4</td>
<td>108,312</td>
<td>23.5</td>
</tr>
</tbody>
</table>

| Rarer genotypes$^c$ | – | – | 19 | 0.004 |

$^a$ Fasting TG was available in OBB. Non-fasting TG was available in UKB.

$^b$ E1/E3 coded as E2/E4 in UKB, as E1 allele is rare and these are ambiguous genotypes [28].

$^c$ Sum of rarer APOE genotypes: E1/E2 or E2/E1, E1/E4 or E4/E1.
Table 2
Linear regression analysis: The effect of innate and modifiable risk factors on TG in UKB.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>% change in TG (95%CD)$^a$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) ± 1</td>
<td>0.63 (0.62–0.65)</td>
<td>$&lt;10^{-300}$</td>
</tr>
<tr>
<td>Sex (M = 1, F = 0)</td>
<td>21.0 (20.8–21.5)</td>
<td>$&lt;10^{-300}$</td>
</tr>
<tr>
<td>BMI (kg/m$^2$) ± 0.5</td>
<td>3.72 (3.34–4.10)</td>
<td>$&lt;10^{-300}$</td>
</tr>
<tr>
<td>APOE22 (Yes = 1, No = 0)</td>
<td>19.0 (1.0–39.0)</td>
<td>0.0390</td>
</tr>
<tr>
<td>TG.PS (SD) ± 0.15</td>
<td>15.2 (15.0–15.4)</td>
<td>$&lt;10^{-300}$</td>
</tr>
<tr>
<td>Age × APOE22</td>
<td>0 (–0.1 to 0)</td>
<td>0.227</td>
</tr>
<tr>
<td>Sex × APOE22</td>
<td>3.0 (–1.1 to +6)</td>
<td>0.172</td>
</tr>
<tr>
<td>BMI × APOE22</td>
<td>–0.2 (–0.5 to 0)</td>
<td>0.364</td>
</tr>
<tr>
<td>TG.PS × APOE22</td>
<td>–1.9 (–2.0)</td>
<td>0.987</td>
</tr>
</tbody>
</table>

$^a$ % change per unit of covariate.

Analyses dedicated to comparing the effect magnitude of investigated TG risk factors are shown in supplementary 10 and supplementary 11. Notably, BMI ≥ 30.0 kg/m$^2$ (≥ 0.54 SDs) was associated with 32% higher ($p < 10^{-300}$) TG and an OR for hyperlipidaemia of 2.52 (95%CI 2.47–2.58, $p < 10^{-300}$) in UKB. By comparison, a TG.PS ≥ 0.54 SDs (equivalent population distribution point to BMI ≥ 30.0 kg/m$^2$) led to 25% higher ($p < 10^{-300}$) TG in UKB, and was associated with an OR of 2.57 (95%CI 2.51–2.62, $p < 10^{-300}$) for hyperlipidaemia.

Fig. 1 demonstrates that a higher TG.PS was similarly associated with higher TG, in OBB and UKB across the APOE genotypes. Males were more susceptible to the TG.PS effect than females with a further +4.0% (2.0–6.0, $p = 1.4 × 10^{-35}$) on top of the −11.0% (9.0–22.0, $p = 8.2 × 10^{-35}$) in OBB and +1.6% (1.3–1.9, $p = 6.4 × 10^{-25}$) on top of the +14.0% (14.0–15.0, $p < 10^{-300}$) in UKB increase in TG per SD of TG.PS; full results shown in supplementary 12.

3.3. Lipid phenotype of APOE22

APOE22 carriers had a lower plasma apoB concentration (biochemistry) in comparison to the rest of APOE genotypes (OBB estimate(SE) = −0.348 (0.0285), $p = 5.97 × 10^{-34}$, UKB estimate(SE) = −0.414 (4.65 × 10^{-35}), $p < 10^{-300}$, supplementary 13). Fig. 2 illustrates the association between apoB concentration and non-HDL cholesterol by APOE genotype using biochemistry (A) and NMR (B) analyses in 6568 participants in OBB (52 APOE22 carriers, 7 of whom defined as having T3HL). Fig. 2A shows that individuals with APOE22 have a higher non-HDL cholesterol:apoB ratio. NMR analysis in Fig. 2B however, shows an opposite effect on APOE22 genotype status. Linear regression analyses, with these interaction effects, are shown in supplementary 14. A direct comparison between biochemistry and NMR analyses for apoB, total cholesterol, HDL cholesterol and non-HDL cholesterol (shown in supplementary 15) demonstrates comparable NMR lipid measurements with biochemistry for the majority of OBB population, but in individuals with the APOE22 genotype, NMR was discordant with biochemistry, with larger discordance observed in T3HL.

3.4. Cardiovascular disease of APOE22

Cross-sectional and prospective CVD analyses, restricted to individuals with APOE22 and APOE33 genotype, were performed in UKB. Table 3 shows the participants who were identified as having CVD.

3.5. Cross-sectional analyses

In APOE22 carriers, hyperlipidaemia (T3HL) exhibited increased risk of prevalent CVD (OR 1.96, 95%CI 1.47–2.62, $p = 3.1 × 10^{-06}$). In normolipidaemia, APOE22 carriers exhibited lower risk of prevalent CVD than normolipidaemic APOE33 carriers (OR 0.81, 95%CI 0.69–0.95, $p = 0.01$); APOE22 was inversely associated with prevalent CVD. There was no evidence of an association with prevalent CVD in participants with T3HL compared with hyperlipidaemic APOE33 carriers (OR 0.97, 95%CI 0.76–1.25, $p = 0.84$). The protective effect of APOE22 genotype was lost in normolipidaemia when the analysis was adjusted for known atherogenic risk factors, apoB concentration and lipid-lowering medication (OR 1.06, 95%CI 0.90–1.25, $p = 0.49$). When individuals on lipid-lowering medications were excluded and the analysis was adjusted for apoB concentration, CVD was more prevalent in normolipidaemic APOE22 carriers than APOE33 carriers (OR 1.44, 95% CI 1.17–1.76, $p = 3.90 × 10^{-04}$). Adjusted analyses showed no

Fig. 1. TG against TG.PS in study participants, by sex and APOE genotype.
Key: (A) corresponds to OBB participants and (B) to UKB participants. Black circles indicate APOE22 carriers with triglyceride concentration > 3.0 mmol/L (T3HL). Coloured lines are fitted from linear models for the relation between TG.PS and TG. Shaded areas around the lines represent the 95%CI. The horizontal black line on the y-axis is placed at 3.0 mmol/L (indicative of hyperlipidaemia).
In APOE22 carriers, the shape of the hyperlipidaemia exhibited increased incidence of CVD (HR 1.95, 95%CI 1.33–2.75, \( p = 2.65 \times 10^{-06} \)). In normolipidaemia, APOE22 carriers exhibited a similar incidence of CVD to normolipidaemic APOE33 carriers (HR 0.91, 95%CI 0.76–1.08, \( p = 0.28 \)). There was no difference in CVD incidence in participants with T3HL compared with hyperlipidaemic APOE33 carriers (HR 0.99, 95%CI 0.75–1.31, \( p = 0.94 \)). Incidence of CVD was greater in normolipidaemic APOE22 carriers than normolipidaemic APOE33 carriers (HR 1.41, 95%CI 1.17–1.69, \( p = 2.56 \times 10^{-06} \)) in adjusted analyses, an effect which was more extreme when individuals on lipid-lowering medication were excluded and the analysis was adjusted for apoB concentration (HR 1.66, 95%CI 1.35–2.05, \( p = 2.29 \times 10^{-06} \)). Adjusted analyses showed no difference in incidence of CVD in hyperlipidaemic APOE22 carriers compared with hyperlipidaemic APOE33 carriers (full results shown in supplementary 16).

Similar effects were obtained when analysing the male participants alone (supplementary 17).

4. Discussion

Our main findings suggest that TG.PS should be considered as an additive T3HL risk factor. Its lifelong effect is not modified by the APOE22 genotype, and is of comparable effect size to previously described T3HL risk factors such as age and obesity. Therefore, consideration of this background risk may help better understand T3HL manifestation, thus enable timely diagnosis and even tailor preventative management in individuals at risk.

Polygenic risk underlying hypertriglyceridaemia and hyperlipidaemia in general is described in literature [9,20]. Our results support these findings and build on them by quantifying the effect it has on the risk of T3HL. The known difference in plasma lipids between normolipidaemic APOE22 carrier status (here defined as plasma TG \( \leq 3.0 \) mmol/L) and transformation to T3HL can be complexly characterised by drastic equimolar rise in plasma TG and cholesterol (typically defined as an absolute rise in plasma lipids). Our adjusted analyses showed that the effect of another risk factor on TG levels causing greater variance, an observation also observed by others [21]. This fits well with the known clinical picture of T3HL, which can develop rapidly into severe combined hyperlipidaemia, and likewise readily disappear upon adequate treatment. This interaction also has similarities with familial hypercholesterolaemia (FH) and how its disease phenotype can be modulated by an LDL-cholesterol raising polygenic trait. First, an extreme LDL-C polygenic trait can provide a phenocopy of FH [22]. Second, the LDL-C polygenic trait appears to have an additive effect to the LDL phenotype, not only for the plasma LDL-C concentration but also for the polygenic trait can provide a phenocopy of FH [22]. Second, the LDL-C polygenic trait appears to have an additive effect to the LDL phenotype, not only for the plasma LDL-C concentration but also for the...
We investigate effect modification and the results from these analyses demonstrated that while APOE22 did not appear to modify the effect of the risk factors, we observed an additive effect of all of these risk factors on raising TG. Sex modified the TG.PS effect, with males being slightly more prone to its TG-raising effects: an increase in effect by 2% in UKB and 4% in OBB. A reduced prevalence of hyperlipidaemia in pre-menopausal females is observed by others [24]. T3HL is no exception, as in the female population, literature suggests that it is mostly after menopause [25]. Our observed positive associations between BMI and age, with hypertriglyceridaemia, agree with published literature [5,6]. Moreover, we demonstrate that the TG.PS had an effect that was robust and comparable in magnitude to the other established risk factors indicating its potential value in a clinical setting where available.

We used NMR-lipidomics to analyse the lipoprotein composition of individuals with APOE22 and T3HL. Initial analysis using standard biochemistry in OBB, showed that APOE22 carriers have higher non-HDL:ApoB ratio, in comparison to the rest of APOE genotypes, with more extreme values observed in hypertriglyceridaemic APOE22 carriers. This observation was based on a small number of APOE22 carriers (45 normolipidaemic carriers and 7 T3HL) versus 6516 individuals with other APOE genotypes. Notably, when performing the same analysis on the same OBB participants using NMR, we observed an opposite effect. Directly comparing biochemistry and NMR analysis for apoB, total cholesterol, HDL and non-HDL cholesterol, we observed that NMR was paradoxical in quantifying lipid concentrations for APOE22 carriers, in comparison to other APOE genotypes. These results may reflect that the structural changes to the lipoproteins caused by the APOE22 genotype are outside the boundaries of the algorithms used for the lipoprotein subtraction inferences from NMR signals.

APOE22 carriers with TG $\leq$ 3.0 mmol/L, appeared to be more protected against CVD than APOE33 carriers with similar TG, by an almost 20% reduced risk in cross-sectional CVD. Corresponding CVD incidence analyses did not show a statistically significant effect (although directionally consistent). A directionally consistent effect of APOE22 against coronary artery disease was also observed (OR 0.83, 95% CI 0.55–1.25), when compared to APOE33, in a meta-analysis by Bennet et al. [26]. The lack of association in incident CVD analysis in normolipidaemia, could be due to our stricter incident CVD definition, with reduced numbers of individuals having suffered from CVD for the first time after entering UKB (in the absence of any past episodes of atherosclerotic disease). In contrast to the normolipidaemic state, in the hyperlipidaemia, our analysis showed a similar risk of CVD to APOE33 hyperlipidaemic individuals which perhaps is somewhat surprising, as the perception arising from individual T3HL cases would indicate that the condition is highly atherogenic. However, looking at our observation on increased variance of triglycerides amongst APOE22 carriers may suggest that people with this genetic background dip in and out of hyperlipidaemia. This observation argues for more frequent monitoring of patients showing signs of T3HL.

Lower apoB plasma concentrations in APOE22 compared to other APOE genotypes was seen in our analysis. Similarly, Li et al. [27] also observed this lower apoB (in UKB). The substantially lower apoB concentration is likely to be inversely associated with atherosclerosis and coronary artery disease, thereby explaining the reduced CVD risk we observe in normolipidaemia [5]. However, we also observe a reversal of the apolipoprotein B to total cholesterol ratio for incident CVD when adjusting for plasma apoB in APOE22 carriers. This could indicate that the abnormal lipoprotein pattern seen in APOE22 even in normolipidaemia is conveying some risk, independently of the inverse association with apoB, and that highly cholesterol-enriched remnant particles may already be present in abundance in the normolipidaemic state. The adjustment for total lipoprotein particle number (apoB) could therefore bring out a qualitative effect of the lipoprotein composition with a possible negative effect on the build-up of atherosclerotic CVD. Li et al. [27] also observed a potential discordance in ischaemic heart disease (IHD) and found that APOE22 carriers, compared with APOE33, had no significant difference in risk apart from marginally increased risk in younger ($<$60 years) participants.

4.1. Limitations

Only 2617 participants were APOE22 carriers out of 466,989 individuals, in OBB and UKB. 507 (0.1% of the total population in the two cohorts) met the T3HL definition, resulting in a relatively low number of cases despite the large total sample size, thus limiting statistical power. We were unable to account for the type and dose of lipid-lowering medication in our analyses as these data were not available. We therefore assumed equal prevalence between the genotypic groups. It is possible that more people in the APOE33 group are on statins as they have higher total cholesterol which would lead to a potential bias towards the null in the analyses we carried out.

Although T3HL was defined based on APOE22 genotype and concomitant TG $>3.0$ mmol/L, we did not have access to a documented clinical T3HL diagnosis. Whilst we were able to use fasting TG in OBB, UKB analysis was based on non-fasting values. The TG.PS was constructed using 107 independent triglyceride-raising variants, restricted to genome-wide significance, identified in an independent GWAS with minor population overlap. OBB contributed genetic data for $\approx1.5\%$ of the 300,000 individuals in whom data were utilised to identify these variants [9]. The TG.PS alone explained 6.4% of the variance in TG in OBB ($F = 474.8, p < 2.2 \times 10^{-16}$) and 5.9% in UKB ($F = 28,920, p < 2.2 \times 10^{-16}$), indicating relatively strong performance and unlikely to cause concern for bias.

4.2. Conclusion

In conclusion, we described the additive effect of a restricted to genome-wide significance TG.PS in precipitating hyperlipidaemia, of comparable effect size to the other known innate and modifiable risk factors for developing T3HL. Although TG.PS alone is not sufficient in precipitating T3HL, knowledge of the polygenic burden of APOE22 carriers may help identify individuals at increased risk of T3HL. It may enable individualised monitoring and lifestyle modifications, facilitating earlier disease prevention or diagnosis and prompt management to reduce the burden of severe early-onset CVD. Moreover, we observed a potential limitation of NMR-based lipidomic assessment in individuals with APOE22 genotype.

Author contributions

KP performed the analyses and wrote the original manuscript. ET, HT and DB facilitated access to UKB data and advised on analyses. MJN facilitated access to OBB data. FK conceptualised the study, provided supervision and contributed to writing the original manuscript. RWK conceptualised the study, supervised, contributed to analyses and writing the original manuscript. All authors contributed to review and editing of the final manuscript. All authors approved the final manuscript.

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Declaration of Competing Interest

RWK has received consulting fees from Novo Nordisk. The Authors claim no other conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2022.11.024.

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