Transcription factor 7-like 2 single nucleotide polymorphisms rs290487 and rs290481 are associated with dyslipidemia in the Balinese population

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Background. Dyslipidemia is one of the major risks for the development of cardiovascular diseases which has been the leading cause of death in developing countries. Previously, common polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene have been associated with altered lipid profiles. In this study, we investigated the associations of *TCF7L2* SNPs, rs290487 and rs290481, with dyslipidemia and altered lipid profile in the Balinese.

Methods. A total of 565 subjects from four locations in the Bali Province, Indonesia, were recruited. Serum lipid concentrations [triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC)] were measured using standard protocol. SNP genotyping was done using the amplification refractory system mutation polymerase chain reaction (ARMS-PCR) method.

Results. We found the shifted major/minor allele frequencies of both SNPs (0.56 for rs290487 T allele, 0.53 for rs290481 T allele) in the Balinese, as compared to dbSNP. The rs290487 and rs290481 C alleles were significantly associated with dyslipidemia, particularly high TC and high LDL-C. These associations were independent of age, sex, population, obesity, diabetes mellitus, and high TyG index as a proxy of insulin resistance. The haplotype CC also showed similar association with these traits. Our findings indicate that *TCF7L2* polymorphisms are associated with dyslipidemia and altered lipid profile in the Balinese.

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Abstract

22 **Background.** Dyslipidemia is one of the major risks for the development of cardiovascular
23 diseases which has been the leading cause of death in developing countries. Previously, common
24 polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene have been associated with
25 altered lipid profiles. In this study, we investigated the associations of *TCF7L2* SNPs, rs290487
26 and rs290481, with dyslipidemia and altered lipid profile in the Balinese.
27 **Methods.** A total of 565 subjects from four locations in the Bali Province, Indonesia, were
28 recruited. Serum lipid concentrations [triglycerides (TG), low-density lipoprotein cholesterol
29 (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC)] were measured
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31 mutation polymerase chain reaction (ARMS-PCR) method.
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33 allele, 0.53 for rs290481 T allele) in the Balinese, as compared to dbSNP. The rs290487 and
34 rs290481 C alleles were significantly associated with dyslipidemia, particularly high TC and
35 high LDL-C. These associations were independent of age, sex, population, obesity, diabetes
36 mellitus, and high TyG index as a proxy of insulin resistance. The haplotype CC also showed
37 similar association with these traits. Our findings indicate that *TCF7L2* polymorphisms are
38 associated with dyslipidemia and altered lipid profile in the Balinese.
39 Keyword: Dyslipidemia, SNPs, *TCF7L2*, rs290487, rs290481, Lipid Profiles
40

Introduction

41
42 Dyslipidemia is one of the risk factors for the development of cardiovascular diseases, the major
43 cause of death in developing countries. Dyslipidemia refers to a condition of abnormal lipid
44 levels, including high triglycerides (TG), high low-density lipoprotein-cholesterol (LDL-C), low
45 high-density lipoprotein-cholesterol (HDL-C), and high total cholesterol (TC) (Lin et al., 2018).
46 According to the 2018 National Basic Health Survey, the national prevalence of high TG, high
47 LDL-C, low HDL-C, high TC in Indonesia were 13.3%, 24.9%, 24.3%, 21.2%, respectively
48 (Kementerian Kesehatan RI, 2019a). As a multifactorial disorder, dyslipidemia can occur due to
49 interactions between genetic and environmental factors, such as dietary intake and lifestyle
50 (Cole, Nikpay & McPherson, 2015).
51 One of the potential genetic risk factors for dyslipidemia is transcription factor 7-like 2
52 (*TCF7L2)* gene polymorphisms. *TCF7L2* encodes for a transcription factor containing the HMG-
53 Box domain which plays a crucial role as the main effector of canonical wingless-type (Wnt)
54 signaling pathway. The Wnt signaling pathway mostly regulates the expression of a wide array
55 of metabolic genes, such as *PDK1* (Pate et al., 2014), *LGR4/5/6* (Chen & Wang, 2018), *Gcg* (Yi,
56 Brubaker & Jin, 2005). The *TCF7L2* pre-mRNA has 17 exons, including 5 alternative exons
57 (exon 4, 13, 14, 15, 16) that will undergo alternative splicing mechanism, resulting in various
58 mature tissue-specific mRNA isoforms (Hansson et al., 2010). *TCF7L2* has been widely studied
59 for its association with metabolic-related diseases, such as type 2 diabetes mellitus (Mayans et
60 al., 2007; Villareal et al., 2010; Shokouhi et al., 2014; Zhu et al., 2017; Zhou et al., 2019),

61   obesity (Al-Daghri et al., 2014), metabolic syndrome (DeMenna et al., 2014), and cancers
62   (Folsom et al., 2008; Chen et al., 2013) in various populations.
63   Due to westernization and urbanization in the past three decades, the Balinese population has
64   undergone lifestyle changes that raised the prevalence of metabolic syndrome (MetS) and
65   metabolic disorders, such as obesity and diabetes mellitus (DM). These traits were known to be
66   closely associated with dyslipidemia through insulin resistance (Athyros et al., 2011; Suastika et
67   al., 2011, 2019), which also increase the Balinese's risk for dyslipidemia. A cross-sectional
68   survey study carried out in Bali Island had reported the frequency of dyslipidemia in normal
69   glucose tolerance subject group as follows, high LDL-C (73.8%), high non-HDL-C (53.9%), low
70   HDL-C (31.3%), and high TG (20.4%). Meanwhile, both impaired fasting glycemia (IFG) and
71   DM subject groups had relative higher percentage in all parameters, showing a positive
72   correlation between glucose impairment with dyslipidemia (Suastika et al., 2019).
73   A prior study had found associations of *TCF7L2* common polymorphisms (rs7903146,
74   rs1225372, and rs10885406) with altered lipid profiles in the Balinese. These findings indicated
75   a relationship between *TCF7L2* and dyslipidemia. However, the minor allele frequencies of these
76   three SNPs were rather low in Balinese, and thus they were hard to detect (Oktavianthi et al.,
77   2018). Therefore, in the present study, we investigated the associations of other *TCF7L2* intronic
78   SNPs, rs290487 (C>T, intron 8) (NCBI, 2021a) and rs290481 (C>T, intron 16) (NCBI, 2021b),
79   which have higher allele frequencies in East-Asian (MAF >0.60). Previously, rs290487 and
80   rs290481 were reported to be associated with type 2 diabetes mellitus in the East-Asian
81   population (Chang et al., 2010; Luo et al., 2009; Zhu et al., 2017), but their associations with
82   dyslipidemia have not been well-studied. We hypothesize that *TCF7L2* SNPs rs290487 and
83   rs290481 might also be associated with dyslipidemia in Balinese population and could be used to
84   predict the risk of dyslipidemia.
85

86   **Materials & Methods**
87   **Subjects, study design, and measurements**
88   A cross-sectional study enrolling 565 unrelated subjects from four locations (Nusa Ceningan,
89   Pedawa, Penglipuran, Legian) in Bali Province, Indonesia was conducted in 2008 – 2015 with
90   written informed consent. Prior to the study, ethical approvals were obtained by the Eijkman
91   Institute Research Ethics Commission (No. 32 on 27 October 2008 and No. 80 on 24 December
92   2014), and the Faculty of Medicine Ethic Committee, Udayana University (No.
93   690a/SKRT/X/2010 on 28 October 2010 and No. 1286/UN.14.2/Litbang/2014 on 18 September
94   2014).
95   The demographic data and anthropometric measurements were obtained including age, sex, body
96   mass index (BMI) which was calculated by dividing weight (kg) by square of height (m2),
97   fasting plasma glucose (FPG), and serum lipid concentrations after overnight fasting for at least
98   10 h [triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein
99   cholesterol (HDL-C), total cholesterol (TC)], and triglyceride glucose (TyG) index which was
100   calculated as Ln[fasting TG (mg/dL) FPG (mg/dL)/2]. TyG index has been widely used as a

101 surrogate marker for homeostasis of model assessment-insulin resistance (HOMA-IR) to
102 evaluate insulin resistance, as both markers were positively correlated (Aman et al., 2021).
103 Obesity was defined as having BMI ≥25 kg/m2 (WHO, 2000). Diabetes mellitus-FG was defined
104 as having FPG ≥126 mg/dL (American Diabetes Association, 2010). Dyslipidemia was classified
105 as having at least one of these following traits; high TG (≥200 mg/dL), high LDL-C (≥160
106 mg/dL), low HDL-C (<40 mg/dL), high TC (≥240 mg/dL) (NCEP, 2002). Clinical dyslipidemia
107 phenotypes following Fredrickson's classification were defined based on TG and TC levels, as
108 follows: low TG, high TC (equal to IIa phenotype); high TG, low TC (equal to IV, I phenotype);
109 high TG, high TC (equal to IIb, III, IV, V); and additional classification based on LDL-C and
110 HDL-C levels, as follows: high LDL-C only; low HDL-C only (Joint committee for guideline
111 version, 2018).
112 **DNA extraction and genotyping**
113 Genomic DNA extraction was performed as described elsewhere (Malik et al., 2011). In this
114 study, we selected two *TCF7L2* intronic SNPs, rs290487 and rs290481, which were common in
115 East Asian (MAF >0.60). Common SNPs were known to have lower false-positive rates than
116 rare SNPs and produce more reliable results (Tabangin, Woo & Martin, 2009). The rs290487 and
117 rs290481 were genotyped using the amplification refractory system mutation polymerase chain
118 reaction (ARMS-PCR), a robust SNP genotyping method with high sensitivity (>80%) and
119 specificity (>90%) (Ye et al., 2001; Nanfack et al., 2015), with novel set of primer pairs. Primer
120 design was done using web-based primer design tool for ARMS-PCR, Primer1
121 (http://primer1.soton.ac.uk/primer1.html) (Collins & Ke, 2012) and edited using BioEdit®
122 Sequence Alignment Editor (Ibis Bioscience, Carlsbad, CA, USA). Primer sequences are
123 presented in Table S1.
124 The optimal annealing temperature was determined using the PCR gradient method (Bioline
125 MyTaq™ HS DNA Polymerase) using the Veriti® thermal cycler (Applied Biosystem, Foster
126 City, CA, USA), followed by visualization using 1% agarose gel electrophoresis (Lonza, Basel,
127 Switzerland). Three samples that represented each genotype of rs290487 and rs290481 from
128 ARMS-PCR results were randomly selected and confirmed by Sanger DNA sequencing using
129 BigDye® Terminator v.3.1 Cycle Sequencing Kits, with ABI 3130xl Genetic Analyzer (Applied
130 Biosystem, Foster City, CA, USA). Genotyping of rs290487 and rs290481 in DNA samples was
131 performed using SimpliAmp™ Thermal Cycler (Applied Biosystem, Foster City, CA, USA) and
132 resolved in 2% agarose gel electrophoresis (Lonza, Basel, Switzerland).
133 **Statistical Analysis**
134 Data analyses were carried out using R version 4.1.1 (www.r-project.org) with R Studio
135 v1.4.1717 (www.rstudio.com). Continuous variables were presented as median (IQR) and
136 compared by performing Wilcoxon-Mann Whitney U test. Categorical variables were presented
137 as percentages and compared by performing Pearson's chi-squared test. The departure of
138 genotype distribution from Hardy-Weinberg equilibrium was tested using Pearson's chi-squared
139 test, and the r^2 and D' measure of linkage disequilibrium was evaluated, as implemented in the
140 "genetics" package (Warnes et al., 2021). Age, sex, population, obesity, diabetes mellitus-FG,

141 and high TyG index were used as adjustments for association analysis. The optimal cutoff point
142 for a high TyG index (8.85) for dyslipidemia was analyzed using the Youden index in
143 "OptimalCutpoints" package (Lopez-Raton & Xose Rodriguez-Alvarez, 2021). Adjusted odds
144 ratios (ORs) with 95% confidence interval (95% CI) for associations of TCF7L2 SNPs with
145 dyslipidemia and individual lipid profiles were estimated using the likelihood ratio test.
146 Associations between dyslipidemia phenotypes based on Fredrickson's classification and each
147 SNP were evaluated using multivariate multinomial logistic regression by implementing the
148 "nnet" package, measured as adjusted relative risk ratios (RRRs) with 95% CI (Ripley &
149 Venables, 2022). The association was significant when the p value is <0.025, following
150 Bonferroni correction (p value = 0.050/2 SNPs) (Cheverud, 2001). The adjusted ORs and 95%
151 CI were illustrated as forest plot using "ggplot2" package (Wickham et al., 2021). Further,
152 inferred haplotypes were estimated with the expectation maximization algorithm, as
153 implemented in the "haplo.stats" package (Sinnwell & Schaid, 2021). The haplotype associations
154 with dyslipidemia and lipid profiles were determined using the generalized linear regression
155 models, adjusted for age, sex, population, obesity, diabetes mellitus-FG, and TyG index.
156 Empirical p values (p sim) at significant level 0.05 were calculated after 10,000 simulations for
157 multiple testing correction (Becker & Knapp, 2004). Finally, the interaction analyses between
158 *TCF7L2* SNPs and obesity status on dyslipidemia probability were investigated using the
159 likelihood ratio test by considering all genetic models. The significant interactions ($p_{interaction}$
160 <0.100) were then plotted using the "interactions" package (Long, 2021).
161

162 Results

163 Baseline characteristics of the subjects

164 Characteristics of the study subjects are presented in Table 1. Subjects were grouped into
165 dyslipidemic (n = 366) and non-dyslipidemic (n = 199) based on NCEP-ATP III criteria of high
166 blood cholesterol (NCEP, 2002). Dyslipidemia was more prevalent in males than in females
167 (73.4% vs. 26.6%, p <0.001). Compared to the non-dyslipidemic subjects, the dyslipidemic
168 subjects had a significantly higher BMI, FPG, TG, LDL-C, TC levels, TyG index (all p <0.050)
169 and lower HDL-C levels (p <0.001). The most prevalent type of dyslipidemia was high TG
170 (49.7%), followed by high TC (41.2%), low HDL-C (37.2%), and high LDL-C (32.7%). Obesity
171 was also prevalent among dyslipidemic subjects (49.7%) (p <0.001).

172 Genotypic and allelic distribution

173 The genotype and allele frequencies, Hardy-Weinberg equilibrium and linkage disequilibrium
174 are shown in Table S2. The T alleles of rs290487 and rs290481, defined as a minor allele
175 according to dbSNP, were presented as the major alleles in Balinese, with the frequencies of 0.56
176 and 0.53, respectively. No significant departure from Hardy-Weinberg equilibrium was found for
177 both SNPs (p >0.050). High LD was found between rs290487 and rs290481 SNPs (D' = 0.90; r^2
178 = 0.72).

179 Genetic associations with dyslipidemia and altered lipid profile

180   The genetic associations of rs290487 and rs290481 with dyslipidemia and lipid profile were
181   presented in Fig. 1 and 2. Both SNPs were significantly associated with dyslipidemia and
182   individual high TC levels. rs290487 was significantly associated with dyslipidemia in additive
183   (OR 1.58, 95% CI 1.14-2.21, p = 0.006) and recessive model (OR 1.91, 95% CI 1.10-3.30, p =
184   0.020), meanwhile rs290481 was significantly associated in additive (OR 1.56, 95% CI 1.14-
185   2.14, p = 0.006) and dominant model (OR 2.08, 95% CI 1.26-3.50, p = 0.005). Further, both
186   SNPs were associated with high TC levels in additive (rs290487 OR 1.95, 95% CI 1.35-2.84, p
187   <0.001; rs290481 OR 1.78, 95% CI 1.22-2.56, p = 0.002) and dominant model (rs290487 OR
188   3.35, 95% CI 1.76-6.88, p <0001; rs290481 OR 2.79, 95% CI 1.48-5.69, p = 0.003).
189   Additionally, rs290487 was also associated with high LDL-C levels in dominant model (OR
190   2.33, 95% CI 1.20-4.92, p = 0.018). Both SNPs did not show any significant associations with
191   individual high TG and low HDL-C. Complete genotypic distribution of both SNPs on non-
192   affected and dyslipidemia affected subjects are shown in Table S3. Further association analyses
193   between each SNP and clinical dyslipidemia phenotypes according to Fredrickson's
194   classification showed the similar effect on high TC, regardless of the TG level (Table 2). Both
195   rs290487 and rs290481 were significantly associated with increase odds for developing
196   combined low TG, high TC (equal to IIa phenotype) (rs290487 additive OR 1.65, 95% CI 1.07-
197   2.55, p = 0.023; rs290481 additive OR 1.67, 95% CI 1.09-2.56, p = 0.018) and high TG, high TC
198   (equal to IIb, III, IV, V phenotypes) (rs290487 additive OR 2.43, 95% CI 1.33-4.43, p = 0.004,
199   dominant OR 17.02, 95% CI 2.23-130.89, p = 0.007; rs290481 additive OR 2.01, 95% CI 1.12-
200   3.61, p = 0.019, dominant OR 6.99, 95% CI 1.59-30.83, p = 0.010).
201   **_TCF7L2_ rs290487 and rs290481 haplotype association with dyslipidemia and altered lipid**
202   **profile**
203   Since the two SNPs had a high LD, we therefore conducted haplotype association analysis with
204   dyslipidemia and altered lipid profile, as shown in Table 3. Only haplotypes with a frequency of
205   ≥0.1 were analysed. We identified one haplotype, CC, carrying risk alleles from both SNPs, was
206   strongly associated with dyslipidemia, high LDL-C, and high TC (p sim< 0.050) under dominant
207   and additive models.
208   **The interactions between _TCF7L2_ rs290487 and rs290481 with obesity towards**
209   **dyslipidemia probability**
210   To investigate the modulatory effect of obesity in influencing SNPs associations with
211   dyslipidemia, we analyzed the interaction between rs290487 and rs290481 SNPs and obesity
212   status by considering all genetic models (Table S4). As shown in Fig. 3, obesity modified the
213   effect of rs290487 and rs290481 genotypes on dyslipidemia risk. In the additive model for both
214   SNPs, subjects carrying the heterozygous CT genotype were more likely to develop dyslipidemia
215   when they had obesity, when compared to the other genotypes carriers (rs290487 $p_{interaction}$ =
216   0.034; rs290481 $p_{interaction}$ = 0.013). When being analysed under the recessive model, the TT+TC
217   genotypes carrier exhibited an increased dyslipidemia probability when co-exist with obesity
218   (rs290487 $p_{interaction}$ = 0.064; rs290481 $p_{interaction}$ = 0.015).
219
220   ## Discussion

221   Our study aimed to investigate the association of *TCF7L2* intronic SNPs rs290487 and rs290481
222   with dyslipidemia in Balinese population. The SNPs' influence on metabolic abnormalities in the
223   selected Indonesian population has not been well-described. In this study, we reported the
224   notable impacts of individual genotypes and a haplotype of the rs290487 and rs290481 SNPs on
225   dyslipidemia and individual lipid profile in the Balinese. Over the past three decades, this
226   population has been exposed to westernization and urbanization brought by rapid tourism
227   development, leading to a high prevalence of obesity and metabolic syndrome, particularly in
228   tourism destination area (Suastika et al., 2011). Our findings support the notion that *TCF7L2*
229   SNPs have a significant impact on lipid metabolism.
230   The rs290487 and rs290481 allele frequencies varied among different populations. In East
231   Asians, these SNPs were commonly found on average MAF of 0.60 (Chang et al., 2007; Liu et
232   al., 2009; Wang et al., 2013; Zhu et al., 2017), but found in much lower frequencies among
233   Caucasians (Delgado-Lista et al., 2010), the population referred by dbSNP. To the best of our
234   knowledge, this study is the first report on MAF of rs290487 and rs290481 in the Balinese
235   population. Our findings showed the frequencies of T allele of rs290487 and rs290481 were 0.56
236   and 0.53, respectively, while C allele of both SNPs automatically became the minor allele.
237   Differences in allelic distribution can occur due to some conditions, such as different genetic
238   background between populations (Ding & Kullo, 2011) and natural selection mechanism,
239   whereas frequency of adaptive alleles in a population tend to be arisen (Kido et al., 2018).
240   In this study, we found dyslipidemia more prevalent in males than females. Changes towards
241   unhealthy lifestyles in the Balinese increase the risk of dyslipidemia in this population.
242   According to the Bali Province Basic Health Survey 2018, Balinese men relatively consumed
243   more sweetened beverages (including energy drink and soft drink) and less vegetables and fruits
244   than women (Kementerian Kesehatan RI, 2019b). In general, risks of dyslipidemia in men were
245   dominantly associated with unhealthy lifestyles, such as high fatty and salty food intake,
246   smoking, hypertension, obesity, and diabetes (Wang et al., 2020; Xi et al., 2020). Meanwhile,
247   dyslipidemia in women was often found at postmenopausal state due to decreased in estrogen
248   level and its lipoprotein maintenance role (Reddy Kilim & Rao Chandala, 2013; Opoku et al.,
249   2019; Xi et al., 2020). Unfortunately, lifestyle and physiological data from dyslipidemic subjects
250   were not available, which is the limitation of this study.
251   Our findings showed that the *TCF7L2* rs290487 and rs290481 C alleles were significantly
252   associated with dyslipidemia and high TC levels. Additional association was found between
253   rs290487 and high LDL-C levels. These associations were consistently found in haplotype CC
254   from both SNPs. Furthermore, we did not find any significant associations between rs290487
255   and rs290481 with individual TG levels, even though our dyslipidemic subjects exhibited 2-fold
256   higher TG levels, compared to non-dyslipidemic subjects. Interestingly, the associations of both
257   SNPs with either low or high TG levels were only detected when it appeared together with high
258   TC levels, indicating a stronger association of these SNPs with TC rather than TG level itself.
259   TC level reflects the total amount of cholesterol, including LDL-C, HDL-C, and other lipids.
260   Therefore, the significant association between *TCF7L2* SNPs with TC might occur due to its

261 major influence on LDL-C. A study performed using MetS subject from 8 European countries
262 have reported that rs290481 C allele, the major allele in that population, also contributed to
263 higher LDL-C levels (Delgado-Lista et al., 2010). Our previous study had also reported the
264 associations of three *TCF7L2* SNPs (rs7903146, rs12255372, rs10885406) with elevated
265 TC/HDL-C ratio (Oktavianthi et al., 2018). TC levels was known to be increased primarily due
266 to elevated LDL-C levels (Kreisberg & Kasim, 1987). High LDL-C and TC level might increase
267 cardiovascular disease risk by promoting atherosclerosis process (Hedayatnia et al., 2020).
268 As a wide-range transcription factor, TCF7L2 takes part in regulating the gene expression by
269 binding to the promoter of its target genes (Norton et al., 2014; Zhao et al., 2016). The presence
270 of intronic polymorphisms may interrupt the alternative splicing mechanism of *TCF7L2*,
271 resulting in changes of mRNA isoforms and leading to dysregulation of its downstream target
272 genes (Mondal et al., 2010; Buroker, 2017). Pradas-Juni *et al*. have found an increase of exon 4-
273 containing mRNA transcripts in pancreatic islets of T2DM patient with rs7903146 TT genotype
274 (Pradas-Juni et al., 2014). The inclusion or exclusion of exon 4 might be influenced by the
275 presence of rs7903146 in intron 4. On the other hand, rs290487 and rs290481 which are located
276 in intron 8 and 16, respectively, might possess different approaches in alternative splicing
277 regulation (Liu et al., 2019). The transition from T to C in rs290487 was suggested to induce
278 altered binding affinity of TCF7L2 binding sites to its target genes (Zhang et al., 2020). Many
279 studies have demonstrated the role of TCF7L2 in regulating genes that are involved in
280 cholesterol and triglyceride biosynthesis, such as *ApoB* (Norton et al., 2014), *Lpl* (He et al.,
281 2020), *Tgh1,* and *Tgh2* (Geoghegan et al., 2019). Geoghegan *et al.* have also found TCF7L2
282 binding sites within 1 kb of the promoter of numerous genes involved in the *de novo* lipogenesis
283 pathway (Geoghegan et al., 2019). Moreover, a specific *TCF7L2* rs7903146 region was known
284 to control *ACSL5* gene expression which is important for lipid biosynthesis and fatty acid
285 degradation by interacting with its promoter as an enhancer (Xia et al., 2016). Therefore, the
286 effect of intronic variants within the *TCF7L2* gene on dyslipidemia might be done through
287 several pathways. A conceptual framework for summarizing the relationship between *TCF7L2*
288 SNPs and dyslipidemia was proposed (Fig. 4).
289 In the current study, we found that obesity modulates the SNPs' association with dyslipidemia,
290 particularly in those who carry heterozygous genotype. Previously, the modulatory effect of
291 obesity in genetic risks towards dyslipidemia has also been reported (Yin et al., 2012; Cole,
292 Nikpay & McPherson, 2015). Obesity and dyslipidemia are linked by insulin resistance, as
293 reviewed in (Vekic et al., 2019). Since we lacked HOMA-IR data, the most common insulin
294 resistance marker, we used TyG index as a surrogate marker (Simental-Mendía, Rodríguez-
295 Morán & Guerrero-Romero, 2008; Khan et al., 2018; Aman et al., 2021). Although the TyG
296 index is a significant determining factor for dyslipidemia, we did not find any direct impact of
297 rs290487 and rs290481 on it (Table S5). This might suggest that the SNPs are rather influencing
298 lipid metabolism. Further studies are warranted to explore rs290487 and rs290481 influence on
299 the other lipid fractions (VLDL, chylomicron, ApoE, etc.), to understand on how *TCF7L2* gene
300 polymorphisms influence on dyslipidemia.

Conclusions

301 **Conclusions**

302 In this study, we have shown that *TCF7L2* rs290487 and rs290481 C allele was significantly
303 associated with dyslipidemia, high LDL-C, and high TC in Balinese population. Despite being
304 minor alleles, the C alleles of both SNPs were relatively high, which raises the assumption that
305 more than 40% of the Balinese were very likely to carry this allele. In addition, the changes
306 towards unhealthy lifestyles (high calorie intake and sedentary lifestyle) increased the Balinese's
307 risk of dyslipidemia even more. As SNPs were unmodifiable risk, lifestyle improvement is
308 needed to lower the risk of dyslipidemia, especially in the Balinese. Indonesian population is
309 very diverse, therefore, future studies to explore these associations in different populations in
310 Indonesia should be conducted.

311

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320

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548

Figure 1

Figure 1: Forest plot of odds ratios for the associations of rs290487 with dyslipidemia and altered lipid profiles.

Forest plot was generated using ggplot2 (▲: p < 0.025); OR (95% CI), Odds Ratio (95% Confidence Interval); TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol. OR was adjusted by age, sex, population, obesity (BMI ≥25 kg/m^2), diabetes mellitus-FG (FPG ≥126 mg/dL), and TyG index (except for High TG).
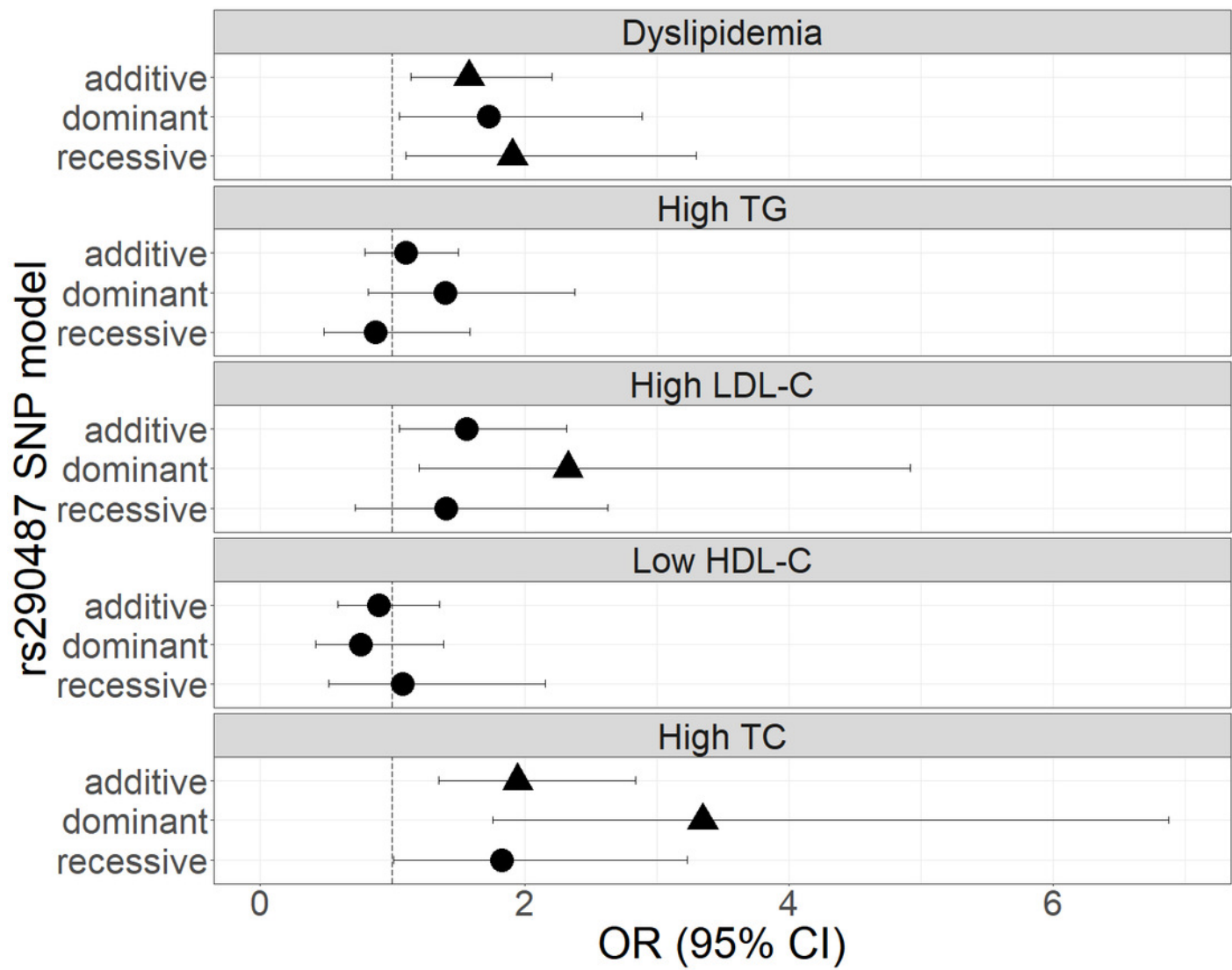
Figure 2

Figure 2: Forest plot of odds ratios for the associations of rs290481 with dyslipidemia and altered lipid profiles.

Forest plot was generated using ggplot2 (▲: $p < 0.025$); OR (95% CI), Odds Ratio (95% Confidence Interval); TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol. OR was adjusted by age, sex, population, obesity (BMI ≥25 kg/m^2), diabetes mellitus-FG (FPG ≥126 mg/dL), and TyG index (except for High TG).
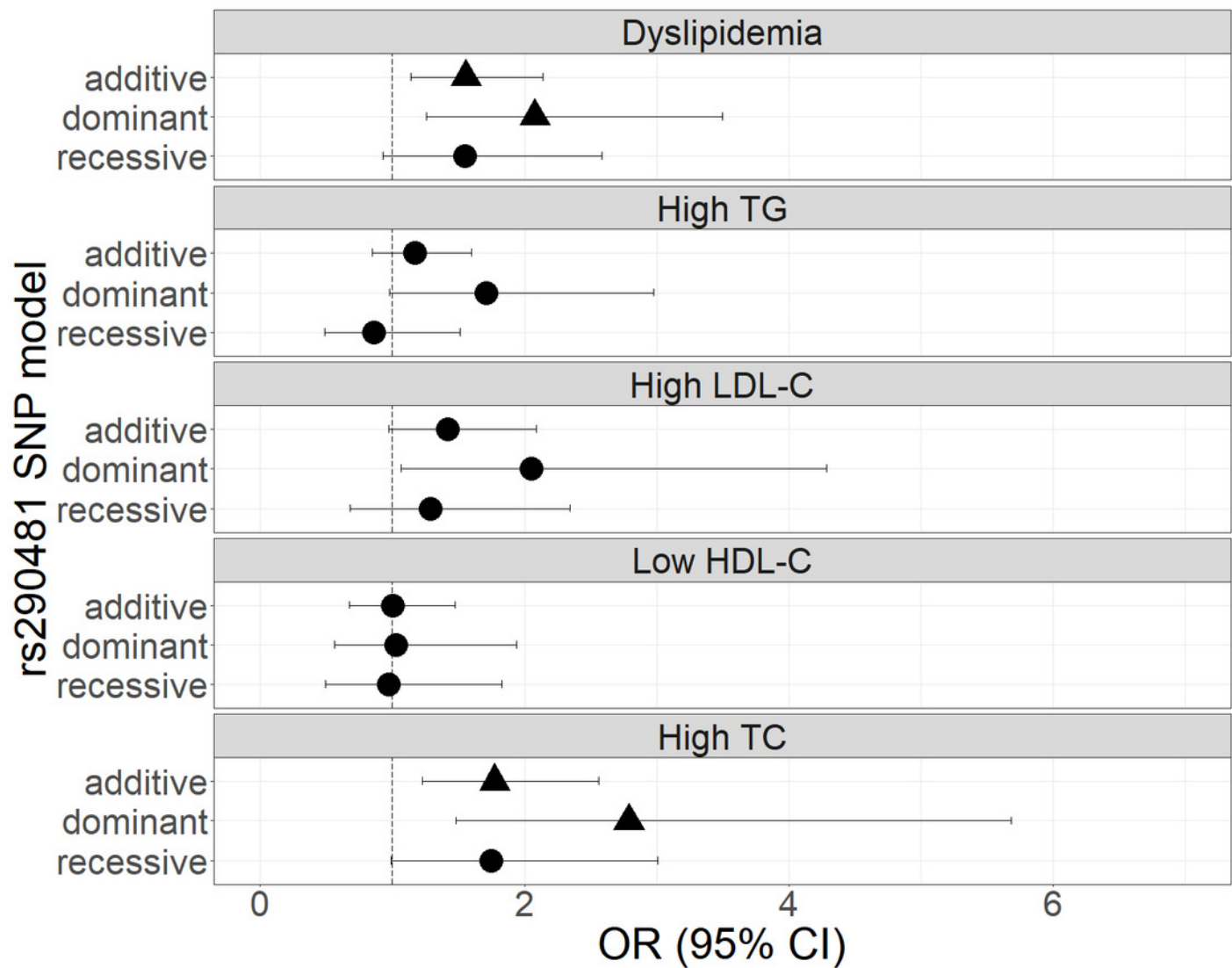
Figure 3

Figure 3: Interaction plot between *TCF7L2* SNPs and obesity status on dyslipidemia risk.

Interaction plot between rs290487 and obesity status on dyslipidemia risk under additive (A) and recessive (B) model. Interaction plot between rs290481 and obesity status on dyslipidemia risk under additive (C) and recessive (D) model.
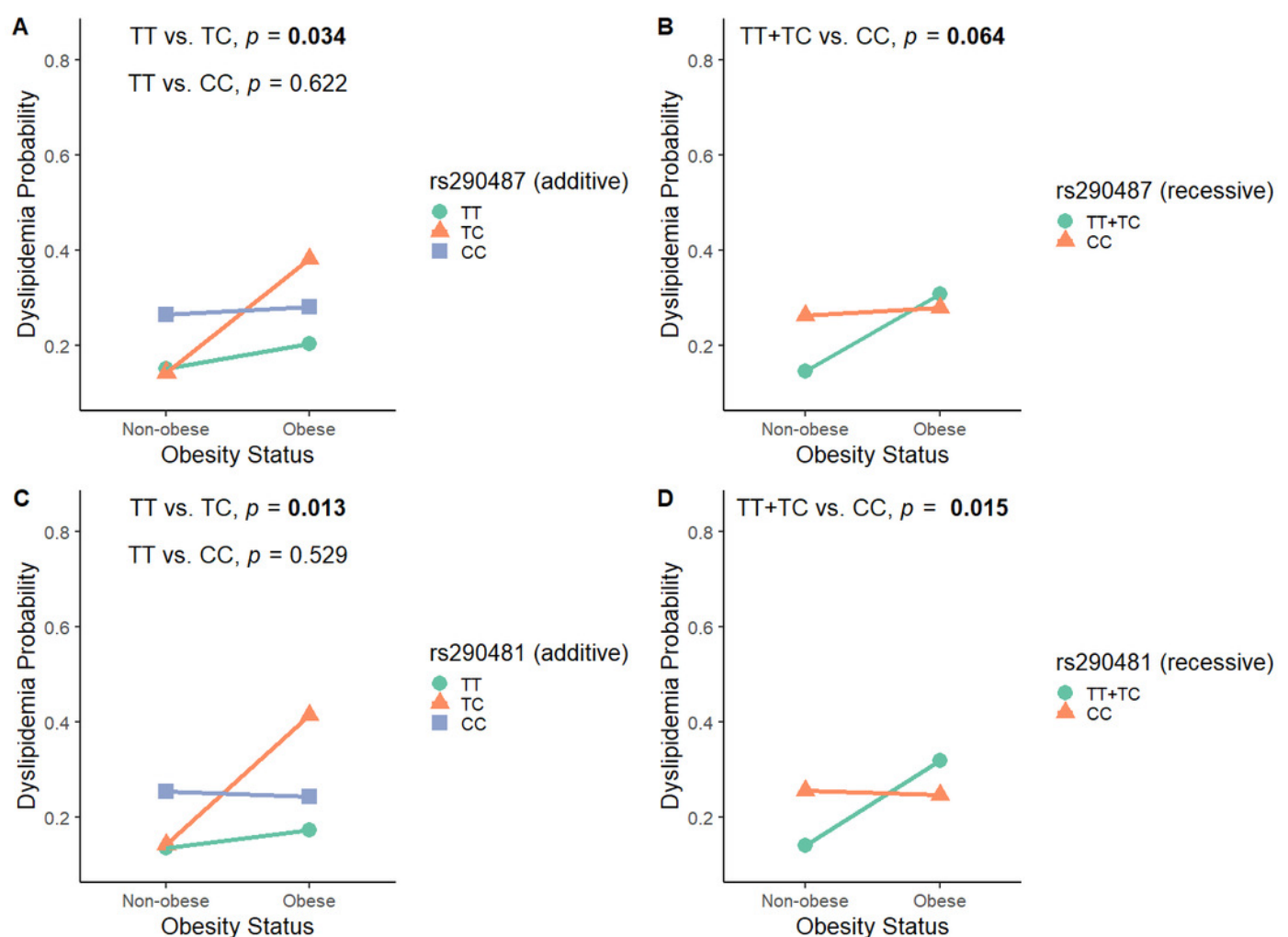
Figure 4

Figure 4: Conceptual framework depicting relationship between *TCF7L2* SNPs with dyslipidemia.
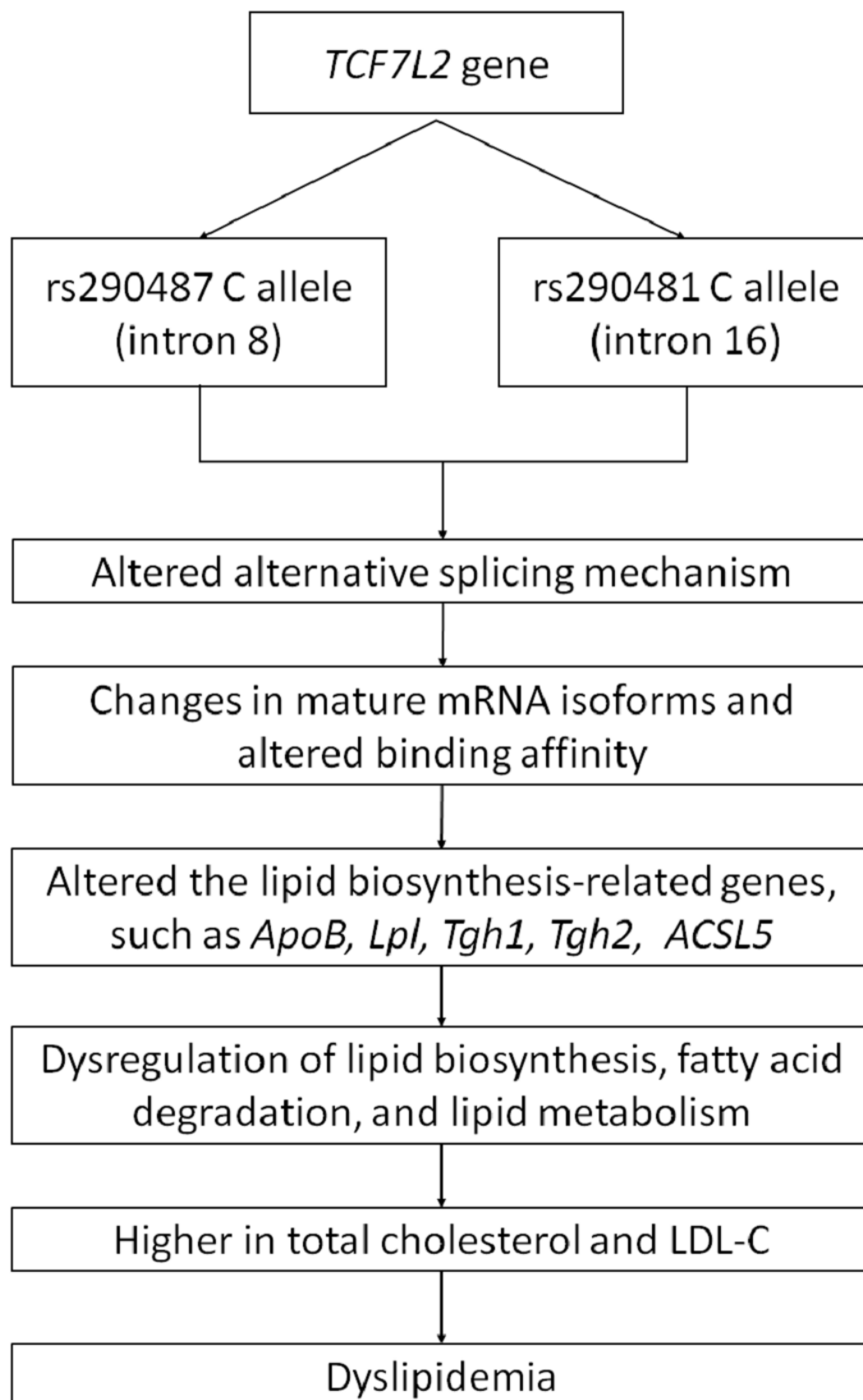
Table 1: Baseline characteristics of non-dyslipidemic and dyslipidemic subjects.

Abbreviations: IQR, interquartile range; BMI, body mass index; FPG, fasting blood glucose; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol ; TyG, triglyceride and glucose . Criteria: obesity, BMI ≥25 kg/m2 (WHO, 2000); diabetes mellitus-FG, FPG ≥126 mg/dL (American Diabetes Association, 2010); high TG, TG ≥200 mg/dL (NCEP, 2002); high LDL-C, LDL-C ≥160 mg/dL (NCEP, 2002); low HDL-C, HDL-C <40 mg/dL (NCEP, 2002); high TC, TC ≥240 mg/dL (NCEP, 2002); dyslipidemia, the presence of at least one altered lipid profile (NCEP, 2002). The p values were calculated using either Wilcoxon-Mann Whitney U test for continuous variables or Pearson's chi-squared test for categorical variables. The significant p values are in bold (p <0.050).

1 **Table 1:**

2 **Baseline characteristics of non-dyslipidemic and dyslipidemic subjects.**

Variable	Non-dyslipidemic (n=366)	Dyslipidemic (n=199)	p
Age [years, median (IQR)]	46.0 (40.0-56.0)	47.0 (39.0-57.0)	0.939
Sex [n(%)]			
Female [244 (43.2)]	191 (52.2)	53 (26.6)	**<0.001**
Male [321 (56.8)]	175 (47.8)	146 (73.4)	
Age per Sex [years, median (IQR)]			
Female Age	45.0 (40.0-55.5)	52.0 (40.0-60.0)	0.053
Male Age	46.0 (40.0-56.5)	44.0 (38.0-54.8)	0.317
Population [n(%)]			
Rural	200 (54.6)	91 (45.7)	0.053
Urban	166 (45.4)	108 (54.3)	
BMI [kg/m^2, median (IQR)]	22.9 (20.4-25.4)	24.9 (22.4-28.2)	**<0.001**
FPG [mg/dL, median (IQR)]	91.0 (83.3-99.0)	93.0 (87.0-100.0)	**0.005**
TG [mg/dL, median (IQR)]	97.5 (77.0-129.8)	199.0 (138.5-237.0)	**<0.001**
LDL-C [mg/dL, median (IQR)]	114.0 (95.3-133.0)	135.0 (101.0-164.0)	**<0.001**
HDL-C [mg/dL, median (IQR)]	54.0 (48.0-61.0)	43.0 (37.5-52.0)	**<0.001**
TC [mg/dL, median (IQR)]	190.0 (170.0-201.8)	225.0 (190.0-250.0)	**<0.001**
TyG index	8.4 (8.1-8.7)	9.1 (8.8-9.4)	**<0.001**
Disease prevalence [n(%)]			
Obesity	107 (29.2)	99 (49.7)	**<0.001**
Diabetes mellitus-FG	17 (4.6)	16 (8.0)	0.145

3 Abbreviations: IQR, interquartile range; BMI, body mass index; FPG, fasting blood glucose; TG,

4 triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein

5 cholesterol; TC, total cholesterol; TyG, triglyceride and glucose. Criteria: obesity, BMI ≥25

6 kg/m2 (WHO, 2000); diabetes mellitus-FG, FPG ≥126 mg/dL (American Diabetes Association,

7 2010); high TG, TG ≥200 mg/dL (NCEP, 2002); high LDL-C, LDL-C ≥160 mg/dL (NCEP,

8 2002); low HDL-C, HDL-C <40 mg/dL (NCEP, 2002); high TC, TC ≥240 mg/dL (NCEP,

9 2002); dyslipidemia, the presence of at least one altered lipid profile (NCEP, 2002). The *p* values

10 were calculated using either Wilcoxon-Mann Whitney U test for continuous variables or

11 Pearson's chi-squared test for categorical variables. The significant *p* values are in bold (*p*

12 <0.050).

Table 2: Multinomial logistic regression results for associations of rs290487 and rs290481 with dyslipidemia phenotypes.

Abbreviations: RRR, relative risk ratio; 95% CI, 95% confidence interval; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol. Altered lipid profiles criteria: high TG (≥200 mg/dL), high LDL-C (≥160 mg/dL), low HDL-C (<40 mg/dL), high TC (≥240 mg/dL) (NCEP, 2002). Dyslipidemia phenotypes were classified as follows: type IIa: high TC and low TG; type IV and I: high TG and low TC; type IIb, III, IV, and V: high TG and high TC, following the Fredrickson's classification (Joint committee for guideline version, 2018); with additional high LDL-C only and low HDL-C only groups. Analyses were performed using multivariate multinomial logistic regression, adjusting for age, sex, population, obesity (BMI ≥25 kg/m^2 (WHO, 2000)) and diabetes mellitus-FG (FPG ≥126 mg/dL (American Diabetes Association, 2010)). The significant p values after Bonferroni's correction (p <0.025) are in bold.

1 **Table 2:**

2 **Multinomial logistic regression results for associations of rs290487 and rs290481 with**

3 **dyslipidemia phenotypes.**

Dyslipidemia Phenotype	Equal to Fredrickson's classification	N	Genotype Frequency			Additive		Dominant		Recessive	
			TT	TC	CC	RRR (95% CI)	*p*	RRR (95% CI)	*p*	RRR (95% CI)	*p*
rs290487											
Non-dyslipidemic		366	0.21	0.47	0.33	Reference		Reference		Reference	
Low TG, High TC	IIa	53	0.30	0.51	0.19	1.65 (1.07-2.55)	**0.023**	1.96 (0.95-4.03)	0.069	1.90 (0.96-3.77)	0.066
High TG, Low TC	IV, I	70	0.19	0.56	0.26	0.92 (0.61-1.38)	0.673	0.93 (0.52-1.67)	0.805	0.84 (0.39-1.79)	0.647
High TG, High TC	IIb, III, IV, V	29	0.28	0.66	0.07	2.43 (1.33-4.43)	**0.004**	17.02 (2.23-130.89)	**0.007**	1.70 (0.66-4.35)	0.271
High LDL-C only		8	0.25	0.63	0.13	1.86 (0.63-5.44)	0.260	3.41 (0.39-29.63)	0.266	1.66 (0.32-8.71)	0.552
Low HDL-C only		39	0.28	0.44	0.28	1.19 (0.71-2.02)	0.510	0.86 (0.41-1.82)	0.692	1.93 (0.86-4.35)	0.110
rs290481											
Non-dyslipidemic		366	0.21	0.47	0.33	Reference		Reference		Reference	
Low TG, High TC	IIa	53	0.30	0.51	0.19	1.67 (1.09-2.56)	**0.018**	2.07 (0.99-4.33)	0.052	1.84 (0.96-3.55)	0.067
High TG, Low TC	IV, I	70	0.19	0.56	0.26	1.08 (0.73-1.58)	0.702	1.36 (0.74-2.51)	0.321	0.84 (0.43-1.68)	0.631
High TG, High TC	IIb, III, IV, V	29	0.28	0.66	0.07	2.01 (1.12-3.61)	**0.019**	6.99 (1.59-30.83)	**0.010**	1.46 (0.59-3.62)	0.419
High LDL-C only		8	0.25	0.62	0.13	1.54 (0.55-4.32)	0.409	3.27 (0.39-27.68)	0.278	1.12 (0.21-5.95)	0.898
Low HDL-C only		39	0.28	0.44	0.28	1.26 (0.77-2.07)	0.359	1.14 (0.53-2.44)	0.739	1.58 (0.73-3.42)	0.249

4 Abbreviations: RRR, relative risk ratio; 95% CI, 95% confidence interval; TG, triglycerides;

5 LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC,

6 total cholesterol. Altered lipid profiles criteria: high TG ($\geq$200 mg/dL), high LDL-C ($\geq$160

7 mg/dL), low HDL-C (<40 mg/dL), high TC ($\geq$240 mg/dL) (NCEP, 2002). Dyslipidemia

8 phenotypes were classified as follows: type IIa: high TC and low TG; type IV and I: high TG and

9 low TC; type IIb, III, IV, and V: high TG and high TC, following the Fredrickson's classification

10 (Joint committee for guideline version, 2018); with additional high LDL-C only and low HDL-C

11 only groups. Analyses were performed using multivariate multinomial logistic regression,

12 adjusting for age, sex, population, obesity (BMI $\geq$25 kg/m^2 (WHO, 2000)) and diabetes mellitus-

13 FG (FPG $\geq$126 mg/dL (American Diabetes Association, 2010)). The significant *p* values after

14 Bonferroni's correction (*p* <0.025) are in bold.

Table 3: Association of *TCF7L2* haplotype with dyslipidemia and altered lipid profile.

Abbreviations: NAS, Non-Affected Subjects; AS, Affected Subjects; SS, Score Statistics; OR, odds ratio; 95%CI, 95% confidence interval; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol. Dyslipidemia was defined by the presence of one of the following criteria: high TG ($\geq$200 mg/dL), high LDL-C ($\geq$160 mg/dL), low HDL-C (<40 mg/dL) or high TC ($\geq$240 mg/dL) (NCEP, 2002). Haplotypes with frequency of $\geq$0.1 were included in the analysis. Association analysis was performed using the adjusted likelihood ratio test, by assuming additive and dominant genetic model and controlling for age, sex, population, obesity (BMI $\geq$25 kg/m^2 (WHO, 2000)), diabetes mellitus-FG (FPG $\geq$126 mg/dL (American Diabetes Association, 2010)), and TyG index (except for High TG). The p_{sim} is a simulated p value after minimal 10,000 simulations. The significant p values (p <0.050) are in bold.

1   **Table 3:**
2   **Association of *TCF7L2* haplotype with dyslipidemia and altered lipid profile.**

Trait	Haplotype		Frequency		Additive				Dominant			
	rs290487	rs290481	NAS	AS	SS	*p* sim	OR (95%CI)	*p*	SS	*p* sim	OR (95%CI)	*p*
Dyslipidemia	T	T	0.53	0.46	-2.674	**0.007**	Reference		-1.980	0.051	Reference	
	C	C	0.39	0.47	2.918	**0.002**	1.62 (1.16-2.26)	**0.003**	2.650	**0.007**	1.93 (1.17-3.19)	**0.008**
High TG	T	T	0.52	0.48	-0.676	0.488	Reference		0.607	0.560	Reference	
	C	C	0.41	0.45	0.812	0.421	1.14 (0.81-1.61)	0.416	1.470	0.144	1.47 (0.87-2.49)	0.141
High LDL-C	T	T	0.52	0.43	-1.908	0.067	Reference		-0.928	0.335	Reference	
	C	C	0.41	0.51	2.125	**0.029**	1.52 (1.02-2.28)	**0.033**	2.363	**0.015**	2.19 (1.11-4.29)	**0.018**
Low HDL-C	T	T	0.51	0.50	-0.004	1.000	Reference		-0.008	0.991	Reference	
	C	C	0.42	0.40	-0.474	0.665	0.94 (0.62-1.44)	0.635	-0.777	0.450	0.83 (0.46-1.51)	0.437
High TC	T	T	0.53	0.40	-3.376	**<0.001**	Reference		-2.350	**0.020**	Reference	
	C	C	0.40	0.53	3.413	**<0.001**	1.94 (1.33-2.85)	**<0.001**	3.650	**<0.001**	3.50 (1.79-6.84)	**<0.001**

3   Abbreviations: NAS, Non-Affected Subjects; AS, Affected Subjects; SS, Score Statistics; OR,

4   odds ratio; 95%CI, 95% confidence interval; TG, triglycerides; LDL-C, low-density lipoprotein

5   cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol. Dyslipidemia

6   was defined by the presence of one of the following criteria: high TG ($\geq$200 mg/dL), high LDL-

7   C ($\geq$160 mg/dL), low HDL-C (<40 mg/dL) or high TC ($\geq$240 mg/dL) (NCEP, 2002). Haplotypes

8   with frequency of $\geq$0.1 were included in the analysis. Association analysis was performed using

9   the adjusted likelihood ratio test, by assuming additive and dominant genetic model and

10 controlling for age, sex, population, obesity (BMI $\geq$25 kg/m^2 (WHO, 2000)), diabetes mellitus-

11 FG (FPG $\geq$126 mg/dL (American Diabetes Association, 2010)), and TyG index (except for High

12 TG). The *p* sim is a simulated *p* value after minimal 10,000 simulations. The significant *p* values

13 (*p* <0.050) are in bold.