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

Prisca C. Limardi1,2, Sukma Oktavianthi1, Lidwina Priliani1, Retno Lestari2, Made Ratna Saraswati3, Ketut Suastika3 and Safarina G. Malik1

1 Genome Diversity and Diseases Laboratory, Eijkman Institute for Molecular Biology, National Research and Innovation Agency, Jakarta, DKI Jakarta, Indonesia
2 Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java, Indonesia
3 Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia

ABSTRACT

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 and altered lipid profile in the Balinese.

INTRODUCTION

Dyslipidemia is one of the risk factors for the development of cardiovascular diseases, the major cause of death in developing countries. Dyslipidemia refers to a condition of
abnormal lipid levels, including high triglycerides (TG), high low-density lipoprotein-cholesterol (LDL-C), low high-density lipoprotein-cholesterol (HDL-C), and high total cholesterol (TC) (Lin et al., 2018). According to the 2018 National Basic Health Survey, the national prevalence of high TG, high LDL-C, low HDL-C, high TC in Indonesia were 13.3%, 24.9%, 24.3%, 21.2%, respectively (Kementerian Kesehatan, 2019a). As a multifactorial disorder, dyslipidemia can occur due to interactions between genetic and environmental factors, such as dietary intake and lifestyle (Cole, Nikpay & McPherson, 2015).

One of the potential genetic risk factors for dyslipidemia is transcription factor 7-like 2 (TCF7L2) gene polymorphisms. TCF7L2 encodes for a transcription factor containing the HMG-Box domain which plays a crucial role as the main effector of canonical wingless-type (Wnt) signaling pathway. The Wnt signaling pathway mostly regulates the expression of a wide array of metabolic genes, such as PDK1 (Pate et al., 2014), LGR4/5/6 (Chen & Wang, 2018), and Gcg (Yi, Brubaker & Jin, 2005). The TCF7L2 pre-mRNA has 17 exons, including five alternative exons (exon 4, 13, 14, 15, 16) that will undergo alternative splicing mechanism, resulting in various mature tissue-specific mRNA isoforms (Hansson et al., 2010). TCF7L2 has been widely studied for its association with metabolic-related diseases, such as type 2 diabetes mellitus (Mayans et al., 2007; Villareal et al., 2010; Shokouhi et al., 2014; Zhu et al., 2017; Zhou et al., 2019), obesity (Al-Daghri et al., 2014), metabolic syndrome (DeMenna et al., 2014), and cancers (Folsom et al., 2008; Chen et al., 2013) in various populations.

Due to westernization and urbanization in the past three decades, the Balinese population has undergone lifestyle changes that raised the prevalence of metabolic syndrome (MetS) and metabolic disorders, such as obesity and diabetes mellitus (DM). These traits are known to be closely associated with dyslipidemia through insulin resistance (Athyros et al., 2011; Suastika et al., 2011, 2019), which also increase the Balinese’s risk for dyslipidemia. A cross-sectional survey study carried out in Bali Island had reported the frequency of dyslipidemia in normal glucose tolerance subject group as follows, high LDL-C (73.8%), high non-HDL-C (53.9%), low HDL-C (31.3%), and high TG (20.4%). Meanwhile, both impaired fasting glycemia (IFG) and DM subject groups had relative higher percentage in all parameters, showing a positive correlation between glucose impairment with dyslipidemia (Suastika et al., 2019).

A prior study had found associations of TCF7L2 common polymorphisms (rs7903146, rs1225372, and rs10885406) with altered lipid profiles in the Balinese. These findings indicated a relationship between TCF7L2 and dyslipidemia. However, the minor allele frequencies of these three SNPs were rather low in Balinese, and thus they were hard to detect (Oktavianthi et al., 2018). Therefore, in the present study, we investigated the associations of other TCF7L2 intronic SNPs, rs290487 (C > T, intron 8) (NCBI, 2021a) and rs290481 (C > T, intron 16) (NCBI, 2021b), which have higher allele frequencies in East-Asian (MAF > 0.60). Previously, rs290487 and rs290481 were reported to be associated with type 2 diabetes mellitus in the East-Asian population (Chang et al., 2010; Luo et al., 2009; Zhu et al., 2017), but their associations with dyslipidemia have not been well-studied. We hypothesize that TCF7L2 SNPs rs290487 and rs290481 might also be
associated with dyslipidemia in Balinese population and could be used to predict the risk of dyslipidemia.

**MATERIALS AND METHODS**

**Subjects, study design, and measurements**

A cross-sectional study enrolling 565 unrelated subjects from four locations (Nusa Ceningan, Pedawa, Penglipuran, Legian) in Bali Province, Indonesia was conducted in 2008–2015 with written informed consent. Prior to the study, ethical approvals were obtained by the Eijkman Institute Research Ethics Commission (No. 32 on 27 October 2008 and No. 80 on 24 December 2014), and the Faculty of Medicine Ethic Committee, Udayana University (No. 690a/SKRT/X/2010 on 28 October 2010 and No. 1286/UN.14.2/Litbang/2014 on 18 September 2014).

The demographic data and anthropometric measurements were obtained including age, sex, body mass index (BMI) which was calculated by dividing weight (kg) by square of height (m$^2$), fasting plasma glucose (FPG), and serum lipid concentrations after overnight fasting for at least 10 h (triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC)), and triglyceride glucose (TyG) index which was calculated as Ln[fasting TG (mg/dL) / FPG (mg/dL)/2]. TyG index has been widely used as a surrogate marker for homeostasis of model assessment-insulin resistance (HOMA-IR) to evaluate insulin resistance, as both markers were positively correlated (Aman et al., 2021). Obesity was defined as having BMI ≥ 25 kg/m$^2$ (World Health Organization, 2000). Diabetes mellitus-FG was defined as having FPG ≥ 126 mg/dL (American Diabetes Association, 2010). Dyslipidemia was classified as having at least one of these following traits; high TG (≥200 mg/dL), high LDL-C (≥160 mg/dL), low HDL-C (<40 mg/dL), high TC (≥240 mg/dL) (NCEP, 2002). Clinical dyslipidemia phenotypes following Fredrickson’s classification were defined based on TG and TC levels, as follows: low TG, high TC (equal to IIa phenotype); high TG, low TC (equal to IV, I phenotype); high TG, high TC (equal to IIb, III, IV, V); and additional classification based on LDL-C and HDL-C levels, as follows: high LDL-C only; low HDL-C only (Joint Committee for Guideline Revision, 2018).

**DNA extraction and genotyping**

Genomic DNA extraction was performed as described elsewhere (Malik et al., 2011). In this study, we selected two TCF7L2 intronic SNPs, rs290487 and rs290481, which were common in East Asian (MAF > 0.60). Common SNPs were known to have lower false-positive rates than rare SNPs and produce more reliable results (Tabangin, Woo & Martin, 2009). The rs290487 and rs290481 were genotyped using the amplification refractory system mutation polymerase chain reaction (ARMS-PCR), a robust SNP genotyping method with high sensitivity (>80%) and specificity (>90%) (Ye et al., 2001; Nanfack et al., 2015), with novel set of primer pairs. Primer design was done using web-based primer design tool for ARMS-PCR, Primer1 (http://primer1.soton.ac.uk/primer1.html) (Collins & Ke, 2012) and edited using BioEdit® Sequence Alignment Editor (Ibis Bioscience, Carlsbad, CA, USA). Primer sequences are presented in Table S1.
The optimal annealing temperature was determined using the PCR gradient method (Bioline MyTaq™ HS DNA Polymerase) using the Veriti® thermal cycler (Applied Biosystem, Foster City, CA, USA), followed by visualization using 1% agarose gel electrophoresis (Lonza, Basel, Switzerland). Three samples that represented each genotype of rs290487 and rs290481 from ARMS-PCR results were randomly selected and confirmed by Sanger DNA sequencing using BigDye® Terminator v.3.1 Cycle Sequencing Kits, with ABI 3130xl Genetic Analyzer (Applied Biosystem, Foster City, CA, USA). Genotyping of rs290487 and rs290481 in DNA samples was performed using SimpliAmp™ Thermal Cycler (Applied Biosystem, Foster City, CA, USA) and resolved in 2% agarose gel electrophoresis (Lonza, Basel, Switzerland).

**Statistical analysis**

Data analyses were carried out using R version 4.1.1 ([www.r-project.org](http://www.r-project.org)) with R Studio v1.4.1717 ([www.rstudio.com](http://www.rstudio.com)). Continuous variables were presented as median (IQR) and compared by performing Wilcoxon-Mann Whitney U test. Categorical variables were presented as percentages and compared by performing Pearson’s chi-squared test. The departure of genotype distribution from Hardy-Weinberg equilibrium was tested using Pearson’s chi-squared test, and the r² and D’ measure of linkage disequilibrium was evaluated, as implemented in the “genetics” package ([Warnes et al., 2021](https://cran.r-project.org/package=genetics)). Age, sex, population, obesity, diabetes mellitus-FG, and high TyG index were used as adjustments for association analysis. The optimal cutoff point for a high TyG index (8.85) for dyslipidemia was analyzed using the Youden index in “OptimalCutpoints” package ([Lopez-Raton & Xose Rodriguez-Alvarez, 2021](https://cran.r-project.org/package=OptimalCutpoints)). Adjusted odds ratios (ORs) with 95% confidence interval (95% CI) for associations of TCF7L2 SNPs with dyslipidemia and individual lipid profiles were estimated using the likelihood ratio test. Associations between dyslipidemia phenotypes based on Fredrickson’s classification and each SNP were evaluated using multivariate multinomial logistic regression by implementing the “nnet” package, measured as adjusted relative risk ratios (RRRs) with 95% CI ([Ripley & Venables, 2022](https://cran.r-project.org/package=nnet)). The association was significant when the p value is < 0.025, following Bonferroni correction (p value = 0.050/2 SNPs) ([Cheverud, 2001](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0001742)). The adjusted ORs and 95% CI were illustrated as forest plot using “ggplot2” package ([Wickham et al., 2011](https://ggplot2.tidyverse.org)). Further, inferred haplotypes were estimated with the expectation maximization algorithm, as implemented in the “haplo.stats” package ([Sinnwell et al., 2021](https://doi.org/10.1002/.contentType)). The haplotype associations with dyslipidemia and lipid profiles were determined using the generalized linear regression models, adjusted for age, sex, population, obesity, diabetes mellitus-FG, and TyG index. Empirical p values (p_sim) at significant level 0.050 were calculated after 10,000 simulations for multiple testing correction ([Becker & Knapp, 2004](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2282858/)). Finally, the interaction analyses between TCF7L2 SNPs and obesity status on dyslipidemia probability were investigated using the likelihood ratio test by considering all genetic models. The significant interactions (p_interaction < 0.100) were then plotted using the “interactions” package ([Long, 2021](https://cran.r-project.org/package=interactions)).
RESULTS

Baseline characteristics of the subjects

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

Genotypic and allelic distribution

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

Genetic associations with dyslipidemia and altered lipid profile

The genetic associations of rs290487 and rs290481 with dyslipidemia and lipid profile were presented in Figs. 1 and 2. Both SNPs were significantly associated with dyslipidemia and individual high TC levels. rs290487 was significantly associated with dyslipidemia in additive (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 = 0.020), meanwhile rs290481 was significantly associated in additive (OR 1.56, 95% CI [1.14–2.14], p = 0.006) and dominant model (OR 2.08, 95% CI [1.26–3.50], p = 0.005). Further, both SNPs were associated with high TC levels in additive (rs290487 OR 1.95, 95% CI [1.35–2.84], p < 0.001; rs290481 OR 1.78, 95% CI [1.22–2.56], p = 0.002) and dominant model (rs290487 OR 3.35, 95% CI [1.76–6.88], p < 0.001; rs290481 OR 2.79, 95% CI [1.48–5.69], p = 0.003). Additionally, rs290487 was also associated with high LDL-C levels in dominant model (OR 2.33, 95% CI [1.20–4.92], p = 0.018). Both SNPs did not show any significant associations with individual high TG and low HDL-C. Complete genotypic distribution of both SNPs on non-affected and dyslipidemia affected subjects are shown in Table S3. Further association analyses between each SNP and clinical dyslipidemia phenotypes according to Fredrickson’s classification showed the similar effect on high TC, regardless of the TG level (Table 2). Both rs290487
and rs290481 were significantly associated with increase odds for developing combined low TG, high TC (equal to IIa phenotype) (rs290487 additive OR 1.65, 95% CI [1.07–2.55], \( p = 0.023 \); rs290481 additive OR 1.67, 95% CI [1.09–2.56], \( p = 0.018 \)) and high TG, high TC (equal to IIb, III, IV, V phenotypes) (rs290487 additive OR 2.43, 95% CI [1.33–4.43], \( p = 0.004 \), dominant OR 17.02, 95% CI [2.23–130.89], \( p = 0.007 \); rs290481 additive OR 2.01, 95% CI [1.12–3.61], \( p = 0.019 \), dominant OR 6.99, 95% CI [1.59–30.83], \( p = 0.010 \)).

**TCF7L2 rs290487 and rs290481 haplotype association with dyslipidemia and altered lipid profile**

Since the two SNPs had a high LD, we therefore conducted haplotype association analysis with dyslipidemia and altered lipid profile, as shown in Table 3. Only haplotypes with a frequency of \( \geq 0.1 \) were analyzed. We identified one haplotype, CC, carrying risk alleles from both SNPs, was strongly associated with dyslipidemia, high LDL-C, and high TC (\( p_{\text{sim}} < 0.050 \)) under dominant and additive models.

**The interactions between TCF7L2 rs290487 and rs290481 with obesity towards dyslipidemia probability**

To investigate the modulatory effect of obesity in influencing SNPs associations with dyslipidemia, we analyzed the interaction between rs290487 and rs290481 SNPs and obesity status by considering all genetic models (Table S4). As shown in Fig. 3, obesity modified the effect of rs290487 and rs290481 genotypes on dyslipidemia risk. In the additive model for both SNPs, subjects carrying the heterozygous CT genotype were more...
### Table 2: Multinomial logistic regression results for associations of rs290487 and rs290481 with dyslipidemia phenotypes.

| Dyslipidemia phenotype | Equal to Fredrickson's classification | GENOTYPE | FREQUENCY | ADDITIVE | DOMINANT | RECESSIVE |
|------------------------|---------------------------------------|----------|-----------|----------|----------|----------|
|                        |                                       | TT       | TC        | CC       |          |          |
| rs290487               |                                       |          |           |          |          |          |
| Non-dyslipidemic       |                                       | 366      | 0.21      | 0.47     | 0.33     |          |          |
| Low TG, High TC        | IIa                                   | 53       | 0.30      | 0.51     | 0.19     |          |          |
| High TG, Low TC        | IV, I                                 | 70       | 0.19      | 0.56     | 0.26     |          |          |
| High TG, High TC       | IIb, III, IV, V                       | 29       | 0.28      | 0.66     | 0.07     |          |          |
| High LDL-C only        | 8                                     | 0.25     | 0.63     | 0.13     |          |          |
| Low HDL-C only         | 39                                    | 0.28     | 0.44     | 0.28     |          |          |
| rs290481               |                                       |          |           |          |          |          |
| Non-dyslipidemic       |                                       | 366      | 0.21      | 0.47     | 0.33     |          |          |
| Low TG, High TC        | IIa                                   | 53       | 0.30      | 0.51     | 0.19     |          |          |
| High TG, Low TC        | IV, I                                 | 70       | 0.19      | 0.56     | 0.26     |          |          |
| High TG, High TC       | IIb, III, IV, V                       | 29       | 0.28      | 0.66     | 0.07     |          |          |
| High LDL-C only        | 8                                     | 0.25     | 0.62     | 0.13     |          |          |
| Low HDL-C only         | 39                                    | 0.28     | 0.44     | 0.28     |          |          |

Notes:
- 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.
- 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 Revision, 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/m²) (American Diabetes Association, 2010), and diabetes mellitus-FG (FPG ≥ 126 mg/dL) and diabetes mellitus-II (FPG ≥ 200 mg/dL) and diabetes mellitus-II (FPG ≥ 240 mg/dL) (American Diabetes Association, 2010). The significant p values after Bonferroni’s correction (p < 0.025) are in bold.

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

| Trait         | Haplotype | rs290487 | rs290481 | Frequency | Additive | Dominant | p  | Dominant | p  |
|---------------|-----------|----------|----------|-----------|----------|----------|----|----------|----|
|               |           | SS       |          |           |          |          |    |          |    |
|               |            | NA       | AS       |           |          |          |    |          |    |
| Dyslipidemia  | T          | 0.53     | 0.46     | <2.674    | 0.007    | Reference|    |          |    |
|               | 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 |
|               | T          | 0.52     | 0.48     | <0.676    | 0.488    |          |    |          |    |
|               | 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 |
|               | T          | 0.52     | 0.43     | <1.908    | 0.067    |          |    |          |    |
|               | 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          | 0.51     | 0.50     | <0.004    | 1.000    |          |    |          |    |
|               | 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          | 0.53     | 0.40     | <3.376    | 0.001    |          |    |<0.001    | 3.650|<0.001 | 3.50     | [1.79–6.84]  |<0.001 |

Notes:
- 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 (≥200 mg/dL), high LDL-C (≥160 mg/dL), low HDL-C (<40 mg/dL), high TC (≥240 mg/dL). Haplotypes with frequency of ≥20.1 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 ≥ 25 kg/m²) (WHO, 2000), diabetes mellitus-FG (FPG ≥ 126 mg/dL), diabetes mellitus-II (FPG ≥ 200 mg/dL), diabetes mellitus-II (FPG ≥ 240 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.
likely to develop dyslipidemia when they had obesity, when compared to the other genotypes carriers (rs290487 $p_{\text{interaction}} = 0.034$; rs290481 $p_{\text{interaction}} = 0.013$). When being analysed under the recessive model, the TT+TC genotypes carrier exhibited an increased dyslipidemia probability when co-exist with obesity (rs290487 $p_{\text{interaction}} = 0.064$; rs290481 $p_{\text{interaction}} = 0.015$).

**DISCUSSION**

Our study aimed to investigate the association of $TCF7L2$ intronic SNPs rs290487 and rs290481 with dyslipidemia in Balinese population. The SNPs’ influence on metabolic abnormalities in the selected Indonesian population has not been well-described. In this study, we reported the notable impacts of individual genotypes and a haplotype of the rs290487 and rs290481 SNPs on dyslipidemia and individual lipid profile in the Balinese. Over the past three decades, this population has been exposed to westernization and urbanization brought by rapid tourism development, leading to a high prevalence of obesity and metabolic syndrome, particularly in tourism destination area (Suastika et al., 2011). Our findings support the notion that $TCF7L2$ SNPs have a significant impact on lipid metabolism.

The rs290487 and rs290481 allele frequencies varied among different populations. In East Asians, these SNPs were commonly found on average MAF of 0.60 (Chang et al., 2007;
Liu et al., 2009; Wang et al., 2013; Zhu et al., 2017), but found in much lower frequencies among Caucasians (Delgado-Lista et al., 2010), the population referred by dbSNP. To the best of our knowledge, this study is the first report on MAF of rs290487 and rs290481 in the Balinese population. Our findings showed the frequencies of T allele of rs290487 and rs290481 were 0.56 and 0.53, respectively, while C allele of both SNPs automatically became the minor allele. Differences in allelic distribution can occur due to some conditions, such as different genetic background between populations (Ding & Kullo, 2011) and natural selection mechanism, whereas frequency of adaptive alleles in a population tend to be arisen (Kido et al., 2018).

In this study, we found dyslipidemia more prevalent in males than females. Changes towards unhealthy lifestyles in the Balinese increase the risk of dyslipidemia in this population. According to the Bali Province Basic Health Survey 2018, Balinese men relatively consumed more sweetened beverages (including energy drink and soft drink) and less vegetables and fruits than women (Kementerian Kesehatan, 2019b). In general, risks of dyslipidemia in men were dominantly associated with unhealthy lifestyles, such as high fatty and salty food intake, smoking, hypertension, obesity, and diabetes (Wang et al., 2020; Xi et al., 2020). Meanwhile, dyslipidemia in women was often found at postmenopausal state due to decreased in estrogen level and its lipoprotein maintenance role (Reddy Kilim & Rao Chandala, 2013; Opoku et al., 2019; Xi et al., 2020). Unfortunately, lifestyle and physiological data from dyslipidemic subjects were not available, which is the limitation of this study.

Our findings showed that the TCF7L2 rs290487 and rs290481 C alleles were significantly associated with dyslipidemia and high TC levels. Additional association was found between rs290487 and high LDL-C levels. These associations were consistently found in haplotype CC from both SNPs. Furthermore, we did not find any significant associations between rs290487 and rs290481 with individual TG levels, even though our dyslipidemic subjects exhibited 2-fold higher TG levels, compared to non-dyslipidemic subjects. Interestingly, the associations of both SNPs with either low or high TG levels were only detected when it appeared together with high TC levels, indicating a stronger association of these SNPs with TC rather than TG level itself. TC level reflects the total amount of cholesterol, including LDL-C, HDL-C, and other lipids. Therefore, the significant association between TCF7L2 SNPs with TC might occur due to its major influence on LDL-C. A study performed using MetS subject from eight European countries have reported that rs290481 C allele, the major allele in that population, also contributed to higher LDL-C levels (Delgado-Lista et al., 2010). Our previous study had also reported the associations of three TCF7L2 SNPs (rs7903146, rs12255372, rs10885406) with elevated TC/HDL-C ratio (Oktavianthi et al., 2018). TC levels was known to be increased primarily due to elevated LDL-C levels (Kreisberg & Kasim, 1987). High LDL-C and TC level might increase cardiovascular disease risk by promoting atherosclerosis process (Hedayatnia et al., 2020).

As a wide-range transcription factor, TCF7L2 takes part in regulating the gene expression by binding to the promoter of its target genes (Norton et al., 2014; Zhao et al., 2016). The presence of intronic polymorphisms may interrupt the alternative splicing
mechanism of TCF7L2, resulting in changes of mRNA isoforms and leading to dysregulation of its downstream target genes (Mondal et al., 2010; Buroker, 2017). Pradas-Juni et al. (2014) have found an increase of exon four-containing mRNA transcripts in pancreatic islets of T2DM patient with rs7903146 TT genotype. The inclusion or exclusion of exon 4 might be influenced by the presence of rs7903146 in intron 4. On the other hand, rs290487 and rs290481 which are located in intron 8 and 16, respectively, might possess different approaches in alternative splicing regulation (Liu et al., 2009). The transition from T to C in rs290487 was suggested to induce altered binding affinity of TCF7L2 binding sites to its target genes (Zhang et al., 2020). Many studies have demonstrated the role of TCF7L2 in regulating genes that are involved in cholesterol and triglyceride biosynthesis, such as ApoB (Norton et al., 2014), Lpl (He et al., 2020), Tgh1, and Tgh2 (Geoghegan et al., 2019). Geoghegan et al. (2019) have also found TCF7L2 binding sites within 1 kb of the promoter of numerous genes involved in the de novo lipogenesis pathway. Moreover, a specific TCF7L2 rs7903146 region was known to control ACSL5 gene expression which is important for lipid biosynthesis and fatty acid degradation by interacting with its promoter as an enhancer (Xia et al., 2016). Therefore, the effect of intronic variants within the TCF7L2 gene on dyslipidemia might be done through several pathways. A conceptual framework for summarizing the relationship between TCF7L2 SNPs and dyslipidemia was proposed (Fig. 4).
In the current study, we found that obesity modulates the SNPs’ association with dyslipidemia, particularly in those who carry heterozygous genotype. Previously, the modulatory effect of obesity in genetic risks towards dyslipidemia has also been reported (Yin et al., 2012; Cole, Nikpay & McPherson, 2015). Obesity and dyslipidemia are linked by insulin resistance, as reviewed in Vekic et al. (2019). Since we lacked HOMA-IR data, the most common insulin resistance marker, we used TyG index as a surrogate marker (Simental-Mendia, Rodríguez-Morán & Guerrero-Romero, 2008; Khan et al., 2018; Aman et al., 2021). Although the TyG index is a significant determining factor for dyslipidemia, we did not find any direct impact of rs290487 and rs290481 on it (Table S5). This might suggest that the SNPs are rather influencing lipid metabolism. Further studies are warranted to explore rs290487 and rs290481 influence on the other lipid fractions (VLDL, chylomicron, ApoE, etc.), to understand on how TCF7L2 gene polymorphisms influence on dyslipidemia.

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

ACKNOWLEDGEMENTS
This study was conducted as a collaborative initiative between Department of Internal Medicine, Udayana University/Sanglah Hospital, Denpasar, Bali and Eijkman Institute for Molecular Biology, Jakarta. The authors sincerely acknowledge the participation and support of all volunteers, field medical doctors, medical faculty students, clinical pathology laboratory and research assistants. We thank Pande Dwipayana, Desak Made Wihandani, I Wayan Weta for their support during sample collections and Dr. Ni Luh Made Agustini Leonita and Clarissa Asha Febinia for their help in DNA isolation.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This research was supported by a block grant from the Government of Republic of Indonesia through the Ministry of Research and Technology for the Eijkman Institute for Molecular Biology. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Grant Disclosures
The following grant information was disclosed by the authors:
Government of Republic of Indonesia through the Ministry of Research and Technology for the Eijkman Institute for Molecular Biology.

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Prisca C Limardi performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Sukma Oktavianthi conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Lidwina Priliani conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Retno Lestari analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Made Ratna Saraswati analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Ketut Suastika analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Safarina G Malik conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The Eijkman Institute Research Ethics Commission (No. 32 on 27 October 2008 and No. 80 on 24 December 2014)

and

The Faculty of Medicine Ethic Committee, Universitas Udayana (No. 690a/SKRT/X/2010 on 28 October 2010 and No. 1286/UN.14.2/Litbang/2014 on 18 September 2014).

Field Study Permissions
The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

The Faculty of Medicine Ethic Committee, Udayana University for field work.

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

We received permission from the following leaders of villages and regencies: I Made Madia Suryanatha S.S.T.P (Legian Village of the Badung Regency), I Wayan Supat
(Penglipuran Village of the Bangli Regency), Ketut Gede Arjaya (Nusa Ceningan Village of the Klungkung Regency) and I Putu Sudarmaja (Pedawa Village of the Buleleng Regency).

**Data Availability**
The following information was supplied regarding data availability:

The data is available at Mendeley: Priliani, Lidwina (2021), “TCF7L2 dataset”, Mendeley Data, V1, DOI 10.17632/xv5jwz3hhv.1.

**Supplemental Information**
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13149#supplemental-information.

**REFERENCES**

Al-Daghri NM, Alkharfy KM, Al-Attas OS, Krishnaswamy S, Mohammed AK, Albagha OM, Alenad AM, Chrousos GP, Alokail MS. 2014. Association between type 2 diabetes mellitus-related SNP variants and obesity traits in a Saudi population. *Molecular Biology Reports* 41(3):1731–1740 DOI 10.1007/s11033-014-3022-z.

Aman M, Resnawita D, Rasyid H, Kasim H, Bakri S, Umar H, Daud NA, Seweng A. 2021. The concordance of triglyceride glucose index (TyG index) and homeostatic model assessment for insulin resistance (Homa-IR) in non-diabetic subjects of adult Indonesian males. *Clinical Epidemiology and Global Health* 9(5):227–230 DOI 10.1016/j.cegh.2020.09.003.

American Diabetes Association. 2010. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 33:S62 DOI 10.2337/DC10-S062.

Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP. 2011. Dyslipidaemia of obesity, metabolic syndrome and type 2 diabetes mellitus: the case for residual risk reduction after statin treatment. *The Open Cardiovascular Medicine Journal* 5(1):24–34 DOI 10.2174/1874192401105010024.

Becker T, Knapp M. 2004. A powerful strategy to account for multiple testing in the context of haplotype analysis. *American Journal of Human Genetics* 75(4):561–570 DOI 10.1086/424390.

Buroker NE. 2017. SNPs, transcriptional factor binding sites and disease. *Review Article Biomedical Genetics and Genomics Biomed Genet Genomics* 2(2):1–9 DOI 10.15761/BGG.1000132.

Chang Y-C, Chang T-J, Jiang Y-D, Kuo S-S, Lee K-C, Chiu KC, Chuang I-M. 2007. Association study of the genetic polymorphisms of the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes in the Chinese population. *Diabetes* 56(10):2631–2637 DOI 10.2337/DB07-0421.

Chang YC, Chiu YF, Low-Tone Ho L, Ting CT, Shih KC, David Curb J, Ida Chen YD, Li HY, Chuang LM. 2010. TCF7L2 genetic variants and progression to diabetes in the Chinese population: pleiotropic effects on insulin secretion and insulin resistance. *Journal of Molecular Medicine* 88(2):183–192 DOI 10.1007/s00109-009-0542-4.

Chen N, Wang J. 2018. Wnt/β-catenin signaling and obesity. *Frontiers in Physiology* 9:317 DOI 10.3389/FPHYS.2018.00792.

Chen J, Yuan T, Liu M, Chen P. 2013. Association between TCF7L2 gene polymorphism and cancer risk: a meta-analysis. *PLOS ONE* 8(8):71730 DOI 10.1371/JOURNAL.PONE.0071730.

Cheverud JM. 2001. A simple correction for multiple comparisons in interval mapping genome scans. *Heredity* 87(1):52–58 DOI 10.1046/j.1365-2540.2001.00901.x.
Cole CB, Nikpay M, McPherson R. 2015. Gene-environment interaction in dyslipidemia. *Current Opinion in Lipidology* 26(2):133–138 DOI 10.1097/MOL.0000000000000160.

Collins A, Ke X. 2012. Primer1: primer design web service for tetra-primer ARMS-PCR. *The Open Bioinformatics Journal* 6(1):55–58 DOI 10.2174/1875036201206010055.

Delgado-Lista J, Perez-Martinez P, Garcia-Rios A, Phillips CM, Williams CM, Gulseth HL, Helal O, Blaak EE, Kiec-Wilk B, Basu S, Drevon CA, Defoort C, Saris WH, Wybranska I, Riserus U, Lovegrove JA, Roche HM, Lopez-Miranda J. 2010. Pleiotropic effects of TCF7L2 gene variants and its modulation in the metabolic syndrome: from the LIPGENE study. *Atherosclerosis* 214(1):110–116 DOI 10.1016/j.atherosclerosis.2010.10.027.

DeMenna J, Puppala S, Chittoor G, Schneider J, Kim JY, Shaibi GQ, Mandarino LJ, Duggirala R, Coletta DK. 2014. Association of common genetic variants with diabetes and metabolic syndrome related traits in the Arizona Insulin Resistance registry: a focus on Mexican American families in the Southwest. *Human Heredity* 78(1):47–58 DOI 10.1159/000363411.

Ding K, Kullo IJ. 2011. Geographic differences in allele frequencies of susceptibility SNPs for cardiovascular disease. *BMC Medical Genetics* 12(1):1–9 DOI 10.1186/1471-2350-12-15.

Folsom AR, Pankow JS, Peacock JM, Bielinski SJ, Heiss G, Boerwinkle E. 2008. Variation in TCF7L2 and increased risk of colon cancer: the atherosclerosis risk in communities (ARIC) study. *Diabetes Care* 31:905 DOI 10.2337/DC07-2131.

Geoghegan G, Simcox J, Seldin MM, Parnell TJ, Stubben C, Just S, Begaye I, Luis AJ, Villanueva CJ. 2019. Targeted deletion of Tcf7l2 in adipocytes promotes adipocyte hypertrophy and impaired glucose metabolism. *Molecular Metabolism* 24(5307):44–63 DOI 10.1016/j.molmet.2019.03.003.

Hansson O, Zhou Y, Renström E, Osmark P. 2010. Molecular function of TCF7L2: consequences of TCF7L2 splicing for molecular function and risk for type 2 diabetes. *Current Diabetes Reports* 10(6):444–451 DOI 10.1007/s11892-010-0149-8.

He LH, Gao JH, Yu XH, Wen FJ, Luo JJ, Qin YS, Chen MX, Zhang DW, Wang ZB, Tang CK. 2020. Artesunate inhibits atherosclerosis by upregulating vascular smooth muscle cells-derived LPL expression via the KLF2/NRF2/TCF7L2 pathway. *European Journal of Pharmacology* 884:173408 DOI 10.1016/j.ejphar.2020.173408.

Hedayatnia M, Asadi Z, Zare-Feyzabadi R, Yaghoobi-Khorasani M, Ghazizadeh H, Ghaffarian-Zirak R, Nosrati-Tirkani A, Mohammad-Bajgiran M, Rohban M, Sadabadi F, Rahimi HR, Ghalandari M, Ghaffari MS, Yousefi A, Pourseimaelli E, Esharhatloou MR, Moohebati M, Ferns GA, Esmaily H, Ghayour-Mobarhan M. 2020. Dyslipidemia and cardiovascular disease risk among the MASHAD study population. *Lipids in Health and Disease* 19(1):1–11 DOI 10.1186/s12944-020-01204-y.

Joint Committee for Guideline Revision. 2018. 2016 Chinese guidelines for the management of dyslipidemia in adults. *Journal of Geriatric Cardiology : JGC* 15:1–29 DOI 10.11909/J.ISSN.1671-5411.2018.01.011.

Kementerian Kesehatan RI. 2019a. Laporan nasional riskesdas 2018. Available at [http://repository.litbang.kemkes.go.id/3514/1/LaporanRiskesdas%202018%20Nasional.pdf](http://repository.litbang.kemkes.go.id/3514/1/LaporanRiskesdas%202018%20Nasional.pdf) (accessed 8 November 2021).

Kementerian Kesehatan RI. 2019b. Laporan provinsi bali riskesdas 2018. Available at [https://ejournal2.litbang.kemkes.go.id/index.php/lpb/issue/view/236](https://ejournal2.litbang.kemkes.go.id/index.php/lpb/issue/view/236) (accessed 8 November 2021).

Khan SH, Sobia F, Niazi NK, Manzoor SM, Fazal N, Ahmad F. 2018. Metabolic clustering of risk factors: evaluation of Triglyceride-glucose index (TyG index) for evaluation of insulin resistance. *Diabetology & Metabolic Syndrome* 10(1):74 DOI 10.1186/s13098-018-0376-8.
Kido T, Sikora-Wohlfeld W, Kawashima M, Kikuchi S, Kamatani N, Patwardhan A, Chen R, Sirota M, Kodama K, Hadley D, Butte AJ. 2018. Are minor alleles more likely to be risk alleles? *BMC Medical Genomics* 11(1):1–11 DOI 10.1186/s12920-018-0322-5.

Kreisberg RA, Kasim S. 1987. Cholesterol metabolism and aging. *The American Journal of Medicine* 82(1):54–60 DOI 10.1016/0002-9343(87)90227-5.

Lin CF, Chang YH, Chien SC, Lin YH, Yeh HY. 2018. Epidemiology of dyslipidemia in the Asia Pacific Region. *International Journal of Gerontology* 12(1):2–6 DOI 10.1016/j.jige.2018.02.010.

Liu PH, Chang YC, Der JY, Chen WJ, Chang TJ, Kuo SS, Lee KC, Hsiao PC, Chiu KC, Chuang LM. 2009. Genetic variants of TCF7L2 are associated with insulin resistance and related metabolic phenotypes in taiwanese adolescents and caucasian young adults. *The Journal of Clinical Endocrinology & Metabolism* 94(9):3575–3582 DOI 10.1210/jc.2009-0609.

Long JA. 2021. Comprehensive, user-friendly toolkit for probing interactions. *Multivariate Behavioral Research* 40(3):373–400 DOI 10.1207/s15327906mbr4003_5.

Lopez-Raton M, Xose Rodriguez-Alvarez M. 2021. Computing optimal cutpoints in diagnostic tests. Available at https://cran.r-project.org/web/packages/OptimalCutpoints/OptimalCutpoints.pdf.

Luo Y, Wang H, Han X, Ren Q, Wang F, Zhang X, Sun X, Zhou X, Ji L. 2009. Meta-analysis of the association between SNPs in TCF7L2 and type 2 diabetes in East Asian population. *Diabetes Research and Clinical Practice* 85(2):139–146 DOI 10.1016/j.diabres.2009.04.024.

Malik SG, Saraswati MR, Suastika K, Trimarsanto H, Oktavianthi S, Sudoyo H. 2011. Association of beta3-adrenergic receptor (ADRB3) Trp64Arg gene polymorphism with obesity and metabolic syndrome in the Balinese: a pilot study. *BMC Research Notes* 4(1):1–7 DOI 10.1186/1756-0500-4-167.

Mayans S, Lackovic K, Lindgren P, Ruikka K, Ågren Å, Eliasson M, Holmberg D. 2007. TCF7L2 polymorphisms are associated with type 2 diabetes in northern Sweden. *European Journal of Human Genetics* 15(3):342–346 DOI 10.1038/sj.ejhg.5201773.

Mondal AK, Das SK, Baldini G, Chu WS, Sharma NK, Hackney OG, Zhao J, Grant SFA, Elbein SC. 2010. Genotype and tissue-specific effects on alternative splicing of the transcription factor 7-like 2 gene in humans. *The Journal of Clinical Endocrinology & Metabolism* 95(3):1450–1457 DOI 10.1210/jc.2009-2064.

Nanfack AJ, Agyingi L, Noubiap JJN, Ngai JN, Colizzi V, Nyambi PN. 2015. Use of amplification refractory mutation system PCR assay as a simple and effective tool to detect HIV-1 drug resistance mutations. *Journal of Clinical Microbiology* 53(5):1671 DOI 10.1128/JCM.00114-15.

NCBI. 2021a. rs290487 RefSNP report-dbSNP. Available at https://www.ncbi.nlm.nih.gov/snp/rs290487?vertical_tab=true#frequency_tab (accessed 9 November 2021).

NCBI. 2021b. rs290481 RefSNP report-dbSNP. Available at https://www.ncbi.nlm.nih.gov/snp/rs290481?vertical_tab=true#frequency_tab (accessed 9 November 2021).

NCEP. 2002. Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 106(25):3143–3421 DOI 10.1161/01.cir.106.25.3143.

Norton L, Chen X, Fourcaudot M, Acharya NK, De Fronzo RA, Heikkinen S. 2014. The mechanisms of genome-wide target gene regulation by TCF7L2 in liver cells. *Nucleic Acids Research* 42(22):13646–13661 DOI 10.1093/nar/gku1225.

Oktavianthi S, Saraswati MR, Suastika K, Dwipayana P, Sulianti A, Hayati RF, Trimarsanto H, Febinia CA, Sudoyo H, Malik SG. 2018. Transcription factor 7-like 2 single nucleotide polymorphisms are associated with lipid profile in the Balinese. *Molecular Biology Reports* 45(5):1135–1143 DOI 10.1007/s11033-018-4265-x.
Opoku S, Gan Y, Fu W, Chen D, Addo-Yobo E, Trofimovitch D, Yue W, Yan F, Wang Z, Lu Z. 2019. Prevalence and risk factors for dyslipidemia among adults in rural and urban China: findings from the China National Stroke Screening and prevention project (CNSSPP). BMC Public Health 19(1):1–15 DOI 10.1186/s12889-019-7827-5.

Pate KT, Stringari C, Sprowl-Tanio S, Wang K, TeSlaa T, Hoventh NP, McQuade MM, Garner C, Digman MA, Teitell MA, Edwards RA, Gratton E, Waterman ML. 2014. Wnt signaling directs a metabolic program of glycolysis and angiogenesis in colon cancer. The EMBO Journal 33(13):1454–1473 DOI 10.15252/embj.201488598.

Pradas-Juni M, Nicod N, Fernández-Rebollo E, Gomis R. 2014. Differential transcriptional and posttranslational transcription factor 7-like regulation among nondiabetic individuals and type 2 diabetic patients. Molecular Endocrinology 28(9):1558–1570 DOI 10.1210/me.2014-1065.

Reddy Kilim S, Rao Chandala S. 2013. A comparative study of lipid profile and oestradiol in pre- and post-menopausal women. Journal of Clinical and Diagnostic Research: JCDR 7:1596 DOI 10.7860/JCDR/2013/6162.3234.

Ripley B, Venables W. 2022. Feed-forward neural networks and multinomial log-linear models. Available at https://cran.r-project.org/web/packages/nnet/nnet.pdf.

Shokouhi S, Delpisheh A, Haghani K, Mahdizadeh M, Bakhtiyari S. 2014. Association of rs7903146, rs12255372, and rs290487 polymorphisms in TCF7L2 gene with type 2 diabetes in an Iranian Kurdish ethnic group. Clinical Laboratory 60(08/2014):1269–1276 DOI 10.7754/Clin.Lab.2013.130809.

Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. 2008. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. Metabolic Syndrome and Related Disorders 6(4):299–304 DOI 10.1089/met.2008.0034.

Sinnwell J, Schaid D, Dwipayana PK, Ratna Saraswati IM, Kuswardhani T, Astika N, Putrawan IB, Matsumoto K, Kajiwara N, Taniguchi H. 2021. Statistical analysis of haplotypes with traits and covariates when linkage phase is ambiguous, relationship between age and metabolic disorders in the population of bali. Journal of Clinical Gerontology and Geriatrics 2(2):47–52 DOI 10.1016/J.JCGG.2011.03.001.

Suastika K, Dwipayana P, Saraswati IMR, Gotera W, Buddhiarta AAG, Sutanegara IND, Gunadi IGN, Nadha KB, Wita W, Rina K, Santosso A, Matsumoto K, Kajiwara N, Taniguchi H. 2011. Prevalence of obesity, metabolic syndrome, impaired fasting glycemia, and diabetes in selected villages of Bali, Indonesia. Journal of the ASEAN Federation of Endocrine Societies 26:159.

Suastika K, Semadi IMS, Dwipayana IMP, Saraswati MR, Gotera W, Buddhiarta AAG, Matsumoto K, Kajiwara N, Taniguchi H. 2019. Dyslipidemia in diabetes: a population-based study in Bali. International Journal of General Medicine 12:313 DOI 10.2147/IJGM.

Tabangin ME, Woo JG, Martin JJ. 2009. The effect of minor allele frequency on the likelihood of obtaining false positives. BMC Proceedings 3(S7):S41 DOI 10.1186/1753-6561-3-S7-S41.

Vekic J, Zeljkovic A, Stefanovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. 2019. Obesity and dyslipidemia. Metabolism 92:71–81 DOI 10.1016/J.METABOL.2018.11.005.

Villarel DT, Robertson H, Bell GI, Patterson BW, Tran H, Wice B, Polonsky KS. 2010. TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. Diabetes 59:479 DOI 10.2337/db09-1169.

Wang J, Li L, Zhang J, Xie J, Luo X, Yu D, Zhao J, Feng T, Pang C, Yin L, Hu F, Zhang J, Wang Y, Wang Q, Zhai Y, You H, Zhu T, Hu D. 2013. Association of rs7903146 (IVS3C/T) and rs290487 (IVS3C/T) polymorphisms in TCF7L2 with type 2 diabetes in 9,619 Han Chinese population. PLoS ONE 8(3):e59053 DOI 10.1371/journal.pone.0059053.
Wang M, Liu M, Li F, Guo C, Liu Z, Pan Y, Liu Y, Liu F, Cai H, Wu Y, He Z, Ke Y. 2020. Gender heterogeneity in dyslipidemia prevalence, trends with age and associated factors in middle age rural Chinese. *Lipids in Health and Disease* 19:1–11
DOI 10.1186/S12944-020-01313-8/TABLES/3.

Warnes G, Gorjanc G, Leisch F, Man M. 2021. Population genetics. *Available at* https://cran.r-project.org/web/packages/genetics/genetics.pdf.

World Health Organization. 2000. The Asia-Pacific perspective: redefining obesity and its treatment. *Available at* https://apps.who.int/iris/handle/10665/206936.

Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K, Yutani H, Dunnington D. 2021. Create elegant data visualisations using the grammar of graphics. *Available at* https://cran.r-project.org/web/packages/ggplot2/ggplot2.pdf.

Xi Y, Niu L, Cao N, Bao H, Xu X, Zhu H, Yan T, Zhang N, Qiao L, Han K, Hang G, Wang W, Zhang X. 2020. Prevalence of dyslipidemia and associated risk factors among adults aged ≥35 years in northern China: a cross-sectional study. *BMC Public Health* 20:1–9
DOI 10.1186/S12889-020-09172-9/FIGURES/2.

Ye S, Dhillon S, Ke X, Collins AR, Day INM. 2001. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Research* 29(17):e88 DOI 10.1093/nar/29.17.e88.

Zhang X, Ye P, Huang H, Wang B, Dong F, Ling Q. 2020. TCF7L2 rs290487 C allele aberrantly enhances hepatic gluconeogenesis through allele-specific changes in transcription and chromatin binding. *Sedentary Life and Nutrition* 12(13):13365–13387
DOI 10.18632/aging.103442.

Zhao C, Deng Y, Liu L, Yu K, Zhang L, Wang H, He X, Wang J, Lu C, Wu LN, Weng Q, Mao M, Li J, Van Es JH, Xin M, Parry L, Goldman SA, Clevers H, Lu QR. 2016. Dual regulatory switch through interactions of Tcf7l2/Tcf4 with stage-specific partners propels oligodendrogial maturation. *Nature Communications* 7(1):271 DOI 10.1038/ncomms10883.

Zhu L, Xie Z, Lu J, Hao Q, Kang M, Chen S, Tang W, Ding H, Chen Y, Liu C, Wu H. 2017. TCF7L2 rs290481 T>C polymorphism is associated with an increased risk of type 2 diabetes mellitus and fasting plasma glucose level. *Oncotarget* 8(44):77000–77008
DOI 10.18632/oncotarget.20300.