Association between *IL-1B (-511)/IL-1RN* (VNTR) polymorphisms and Type 2 Diabetes: a systematic review and meta-analysis

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Interleukin-1 (IL-1) plays an essential role in the immune pro-inflammatory process, which is regarded as one of many factors in the development of type 2 diabetes mellitus (T2DM). Several case-control studies have illustrated the association of the *IL-1B* (-511) (rs16944, Chr 2:112,837,290, C/T Intragenic, Transition Substitution) and *IL-1RN* (VNTR) (gene for IL-1 receptor antagonist, IL-1RA, 86 bp tandem repeats in intron 2) polymorphisms with T2DM risk. However, the results were inconsistent and inconclusive. We performed a meta-analysis (registry number: CRD42021268494) to assess the association of the *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms with T2DM risk. Random-effects models were applied to calculate the pooled ORs (odds ratios) and 95% CIs (confidence intervals) to test the strength of the association in the overall group and subgroups stratified by ethnicity, respectively. Between-study heterogeneity and publication bias were evaluated by the *Q*-test, I^2 statistic, Harbord test, and Peters test accordingly. Sensitivity analyses were also performed. A total of 12 publications evaluating the association of *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms with the risk of T2DM development were included. The meta-analysis showed that *IL-1RN* (VNTR) was related to the increasing development of T2DM risk in the recessive model (OR=1.62, 95% CI: 1.09-2.42, P_{het}=0.377, P_z=0.018) and in the homozygous model (OR=2.02, 95% CI: 1.07-3.83, P_{het}=0.085, P_z=0.031), and the *IL-1RN 2** allele was found a significant association with evaluated T2DM risk in all ethnicities (OR=2.08, 95% CI: 1.43-3.02, P_{het}⎕0.001, P_z⎕0.001) and in EA (OR=2.01, 95% CI: 1.53-2.66, P_{het}=0.541, P_z⎕0.001). Moreover, stratification by ethnicity revealed that *IL-1B (-511)* was associated with a decreased risk of T2DM in the dominant model (OR=0.76, 95% CI:

0.59–0.97, P_{het}=0.218, P_z=0.027) and codominant model (OR=0.73, 95% CI: 0.54–0.99, P_{het}=0.141, P_z=0.040) in the East Asian (EA) subgroup. Our results suggest that the *IL-1RN 2** allele and *2*2** homozygous polymorphism are strongly associated with increasing T2DM risk and that the *IL-1B (-511) T* allele polymorphism is associated with decreasing T2DM risk in the EA subgroup.

1   **Association between *IL-1B (-511)/IL-1RN* (VNTR) polymorphisms and Type 2**

2   **Diabetes: a systematic review and meta-analysis**

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29 **Abbreviation:**

T2DM	type 2 diabetes mellitus
IL-1	Interleukin-1
IL-1Ra	IL-1 receptor antagonist
IL-1β	interleukin-1β
HWE	Hardy–Weinberg Equilibrium
OR	odds ratios
CIs	confidence intervals
EA	East Asian

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54   **Abstract:**

55   Interleukin-1 (IL-1) plays an essential role in the immune pro-inflammatory process, which is

56   regarded as one of many factors in the development of type 2 diabetes mellitus (T2DM). Several

57   case-control studies have illustrated the association of the *IL-1B (-511)* (rs16944, Chr

58   2:112,837,290, C/T Intragenic, Transition Substitution) and *IL-1RN* (VNTR) (gene for IL-1

59   receptor antagonist, IL-1RA, 86 bp tandem repeats in intron 2) polymorphisms with T2DM risk.

60   However, the results were inconsistent and inconclusive. We performed a meta-analysis (registry

61   number: CRD42021268494) to assess the association of the *IL-1B (-511)* and *IL-1RN* (VNTR)

62   polymorphisms with T2DM risk. Random-effects models were applied to calculate the pooled

63   ORs (odds ratios) and 95% CIs (confidence intervals) to test the strength of the association in the

64   overall group and subgroups stratified by ethnicity, respectively. Between-study heterogeneity

65   and publication bias were evaluated by the *Q*-test, I^2 statistic, Harbord test, and Peters test

66   accordingly. Sensitivity analyses were also performed. A total of 12 publications evaluating the

67   association of *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms with the risk of T2DM

68   development were included. The meta-analysis showed that *IL-1RN* (VNTR) was related to the

69   increasing development of T2DM risk in the recessive model (OR=1.62, 95% CI: 1.09-2.42,

70   P_{het}=0.377, P_z=0.018) and in the homozygous model (OR=2.02, 95% CI: 1.07-3.83, P_{het}=0.085,

71   P_z=0.031), and the *IL-1RN 2** allele was found a significant association with evaluated T2DM

72   risk in all ethnicities (OR=2.08, 95% CI: 1.43-3.02, P_{het}<0.001, P_z<0.001) and in EA

73   (OR=2.01, 95% CI: 1.53-2.66, P_{het}=0.541, P_z<0.001). Moreover, stratification by ethnicity

74   revealed that *IL-1B (-511)* was associated with a decreased risk of T2DM in the dominant model

75   (OR=0.76, 95% CI: 0.59–0.97, P_{het}=0.218, P_z=0.027) and codominant model (OR=0.73, 95% CI:

76   0.54–0.99, P_{het}=0.141, P_z=0.040) in the East Asian (EA) subgroup. Our results suggest that the

77   *IL-1RN 2** allele and *2*2** homozygous polymorphism are strongly associated with increasing

78   T2DM risk and that the *IL-1B (-511) T* allele polymorphism is associated with decreasing T2DM

79   risk in the EA subgroup.

80   **1. Introduction:**

81   The latest data from the 9th edition of the IDF Diabetes Atlas show that approximately 463

82   million adults (20-79 years) have diabetes, and by 2045, the number will rise to 700 million.

83   Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes (> 90% of diabetes). The

84   proportion of T2DM patients is increasing in most countries, especially in low- and middle-

85   income countries such as China and India (https://www.idf.org/aboutdiabetes/what-is-

86   diabetes/facts-figures.html). Growing evidence suggests that the risk of T2DM is associated with

87   various factors, such as genetics, ethnicity, environment, and lifestyle. In particular, genetic

88   susceptibility seems to play an essential role in the pathogenesis of T2DM(Hivert et al. 2014).

89   T2DM occurs when β-cell function progressively deteriorates and fails to compensate for insulin

90   resistance, partly due to the demise of pancreatic β-cells through apoptosis(Donath & Halban

91   2004). The chronic and low-grade inflammation in metabolic organs including the liver, brain,

92   pancreas and adipose tissue is known as metaflammation (metabolism-induced inflammation),

93   which can be widely observed in T2DM(Hotamisligil 2006; Hotamisligil 2017). Increasing

94   evidence has shown that metaflammation plays a vital role in the development of T2DM and its

95   cardiovascular complications(Hotamisligil 2017) and pro-inflammatory cytokines, especially

96   interleukin-1β (IL-1β), play a key role in this process(Dinarello et al. 2010; Fève & Bastard 2009;

97   Sathyapalan & Atkin 2011). Moreover, the increased level of IL-1β in human pancreatic cells

98   due to the elevated glucose concentrations and decreased level of IL-1 receptor antagonist (IL-

99   1Ra) in islets of T2DM patients result in impaired insulin secretion, decreased cell proliferation,

100   and apoptosis of β-cells(Böni-Schnetzler & Donath 2013). A previous observational study

101   demonstrated that a combined elevation of IL-1β and IL-6 was associated with a roughly

102   threefold increased risk of T2DM, and IL-1β might induce insulin resistance via activating the

103   IκB kinase β(Spranger et al. 2003). Furthermore, CANTOS and TRACK trials have evaluated

104   the role of IL-1 inhibition in the treatment of several inflammatory disorders, such as coronary

105   artery disease, T2DM and rheumatoid arthritis(Qamar & Rader 2012; Ruscitti et al. 2019).

106   According to the result from CANTOS trials, canakinumab (a fully humanized monoclonal

107   antibody) could selectively block IL-1β, and its efficacy in suppressing both levels of

108   inflammatory markers and clinical symptoms in patients with autoinflammatory disease such as

109   T2DM and impaired glucose tolerance has been observed in this trial(Aday & Ridker 2018;

110   Rissanen et al. 2012). Therefore, the genetic polymorphisms that regulate the expression levels

111   of IL-1β and IL-1Ra might have an essential impact on the interindividual differences in T2DM.

112   Consisting of 3 linked genes and mapping to chromosome 2q13-24, IL-1 cytokine genes encode

113   IL-1α, IL-1β and IL-1Ra. Both IL-1α and IL-1β are pro-inflammatory cytokines, while IL-1Ra

114   can inhibit inflammation by competing for receptor binding(Dinarello 2000). In the promoter

115   region, *IL-1B (-511)* (rs16944, Chr 2:112,837,290, C/T Intragenic, Transition Substitution) has a

116   bi-allelic polymorphism at position -511, representing the C/T transition(Kristiansen et al. 2000).

117   *IL-1RN* (VNTR) (gene for IL-1 receptor antagonist, IL-1RA, 86 bp tandem repeats in intron 2)

118   has 5 different alleles: *1** (4 repeats), *2** (2 repeats), *3** (5 repeats), *4** (3 repeats) and *5** (6

119   repeats)(Tarlow et al. 1993). *IL-1RN 1** (4 repeats) and *IL-1RN 2** (2 repeats) are the most

120   common, whereas the others occur much less frequently (< 5%)(Santtila et al. 1998). Many

121   genetic association studies have been performed to estimate the relationship of the *IL-1B (-511)*

122   and *IL-1RN* (VNTR) polymorphisms with T2DM risk(Achyut et al. 2007; Muktabhant et al.

123   2013). Several results suggest that there is no relationship between them(Borilova Linhartova et

124   al. 2019; Muktabhant et al. 2013), whereas B.R Achyut *et al.* showed that both the *IL-1B (-511)*

125   and *IL-1RN* (VNTR) polymorphisms were associated with susceptibility to T2DM as well as

126   complications, and he indicated that *T2 (IL-1β −511T/IL-1RN*2)* haplotype was associated with

127   a roughly twofold increased risk of T2DM(Achyut et al. 2007). Considering the small sample

128   sizes (n＜600) and varying population characteristics (ethnic differences) in different studies, the

129   results are conflicting. To illustrate the potential association of the *IL-1B (-511)* and *IL-1RN*

130   (VNTR) polymorphisms with T2DM risk, we conducted a meta-analysis including 12 reported

131   publications.

132 **2. Materials and Methods:**

133 This systematic review was conducted following the Preferred Reporting Items for Systematic

134 Reviews and Meta-Analysis (PRISMA) statement guidelines(Moher et al. 2009). A protocol was

135 registered before commencing this review on PROSPERO (CRD42021268494).

136 **2.1. The search strategy**

137 The online databases we searched included PubMed, Web of Science, CNKI (China National

138 Knowledge Infrastructure) and Wanfang. We identified relevant articles reporting the association

139 of *IL-1RN* (VNTR) and *IL-1B (-511)* polymorphisms with the risk of T2DM in the medical

140 literature through the end of September 12, 2020. This study used a combination of the following

141 search terms: "Type 2 Diabetes Mellitus" or "T2DM" or "interleukin-1" or "IL-1" or

142 "polymorphism" or "genetics" or "association". In addition, we also conducted a hand search to

143 identify relevant data in references from retrieved articles. Juan Jiao and Zhaoping Wang

144 performed the Search Strategy. In case of disagreement, it will be settled by a third assessor's

145 evaluation and discussed until a consistent result was reached.

146 **2.2. The inclusion and exclusion criteria**

147 All studies included in this analysis met the following criteria: 1) the study revealed the

148 relationship of *IL-1B (-511)* and *IL-1RN* (VNTR) with T2DM risk; 2) the design was a "case-

149 control study"; 3) the research subjects were humans with T2DM; 4) the publication language

150 was English or Chinese; and 5) the study provided sufficient data to estimate ORs (odds ratios)

151 and 95% CIs (confidence intervals). The major exclusion criteria were as follows: 1) duplicate

152 data; 2) randomly chosen controls; 3) non-research articles; and 4) insufficient reporting of data.

153 **2.3. Data extraction**

154 All data were systematically reviewed and extracted by two investigators (ZPW and DNG)

155 according to a standardized form, and then all of the following information was collected in an

156 electronic database: the first author's name, year of publication, country of origin, ethnicity, total

157 number of cases/controls, genotyping method, diagnostic criteria of T2DM, genotypic

158 frequencies and language of the report. Disagreement was settled by a third assessor's evaluation

159 and discussed until a consistent conclusion was agreed.

160 **2.4. Assessment of quality score**

161 We assessed the quality score of identified publications based on the Newcastle Ottawa Scale

162 (NOS) (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). And the NOS involved

163 "selection", "comparability", and "estimation of outcomes or exposures". The scores ranged

164 from 0 to 9, and the score of "high quality study" was ≥ 6 (detailed scores see Table S1)(Lo et al.

165 2014). And the STREGA (Strengthening the REporting of Genetic Association) system was also

166 performed to assessed the methodological quality of all included studies, which includes 22

167 items with scoring from 0 to 22 (detailed scores see Table S2)(Little et al. 2009). And the score

168 of high quality was 18-22; the score of moderate-high quality was 13-17; the score of low quality

169 was 0-12(Duan et al. 2018).

170 **2.5. Data analysis**

171 We conducted this analysis and entered data with Stata software, version 15.0 (Stata Corp.,

172 College Station, TX, USA). We used original genotypic distribution data without any adjustment

173 to measure the strength of the association between *IL-1B (-511)/IL-1RN* and T2DM risk by ORs

174 (odds ratios) with 95% CIs (confidence intervals) under dominant, recessive, additive

175 (overdominant+codominant) and homozygous models. The distributions of genotype frequencies

176 of controls were all consistent with Hardy-Weinberg Equilibrium (*HWE*) (*P*>0.05, Table 1).

177 Between-study heterogeneity among the studies was tested with Cochran's Q and the I^2 test

178 statistics. I^2 was calculated based on the formula $I^2=100\%\times(Q\text{-}df)/Q$. Heterogeneity was regarded

179 as significant when $P_{het} < 0.1$ or $I^2 > 50\%$. For those with significant heterogeneity, we

180 performed stratified analyses to examine the statistical significance of the difference in ORs

181 according to ethnicity (East Asian, South Asian, North African and Caucasian). We used the

182 random-effects model (DerSimonian and Laird method) which was combined by applying

183 inverse variance-weighted meta-analysis to calculate the pooled OR and 95% CI, with $P_z < 0.05$

184 considered statistically significant(DerSimonian & Laird 1986). When the heterogeneity was

185 significant, sensitivity analyses were conducted to evaluate the influence of each single study by

186 omitting one study at a time and checking the pool effect size for the remainder of the studies.

187 Finally, the Harbord test and Peters test were used when fewer than ten articles were included, as

188 they are more sensitive for assessing publication bias, with $P < 0.1$ assuming that the bias was

189 statistically significant.

190 **3. Results:**

191 **3.1. Included studies**

192 Figure 1 shows the detailed process of selecting and assessing eligible studies. We identified 450

193 publications via the initial keyword search. After screening, 12 publications met our inclusion

194 criteria(Achyut et al. 2007; Blakemore et al. 1996; Borilova Linhartova et al. 2019; Cao et al.

195 2013; Doody et al. 2017; Lin et al. 2016; Liu et al. 2014; Muktabhant et al. 2013; Tayel et al.

196 2018; Zhang et al. 2004a; Zhang et al. 2004b; Zhou et al. 2010), and 438 studies were excluded

197 for containing duplicate data, missing critical data, and being review papers or studies that were

198 not related to T2DM. Table 1 illustrates the characteristics of all included publications in this

199 meta-analysis. In terms of polymorphisms and diseases, 6 identified case-control studies,

200 comprising 981 cases and 1225 controls, regarded *IL-1RN* (VNTR) to evaluate its association

201 with T2DM risk(Achyut et al. 2007; Blakemore et al. 1996; Borilova Linhartova et al. 2019;

202 Zhang et al. 2004a; Zhang et al. 2004b; Zhou et al. 2010), and 7 identified case-control studies,

203 including 1679 cases and 1442 controls, tested the association between *IL-IB (-511)* and T2DM

204 risk(Achyut et al. 2007; Cao et al. 2013; Doody et al. 2017; Lin et al. 2016; Liu et al. 2014;

205 Muktabhant et al. 2013; Tayel et al. 2018). Stratified by ethnicity, six publications were

206 performed in East Asian (EA) populations(Cao et al. 2013; Lin et al. 2016; Liu et al. 2014;

207 Zhang et al. 2004a; Zhang et al. 2004b; Zhou et al. 2010), two studies were undertaken in South

208 Asian (SA) populations(Achyut et al. 2007; Doody et al. 2017), two studies were conducted in

209 Caucasian populations(Blakemore et al. 1996; Borilova Linhartova et al. 2019), and the

210 remaining studies were completed in Southeast Asian (SA)(Muktabhant et al. 2013) and North

211 African (NA$_1$) populations(Tayel et al. 2018).

212 **3.2. Individual polymorphism meta-analysis**

213 The genotypic distributions of the *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms are shown

214 in Table 2. Table 3 shows the meta-analysis results for these two polymorphisms.

215 For the *IL-1B (-511)* polymorphism, seven studies with 1679 cases and 1442 controls were

216 included in the meta-analysis. The results of the pooled analysis suggest that the *IL-1B (-511)*

217 polymorphism is not significantly associated with T2DM risk in all study subjects under the

218 dominant model (OR=0.84, 95% CI: 0.57–1.25, P_{het}=0.001, P_z=0.395) (Table 3 and Figure 2A),

219 recessive model (OR=0.89, 95% CI: 0.61–1.31, P_{het}<0.001, P_z=0.561) (Table 3), and

220 homozygous model (OR=0.82, 95% CI: 0.48–1.39, P_{het}<0.001, P_z=0.451) (Table 3). In the

221 stratification analyses by ethnicity, we found that there was a significant association between the

222 *IL-1B (-511)* polymorphism and decreasing T2DM risk in the EA population under the dominant

223 model (OR=0.76, 95% CI: 0.59–0.97, P_{het}=0.218, P_z=0.027) (Table 3 and Figure 2A) and

224 codominant model (OR=0.73, 95% CI: 0.54–0.99, P_{het}=0.141, P_z=0.040) (Table 3), but no

225 association was observed under the recessive model (OR=0.97, 95% CI: 0.79–1.20, P_{het}=0.589,

226 P_z=0.789) or homozygous model (OR=0.80, 95% CI: 0.62–1.03, P_{het}=0.571, P_z=0.081). In other

227 ethnic groups, no association between the *IL-1B (-511)* polymorphism and T2DM risk was found.

228 Between-study heterogeneity was found in the dominant, recessive and homozygous models

229 (P_{het}=0.001, P_{het}<0.001, P_{het}<0.001) (Table 3).

230 For the *IL-1RN* (VNTR) polymorphism, six publications were involved in the meta-analysis (980

231 cases and 1225 controls). Overall, a significantly increased T2DM risk was found to be

232 associated with *2** allele in all ethnicities (OR=2.08, 95% CI: 1.43-3.02, P_{het}<0.001, P_z<0.001)

233 and in EA (OR=2.01, 95% CI: 1.53-2.66, P_{het}=0.541, P_z<0.001) (Table 3). Pooling data also

234 revealed that this polymorphism is strongly associated with an increasing T2DM risk in the

235 recessive model (OR=1.62, 95% CI: 1.09-2.42, P_{het}=0.377, P_z=0.018) (Table 3 and Figure 3A)

236 and homozygous model (OR=2.02, 95% CI: 1.07-3.83, P_{het}=0.085, P_z=0.031) (Table 3 and

237 Figure 3B), but there was no association in the dominant model (OR=1.35, 95% CI: 0.79-2.31,

238 P_{het}<0.001, P_z=0.275) (Table 3). Between-study heterogeneity was found in the dominant model

239 (P_{het}<0.001) (Table 3).

240 **3.3. Sensitivity and publication bias analysis**

241 We next performed sensitivity analysis on the association between IL-1β (-511) and T2DM. The

242 results showed that Achyut's study had an influence on the pooled OR in the IL-1β (-511)-

243 dominant model (OR=0.71, 95% CI: 0.53-0.96) and homozygous model (OR=0.65, 95% CI:

244 0.44-0.97) (Figure 2B, D) but did not affect the pooled OR in the recessive model (Figure 2C).

245 Achyut's study was performed in a SA population; thus, there was no influence on the pooled

246 OR in the EA population. Due to the lack of heterogeneity in IL-1RN recessive and homozygous

247 models, sensitivity analysis was not performed.

248 The results of the Harbord test and Peters test showed no publication bias for *IL-1B (-511)* in the

249 dominant model (P_{har}=0.759 and P_{pet}=0.881, respectively) (Figure 4A). Moreover, no evidence

250 of publication bias was found for *IL-1RN* (VNTR) in the recessive (P_{har}=0.498 and P_{pet}=0.495,

251 respectively) or homozygous models (P_{har}=0.634 and P_{pet}=0.481, respectively) (Figure 4B, C).

252 **4. Discussion:**

253 The *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms have been reported to be related to the

254 pathogenesis of T2DM(Ehses et al. 2009; Masters et al. 2010), and many case-control studies

255 have illustrated the association of *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms with T2DM

256 risk. However, each individual study may not have been powerful, causing their results to be

257 controversial. Given the above, we conducted the present meta-analysis to derive a more precise

258 evaluation of the association of *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms with T2DM

259 risk. We found that i) the *T* allele of *IL-1B (-511)* was related with a decreased T2DM risk in the

260 EA subgroup; ii) significantly elevated risk of developing T2DM was observed to be associated

261 with the *2** allele in all ethnicities and EA subgroup, and its carriers of the *IL-1RN* (VNTR)

262 polymorphism had an increased risk of developing T2DM among all ethnicities; iii) compared to

263 the *1*/1** homozygotes, the *2*/2** homozygotes of the *IL-1RN* (VNTR) polymorphism had an

264 increased risk of T2DM development; and iv) no significant association was observed between

265 the *IL-1RN* (VNTR) polymorphism and T2DM risk in the EA subgroup. The results of

266 sensitivity analysis showed that Achyut's study affected the pooled ORs in the IL-1β (-511)

267 dominant and homozygous models. However, Achyut's study did not influence the pooled OR in

268 the EA subgroup after stratification by ethnicity. Therefore, the result of the association between

269 *IL-1B (-511)* and the decreased risk of T2DM in the dominant model in the EA subgroup was

270 convincing.

271 First, IL-1β was considered a mediator of fever, and rapidly, it was found that IL-1β induced

272 innate immunity to defend against pathogens. However, chronic overexpression of IL-1β has

273 been related to multiple immune diseases, including T2DM(Mandrup-Poulsen 1996). Eventually,

274 it was suggested that IL-1β can induce the inflammatory microenvironment of islets, leading to

275 impaired insulin secretion, decreased cell proliferation and apoptosis of β-cells, ultimately

276 contributing to the development of T2DM(Rhodes 2005). Donath, M Y *et al.* extensively

277 described the development of islet inflammation in GK rats, suggesting that the expression of

278 pro-inflammatory cytokines IL-1β and others (IL-6, TNFα) was increased in islets, while

279 elevated expression levels of many chemokines (CXCL1/KC, MCP-1, MIP-1α) and infiltration

280 of immune cells in islets were observed(Ehses et al. 2009). In all cases, macrophage infiltration

281 is increased in the islet inflammatory process(Ehses et al. 2007). Moreover, two independent

282 studies have now indicated that the numbers of islet-related CD68^{+} cells are increased in T2DM

283 patients(Ehses et al. 2007; Richardson et al. 2009). In addition, data obtained from laser-captured

284 β-cells from T2DM patients have shown that evaluated expression levels of IL-1β and

285 chemokines possibly led to this immune cell infiltration(Böni-Schnetzler et al. 2008). Since it

286 can regulate various inflammatory processes, any change in the level of IL-1β in blood or tissue

287 possibly affects these processes. Larsen et al. suggested that blocking IL-1β is a potential therapy

288 in the treatment of T2DM(Larsen et al. 2007). The *IL-1B (-511) C* allele is associated with higher

289 expression of IL-1β and with severe inflammation in the liver, while the *(-511) T* allele is

290 associated with lower levels of IL-1β(Hirankarn et al. 2006; Vishnoi et al. 2008). In this meta-

291 analysis, we found that the *IL-1B (-511) T* allele had a decreased T2DM risk in the EA subgroup,

292 suggesting an influence of the *IL-1B (-511)* polymorphism system in this ethnic group.

293   The *IL-1RN 2** allele (two repeats) is associated with increased IL-1Ra levels(Danis et al. 1995),

294   which can compete with an inhibitor of IL-1β by binding to the IL-1 receptor. The *2** allele

295   significantly increases the IL-1β secretion in vitro and balances the expression of IL-1β and IL-

296   1Ra(Santtila et al. 1998), and their ratio (IL-1Ra/IL-1β) determines the severity of inflammation.

297   It has been shown that the *IL-1RN 2** allele is associated with a low ratio (IL-1Ra/IL-1β),

298   thereby inducing a longer and more severe pro-inflammation(Witkin et al. 2002). The frequency

299   of the *2** allele is increased in inflammatory conditions or autoimmune diseases(Tountas et al.

300   1999; van der Paardt et al. 2002). Consistently, this meta-analysis suggested that the *IL-1RN 2**

301   allele and *2*2** homozygotes increased T2DM risk, which indicated that the *IL-1RN 2** allele

302   and *2*2** homozygote polymorphism play critical roles in the development of T2DM.

303   Although we have devoted considerable efforts and resources to testing the potential association

304   of the *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms with T2DM risk, this analysis still has

305   some limitations. First, significant heterogeneity was found in pooled analyses between the *IL-*

306   *1B (-511)* polymorphism and T2DM risk. Due to the limited number of included publications, we

307   conducted further ethnic stratification analysis and meta-regression to identify the exact sources

308   of between-study heterogeneity. The meta-regression results showed that ethnicity was not the

309   source of heterogeneity (both values of tau^2 were not much different (0.34 vs 0.40)). Some

310   possible relevant factors (genotyping method, sex) may lead to heterogeneity. However,

311   subgroup analysis can significantly reduce heterogeneity. Second, the literature articles were

312   primarily published in English or Chinese; thus, some eligible publications may be missing,

313   causing some bias. Third, the publications included have some confounders, such as age, sex,

314   diet, and exercise, whereas we used only the raw data to conduct pooled analysis; therefore, we

315   were unable to control these possible confounders or test the potential gene-environment

316   interactions. Fourth, the number of included publications was limited; thus, additional studies

317   with large sample sizes and a wider variety of ethnicities are needed in the future to evaluate the

318   association.

319   In conclusion, our meta-analysis first indicated that the *IL-1B (-511) T* allele polymorphism is

320   associated with decreased T2DM risk in the EA population and that the *IL-1RN* 2* allele and

321   *2*2** homozygote polymorphism are strongly associated with increased T2DM risk. Further

322   well-designed studies including different ethnicities with large sample sizes are needed to verify

323   this conclusion.

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481 **Figure Legends**

482 **Figure 1. Flowchart of study identification process.**

483 **Figure 2. An overall forest plot of *IL-1B (-511)* associated with T2DM risk in the dominant**

484 **model (*TT* + *TC* vs *CC*) and sensitivity analyses.**

485 A. Forest plot of *IL-1B (-511)* associated with T2DM in the dominant model; (SA: South Asian,

486 SEA: South East Asian, EA: East Asian, NA: North Africa);

487 B. Sensitivity analysis for *TT* + *TC* vs *CC* associated with T2DM in the overall meta-analysis.

488 C. Sensitivity analysis for *TT* vs *TC* + *CC* associated with T2DM in the overall meta-analysis.

489 D. Sensitivity analysis for *TT* vs *CC* associated with T2DM in the overall meta-analysis.

490 **Figure 3. Forest plot of meta-analysis on *IL-1RN* (VNTR) and the risk of T2DM in the**

491 **recessive model (*2*2** vs *2*1** + *1*1**) and homozygous model (*2*2** vs *1*1**).**

492 A. Forest plot of *IL-1RN* (VNTR) associated with T2DM in the recessive model.

493 B. Forest plot of *IL-1RN* (VNTR) associated with T2DM in the homozygous model

494 **Figure 4. Harbord test for publication bias testing.**

495 A. **Publication** bias of *IL-1B (-511)* associated with T2DM in the dominant model (*TT* + *TC* vs

496 *CC*);

497 B. **Publication** bias of *IL-1RN* (VNTR) associated with T2DM in the recessive model (*2*2** vs

498 *2*1** + *1*1**);

499 C. **Publication** bias of *IL-1RN* (VNTR) associated with T2DM in the homozygous model (*2*2**

500 vs *1*1**).

Figure 1

Flowchart of study identification process.

Figure 1 shows the flowchart of study identification process.

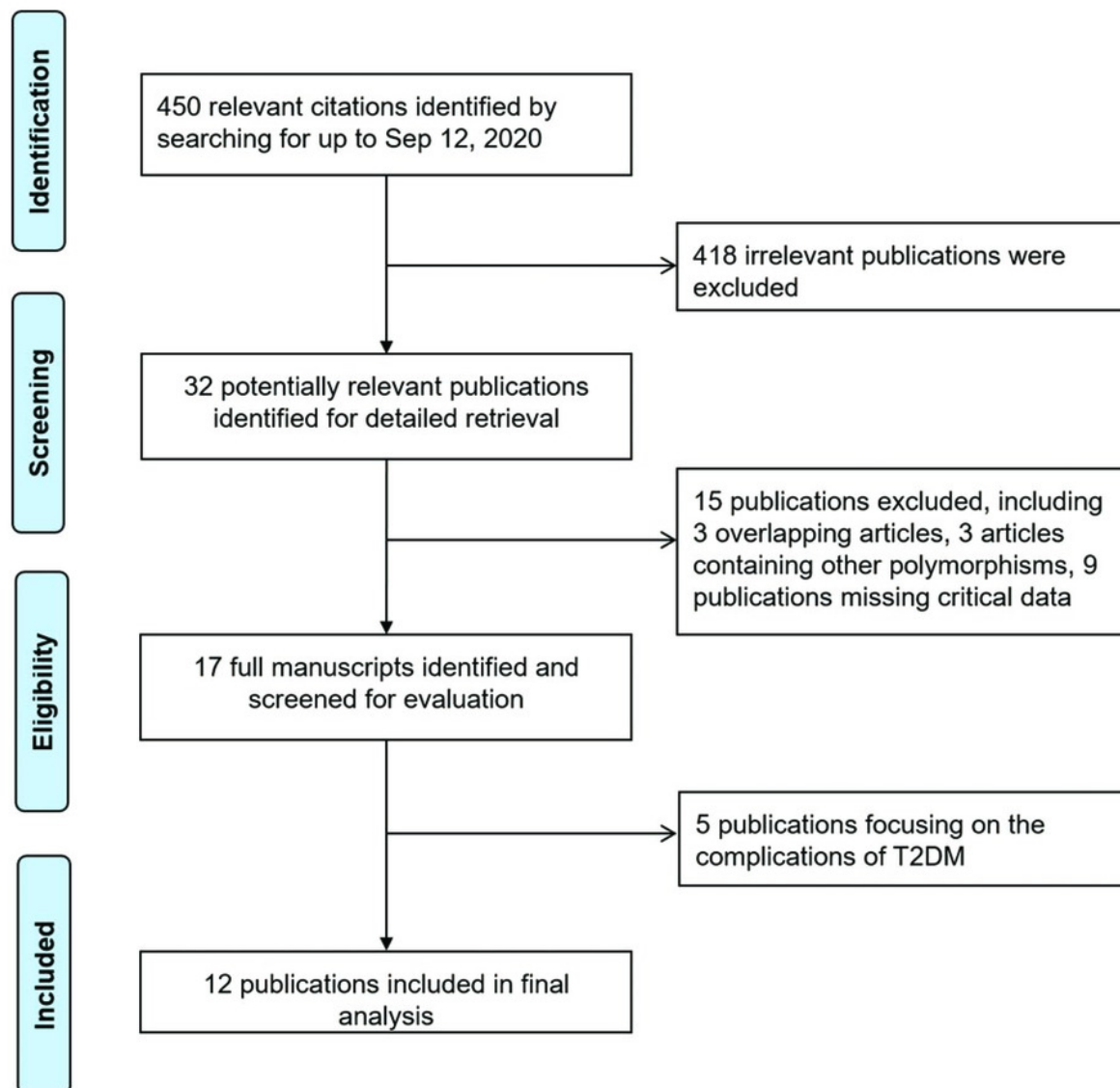



Figure 2

An overall forest plot of *IL-1B (-511)* associated with T2DM risk in the dominant model (*TT + TC* vs *CC*) and sensitivity analyses .

'(A) Forest plot of *IL-1B (-511)* associated with T2DM in the dominant model; (SA: South Asian, SEA: South East Asian, EA: East Asian, NA: North Africa). (B) Sensitivity analysis for *TT + TC* vs *CC* associated with T2DM in the overall meta-analysis. (C) Sensitivity analysis for *TT* vs *TC + CC* associated with T2DM in the overall meta-analysis. (D) Sensitivity analysis for *TT* vs *CC* associated with T2DM in the overall meta-analysis.'

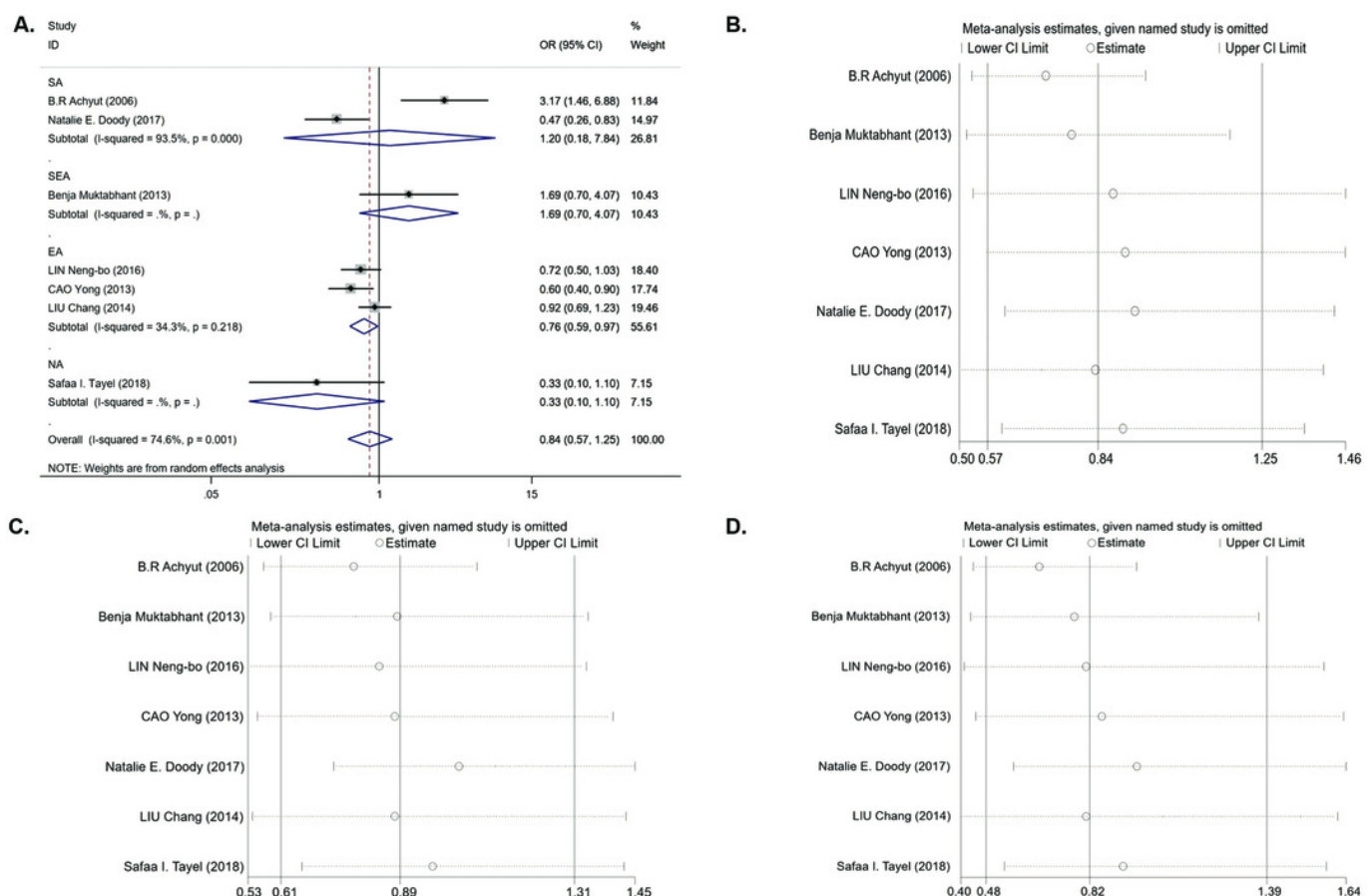



Figure 3

Forest plot of meta-analysis on *IL-1RN* (VNTR) and the risk of T2DM in the recessive model (*2*2** vs *2*1* + 1*1**) and homozygous model (*2*2** vs *1*1**) .

'(A) Forest plot of *IL-1RN* (VNTR) associated with T2DM in the recessive model. (B) Forest plot of *IL-1RN* (VNTR) associated with T2DM in the homozygous model.'

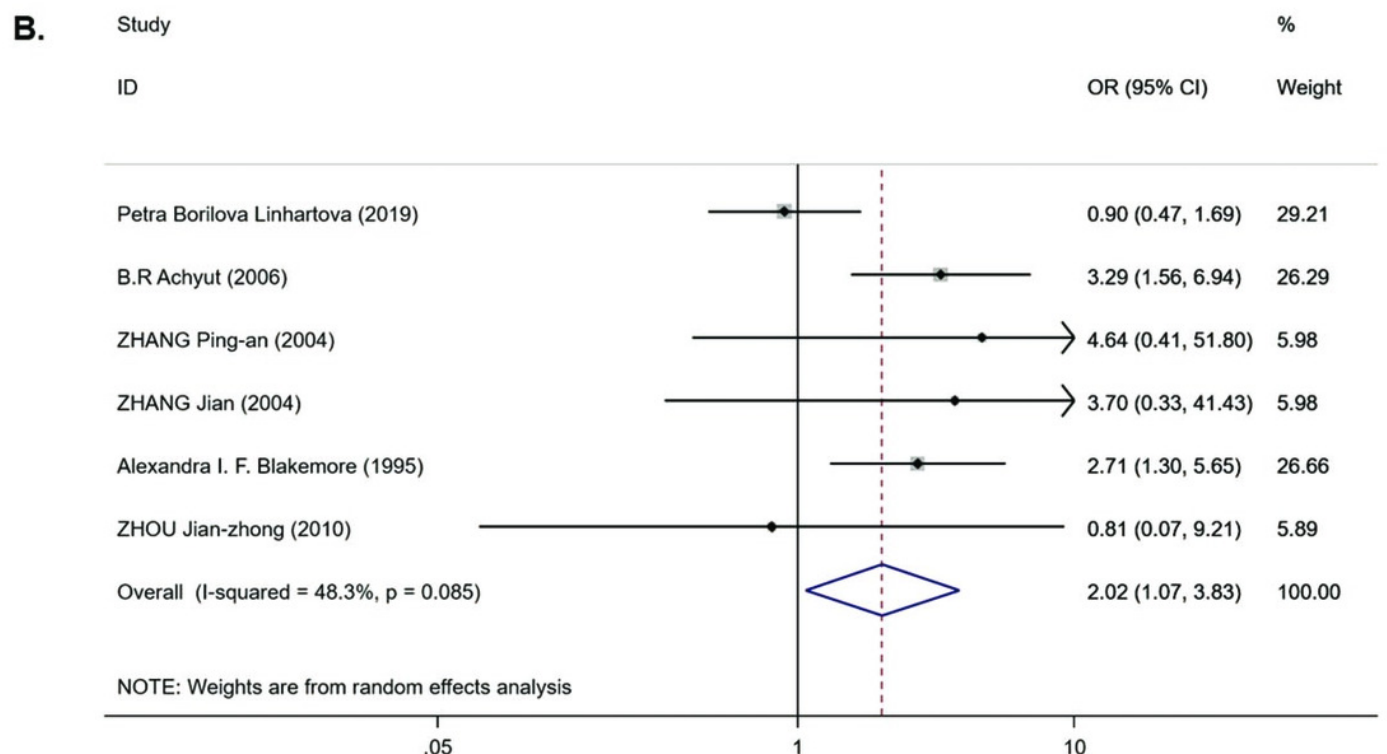
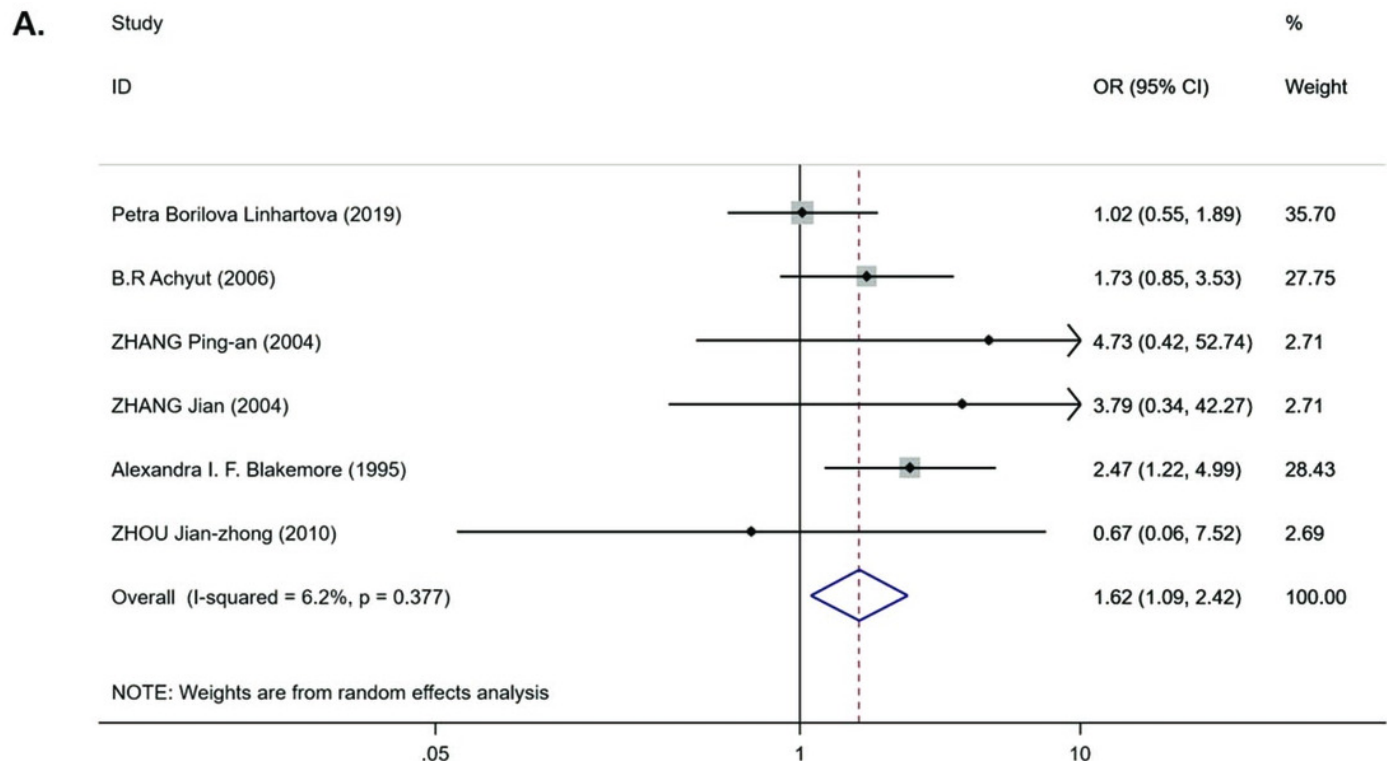



Figure 4

Harbord test for publication bias testing.

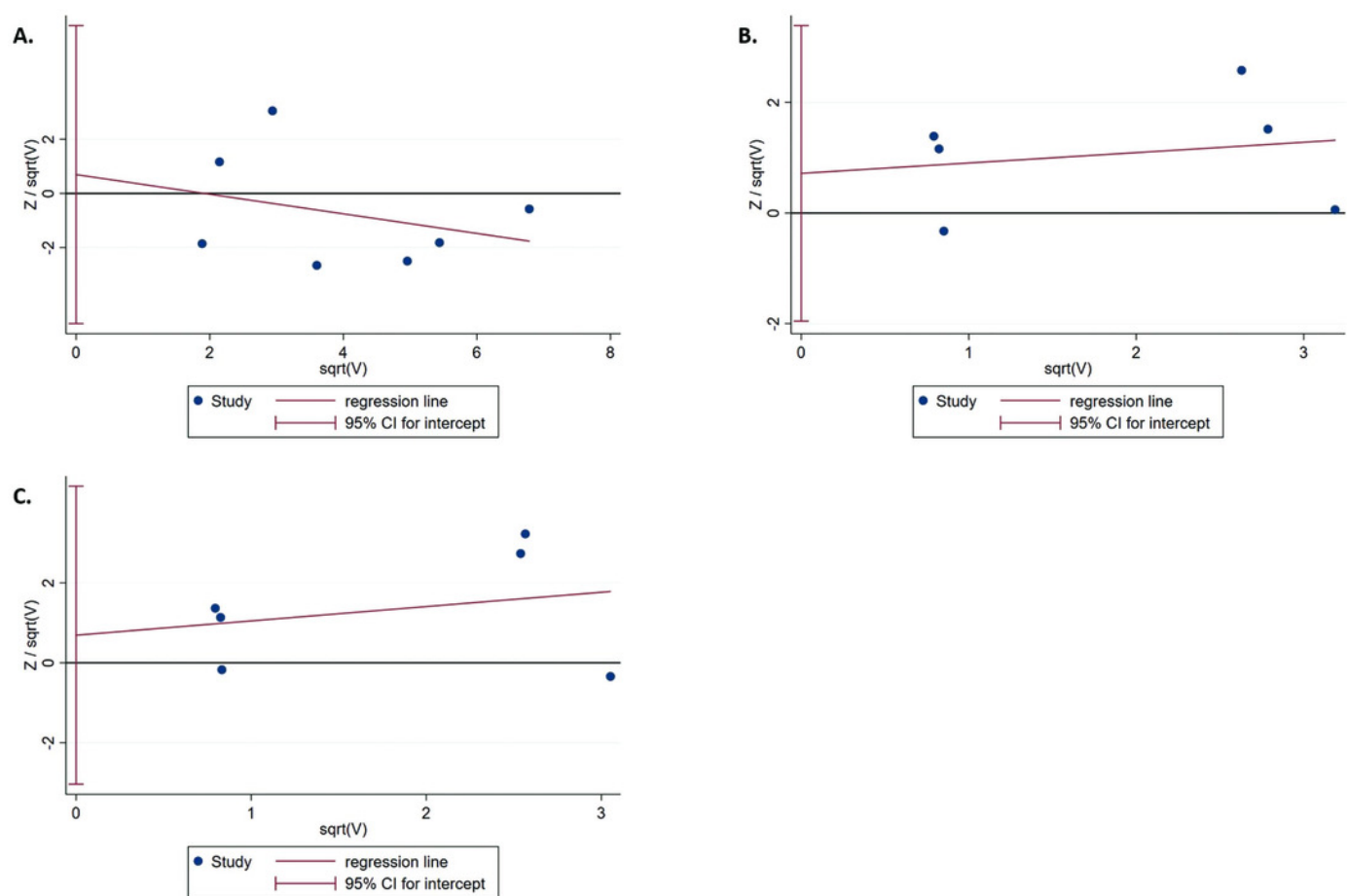
'(A) **Publication** bias of *IL-1B (-511)* associated with T2DM in the dominant model (*TT + TC* vs *CC*); (B) **Publication** bias of *IL-1RN* (VNTR) associated with T2DM in the recessive model (*2*2** vs *2*1* + 1*1**); (C) **Publication** bias of *IL-1RN* (VNTR) associated with T2DM in the homozygous model (*2*2** vs *1*1**).'

Characteristics of Identified Studies on *IL-1B (-511)/IL-1RN(VNTR)* polymorphism and the risks of T2DM.

Table 1 illustrates the characteristics of all included publications in this meta-analysis.

1 **Table 1. Characteristics of Identified Studies on *IL-1B (-511)/IL-1RN(VNTR)* polymorphism and the risks of T2DM.**

Author	Year	Country/Ethnicity	Diagnostic Criteria	Genotyping method	Number Cases	Number Controls	Language	IL-1 gene	QA	HWE
Zhang Jian	2004	China/EA	NA	PCR-RFLP	106	198	Chinese	*IL-1RN*	7	1.00
Zhang Ping-an	2004	China/EA	WHO, 1999	PCR-RFLP	106	247	Chinese	*IL-1RN*	8	0.70
Zhou Jian-zhong	2010	China/EA	WHO, 1999	PCR-RFLP	72	97	Chinese	*IL-1RN*	7	0.23
Petra Borilova Linhartova	2019	Czech Republic/ Caucasian	NA	PCR-RFLP	380	212	English	*IL-1RN*	8	1.00
Alexandra I. F. Blakemore	1995	North of England/ Caucasian	WHO, NA	PCR	117	248	English	*IL-1RN*	7	0.61
B.R Achyut	2006	North Indian/SA	WHO, 1999	PCR-RFLP	200	223	English	*IL-1RN* / *IL-1B (-511)*	8	0.10 / 0.16
Liu Chang	2014	China/EA	WHO, 1999	PCR-RFLP	583	366	Chinese	*IL-1B (-511)*	7	1.00
Cao Yong	2013	China/EA	WHO, 1999	PCR-RFLP	268	263	Chinese	*IL-1B (-511)*	8	0.06
Lin Neng-bo	2016	China/EA	WHO, 1999	PCR-RFLP	286	327	Chinese	*IL-1B (-511)*	8	0.08
Natalie E. Doody	2017	North Indian/SA	WHO, 1999	PCR-RFLP	202	203	English	*IL-1B (-511)*	8	0.87
Benja Muktabhant	2013	Thailand/SEA	NA	PCR-RFLP	90	30	English	*IL-1B (-511)*	7	0.16
Safaa I. Tayel	2018	Egypt/NA$_1$	ADA, NA	TaqMan	50	30	English	*IL-1B (-511)*	8	1.00

2   T2DM=Type 2 diabetes mellitus; EA=East Asian; SA=South Asian; SEA=South East Asian; NA=Not Available (the diagnosis of T2DM was

3   originally based on the presence of clinical symptoms and biochemical); NA1=North African; ADA=American Diabetes Association (fasting blood

4   glucose ≥126mg/dL or 2 h blood glucose after overload with 75 g of glucose≥ 200mg/dL in oral glucose tolerance test (OGTT) or glycated

5   hemoglobin (HbA1c) ≥6.5 in patients with classic symptoms of hyperglycemia); WHO=World Health Organization (fasting glucose levels [7.0

6   mmol/L or [126 mg/dL after a minimum 12-h fast or 2-h post glucose level (oral glucose tolerance test or 2-h OGTT) [11.1 mmol/L or 200 mg/dL]

7   on more than one occasion); QA=Quality Assessment; HWE=Hardy-Weinberg Equilibrium.

The IL-1 polymorphism distribution in cases and controls.

Table 2 shows the genotypic distributions of the *IL-1B (-511)* and *IL-1RN* (VNTR) polymorphisms .

1   **Table 2. The IL-1 polymorphism distribution in cases and controls.**

Gene	Case					Control				
IL-1B (-511)	*C*	*T*	*CC*	*TC*	*TT*	*C*	*T*	*CC*	*TC*	*TT*
Cao Yong	302	270	78	129	61	332	322	52	147	64
Liu Chang	634	532	171	292	120	385	347	101	183	82
B.R Achyut	108	292	9	90	101	175	271	29	117	77
Lin Neng-bo	302	270	85	132	69	332	322	76	180	71
Safaa I. Tayel	55	45	16	23	11	21	39	4	13	13
Natalie E. Doody	186	218	40	106	56	133	273	21	91	91
Benja Muktabhant	90	90	23	44	23	33	27	11	11	8
IL-1RN	*1**	*2**	*1*/1**	*1*/2**	*2*/2**	*1**	*2**	*1*/1**	*1*/2**	*2*/2**
B.R Achyut	227	234	60	107	20	340	92	138	64	14
Zhang Jian	192	34	88	16	2	360	36	163	34	1
Zhang Ping-an	192	34	88	16	2	450	44	204	42	1
Zhou Jian-zhong	107	71	36	35	1	153	41	58	37	2
Alexandra I. F. Blakemore	154	106	55	44	18	372	124	141	90	17
Petra Borilova Linhartova	569	289	220	129	31	303	121	108	87	17

Meta-analysis of the relationship of the IL-1 polymorphisms with the risks of T2DM.

Table 3 shows the meta-analysis results for these two polymorphisms.

1 **Table 3. Meta-analysis of the relationship of the IL-1 polymorphisms with the risks of T2DM.**

Gene	No. studies	Allele comparison			Genetic model comparison			
		OR (95% CI)	P_{het}	P_z		OR (95% CI)	P_{het}	P_z
IL-1B (-511)								
Total	7	0.89(0.68-1.17)	<0.001	0.406	Dominant	0.84(0.57–1.25)	0.001	0.395
					Recessive	0.89(0.61-1.31)	<0.001	0.561
					Overdominant	0.92(0.74-1.14)	0.065	0.422
					Codominant	0.85(0.6-1.21)	0.011	0.375
					Codominant#	0.96(0.69-1.34)	0.003	0.803
					Homozygote	0.82(0.48-1.39)	<0.001	0.451
EA	3	0.89(0.79-1.01)	0.606	0.072	**Dominant**	**0.76(0.59-0.97)**	**0.218**	**0.027**
					Recessive	0.97(0.79-1.20)	0.589	0.789
					Overdominant	0.82(0.64-1.04)	0.162	0.094
					Codominant	**0.73(0.54-0.99)**	**0.141**	**0.040**
					Codominant#	1.07(0.86-1.34)	0.385	0.531
					Homozygote	0.80(0.62-1.03)	0.571	0.081
SA	2	1.00(0.33-2.98)	<0.001	0.997	Dominant	1.20(0.18-7.84)	<0.001	0.853
SEA	1	1.22(0.68-2.20)	-	0.503	Dominant	1.69(0.70-4.07)	-	0.245
NA	1	**0.44(0.23-0.85)**	-	**0.015**	Dominant	0.33(0.10-1.10)	-	0.070
IL-1RN								
Total	6	**2.08(1.43-3.02)**	**<0.001**	**<0.001**	Dominant	1.35(0.79-2.31)	<0.001	0.275
					Recessive	**1.62(1.09-2.42)**	**0.377**	**0.018**
					Overdominant	1.20(0.72-1.99)	<0.001	0.485
					Codominant	1.28(0.73-2.24)	<0.001	0.398
					Codominant#	1.38(0.89-2.13)	0.351	0.155
					Homozygote	**2.02(1.07-3.83)**	**0.085**	**0.031**
EA	3	**2.02(1.53-2.66)**	**0.541**	**<0.001**	Dominant	1.11(0.78-1.59)	0.525	0.558
					Recessive	2.30(0.57-9.25)	0.470	0.243
					Overdominant	1.05(0.72-1.54)	0.337	0.790

					Codominant	1.06(0.74-1.53)	0.371	0.749
					Codominant#	2.25(0.53-9.55)	0.356	0.270
					Homozygote	2.42(0.60-9.78)	0.554	0.216
SA	1	**3.81(2.84-5.11)**	-	**<0.001**	Dominant	3.75(2.48-5.67)	-	<0.001
Caucasian	2	1.60(0.998-2.58)	0.02	0.051	Dominant	1.04(0.54-2.02)	0.017	0.901

CI=confidence interval; IL-1=interleukin-1; OR=odds ratio; P_{het}=P-value for heterogeneity; P_z=P-value for overall effect; SA: South Asian, SEA:

South East Asian, EA: East Asian, NA: North Africa.

For *IL1B (-511)* polymorphism: dominant (*TT + TC* vs *CC*), recessive (*TT* vs *TC + CC*), overdominant (*CT vs CC+TT*), codominant (*CT vs CC*),

codominant# (*CT vs TT*) and homozygote (*TT* vs *CC*).

For *IL1RN* (VNTR) polymorphism: dominant (*2*2* + *2*1** vs *1*1**), recessive (*2*2** vs *2*1* + 1*1**), overdominant (*2*1** vs *1*1*+2*2**),
codominant (*2*1* vs 1*1**), codominant# (*2*1*vs 2*2**) and homozygote (*2*2** vs *1*1**).