Association of hypoxia inducible factor 1-Alpha gene polymorphisms with multiple disease risks: A comprehensive meta-analysis

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Abstract

HIF1A gene polymorphisms have been confirmed the association with cancer risk through the statistical meta-analysis based on single genetic association (SGA) studies. A good number SGA studies also investigated the association of HIF1A gene with several other diseases, but no researcher yet performed statistical meta-analysis to confirm this association more accurately. Therefore, in this paper, we performed a statistical meta-analysis to draw a consensus decision about the association of HIF1A gene polymorphisms with several diseases except cancers giving the weight on large sample size. This meta-analysis was performed based on 41 SGA study’s findings, where the polymorphisms rs11549465 (1772 C/T) and rs11549467 (1790 G/A) of HIF1A gene were analyzed based on 11544 and 7426 cases and 11494 and 7063 control samples, respectively. Our results showed that the 1772 C/T polymorphism is not significantly associated with overall disease risks. The 1790 G/A polymorphism was significantly associated with overall diseases under recessive model (AA vs. AG + GG), which indicates that the A allele is responsible for overall diseases though it is recessive. The subgroup analysis based on ethnicity showed the significant association of 1772 C/T polymorphism with overall disease for Caucasian population under the all genetic models, which indicates that the C allele controls overall diseases. The ethnicity subgroup showed the significant association of 1790 G/A polymorphism with overall disease for Asian population under the recessive model (AA vs. AG + GG), which indicates that the A allele is responsible for overall diseases. The subgroup analysis based on disease types showed that 1772 C/T is significantly associated with chronic obstructive pulmonary disease (COPD) under two genetic models (C vs. T and CC vs. CT + TT), skin disease under two genetic models (CC vs. TT and CC + CT vs. TT), and diabetic complications under three genetic models (C vs. T, CT vs. TT and CC + CT vs. TT), where C allele is high risk factor for skin disease and diabetic complications (since, ORs > 1), but low risk factor for COPD (since, ORs < 1). Also the 1790 G/A variant significantly associated with the subgroup of cardiovascular disease (CVD) under homozgyote model, diabetic complications under allelic and homozgyote models, and other disease under four genetic models, where the A is high risk factor for diabetic complications and low risk factor for CVD. Thus, this study provided more evidence that the HIF1A gene is significantly associated with COPD, CVD, skin...
Introduction

In the scientific community, hypoxia-inducible factor 1α (HIF1A), a transcription factor, has been a research focus to explain its role in oxygen sensing under normal and hypoxic conditions. Many aspects of Human physiology need to match oxygen supply to cellular metabolism and presumably regulate gene expression by sensing oxygen [1]. HIF1A regulates the expression of hundreds of genes [2, 3] involved in many biological processes, including neovascularization, angiogenesis, cytoskeletal structure, apoptosis, adhesion, migration, invasion, metastasis, glycolysis, and metabolic bioenergetics [4–6]. Low oxygen levels or hypoxia represent an important microenvironment condition to affect the pathology of many human diseases, including cancer, diabetes, aging, and stroke/ischemia [7, 8]. HIF1A 1772 C/T (rs11549465) and 1790 G/A (rs11549467) single nucleotide polymorphisms (SNPs) have been identified in association with different types of cancers [9–14]. In recent years, a study also reviewed the association of HIF1A 1772 C/T and 1790 G/A polymorphisms with different types of cancers and found that both polymorphisms are significantly associated with overall cancers [15]. The subgroup analyses indicated 1772 C/T polymorphism in association with decreasing the risk of renal cell carcinoma and the 1790 G/A polymorphism with significantly increased cancer risk in the Asian and Caucasian population [15]. However, a good number of single genetic association (SGA) studies also reported the association of these two polymorphisms with other diseases, including type 2 diabetes (T2D), cardiovascular diseases (CVD), lung disease, autoimmune diseases, inflammatory diseases, preeclampsia, osteoarthritis, lumbar disc degeneration, high altitude polycythemia, age-related macular degeneration and many more [16–54]. The SGA study of Hernández-Molina et al. [18] reported that HIF1A 1772 C/T is a significant genetic factor for autoimmune disease, whereas some other studies [25, 31] found its insignificant association. Similarly, some authors showed the significant association of HIF1A (1772 C/T and 1790 G/A) with cardiovascular diseases (CVD) [21, 40], though some authors did not find the significant effect in the same question [22, 26]. Again for inflammatory diseases, a significant association was claimed by [20, 38], and an insignificant association by [27, 32, 41]. For Chronic obstructive pulmonary disease (COPD), Yu et al. [17] and Putra et al. [39] claimed the significant and insignificant association with HIF1A gene polymorphisms, respectively. Wei et al. [37] showed significant association of 1772 C/T and insignificant association of 1790 G/A polymorphisms of HIF1A with COPD. The both SNPs of HIF1A gene were significantly associated with preeclampsia [16, 24], but another study found their insignificant association [34]. Likewise, Geza et al. [29] reported the significant association of diabetes (type 1 & 2) with HIF1A 1772 C/T polymorphism, and Yamada et al. [35] also suggested that HIF1A 1772 C/T is significantly associated with type 2 diabetes (T2D) and HIF1A 1790 G/A is not. Another two studies claimed the insignificant association between HIF1A gene polymorphisms and type 2 diabetes [45, 50]. Ekberg et al. [51], and Bi et al. [52] both found the significant association of HIF1A gene polymorphisms with diabetic complication diseases, but Liu et al. (a) [45] and Pichu et al. (b) [50] found no relation. Also, Lin et al. [33] reported that the HIF1A 1790 G/A might be played a protecting role significantly to develop the lumbar disc degeneration (LDD), and HIF1A 1772 C/T did not play any role with the severity of LDD. Some authors also checked the association of the HIF1A gene with
cellulite [28], hemodialysis patients [30], high-altitude polycythemia (HAPC) [36], and age-related macular degeneration (AMD) [23]. They found the significant association of HIF1A with cellulite, and insignificant association of HIF1A with hemodialysis patients, HAPC and AMD risk.

Thus, we observed that different SGA studies produce inconsistent results about the association of HIF1A gene polymorphisms with multiple disease risks beyond cancers. This type of inconsistent results may be produced due to the small sample size and/or heterogeneous population in each of the individual SGA studies. Therefore, a consensus decision about the association of HIF1A gene polymorphisms with multiple disease risk is required to make a treatment plan against this genetic effect. To make a consensus decision about the contradictory findings of different studies more accurately, researchers usually consider statistical meta-analysis [15, 55–60]. The meta-analysis makes a decision about the association more accurately compared to SGA studies. Therefore, in this study, we considered statistical meta-analysis to make a consensus decision about the association of HIF1A gene (1772 C/T and 1790 G/A) polymorphisms with several disease risks excluding cancers, giving the weight on large sample size and appropriate statistical modeling.

**Material and methods**

**Search strategy**

PubMed, PubMed Central and Google Scholar were searched to retrieve relevant articles published between 2001 to October 2021 in the English language for this Meta-analysis. For searching the following terminologies were considered: (i) HIF1A, (ii) genetic association, (iii) SNPs, (iv) HIF1A, polymorphisms, (v) rs11549465 or 1772 C/T or P582S, (vi) rs11549467 or 1790 A/G or A588T, (vii) case-control study, (viii) disease, (ix) HIF1A, diseases (x) HIF1A, disorders.

**Eligibility criteria**

The title and abstract of the primarily selected relevant studies were independently investigated by two authors. For the final review some important inclusion-exclusion criteria were used to extract data and only included if the studies were (i) designed to examine the association between HIF1A gene polymorphisms (C1772T, A1790G) and disease/disorder risk; (ii) Human case-control studies; (iii) sufficient to provide significant data of genotype frequency.

**Data extraction**

For the final review, the following information from each of the included studies was extracted, like; first author, year of publication, country of origin, ethnicity of the study subject, number of cases and control, disease type, allelic and genotypic distribution, and so on according to the PRISMA statement [61]. To confirm the validity of a selected SGA study for inclusion in the meta-analysis, the Hardy-Weinberg equilibrium (HWE) test was performed using the Chi-square statistic. A study was considered suitable for meta-analysis only if Pr(χ²ōbs ≤ χ²) ≥ .05 exist (Table 1).

**Quality assessment**

Two authors independently checked the assessment of individual study quality by using the Newcastle-Ottawa Scale (NOS) [62]. The total Nine point NOS score was generated through the categories of selection (4 points), comparability (2 points), and exposure (3 points).
Table 1. Characteristic of 38 and 24 studies included in the meta-analysis of HIF1A 1772 C/T and 1790 G/A polymorphisms, respectively.

| Author                  | Year | Country | Ethnicity | Diseases                                      | Case/Control | $P_{HWE}$ |
|-------------------------|------|---------|-----------|-----------------------------------------------|--------------|-----------|
| Harati-Sadegh et al.    | 2018 | Iran    | Mixed     | Preeclampsia                                  | 203/202      | 0.038     |
| Yu et al.               | 2017 | China   | Asian     | Chronic obstructive pulmonary disease (COPD)  | 164/161      | 0.025     |
| Hernandez-Molina et al. | 2017 | Mexico  | Mixed     | Primary Sjogren syndrome                      | 106/135      | 0.038     |
| Fernandez-Torres et al.| 2015 | Mexico  | Mixed     | Osteoarthritis                                | 70/66        | 0.230     |
| Hlatky et al.           | 2007 | USA     | Caucasian | Coronary artery disease (CAD)                 | 909/466      | 0.157     |
| Duran et al.            | 2015 | Spain   | Caucasian | Coronary artery disease (CAD)                 | 518/112      | 0.994     |
| Okur et al.             | 2014 | Turkey  | Caucasian | Age-related macular degeneration (AMD)        | 87/80        | 0.779     |
| Andraweera et al.       | 2014 | Sri Lanka | Asian    | Preeclampsia                                  | 174/168      | 0.262     |
| Feng et al.             | 2014 | China   | Asian     | Systemic lupus erythematosus                  | 1495/2294    | 0.397     |
| Torres et al.           | 2010 | Spain   | Caucasian | Gaint cell arteritis                          | 215/470      | 0.064     |
| Emanuele et al.         | 2010 | Italy   | Caucasian | Cellulitis                                    | 200/200      | 0.000     |
| Geza et al. (a)         | 2009 | Hungary | Caucasian | Type 1 diabetes                               | 166/354      | 0.203     |
| Geza et al. (b)         | 2009 | Hungary | Caucasian | Type 2 diabetes                               | 370/354      | 0.203     |
| Zheng et al.            | 2009 | Korea   | Asian     | Hemodialysis                                  | 14/360       | 0.257     |
| Wipff et al.            | 2009 | France  | Caucasian | Systemic sclerosis                            | 640/463      | 0.730     |
| Chacchami et al.        | 2013 | Greece  | Caucasian | Osteoarthritis                                | 134/63       | 0.777     |
| Lin et al.              | 2013 | China   | Asian     | Lumbar disc degeneration (LDD)                | 274/301      | 0.193     |
| Nava-Salazar et al.     | 2011 | Mexico  | Mixed     | Preeclampsia                                  | 150/105      | 0.608     |
| Yamada et al.           | 2005 | Japan   | Asian     | Type 2 diabetes                               | 440/572      | 0.084     |
| Chen et al.             | 2016 | China   | Asian     | High altitude polycythemia (HAPC)            | 234/250      | 0.446     |
| Wei et al.              | 2015 | China   | Asian     | Chronic obstructive pulmonary disease (COPD)  | 120/112      | 0.733     |
| de Carvalho Fraga et al.| 2013 | Brazil  | Mixed     | Oral lichen planus (OLP)                      | 32/88        | 0.000     |
| Putra et al.            | 2013 | Japan   | Asian     | Chronic obstructive pulmonary disease (COPD)  | 48/110       | 0.545     |
| Q. Liu et al.           | 2013 | China   | Asian     | Coronary artery disease (CAD)                 | 356/213      | 0.862     |
| Zafar et al.            | 2021 | Pakistan| Asian     | Metabolic syndrome                            | 200/200      | 0.031     |
| Sheng et al.            | 2019 | China   | Asian     | Left ventricular hypertrophy                  | 198/385      | 0.097     |
| Urganci et al.          | 2019 | Turkey  | Asian     | Psoriasis                                    | 150/150      | 0.576     |
| Liu et al.              | 2021 | China   | Asian     | Type 2 diabetes                               | 150/144      | 0.397     |
| Liu et al. (b)          | 2021 | China   | Asian     | Diabetic retinopathy                          | 149/144      | 0.397     |
| Takagi et al.           | 2020 | Japan   | Asian     | Systemic sclerosis                            | 182/178      | 0.468     |
| Saravani et al.         | 2019 | Iran    | Asian     | Multiple sclerosis                            | 150/150      | 0.014     |
| Qin et al.              | 2020 | China   | Asian     | Parkinson’s disease                           | 1692/1419    | 0.483     |
| Tsukatani et al.        | 2021 | Japan   | Asian     | Pressure injury                               | 130/48       | 0.883     |
| Pichu et al. (a)        | 2015 | India   | Asian     | Type 2 diabetes                               | 79/66        | 0.000     |
| Pichu et al. (b)        | 2015 | India   | Asian     | Diabetic food ulcer                           | 79/66        | 0.000     |
| Ekberg et al.           | 2019 | Sweden  | Caucasian | Diabetic retinopathy                          | 555/148      | 0.000     |
| Bi et al.               | 2015 | China   | Asian     | Diabetic nephropathy                          | 140/104      | 0.395     |
| Gu et al.               | 2013 | USA     | Caucasian | Diabetic nephropathy                          | 571/594      | 0.159     |

Continued...
NOS score of an individual study is considered poor (0–3), fair (4–6) and excellent (7–9) quality. In our meta-datasets, 38 studies showed excellent quality and 3 were fair quality (S1 Table).

Statistical meta-analysis

To perform meta-data on SGA studies, we used the following statistical analysis. The HWE test was performed using the Chi-square statistic to confirm the suitability of a selected study for inclusion in the meta-analysis. The consistency of genotypic ratio under the control population was used as the null hypothesis (Ho) for the HWE test. The test statistic of Ho is defined as

$$\chi^2 = \sum_{i=1}^{3} \frac{(O_i - E_i)^2}{E_i} \sim \chi^2_{(1)}$$

which follows chi-square distribution with 1 degree of freedom, where $O_i$ and $E_i$ denote observe and expected frequency of the genotype, respectively. If $p$ and $q$ denote the probabilities of two alleles (e.g. C and T), respectively and $O_i = obs(i)$ is observed frequency of $i$th genotype among the 3 genotypes CC, CT and TT. Then $p$ and $q$ are defined as follows

$$p = \frac{2 \times obs(CC) + obs(CT)}{2 \times (obs(CC) + obs(CT) + obs(TT))}; \quad q = 1 - p$$

Then the expected frequency of $i$th genotype is $E_i = E(i)$, which defined as $E(CC) = p^2n$, $E(C) = 2pq$, $E(TT) = q^2n$, where $n$ is total number of observations.

To investigate the association of SNPs with multiple diseases based on pooled odds ratios (ORs), the individual OR of $k$th SGA study was calculated as follows

$$OR_k = \frac{\frac{b_{ik}}{b_{ik}}}{\frac{b_{ik}}{b_{ik}}} = \frac{b_{ik}b_{ik}}{b_{ik}b_{ik}}$$

Table 1. (Continued)

| Author            | Year | Country | Ethnicity | Diseases                        | Case/ Control | $P_{HWE}$ |
|-------------------|------|---------|-----------|---------------------------------|---------------|-----------|
| Torres et al. [27] | 2010 | Spain   | Caucasian | Giant cell arteritis            | 215/470       | 0.908     |
| Chachami et al. [32] | 2013 | Greek   | Caucasian | Osteoarthritis                  | 134/63        | 0.846     |
| Lin et al. [33]   | 2013 | China   | Asian     | Lumbar disc degeneration (LDD)  | 274/301       | 0.062     |
| Nava-Salazar et al. [34] | 2011 | Mexico  | Mixed     | Preeclampsia                    | 150/105       | 0.961     |
| Yamada et al. [35] | 2005 | Japan   | Asian     | Type 2 diabetes                 | 440/572       | 0.364     |
| Chen et al. [36]  | 2016 | China   | Asian     | High altitude polycythemia (HAPC) | 234/250       | 0.092     |
| Wei et al. [37]   | 2015 | China   | Asian     | Chronic obstructive pulmonary disease (COPD) | 120/112       | 0.585     |
| Putra et al. [39] | 2013 | Japan   | Asian     | Chronic obstructive pulmonary disease (COPD) | 48/110        | 0.655     |
| Q. Liu et al. [40] | 2013 | China   | Asian     | Coronary artery disease (CAD)   | 356/213       | 0.753     |
| Sheng et al. [43] | 2019 | China   | Asian     | Left ventricular hypertrophy    | 198/385       | 0.058     |
| Liu et al. (a) [45] | 2021 | China   | Asian     | Type 2 diabetes                 | 150/144       | 0.765     |
| Liu et al. (b) [45] | 2021 | China   | Asian     | Diabetic retinopathy            | 149/144       | 0.765     |
| Takagi et al. [46] | 2020 | Japan   | Asian     | Systemic sclerosis              | 182/174       | 0.409     |
| Qin et al. [48]   | 2020 | China   | Asian     | Parkinson’s disease             | 1692/1419     | 0.173     |
| Tsukatani et al. [49] | 2021 | Japan   | Asian     | Pressure injury                 | 130/48        | 0.883     |
| Pichu et al. (a) [54] | 2018 | India   | Asian     | Type 2 diabetes                 | 185/145       | 0.000     |
| Pichu et al. (b) [54] | 2018 | India   | Asian     | Diabetic food ulcer             | 199/145       | 0.000     |

$P_{HWE}$ $P$-value of the chi-square goodness-of-fit test for Hardy-Weinberg equilibrium in control population; $P_{HWE} > 0.05$ means satisfied HWE, otherwise not

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where \( b_{1k} \) and \( b_{2k} \) stands for exposures and \( b_{1k} \) and \( b_{4k} \) non-exposures frequencies, in case-control groups of \( k \)th study, respectively (for example, the genetic model \( C \) vs. \( T \), where \( C \) is exposer and \( T \) is non-exposer). Then the pooled ORs under the each of five different genetic models (dominant model \([CC + CT \ vs. TT \ or \ AA + AG \ vs. GG]\), homozygote model \([CC \ vs. TT \ or \ AA \ vs. GG]\), heterozygote model \([CT \ vs. TT \ or \ AG \ vs. GG]\), recessive model \([CC \ vs. CT + TT \ or \ AA \ vs. AG + GG]\), and allelic contrast model \([C \ vs. T \ or \ A \ vs. G]\)) was calculated by using the random effects model (REF) for the highly significant heterogeneity (p-value < 0.10) among SGA studies, otherwise, fixed effects model (FEM) was used as suggested by other researchers [63, 64]. This heterogeneity was tested using Cochran’s Q statistic which will be introduced later. To calculate pooled ORs based on FEM, the Mantel-Haenszel (MH) method was used as follows.

The FEM for \( k \)th SGA study is defined as

\[
\hat{\beta}_k = \beta_F + \epsilon_k, \tag{4}
\]

where,

\[
\hat{\beta}_F = \hat{OR}_{MH} = \frac{\sum_{k=1}^{K} \left( \frac{b_{1k} b_{2k}}{N_k} \right)}{\sum_{k=1}^{K} \left( \frac{b_{1k} b_{4k}}{N_k} \right)} = \sum_{k=1}^{K} \left( \frac{b_{1k} b_{2k}}{\sum_{k=1}^{K} b_{1k} b_{4k} \frac{b_{2k}}{N_k}} \right) \times OR_i,
\tag{5}
\]

\[
\text{Var}(\hat{\beta}_F) = \frac{1}{\sum_{k=1}^{K} \left( \frac{b_{1k} b_{4k}}{N_k} \right)}.
\]

\( \hat{\beta}_k = \ln(\text{OR}_k), N_k = b_{1k} + b_{2k} + b_{3k} + b_{4k} \) and the error term \( \epsilon_k \sim N(0, \sigma_k^2) \).

Again, to calculate pooled ORs based on REM, the inverse variance method was used as follows.

The REM for \( k \)th SGA study is defined as

\[
\hat{\beta}_k = \beta_k + v_k + \epsilon_k, \tag{6}
\]

where, \( v_k \sim N(0, \tau^2), \beta_k = \frac{\sum_{k=1}^{K} \left( \frac{b_{1k} b_{4k}}{w_k} \right)}{\sum_{k=1}^{K} \left( \frac{b_{1k} b_{4k}}{w_k} \right)} \), \( se(\hat{\beta}_k) = \sqrt{\text{Var}(\hat{\beta}_k)} = \sqrt{\frac{1}{\sum_{k=1}^{K} \left( \frac{w_k}{w_k} \right) w_k} = \frac{1}{\sigma_k^2}, \tag{7}
\]

\[
\tau^2 = \frac{Q - (K - 1)}{\sum w_k - \left( \sum \frac{\epsilon_k^2}{w_k} \right)}, w_k = \frac{1}{\sigma_k^2}, \text{ and }
\]

\[
\sigma_k^2 = \text{var}(\ln(\text{OR}_k)) = \frac{1}{b_{1k}} + \frac{1}{b_{2k}} + \frac{1}{b_{4k}}.
\]

The 95% confidence interval (CI) for pooled ORs can be obtained based on z-statistic as follows

\[
\text{Pro}\{\hat{\beta}_F - 1.96\sqrt{\text{Var}(\hat{\beta}_F)} \leq z \leq \hat{\beta}_F + 1.96\sqrt{\text{Var}(\hat{\beta}_F)}\} = 0.95, \quad \text{for FEM}
\]

\[
\text{Pro}\{\hat{\beta}_k - 1.96\sqrt{\text{Var}(\hat{\beta}_k)} \leq z \leq \hat{\beta}_k + 1.96\sqrt{\text{Var}(\hat{\beta}_k)}\} = 0.95, \quad \text{for REM}
\]
where

\[
z = \begin{cases} 
\frac{\sum_{k} w_k \hat{\beta}_k}{\sqrt{\sum w_k}}, & \text{for FEM} \\
\frac{\sum_{k} w_{kr} \hat{\beta}_k}{\sqrt{\sum w_{kr}}}, & \text{for REM} 
\end{cases}
\]  

(8)

Then the Cochran’s Q statistic [65] is defined as

\[
Q = \sum_{k=1}^{K} w_k \left( \hat{\beta}_k - \frac{\sum_{k=1}^{K} w_k \hat{\beta}_k}{\sum_{k=1}^{K} w_k} \right)^2 \sim \chi^2_{(K-1)}
\]

(9)

and its extended Higgin’s and Thompson I^2—statistic [66] was also used to check the heterogeneity of SGA studies. The I^2-statistic is defined as

\[
I^2 = \max \left\{ 0, \frac{Q - (K - 1)}{Q} \times 100 \right\}
\]

(10)

The I^2 values > 25%, > 50% and > 75% defined as low, moderate, and high heterogeneity, respectively.

Subgroup analyses were performed based on ethnicity and disease types. Sensitivity analysis was carried out using both the full data and reduced data, where the reduced dataset did not include the SGA studies that were rejected by the HWE validation test.

To investigate the publication bias on the included SGA studies in the meta-analysis, we constructed the funnel plot, where the standard error (se) of the estimated effect was plotted against the ORs [63, 64, 67]. Also, Egger’s regression test and Begg’s test [68, 69] was performed for quantitative evaluation (p-value < 0.05 indicates the existence of publication bias). The Egger regression test was performed under H_0: \alpha = 0 (absence of publication bias) and the test statistic follows as

\[
T = \frac{\theta}{se(\theta)} \sim t_{(K-2)}
\]

(11)

where \( \theta \) is estimated by the least square estimation with the respective following models

\[
\hat{\beta}_k \sqrt{w_k} = \theta + \mu \sqrt{w_k} + \varepsilon_k, \quad \text{for FEM, and}
\]

\[
\hat{\beta}_{kr} \sqrt{w_{kr}} = \theta + \mu \sqrt{w_{kr}} + \varepsilon_k, \quad \text{for REM,}
\]

(12)

(13)

with \( \varepsilon_k \sim iid N(0, \sigma^2) \). The Begg’s test was performed under H_0: \alpha = 0 (absence of publication bias) and the test statistic follows

\[
Z = \frac{C - D}{\sqrt{K(K - 1)(2K + 5)/18}} \sim N(0, 1)
\]

(14)

where C and D represents concordant and discordant number, respectively, and obtained by using the Kendall’s ranking of \( t^*_k \) and \( \hat{\sigma}^*_k \) or \( \hat{\sigma}^*_{kr} \). Here

\[
t^*_k = \frac{t_k - \bar{t}}{\sqrt{\hat{\sigma}^*_k}}
\]

(15)
where, \( t_k = \text{OR}_k \) is denoted the OR of \( k \)th study, and

\[
\bar{t} = \begin{cases} \frac{\sum_k w_k t_k}{\sqrt{\sum_k w_k}}, & \text{for FEM} \\ \frac{\sum_k w_k p_k t_k}{\sqrt{\sum_k w_k p_k}}, & \text{for REM} \end{cases}
\] (16)

\[
\hat{g}_k' = \begin{cases} \frac{\hat{g}_k^2 - 1}{\sum w_k}, & \text{for FEM} \\ \frac{\hat{g}_k^2 - 1}{\sum w_k}, & \text{for REM} \end{cases}
\] (17)

Also, we studied a false positive report probability (FPRP) to verify whether the findings could be regarded as false positives or not [70]. We computed the statistical power and FPRP based on our significant ORs using the following mechanism,

\[
\text{FPRP} = \frac{\alpha(1 - \pi)}{\alpha(1 - \pi) + (1 - \beta)\pi}
\] (18)

where, \( \alpha \) is the level of significance, \( \pi \) is the prior probability and \( (1 - \beta) \) is statistical power.

To implement all the statistical analysis, we used 'meta' package in R program (http://meta-analysis-with-r.org/).

**Results**

**Study characteristics**

Initially, 187 studies were selected through text mining that included the HIF1A gene and polymorphisms in their title or abstracts. After screening of the duplications, excluding the studies that did not match with the eligibility criteria or had incomplete information, a total of 41 studies were selected based on the PRISMA statement for the final review (Fig 1). In this study, 35 studies comprised 38 datasets of the HIF1A 1772 C/T polymorphism with a sample size of 23038 (comprising 11544 cases and 11494 controls), and 22 studies comprised 24 datasets for the HIF1A 1790 G/A polymorphism with a sample size of 14489 (comprising 7426 cases and 7063 controls) were incorporated. For Meta-analysis of HIF1A 1772 C/T and 1790 G/A polymorphisms, the types of diseases included (after excluding all types of cancer) were grouped as cardiovascular diseases (CVDs), type 2 diabetes (T2D), autoimmune diseases, inflammatory diseases, chronic obstructive pulmonary disease (COPD), preeclampsia, skin disease, diabetic complications, and other (age-related macular degeneration (AMD), Hemodialysis, lumbar disc degeneration (LDD), high altitude polycythemia (HAPC), metabolic syndrome, pressure injury). The 'other' disease group was made in case of a single study of each disease to perform this meta-analysis. The subgroup of respective diseases was shown in S4 Table.

**Quantitative synthesis of HIF1A 1772 C/T polymorphism.** Results generated through this meta-analysis indicated that the HIF1A 17772 C/T polymorphism was insignificantly associated with the overall disease risk under all genetic models [C vs. T: OR = 1.12, 95% CI = 0.97–1.29, \( p \)-value = 0.113]; [CC vs. TT: OR = 1.16, 95% CI = 0.94–1.44, \( p \)-value = 0.154]; [CT vs. TT: OR = 1.15, 95% CI = 0.83–1.59, \( p \)-value = 0.395]; [CC + CT vs. TT: OR = 1.14, 95% CI = 0.86–1.51, \( p \)-value = 0.375]; and [CC vs. CT + TT: OR = 1.10, 95% CI = 0.93–1.31, \( p \)-value = 0.257; (Table 2; Fig 2; S1-S5 Figs in S1 File).

The subgroup analyses results based on disease type showed that the HIF1A 1772 C/T polymorphism is significantly associated with increasing the risk of diabetic complications under three genetic models: [C vs. T: OR = 1.34, 95% CI = 1.12–1.61, \( p \)-value = 0.001]; [CT vs. TT:
OR = 2.43, 95% CI = 1.41–4.18, p-value = 0.001; [CC + CT vs. TT: OR = 2.11, 95% CI = 1.29–3.43, p-value = 0.003]. For skin diseases group, this polymorphism was also significantly increasing the risk of disease under two genotypic models [CC vs. TT: OR = 3.01, 95% CI = 1.09–8.32, p-value = 0.034] and [CC + CT vs. TT: OR = 2.71, 95% CI = 0.99–7.49, p-value = 0.055]. Interestingly, the polymorphism significantly decreasing the risk of chronic obstructive pulmonary disease (COPD) under two genetic models [C vs. T: OR = 0.46, 95% CI = 0.30–0.71, p-value = 0.000] and [CC vs. CT + TT: OR = 0.43, 95% CI = 0.27–0.67, p-value = 0.000]. However, the subgroup analyses of autoimmune diseases, inflammation, preeclampsia, CVD, T2D and other showed insignificant association with the HIF1A 1772 C/T polymorphism.

The subgroup analyses by ethnicity for the HIF1A 1772 C/T polymorphism exhibited that this polymorphism was strongly associated with overall disease risk in Caucasian populations under all genetic models [C vs. T: OR = 1.27, 95% CI = 1.05–1.54, p-value = 0.013]; [CC vs. TT: OR = 2.00, 95% CI = 1.40–2.87, p-value = 0.000]; [CT vs. TT: OR = 1.64, 95% CI = 1.12–2.40, p-value = 0.011]; [CC + CT vs. TT: OR = 1.93, 95% CI = 1.35–2.77, p-value = 0.000]; and [CC vs. CT + TT: OR = 1.24, 95% CI = 1.02–1.52, p-value = 0.032]. This polymorphism showed a low significant association with overall disease risk in mixed population under dominant
Table 2. Meta-analysis of the HIF1A rs11549465 C/T and T/C polymorphisms in association with different diseases.

| Subgroup                                           | Study number | C vs. T | OR (95% CI) | p-val | CC vs. TT | OR (95% CI) | p-val | CT vs. TT | OR (95% CI) | p-val | CC + CT vs. TT | OR (95% CI) | p-val | CC vs. CT + TT | OR (95% CI) | p-val |
|----------------------------------------------------|--------------|---------|-------------|-------|-----------|-------------|-------|-----------|-------------|-------|----------------|-------------|-------|----------------|-------------|-------|
| Overall                                            | 38           | 1.12 [0.97; 1.29] | 0.113    | 1.16 [0.94; 1.44] | 0.154    | 1.15 [0.83; 1.59] | 0.395 | 1.14 [0.86; 1.51] | 0.375 | 1.10 [0.93; 1.31] | 0.257 |
| Preeclampsia                                       | 3            | 1.03 [0.62; 1.70] | 0.911    | 0.70 [0.26; 1.91] | 0.483    | 0.78 [0.27; 2.29] | 0.649 | 0.70 [0.26; 1.92] | 0.491 | 1.07 [0.77; 1.48] | 0.694 |
| Chronic obstructive pulmonary disease (COPD)       | 3            | 0.46 [0.30; 0.71] | 0.000    | 0.59 [0.13; 2.67] | 0.492    | 1.52 [0.31; 7.43] | 0.603 | 0.68 [0.15; 3.09] | 0.620 | 0.43 [0.27; 0.67] | 0.000 |
| Autoimmune disease                                 | 7            | 1.08 [0.75; 1.56] | 0.672    | 1.02 [0.62; 1.67] | 0.938    | 1.15 [0.68; 1.95] | 0.597 | 1.05 [0.64; 1.72] | 0.842 | 1.08 [0.72; 1.64] | 0.703 |
| Inflammatory disease                               | 5            | 1.19 [0.80; 1.77] | 0.386    | 1.40 [0.60; 3.28] | 0.437    | 0.41 [0.05; 3.06] | 0.383 | 0.60 [0.15; 2.34] | 0.458 | 2.95 [0.56; 15.54] | 0.201 |
| Cardiovascular disease (CVD)                       | 4            | 1.03 [0.71; 1.48] | 0.887    | 3.01 [1.09; 8.32] | 0.034    | 1.68 [0.56; 5.11] | 0.357 | 2.71 [0.98; 7.49] | 0.055 | 0.81 [0.09; 6.85] | 0.844 |
| Skin disease                                        | 2            | 0.83 [0.11; 6.59] | 0.863    | 0.95 [0.59; 1.52] | 0.822    | 0.95 [0.61; 1.50] | 0.841 | 0.96 [0.62; 1.48] | 0.852 | 1.18 [0.93; 1.49] | 0.179 |
| Type 2 diabetes                                     | 4            | 1.31 [0.85; 2.00] | 0.218    | 2.06 [0.47; 9.08] | 0.340    | 1.65 [0.84; 3.26] | 0.147 | 2.05 [0.53; 7.96] | 0.301 | 1.33 [0.90; 1.95] | 0.148 |
| Diabetic complications                              | 5            | 1.34 [1.12; 1.61] | 0.001    | 1.59 [0.94; 2.69] | 0.085    | 1.24 [1.14; 4.18] | 0.001 | 2.11 [1.29; 3.18] | 0.003 | 1.24 [0.88; 1.75] | 0.216 |
| Others                                             | 5            | 1.10 [0.91; 1.32] | 0.399    | 0.95 [0.59; 1.52] | 0.822    | 0.95 [0.61; 1.50] | 0.841 | 0.96 [0.62; 1.48] | 0.852 | 1.18 [0.93; 1.49] | 0.179 |
| Ethnicity                                          |              |         |             |       |           |             |       |           |             |       |                 |             |       |
| Asian                                              | 22           | 0.97 [0.81; 1.17] | 0.785    | 0.87 [0.66; 1.14] | 0.305    | 1.19 [0.99; 1.45] | 0.067 | 1.10 [0.92; 1.32] | 0.300 | 0.92 [0.73; 1.15] | 0.455 |
| Caucasian                                          | 11           | 1.27 [1.05; 1.54] | 0.013    | 2.00 [1.40; 2.87] | 0.000    | 1.64 [1.12; 2.40] | 0.011 | 1.93 [1.35; 2.77] | 0.000 | 1.24 [1.02; 1.52] | 0.032 |
| Mixed                                              | 5            | 1.61 [0.83; 3.12] | 0.160    | 0.92 [0.37; 2.30] | 0.866    | 0.18 [0.03; 1.31] | 0.091 | 0.24 [0.11; 0.54] | 0.001 | 3.38 [0.79; 14.41] | 0.100 |
| HWE tested data                                     |              | 28       | 1.11 [0.97; 1.27] | 0.144 | 1.19 [0.99; 1.55] | 0.201    | 1.19 [0.98; 1.43] | 0.072 | 1.20 [0.99; 1.44] | 0.058 | 1.10 [0.94; 1.28] | 0.255 |

OR (95% CI) is Odds Ratio (95% Confidence Interval); The bold results indicates the statistical significance.

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model [CC + CT vs. TT: OR = 0.24, 95% CI = 0.11–0.54, p-value = 0.001] and insignificant association for Asian patients.

**Sources of heterogeneity.** According to the results of heterogeneity analysis, we found the significant heterogeneity of HIF1A 1772 C/T polymorphism with overall disease risk under the four genetic models: [C vs. T: Q = 119.65, df = 37, p-value = 0.0001, I² = 69.1%]; [CT vs. TT: Q = 55.55, df = 37, p-value = 0.026, I² = 33.4%]; [CC + CT vs. TT: Q = 48.97, df = 37, p-value = 0.090, I² = 24.4%]; and [CC vs. CT + TT: Q = 128.09, df = 37, p-value = 0.0001, I² = 71.1%]. Also in subgroup analysis, some genetic model showed the significant heterogeneity in cases of autoimmune disease, inflammatory disease, CVD, skin disease, T2D disease group and Asian, Caucasian and mixed ethnic populations (S2 Table). That subgroup may be the main sources of heterogeneity for conducting the meta-analysis of HIF1A 1772 C/T polymorphism.

**Quantitative synthesis of HIF1A 1790 G/A polymorphism**

The pooled estimate of HIF1A 1790 G/A polymorphism showed a significant association with decrease the risk of overall disease under recessive model [AA vs. GA + GG: OR = 0.78, 95% CI = 0.67–0.91, p-value = 0.002]. (Table 3; Fig 3; S6-S10 Figs in S1 File).
The subgroup analyses based on disease type showed that the HIF1A 1790 G/A polymorphism is significantly associated with increasing the risk of diabetic complications under the allelic contrast model \(A vs. G: OR = 1.71, 95\% CI = 1.27–2.28, p\)-value = 0.000\] and

![Forest plot of HIF1A 1772 C/T polymorphism and overall disease risk for different ethnic populations under allelic model.](https://doi.org/10.1371/journal.pone.0273042.g002)

In the forest plot, the square of the horizontal line represents the individual study-specific odds ratios (ORs) with 95% confidence intervals (CIs) and the black area of the squares represents the corresponding study weight. The black diamond reflects the pooled OR and the lateral points of the diamond represent the CI of the overall analyses. The solid vertical lines are the OR of 1 indicates no effect. The dashed vertical line shows the corresponding pooled OR of the analyses.

The subgroup analyses based on disease type showed that the HIF1A 1790 G/A polymorphism is significantly associated with increasing the risk of diabetic complications under the allelic contrast model \(A vs. G: OR = 1.71, 95\% CI = 1.27–2.28, p\)-value = 0.000\] and
### Table 3. Summary results of ORs and 95% CI of HIF1A rs11549467 G/A polymorphism association with diseases.

| Subgroup                                | Study number | A vs. G (OR [95% CI]) | p-val | AA vs. GG (OR [95% CI]) | p-val | AG vs. GG (OR [95% CI]) | p-val | AA + AG vs. GG (OR [95% CI]) | p-val | AA vs. AG + GG (OR [95% CI]) | p-val |
|------------------------------------------|--------------|------------------------|-------|-------------------------|-------|------------------------|-------|-----------------------------|-------|-----------------------------|-------|
| Overall                                  | 24           | 1.03 [0.81; 1.31]      | 0.795 | 0.96 [0.75; 1.23]       | 0.753 | 1.18 [0.8; 1.72]        | 0.402 | 1.10 [0.79; 1.53]            | 0.572 | 0.78 [0.67; 0.91]            | 0.002 |
| Preeclampsia                             | 2            | 0.63 [0.27; 1.43]       | 0.269 | NA [NA; NA]             | NA    | 0.62 [0.27; 1.43]       | 0.263 | 0.62 [0.27; 1.43]            | 0.263 | 0.84 [0.05; 13.43]           | 0.900 |
| Inflammatory disease                     | 5            | 0.86 [0.70; 1.06]       | 0.166 | 0.99 [0.21; 4.63]       | 0.992 | 0.98 [0.65; 1.46]       | 0.904 | 0.98 [0.66; 1.44]            | 0.904 | 0.81 [0.62; 1.05]            | 0.106 |
| Chronic obstructive pulmonary disease (COPD) | 3           | 1.54 [0.32; 7.34]      | 0.588 | 2.61 [0.51; 13.29]      | 0.249 | 1.73 [0.29; 10.55]      | 0.550 | 1.67 [0.30; 9.41]            | 0.562 | 1.82 [0.43; 7.79]           | 0.417 |
| Cardiovascular disease (CVD)             | 4            | 0.83 [0.67; 1.02]       | 0.080 | 0.46 [0.25; 0.84]       | 0.012 | 0.82 [0.50; 1.35]       | 0.441 | 0.79 [0.47; 1.34]            | 0.385 | 0.73 [0.52; 1.03]            | 0.076 |
| Type 2 diabetes                          | 3            | 1.26 [0.99; 1.60]       | 0.062 | 1.48 [0.89; 2.46]       | 0.128 | 1.86 [0.71; 4.85]       | 0.207 | 1.54 [0.85; 2.79]            | 0.155 | 0.79 [0.50; 1.23]            | 0.299 |
| Diabetic complications                    | 2            | 1.71 [1.27; 2.28]       | 0.000 | 2.34 [1.40; 3.89]       | 0.001 | 2.83 [0.65; 12.31]      | 0.166 | 2.30 [0.89; 5.96]            | 0.087 | 1.07 [0.69; 1.66]            | 0.759 |
| Others                                   | 3            | 0.72 [0.58; 0.89]       | 0.003 | 0.50 [0.32; 0.78]       | 0.002 | 0.82 [0.58; 1.15]       | 0.246 | 0.72 [0.52; 0.99]            | 0.042 | 0.60 [0.41; 0.87]            | 0.008 |
| Ethnicity                                |              |                        |       |                         |       |                        |       |                             |       |                             |       |
| Asian                                    | 15           | 1.13 [0.84; 1.52]       | 0.430 | 0.94 [0.49; 1.80]       | 0.850 | 1.47 [0.92; 2.37]       | 0.111 | 1.30 [0.86; 1.99]            | 0.217 | 0.78 [0.67; 0.91]            | 0.002 |
| Caucasian                                | 4            | 0.94 [0.63; 1.39]       | 0.749 | 4.27 [0.06; 294.6]      | 0.502 | 0.57 [0.21; 1.54]       | 0.268 | 0.63 [0.26; 1.52]            | 0.308 | 1.17 [0.19; 7.34]            | 0.867 |
| Mixed                                    | 5            | 0.96 [0.68; 1.34]       | 0.795 | 0.79 [0.15; 4.15]       | 0.785 | 0.97 [0.67; 1.41]       | 0.869 | 0.96 [0.67; 1.39]            | 0.831 | 0.81 [0.22; 2.93]            | 0.747 |
| HWE tested data                          | Overall      | 21                      | 0.83 [0.74; 0.93]       | 0.001 | 0.96 [0.75; 1.23]       | 0.753 | 0.92 [0.78; 1.08]            | 0.295 | 0.87 [0.74; 1.02]            | 0.091 | 0.73 [0.61; 0.87]            | 0.001 |

OR (95% CI) is Odds Ratio (95% Confidence Interval); The bold results indicate the statistical significance.

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HIF1A rs11549467 G/A polymorphism also significantly associated with decreasing the risk of CVD under homozygote model [AA vs. GG: OR = 0.46, 95% CI = 0.25–0.84, p-value = 0.012] and other disease group under four genetic models [A vs. G: OR = 0.72, 95% CI = 0.58–0.89, p-value = 0.003] [AA vs. GG: OR = 0.50, 95% CI = 0.32–0.78, p-value = 0.002] [AA + AG vs. GG: OR = 0.72, 95% CI = 0.52–0.99, p-value = 0.042] [AA vs. AG + GG: OR = 0.60, 95% CI = 0.41–0.87, p-value = 0.008] (Table 3).

The subgroup analyses by ethnicity of the HIF1A 1790 G/A polymorphism indicated that in the Asian population this polymorphism was significantly associated with decreasing overall disease risk under the recessive model [AA vs. GA + GG: OR = 0.78, 95% CI = 0.67–0.91, p-value = 0.002]. However, this polymorphism revealed an insignificant association with overall disease risk for the Caucasian and mixed populations (Table 3).

### Sources of heterogeneity

In this Meta-analysis, significant heterogeneity was observed in different studies of HIF1A 1790 G/A polymorphism for overall analysis under three genetic models [A vs. G: Q = 75.04, df = 23, p-value = 0.0001, I² = 69.4%] [GA vs. GG: Q = 99.55, df = 23, p-value = 0.0001, I² = 76.9%] [AA + GA vs. GG: Q = 89.84, df = 23, p-value = 0.0001, I² = 74.4%]. The subgroup
analysis suggested that some genetic model showed significant heterogeneity in the cases of COPD, CVD, T2D, Diabetic complications, Asian, and Caucasian populations. (S2 Table).

Publication bias checking

The funnel plot was used to check publication bias of HIF1A gene 1772 C/T, and 1790 G/A polymorphisms with allelic model C vs. T, and A vs. G, respectively. The conventionally constructed plots confirmed the symmetric distribution of ORs based on standard error and suggested no evidence of publication bias (Fig 4). Also, the Begg's test and Egger's linear regression test analysis data confirmed no significant publication bias under the allelic model of HIF1A 1772 C/T polymorphism [C vs. T allele; p-value = 0.9900 and 0.5052 respectively], and for the 1790 G/A [A vs. G allele; p-value = 0.7284 and 0.8537 respectively] polymorphisms (S3 Table).
Sensitivity analysis

In this study, sensitivity analysis was performed to increase the reliability of meta-analysis results. Studies that do not qualify HWE were excluded to investigate the existence of the attained results. The statistical associations of the results were not altered after excluding the respective studies, which confirmed the reliability of this meta-analysis (Tables 2 and 3).

False positive report probability (FPRP) and power analyses

We performed false-positive report probability (FPRP) to assess whether associations reported previously were false positives. We preset FPRP at 0.2 as the threshold for biological importance and a prior probability \( \pi \) at 0.01 to detect the significant OR [71]. We computed the statistical power and FPRP by fixing the odds ratio at 1.5 (or, 1/1.5 for protective effect) for identifying important biologic effects [70]. It should be mentioned here that an OR value at 1.5 is considered as a plausible value for a significant biologic effects [72, 73]. The association was considered significant, when the FPRP value was less than 0.2 [74]. Based on the above discussion, the rs11549465 SNP significantly increased the overall disease risk in Caucasian patients. Also, the rs11549465 SNP significantly decreased the overall disease risk for Asian patients and subgroup of CVD risk (Table 4). The rs11549467 SNP significantly decreased the overall disease risk for Asian patients and subgroup of CVD risk (Table 4).

Discussion and conclusion

We performed a statistical meta-analysis to investigate the association of HIF1A gene polymorphisms with multiple diseases risks more accurately compare to SGA studies. This analysis was performed based on 41 SGA study’s findings, where the polymorphisms rs11549465 (1772 C/T) and rs11549467 (1790 G/A) of HIF1A gene were analyzed based on 11544 and 7426 cases and 11494 and 7063 control samples, respectively. This study included different types of diseases (i.e. CVD, T2D, autoimmune diseases, inflammatory diseases, COPD, preeclampsia,
parkinson disease, diabetic complications, AMD, Hemodialysis, LDD, HAPC, metabolic syndrome, and pressure injury) and ethnic groups (i.e. Asian, Caucasian and mixed) were considered in this meta-analysis. The allelic alterations in different ethnic population and their association with diseases were carefully evaluated using five different genetic models (i) dominant models: \(CC + CT\) vs. \(TT\) or \(AA + AG\) vs. \(GG\), (ii) homozygote models: \(CC\) vs. \(TT\) or \(AA\) vs. \(GG\), (iii) heterozygote models: \(CT\) vs. \(TT\) or \(AG\) vs. \(GG\), (iv) recessive models: \(CC + CT\) vs. \(TT\) or \(AA\) vs. \(AG + GG\), and (v) allelic contrast models: \(C\) vs. \(T\) or \(A\) vs. \(G\), for each of 1772 C/T and 1790 G/A polymorphisms. The results of this study suggested that the \(HIF1A\) 1772 C/T polymorphism is insignificantly associated with overall disease risks under all the genetic models, which indicates that the \(C\) allele is not associated with overall diseases. The \(HIF1A\) 1790 G/A polymorphism showed a significant association with overall disease under the recessive model (\(AA\) vs. \(AG + GG\)), which indicates that the \(A\) allele is associated with overall diseases.

### Table 4. Results of false positive report probability analysis for significant findings.

| Genotype and Variables | OR (95% CI) | Statistical Power* | FPRP values for prior probabilities at |
|------------------------|------------|-------------------|---------------------------------------|
|                        |            |                   | 0.25 | 0.1  | 0.01  | 0.001 | 0.0001 | 0.00001 |
| rs11549465 and Caucasian |            |                   |      |      |       |       |       |       |
| C vs. T                | 1.27 [1.05; 1.54] | 0.955             | 0.045⁠b | 0.125⁠b | 0.610 | 0.940 | 0.994 | 0.999 |
| CC vs. TT              | 2.00 [1.40; 2.87] | 0.986             | 0.001⁠b | 0.002⁠b | 0.017⁠b | 0.146⁠b | 0.631 | 0.945 |
| CT vs. TT              | 1.64 [1.12; 2.40] | 0.323             | 0.092⁠b | 0.233 | 0.769 | 0.971 | 0.997 | 1.000 |
| CC + CT vs. TT         | 1.93 [1.35; 2.77] | 0.992             | 0.001⁠b | 0.003⁠b | 0.035⁠b | 0.267 | 0.785 | 0.973 |
| CC vs. CT + TT         | 1.24 [1.02; 1.52] | 0.967             | 0.106⁠b | 0.263 | 0.797 | 0.975 | 0.997 | 1.000 |
| rs11549465 and Chronic obstructive pulmonary disease (COPD) | | | | | | | | |
| C vs. T                | 0.46 [0.30; 0.71] | 0.933             | 0.001⁠b | 0.004⁠b | 0.046⁠b | 0.327 | 0.829 | 0.980 |
| CC vs. CT + TT         | 0.43 [0.27; 0.67] | 0.879             | 0.001⁠b | 0.002⁠b | 0.021⁠b | 0.179⁠b | 0.685 | 0.956 |
| rs11549465 and Skin disease |            |                   |      |      |       |       |       |       |
| C vs. TT               | 3.01 [1.09; 8.32] | 0.497             | 0.163⁠b | 0.378 | 0.870 | 0.985 | 0.999 | 1.000 |
| CC + CT vs. TT         | 2.71 [0.98; 7.49] | 0.578             | 0.221 | 0.460 | 0.903 | 0.990 | 0.999 | 1.000 |
| rs11549465 and Diabetic complications | | | | | | | | |
| C vs. T                | 1.34 [1.12; 1.61] | 0.886             | 0.006⁠b | 0.018⁠b | 0.166⁠b | 0.667 | 0.953 | 0.995 |
| CT vs. TT              | 2.43 [1.41; 4.18] | 0.777             | 0.005⁠b | 0.015⁠b | 0.145⁠b | 0.632 | 0.945 | 0.994 |
| CC + CT vs. TT         | 2.11 [1.29; 3.43] | 0.922             | 0.008⁠b | 0.025⁠b | 0.218 | 0.738 | 0.966 | 0.996 |
| rs11549467 and Overall |            |                   |      |      |       |       |       |       |
| AA vs. AG + GG         | 0.78 [0.67; 0.91] | 0.973             | 0.005⁠b | 0.014⁠b | 0.139⁠b | 0.619 | 0.942 | 0.994 |
| rs11549467 and Asian   |            |                   |      |      |       |       |       |       |
| AA vs. AG + GG         | 0.78 [0.67; 0.91] | 0.973             | 0.005⁠b | 0.014⁠b | 0.139⁠b | 0.619 | 0.942 | 0.994 |
| rs11549467 and Cardiovascular disease (CVD) | | | | | | | | |
| AA vs. GG              | 0.46 [0.25; 0.84] | 0.860             | 0.039⁠b | 0.107⁠b | 0.569 | 0.930 | 0.993 | 0.999 |
| rs11549467 and Diabetic complications | | | | | | | | |
| A vs. G                | 1.71 [1.27; 2.28] | 0.857             | 0.001⁠b | 0.003⁠b | 0.029⁠b | 0.231 | 0.750 | 0.968 |
| AA vs. GG              | 2.34 [1.40; 3.89] | 0.831             | 0.004⁠b | 0.011⁠b | 0.111⁠b | 0.557 | 0.926 | 0.992 |
| rs11549467 and Others  |            |                   |      |      |       |       |       |       |
| A vs. G                | 0.72 [0.58; 0.89] | 0.747             | 0.009⁠b | 0.028⁠b | 0.240 | 0.761 | 0.970 | 0.997 |
| AA vs. GG              | 0.50 [0.32; 0.78] | 0.747             | 0.009⁠b | 0.028⁠b | 0.240 | 0.761 | 0.970 | 0.997 |
| AA + AG vs. GG         | 0.72 [0.52; 0.99] | 0.671             | 0.162⁠b | 0.367 | 0.864 | 0.985 | 0.998 | 1.000 |
| AA vs. AG + GG         | 0.60 [0.41; 0.87] | 0.747             | 0.009⁠b | 0.028⁠b | 0.240 | 0.761 | 0.970 | 0.997 |

*aStatistical power was calculated using the number of observations in each subgroup and the corresponding ORs and P values in this table.

bThe level of false-positive report probability threshold was set at 0.2 and noteworthy findings are presented

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though A was recessive. The subgroup analysis based on ethnicity showed significant association between the HIF1A 1772 C/T polymorphism and overall disease for the Caucasian population under all genetic models, which indicates that the C allele is associated with overall diseases. Again, the ethnicity subgroup showed a significant association between HIF1A 1790 G/A polymorphism and overall disease for the Asian population under recessive model only (AA vs. AG + GG), which indicates that the A allele is associated with overall diseases. The subgroup analysis based on disease type showed that HIF1A 1772 C/T is significantly associated with COPD, skin and diabetic complications diseases, where C is high risk factor for skin and diabetic complications (since, ORs > 1), but low risk factor for COPD (since, ORs < 1). This subgroup analysis results goes in favor of Ekberg et al. [65], and Bi et al. [66] for diabetic complications and Yu et al. [17] and Wei et al. [37] for COPD. The association of diabetic complications risk was also supported by a previous meta-analysis report [75]. Also the subgroup analysis results of HIF1A 1772 C/T polymorphism showed insignificant association with autoimmune diseases, inflammatory diseases, and preeclampsia under all five genetic models which goes in favor of Wipff et al. [31] and Feng et al. [25] for autoimmune disease, Torres et al. [27], Chachami et al. [32] and Senhaji et al. [41] for inflammatory disease, and Nava-Salazar et al. [34] for preeclampsia. The subgroup analysis results of HIF1A 1790 G/A polymorphism showed significant association with CVD under homozygote model (AA vs. GG) and diabetic complications under allelic (A vs. G) and homozygote (AA vs. GG) models. Also, the HIF1A 1790 G/A polymorphism showed significantly decreasing the risk of other (LDD, HAPC, Pressure injury) disease group under four genetic models (A vs. G, AA vs. GA + GG, AA vs. GA + GG, AA vs. GA + GG) and insignificant association with inflammatory disease, COPD, autoimmune disease and preeclampsia under all genetic models. The association of diabetic complications contradicted the reports by Ren et al. [75]. The insignificant result of HIF1A 1790 G/A were supported by Bahadori et al. [26] for CVD, Torres et al. [27], Chachami et al. [32], Senhaji et al. [41], and Fernández-Torres et al. [20] for inflammatory disease, Putra et al. [39] for lung and Nava-Salazar et al. [34] for preeclampsia.

Thus the above discussion provided the significant evidence that the HIF1A gene is a risk factor for the development of COPD, CVD, skin disease and diabetic complications. Now it is required to explore the causality of HIF1A gene SNPs (1772 C/T and 1790 G/A) in the development of those disease by their expression analysis. Recently, some researchers studied single or multiple disease causing genes or SNPs by using network analysis or Mendelian randomization [76–82]. These SNPs can act as biological markers to locate the disease-causing genes that are regulated either directly or indirectly by those SNPs [83]. When SNPs occur within a gene or in a regulatory region near a gene, they are known as cis-acting factors, and they may play a more direct role in disease development by affecting the gene’s function. When SNPs occur far away from the disease causing genes, they are known as trans-acting factors. The cis- and trans-acting factors are usually considered as the causal and non-causal risk factors of disease development, respectively. SNPs can be silent due to its occurrence within the noncoding regions or may change the encoded amino acids due to its occurrence within the coding region. They may influence promoter or enhancer activities, messenger RNA (mRNA) stability, and subcellular localization of mRNAs and/or proteins and hence may develop disease. A post-transcriptional modification (PTM) in mRNA, known as N4-acetylcytidine (ac4C) that occurs on cytidine, plays a vital role in the stability and regulation of mRNA translation. There are at least 15 nucleotide modifications found in mRNA of which m6A and N1-methyladenosine (m1A) are similar in function to ac4C. They play a significant role in the translation process of mRNA and its stability that leads to the progression of several human diseases [84–87].

If SNPs (1772 C/T and 1790 G/A) of HIF1A gene data are available for COPD, CVD, skin disease and diabetic complications, and control samples, an effective disease prediction model
may be developed by using a suitable machine learning technique including logistic classifier. For example, some recent studies developed SNPs based diseases prediction model [88, 89]. However, there were some limitations in this study, such as (i) the heterogeneity factors such as gender, age, smoking, drinking, blood pressure, family history, etc was not considered to estimate the combined effect for overall or subgroup analysis like as [56–60]. Because the dataset was generated through multiple diseases excluding cancer, so we cannot focus on specific behavior factor due to the insufficient information of GWAS studies. (ii) the metadata was collected considering the English language only, (iii) some subgroup analysis may be affected due to the small subgroup sample size and unavailable data due to limited GWAS studies.

In conclusion, this study made a consensus decision about the association of HIF1A gene polymorphisms with multiple diseases risks excluding cancers. The meta-analysis results showed that the HIF1A 1772 C/T polymorphism is not significantly associated with overall disease risks. The HIF1A 1790 G/A polymorphism was associated with overall diseases under recessive model, where the allele A controls the diseases though it is recessive. The ethnicity subgroup analysis showed the significant association of HIF1A 1772 C/T polymorphism with overall disease for Caucasian population under all genetic models, where C allele controls the diseases, while HIF1A 1790 G/A polymorphism was significantly associated with overall disease for Asian population under a genetic model due to the influence of A allele. The subgroup analysis based on disease types showed that HIF1A 1772 C/T is significantly associated with chronic obstructive pulmonary disease (COPD), skin and diabetic complications diseases, where C allele is the high risk factor for skin and diabetic complications diseases, and low risk factor for COPD. The HIF1A 1790 G/A polymorphism showed significant association with CVD under homozygote model and diabetic complications under allelic and homozygote models. The rest of diseases showed insignificant association with HIF1A gene under all of five genetic models by the subgroup analysis. Taken together, the results of this study suggest that HIF1A could be a useful prognostic biomarker for COPD, CVD, skin disease and diabetic complication diseases. In future, availability of more SGA studies on the different ethnic populations might shed more lights to unveil and confirm the association of the HIF1A gene polymorphisms with different diseases.

Supporting information
S1 Checklist. PlosOne-meta-analysis-on-genetic-association-studies-checklist. (DOCX)
S2 Checklist. PRISMA checklist. (DOCX)
S1 Data. The dataset of HIF1A gene rs11549465 polymorphism. (XLSX)
S2 Data. The dataset of HIF1A gene rs11549467 polymorphism. (XLSX)
S1 Table. Quality assessment for included study in the meta-analysis. (DOCX)
S2 Table. Heterogeneity analysis of HIF1A gene polymorphisms. (DOCX)
S3 Table. Publication bias checking by using Egger’s linear regression and Begg’s test of HIF1A gene polymorphisms.
(DOCX)

S4 Table. Group of disease conducting this meta-analysis.
(DOCX)

S1 File. Forest plot of HIF1A gene polymorphisms (rs11549465, rs11549467) for four genetic models.
(DOCX)

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References
1. Iglesias P.; Penas C.; Barral-Cagiao L.; Pazos E.; Costoya J.A. A Bio-Inspired Hypoxia Sensor Using HIF1α-Oxygen-Dependent Degradation Domain. Sci. Rep. 2019, 9, https://doi.org/10.1038/s41598-019-43618-4 PMID: 31068630
2. Wenger R.H.; Stiehl D.P.; Camenisch G. Integration of Oxygen Signaling at the Consensus HRE. Sci. STKE 2005, 2005. https://doi.org/10.1126/stke.3062005re12 PMID: 16234508
3. Semenza G.L. Oxygen-Dependent Regulation of Mitochondrial Respiration by Hypoxia-Inducible Factor 1. Biochem. J. 2007, 405. https://doi.org/10.1042/BJ20070389 PMID: 17555402
4. CieSzczyk P.; Kalinski M.; Ostanek M.; Jascaniene N.; Krupec k K.; Ficek K.; et al. Variation in the Hif1a Gene in Elite Rowers. J. Strength Cond. Res. 2012, 26, https://doi.org/10.1519/JSC.0b013e31824b876d PMID: 22476163
5. Semenza G.L. Targeting HIF-1 for Cancer Therapy. Nat. Rev. Cancer 2003, 3. https://doi.org/10.1038/nrc1187 PMID: 13130303
6. Pouyssegur J.; Dayan F.; Mazure N.M. Hypoxia Signalling in Cancer and Approaches to Enforce Tumour Regression. Nature 2006, 441. https://doi.org/10.1038/nature04400 PMID: 16437109
7. Jia X.; Hong Q.; Lei L.; Li D.; Li J.; Mo M.; et al. Basal and Therapy-Driven Hypoxia-Inducible Factor-1α Confers Resistance to Endocrine Therapy in Estrogen Receptor-Positive Breast Cancer. Oncotarget 2015, 6, https://doi.org/10.18632/oncotarget.3257 PMID: 25929336
4. Analyze the expression patterns of specific genes in early and late onset preeclampsia in Sinhalese women. 

5. Evaluate the role of HIF1-α gene polymorphisms in the development of knee osteoarthritis: A pilot study. 

6. Study the association of HIF1-α gene polymorphisms with multiple disease risks. 

7. Explores the potential implications of HIF1-α gene polymorphisms in the coordination of renal stress response. 

8. Investigate the impact of HIF1-α gene polymorphisms on the progression of chronic obstructive pulmonary disease. 

9. Analyze the relationship between HIF1-α gene polymorphisms and cancer susceptibility in a Chinese population. 

10. Evaluate the association of HIF1-α gene polymorphisms with multiple disease risks. 

11. Examine the role of HIF1-α gene polymorphisms in the modulation of renal stress response. 

12. Study the potential implications of HIF1-α gene polymorphisms in the coordination of renal stress response. 

13. Explore the association of HIF1-α gene polymorphisms with multiple disease risks. 

14. Investigate the potential implications of HIF1-α gene polymorphisms in the coordination of renal stress response. 

15. Analyze the impact of HIF1-α gene polymorphisms on the progression of chronic obstructive pulmonary disease. 

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20. Evaluate the association of HIF1-α gene polymorphisms with multiple disease risks. 

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22. Study the association of HIF1-α gene polymorphisms with multiple disease risks. 

23. Investigate the potential implications of HIF1-α gene polymorphisms in the coordination of renal stress response. 

24. Evaluate the association of HIF1-α gene polymorphisms with multiple disease risks. 

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28. Evaluate the association of HIF1-α gene polymorphisms with multiple disease risks. 

29. Explore the potential implications of HIF1-α gene polymorphisms in the coordination of renal stress response. 

30. Study the association of HIF1-α gene polymorphisms with multiple disease risks.
26. Bahadori B.; Ulitz E.; Mayer A.; Harauer J.; Dam K.; Truschig-Wilders M.; et al. Polymorphisms of the Hypoxia-Inducible Factor 1 Gene and Peripheral Artery Disease. *Vasc. Med.*, 2010, 15, https://doi.org/10.1177/1358863X10379674 PMID: 20926496

27. Torres O.; Palomino-Morales R.; Vazquez-Rodriguez T.R.; Gamallo C.; Morado I.C.; Miranda-Filloy J. A.; et al. Lack of Association between Hypoxia Inducible Factor-1 Alpha Gene Polymorphisms and Bispy-Proven Giant Cell Arteritis. *Clin. Exp. Rheumatol.* 2010, 28, PMID: 20412701

28. Emanuele E.; Bertona M.; Geroldi D. A Multilocus Candidate Approach Identifies ACE and HIF1A as Susceptibility Genes for Cellulite. *J. Eur. Acad. Dermatol Venereol.*, 2010, 24, https://doi.org/10.1111/j.1468-3083.2009.03556.x PMID: 20059631

29. Geza N.; Reka K.N.; Keresztesy E.; Somogyi A.; Szekely A.; Nemeth N.; et al. Association of Hypoxia Inducible Factor-1 Alpha Gene Polymorphism with Both Type 1 and Type 2 Diabetes in a Caucasian (Hungarian) Sample. *BMC Med. Genet.* 2009, 10, https://doi.org/10.1186/1471-2350-10-79 PMID: 19961832

30. Zheng Z.L.; Hwang Y.H.; Kim S.K.; Kim S.; Son M.J.; Ro H.; et al. Genetic Polymorphisms of Hypoxia-Inducible Factor-1 Alpha and Cardiovascular Disease in Hemodialysis Patients. *Nephron—Clin. Pract.* 2009, 113, https://doi.org/10.1159/000228542 PMID: 19602906

31. Wipff J.; Diepue P.; Avouac J.; Tiev K.; Hachulla E.; Granel B.; et al. Association of Hypoxia-Inducible Factor 1A (HIF1A) Gene Polymorphisms with Systemic Sclerosis in a French European Caucasian Population. *Scand. J. Rheumatol.* 2009, 38, https://doi.org/10.1080/03009740802629432 PMID: 19306159

32. Chacharni G.; Kalousi A.; Papatheodorou L.; Lyberopoulos H.; Nasiakas V.; Tanimoto K.; et al. An Association Study between Hypoxia Inducible Factor-1alpha (HIF-1α) Polymorphisms and Osteonecrosis. *PLoS One* 2013, 8, https://doi.org/10.1371/journal.pone.0079647 PMID: 24260273

33. Lin W.P.; Wang X.J.; Wang C.R.; Zhang L.Q.; Li N.; Wang F.S.; et al. Polymorphism in the Hypoxia-Inducible Factor 1alpha Gene May Confer Susceptibility to LDD in Chinese Cohort. *PLoS One* 2013, 8, https://doi.org/10.1371/journal.pone.0073158 PMID: 23991178

34. Nava-Salazar S.; Sánchez-Rodríguez E.N.; Mendoza-Rodríguez C.A.; Moran C.; Romero-Arauz J.F.; Cerbañ M.A. Polymorphisms in the Hypoxia-Inducible Factor 1 Alpha Gene in Mexican Patients with Preeclampsia: A Case-Control Study. *BMC Res. Notes* 2011, 4, https://doi.org/10.1186/1756-0500-4-68 PMID: 21414224

35. Yamada N.; Horikawa Y.; Oda N.; Iizuka K.; Shihara N.; Kishi S.; et al. Genetic Variation in the Hypoxia-Inducible Factor-1a Gene Is Associated with Type 2 Diabetes in Japanese. *J. Clin. Endocrinol. Metab.* 2005, 90, https://doi.org/10.1210/jc.2005-0991 PMID: 16048631

36. Chen Y.; Jiang C.; Luo Y.; Liu F.; Gao Y. Interaction of CARD14, SENP1 and VEGFA Polymorphisms on Susceptibility to High Altitude Polycythemia in the Han Chinese Population at the Qinghai-Tibetan Plateau. *Blood Cells, Mol. Dis.* 2016, 57, https://doi.org/10.1016/j.bcmd.2015.11.005 PMID: 26852650

37. Wei W.T.; Li B.; Chen M.; Jia H.R.; Zhang H.X. Associations between HIF-1α Polymorphisms C1772T and C1790A and Susceptibility to Chronic Obstructive Pulmonary Disease. *Genet. Mol. Res.* 2015, 14, https://doi.org/10.4238/2015.December.21.2 PMID: 26782374

38. de Carvalho Fraga C.A.; Alves L.R.; Marques-Silva L.; de Sousa A.A.; Jorge A.S.B.; de Jesus S.F.; et al. Association of Hypoxia-Inducible Factor-1a Gene Polymorphism with Both Type 1 and Type 2 Diabetes in a Caucasian Population. *Indian J. Dermatol.* 2019, 64, https://doi.org/10.4103/ijd.IJD_422_18 PMID: 31148856
45. Liu Y.-H.; Guo C.; Sun Y.-Q.; Li Q. Polymorphisms in HIF-1α Gene Are Not Associated with Diabetic Retinopathy in China. *World J. Diabetes* 2021, 12, 1304–1311, https://doi.org/10.4239/wjd.v12.i8.1304 PMID: 34512895

46. Takagi K.; Kawamoto M.; Higuchi T.; Tochimoto A.; Harigai M.; Kawaguchi Y. Single Nucleotide Polymorphisms of the HIF1A Gene Are Associated with Pulmonary Arterial Hypertension in Systemic Sclerosis and Contribute to SSC-PAH Disease Severity. *Int. J. Rheum. Dis.* 2020, 23, 674–680, https://doi.org/10.1111/1756-185X.13822 PMID: 32144871

47. Saravani M.; Rokni M.; Mehrbani M.; Amirkhosravi A.; Faramarz S.; Fatemi I.; et al. The Evaluation of VEGF and HIF-1α Gene Polymorphisms and Multiple Sclerosis Susceptibility. *J. Gene Med.* 2019, 21, 1–7, https://doi.org/10.1002/jgm.3132 PMID: 31652374

48. Qin L.; Shu L.; Zhong J.; Pan H.; Guo J.; Sun Q.; et al. Association of HIF1A and Parkinson’s Disease in a Han Chinese Population Demonstrated by Molecular Inversion Probe Analysis. *Neurof. Sci.* 2019, 40, 1927–1931, https://doi.org/10.1007/s10072-019-03905-4 PMID: 31025220

49. Tsukatani T.; Minematsu T.; Dai M.; Tamai N.; Nakagami G.; Sugama J.; et al. Polymorphism Analysis of Candidate Risk Genes for Pressure Injuries in Older Japanese Patients: A Cross-Sectional Study at a Long-Term Care Hospital. *Wound Repair Regen.* 2021, 29, 741–751, https://doi.org/10.1111/wrr.12912 PMID: 33819344

50. Pichu S.; Sathiyamoorthy J.; Krishnamoorthy E.; Umapathy D.; Viswanathan V. Impact of the Hypoxia Inducible Factor-1α (HIF-1α) Pro582Ser Polymorphism and Its Gene Expression on Diabetic Foot Ulcers. *Diabetes Res. Clin. Pract.* 2015, 109, https://doi.org/10.1016/j.diabres.2015.05.014 PMID: 26113285

51. Ekberg N.R.; Eliasson S.; Li Y.W.; Zheng X.; Chatzidionysiou K.; Falhammar H.; et al. Protective Effect of the HIF-1A Pro582Ser Polymorphism on Severe Diabetic Retinopathy. *J. Diabetes Res.* 2019, 2019, https://doi.org/10.1155/2019/2036962 PMID: 31214621

52. Bi Y.X.; Yu L.; Jin G.X. Correlation between Polymorphisms of Hypoxia-Inducible Factor-1α (HIF1A) Pro582Ser and Type 2 Diabetic Nephropathy. *Genet. Mol. Res.* 2015, 14, 14503–14509, https://doi.org/10.4292/2015.13SM.26600909

53. Gu H.F.; Zheng X.; Seman N.A.; Gu T.; Botusan I.R.; Sunkari V.G.; et al. Impact of the Hypoxia-Inducible Factor-1α (HIF1A) Pro582Ser Polymorphism on Diabetes Nephropathy. *Diabetes Care* 2013, 36, 415–421, https://doi.org/10.2337/dc12-1125 PMID: 22991450

54. Xu M.Q.; Ye Z.; Hu F.B.; He L. Quantitative Assessment of the Effect of Angiotensin Gene Polymorphisms on the Risk of Coronary Heart Disease. *Circulation* 2007, 116, 1356–1366, https://doi.org/10.1161/CIRCULATIONAHA.107.728857 PMID: 17846284

55. Xu M.; Sham P.; Ye Z.; Lindpaintner K.; He L. A1166C Genetic Variation of the Angiotensin II Type I Receptor Gene and Susceptibility to Coronary Heart Disease: Collaborative of 53 Studies with 20,435 Cases and 23,674 Controls. *Atherosclerosis* 2010, 213, 191–199, https://doi.org/10.1016/j.atherosclerosis.2010.07.046 PMID: 20732682

56. Xu M.; Lin Z. Genetic Influences of Dopamine Transport Gene on Alcohol Dependence: A Pooled Analysis of 13 Studies with 2483 Cases and 1753 Controls. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* 2011, 35, https://doi.org/10.1016/j.pnpbp.2010.11.001 PMID: 21076357

57. Xu M.; Cao H.; Baranova A.; Huang H.; Li S.; Cai L.; et al. Multi- Trait Analysis for Genome-Wide Association Study of Five Psychiatric Disorders. *Transl. Psychiatry* 2020, 10, https://doi.org/10.1038/s41398-020-00902-6 PMID: 32606422

58. Jiang L.; Wang K.; Lo K.; Zhong Y.; Yang A.; Fang X.; et al. Sex-Specific Association of Circulating Ferritin Level and Risk of Type 2 Diabetes: A Dose-Response Meta-Analysis of Prospective Studies. *J. Clin. Endocrinol. Metab.* 2019, 104, 4539–4551, https://doi.org/10.1210/jc.2019-00495 PMID: 31074798

59. Liberati A.; Altman D.G.; Tetzlaff J.; Mulrow C.; Gøtzsche P.C.; Ioannidis J.P.A.; et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. *Ann. Intern. Med.* 2009, 151.

60. Stang A. Critical Evaluation of the Newcastle-Ottawa Scale for the Assessment of the Quality of Non-randomized Studies in Meta-Analyses. *Eur. J. Epidemiol.* 2010, 25, https://doi.org/10.1007/s10654-010-9491-z PMID: 20652370
63. Liu I.-M.; Agresti A. Mantel-Haenszel-Type Inference for Cumulative Odds Ratios with a Stratified Ordinal Response. *Biometrics* 1996, 52, https://doi.org/10.2307/2532838 PMID: 8962452

64. Ravi S. Book Review: Methods for Meta-Analysis in Medical Research. *Stat. Methods Med. Res.* 2005, 14, https://doi.org/10.1191/0962280205sm401x

65. Cochran W.G. The Combination of Estimates from Different Experiments. *Biometrics* 1954, 10, https://doi.org/10.2307/3001666

66. Higgins J.P.T.; Thompson S.G. Quantifying Heterogeneity in a Meta-Analysis. *Stat. Med.* 2002, 21, https://doi.org/10.1002/sim.1186 PMID: 12111919

67. Valenzuela C. 2 Solutions for Estimating Odds Ratios with Zeros. *Rev. Med. Chil.* 1993, 121. PMID: 8085071

68. Egger M.; Smith G.D.; Schneider M.; Minder C. Bias in Meta-Analysis Detected by a Simple, Graphical Test. *Br. Med. J.* 1997, 315, https://doi.org/10.1136/bmj.315.7109.629 PMID: 9310663

69. Begg C.B.; Mazumdar M. Operating Characteristics of a Rank Correlation Test for Publication Bias. *Biometrics* 1994, 50, https://doi.org/10.2307/2533446

70. Wacholder S.; Chanock S.; Garcia-Closas M.; El Ghormli L.; Rothman N. Assessing the Probability That a Positive Report Is False: An Approach for Molecular Epidemiology Studies. *J. Natl. Cancer Inst.* 2004, 96, 434–442, https://doi.org/10.1093/jnci/djh075 PMID: 15026468

71. Zhou L.; Zheng Y.; Tian T.; Liu K.; Wang M.; Lin S.; et al. Associations of Interleukin-6 Gene Polymorphisms with Cancer Risk: Evidence Based on 49,408 Cancer Cases and 61,790 Controls. *Gene* 2018, 670, 136–147, https://doi.org/10.1016/j.gene.2018.05.104 PMID: 29842912

72. Marcus P.M.; Vines P.; Rothman N. NAT2 Slow Acetylation and Bladder Cancer Risk: A Meta-Analysis of 22 Case-Control Studies Conducted in the General Population. *Pharmacogenomics* 2000, 10, https://doi.org/10.1097/00008571-200003000-00003 PMID: 10761999

73. Engel L.S.; Taioli E.; Pfeiffer R.; Garcia-Closas M.; Marcus P.M.; Lan Q.; et al. Pooled Analysis and Meta-Analyses of Glutathione S-Transferase M1 and Bladder Cancer: A HuGE Review. *Am. J. Epidemiol.* 2002, 156. https://doi.org/10.1093/aje/kwf016 PMID: 12117698

74. He J.; Zou Y.; Liu X.; Zhu J.; Zhang J.; Zhang R.; et al. Association of Common Genetic Variants in Pre-MicroRNAs and Neurobiomarkers Susceptibility: A Two-Center Study in Chinese Children. *Mol. Ther.—Nucleic Acids* 2018, 11, https://doi.org/10.1016/j.omt.2018.01.003 PMID: 29858046

75. Ren H.; Luo J.Q.; Gao Y.C.; Chen M.Y.; Chen X.P.; Zhou H.H.; et al. Genetic Association of Hypoxia Inducible Factor 1-Alpha (HIF1A) Pro582Ser Polymorphism with Risk of Diabetes and Diabetic Complications. *Aging* (Albany NY). 2020, 12; 12783–12798, https://doi.org/10.18632/aging.103215 PMID: 3265866

76. Zhang F.; Baranova A.; Zhou C.; Cao H.; Chen J.; Zhang X.; et al. Causal Influences of Neuroticism on Mental Health and Cardiovascular Disease. *Hum. Genet.* 2021, 140, https://doi.org/10.1007/s00439-021-02288-4 PMID: 33973063

77. Zhang F.; Rao S.; Cao H.; Zhang X.; Wang Q.; Xu Y.; et al. Genetic Evidence Suggests Posttraumatic Stress Disorder as a Subtype of Major Depressive Disorder. *J. Clin. Invest.* 2021, https://doi.org/10.1172/jci145942 PMID: 33905376

78. Wang X.; Fang X.; Zheng W.; Zhou J.; Song Z.; Xu M.; et al. Genetic Support of A Causal Relationship Between Iron Status and Type 2 Diabetes: A Mendelian Randomization Study. *J. Clin. Endocrinol. Metab.* 2021, 106, https://doi.org/10.1210/clinem/dgab454 PMID: 34147035

79. Hou L.; Xu M.; Yu Y.; Sun X.; Liu X.; Liu L.; et al. Exploring the Causal Pathway from Ischemic Stroke to Atrial Fibrillation: A Network Mendelian Randomization Study. *Hum. Mol. Med.* 2020, 26, https://doi.org/10.1186/s10020-019-0133-y PMID: 31941463

80. Zhang F.; Baranova A. Smoking Quantitatively Increases Risk for COVID-19. *Eur. Respir.* J. 2021, https://doi.org/10.1183/13993003.01273-2021 PMID: 34326191

81. Hu P.; Jiao R.; Jin L.; Xiong M. Application of Causal Inference to Genomic Analysis: Advances in Methodology. *Front. Genet.* 2018, 9. https://doi.org/10.3389/fgene.2018.00236 PMID: 30042787

82. Kou N.; Zhou W.; He Y.; Ying X.; Chai S.; Fei T.; et al. A Mendelian Randomization Analysis to Expose the Causal Effect of IL-16 on Osteoporosis Based on Genome-Wide Association Study Data. *Front. Bioeng. Biotechnol.* 2020, 8, https://doi.org/10.3389/fbioe.2020.00201 PMID: 32266232

83. Gray I.C.; Campbell D.A.; Spurr N.K. Single Nucleotide Polymorphisms as Tools in Human Genetics. *Hum. Mol. Genet.* 2000, 9, https://doi.org/10.1093/hmg/9.16.2403 PMID: 11005795

84. Zhou X.; Li Q.; Xu J.; Zhang X.; Zhang H.; Xiang Y.; et al. The Aberrantly Expressed MiR-193b-3p Contributed to Preeclampsia through Regulating Transforming Growth Factor-β Signaling. *Sci. Rep.* 2016, 6, https://doi.org/10.1038/srep19910 PMID: 26822621
85. Yan X.; Zhao X.; Li J.; He L.; Xu M. Effects of Early-Life Malnutrition on Neurodevelopment and Neuropsychiatric Disorders and the Potential Mechanisms. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* 2018, 83. https://doi.org/10.1016/j.pnpbp.2017.12.016 PMID: 29287829

86. Jin G.; Xu M.; Zou M.; Duan S. The Processing, Gene Regulation, Biological Functions, and Clinical Relevance of N4-Acetylcytidine on RNA: A Systematic Review. *Mol. Ther. — Nucleic Acids* 2020, 20.

87. Zheng S.; Zhao T.; Yuan S.; Yang L.; Ding J.; Cui L.; et al. Immunodeficiency Promotes Adaptive Alterations of Host Gut Microbiome: An Observational Metagenomic Study in Mice. *Front. Microbiol.* 2019, 10, https://doi.org/10.3389/fmicb.2019.02415 PMID: 31781050

88. Liu M.; Li F.; Yan H.; Wang K.; Ma Y.; Shen L.; et al. A Multi-Model Deep Convolutional Neural Network for Automatic Hippocampus Segmentation and Classification in Alzheimer’s Disease. *Neuroimage* 2020, 208, https://doi.org/10.1016/j.neuroimage.2019.116459 PMID: 31837471

89. Yu H.; Pan R.; Qi Y.; Zheng Z.; Li J.; Li H.; et al. LEPR Hypomethylation Was Significantly Associated with Gastric Cancer in Males. *Exp. Mol. Pathol.* 2020, 116, https://doi.org/10.1016/j.yexmp.2020.104493 PMID: 32659237