Association of *UCP1* and *UCP2* Variants With Diabetic Retinopathy Susceptibility in Type-2 Diabetes Mellitus Patients: A Meta-analysis

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Abstract

Background: Genetic association of uncoupling proteins (UCPs) variants with the susceptibility of diabetic retinopathy (DR) in diabetes mellites (DM) patients has been reported but with controversy. Here we aimed to conduct a meta-analysis to confirm the association of different UCPs variants with DR.

Methods: Three databases (Medline Ovid, Embase Ovid and CENTRAL) were applied in the literature search. Five genetic models, including allelic, homozygous, heterozygous, dominant and recessive models, were evaluated. Odds ratios (OR) were estimated under the random or fixed-effects models. Subgroup analyses, publication bias and sensitivity analyses were also conducted.

Results: Eleven studies on 2 UCPs variants (*UCP1* rs1800592 and *UCP2* rs659366) were included. Our meta-analysis showed that *UCP1* rs1800592 was not associated with DR in type-2 DM patients, and *UCP2* rs659366 also showed no association with DR. In the subgroup analyses on the stage of DR, allele A of *UCP1* rs1800592 significantly increased the susceptibility of proliferative diabetic retinopathy (PDR) in type-2 DM patients in the allelic (OR = 1.26, $P = 0.03$) and homozygous models (OR = 1.60, $P = 0.04$). Subgroup analysis on ethnicity did not found any significant association of rs1800592 and rs659366 with DR.

Conclusion: Our meta-analysis confirmed the association of *UCP1* rs1800592 variant with PDR in patients with type-2 DM, suggesting its potential as a genetic marker for PDR prediction in population screening.

1. Background

Diabetic retinopathy (DR), a common sight-threatening microvascular complication among patients with diabetes mellites (DM), is the major cause of irreversible blindness and visual impairment in working-age adults [1]. Though the pathophysiological mechanisms of DR remain elusive, increasing evidences suggest that long duration of DM, poor control of blood glucose and high blood pressure mainly contribute to the pathogenesis and development of DR [2]. However, DR could also occur in patients with short duration of DM, well control of blood glucose and normal blood pressure. Besides, epidemiological studies revealed familial inheritance and ethnic variations in DR [3], indicating that genetic factors could play a role in the pathogenesis and development of DR[4].

The elevation of oxidative stress has been suggested contributing to the development of DM complications [5], which is caused by reactive oxygen species (ROS) overproduction, mainly the mitochondrial ROS [5–7]. Excessive ROS resulted from hyperglycemia causes retinal mitochondrial dysfunction and induces capillary endothelial cell apoptosis, which subsequently leads to the diabetic microvascular complications, including DR [8, 9].Uncoupling proteins (UCPs) belong to a group of proton carrier transporters (H^+) in the inner membrane of mitochondria [10]. UCPs are able to uncouple the oxidized substrates and dissipate the potential energy on the inner membrane as heat to reduce ROS overproduction from mitochondria [11–14]. The overproduced ROS could cause increases proton

conductance by UCP1-3, leading to decease in superoxide radicals through the mitochondria respiratory chain reaction [15]. In human genome, there were five different UCPs, named UCP1 to 5, with various tissue distributions and functions [16]. Uncoupling protein 1 (*UCP1*) gene is located on chromosome 4q31.1 and found to be expressed in brown adipose tissue, endothelial cells and pericytes of retina [17]. UCP1 mainly plays a role in the maintenance of body temperature in a cold environment through non-shivering thermogenesis [14]. It has been shown that elevated of glucose levels upregulates UCP1 expression, protecting cells from glucose-induced ROS damage [18]. Uncoupling protein 2 (*UCP2*) and 3 (*UCP3*) genes are both located in the same cluster on chromosome 11q13.4. UCP2 is ubiquitously expressed across different tissues in the body, whereas UCP3 is mainly expressed in the skeletal muscle tissue [19].In *UCP2* knockout mice, ROS production increases in macrophages [20] and pancreatic islets [21], whereas overexpression of UCP2 inhibits mitochondrial death pathway in cardiomyocytes[22], indicating that UCP2 could be involved in cell protection from ROS damage. UCP2 and UCP3, together with *SLC25A27* (UCP4) and *BMCP1* (UCP5), exert cytoprotective effects by reducing oxidative stress under certain conditions [23].

Since UCPs are involved in the pathophysiology of glucose-related ROS cell damage, it is reasonable to hypothesize that the UCPs variants could be related to the susceptibility of DR. Yet, inconsistent results have been reported on the association analysis of UCPs variants with the risk of DR [24–26]. Herein, we aimed to conduct a meta-analysis to clarify the association of different UCPs variants with the susceptibility of DR.

2. Methods

2.1. Study design

The protocol of this meta-analysis has been registered in the international prospective register of systematic reviews (PROSPERO protocol CRD42020173510; available athttps://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020173510).

2.2. Searching strategies and selection criteria

Three databases, including Medline Ovid, Embase Ovid, and CENTRAL, were applied in the literature search for the potential studies. The eligible studies related to the susceptibility of DR and UCPs variants would be included in this meta-analysis. The following terms were used in this search: “diabetic retinopathy”, “uncoupling protein”, and “polymorphisms OR variants”. The detail search strategies and results were shown in *Supplementary document*.

Literature language was not limited to English. For languages other than Chinese and English, Google Translate (http://translate.google.com/) was used to translate the full text. The bibliographies of the screened articles have been carefully browsed to identify the omitted studies.

The inclusion criteria included: 1) studies on the analysis of the association of UCPs variants with DR; 2) the recruited participants were independent and unrelated to each other; 3) sufficient genotype data for the calculation of odd ratio (OR) with 95% confidence interval (C.I.); 4) participants diagnosed with diabetes without retinopathy (DWR) would be served as the control subjects for the Hardy-Weinberg equilibrium (HWE) analysis or the data provided should be able to calculate HWE of the control group; and 5) the type of diabetes in the participants was clearly provided, including type-1 and type-2 DM. The exclusion criteria included: 1) the genotype distributions of the control subjects did not follow HWE (P_{HWE}< 0.05); and 2) the variants reported only by one study would not be included in this analysis.

2.3. Data extraction

Two researchers (X.L. and Z.J.) independently extracted and assessed the full-text reports for all potentially eligible studies. The included studies were evaluated by the Newcastle-Ottawa quality assessment scale (NOS). The extracted items include: first author, year of publication, region of study, ethnicity, number of cases and controls, diagnostic criteria, allele or genotype frequency, Hardy-Weinberg equilibrium (HWE) status, and genotyping method. If there was any disagreement regarding to the eligibility, scores of NOS and extracted items, the judicator (Z.W.) would make the final decision. If any full-text reports have been rejected, the reasons for the rejection would be given. The data extraction form included the following data: 1) the first author and the year of publication; 2) the country and the ethnicity of the studied subjects; 3) the methodology of genotyping; 4) the methodology of DR diagnosis; 5) *P*-value of HWE in the control group; 6) The genotypic count of each variant in the patient and control groups.

Non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) are regarded as different stages of DR, which shows different pathology and pathophysiology. This meta-analysis evaluated three types of case groups: (1) DR, (2) only PDR, and (3) combined NPDR, PDR and DR.

2.4. Statistical analysis

A publicly available program (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to estimate the HWE of the included studies. HWE in the control subjects was evaluated by χ^2 test, and $P < 0.05$ was considered as deviation from HWE.

The association of UCPs variants with DR was evaluated by five genetic models, including the allelic (reference allele versus variant allele), homozygous (homozygous reference genotype versus homozygous variant genotype), heterozygous (homozygous reference genotype versus heterozygous genotype), dominant (homozygous reference genotype versus homozygous variant and heterozygous genotypes), and recessive models (homozygous reference and heterozygous genotypes versus homozygous variant genotype). Subgroup analyses were also conducted based on the stage of DR and the ethnicity.

Heterogeneity was examined by the Q statistic (significance defined as $P < 0.1$) and the I^2 statistic (significant inconsistency defined as $I^2 > 50\%$) [27]. If heterogeneity test showed significance ($P < 0.1$ or I^2

> 50%), the random-effect model was selected to measure the pooled effect value (DerSimonian and Laird method) [28]; otherwise, the fixed-effect model was applied (Mantel-Haenszel method)[29]. The pooled odds ratio (OR) with 95% confidence intervals (C.I.) was calculated to measure the strength of association between the UCPs variants and DR, which was assessed by the Z test (significance defined as $P < 0.05$). Sensitivity analysis was used to measure the stability of the results by excluding one study at a time when there were more than two studies. Egger's test was used to quantitatively evaluate the potential publication bias. All statistical analysis was performed by STATA software (version 14.0; STATA Corporation, College Station, TX).

3. Results

3.1. Studies characteristics

Forty studies were resulted and retrieved from the literature search in the 3 databases. After screening on the abstracts and full-text reports, 11 studies met with the inclusion criteria [17, 24, 25, 30–35]. Jin *et al*. 2020 is comprised of 2 datasets, of which dataset 1 has been reported in Jin *et al*. 2017 [32, 33]. For Jin *et al*. 2020 and Montesanto *et al*. 2018, only the allelic data was able to be extracted [33, 34]. Therefore, dataset 2 of Jin *et al*. 2020 as the data for Jin 2020 and Montesanto *et al*. 2018 were used to calculate the pooled effect in the allelic model. The quality of the included studies was evaluated by NOS, ranging from 5 to 8, and the overall quality was moderate. The flow chart of the study selection was presented in Fig. 1.

A total of 5 UCPs variants, *UCP1* rs1800592, *UCP2rs659366*, *UCP2* rs660339 (p.A55V), *UCP2* 45-bp Ins/Del and *UCP2* rs1800849, were identified from the literature search (Table 1 and Supplementary table). Yet, only rs1800592 and rs659366 were subjected to further analysis as they were reported in more than 3 studies. The 11 included studies for rs1800592 and rs659366 came from 4 countries, including China ($n = 5$), Brazil ($n = 2$), Germany ($n = 3$),Brazil(n = 2) and Italy ($n = 1$). The extracted data (except for Jin *et al*. 2020 and Montesanto *et al*. 2018), HWE and minor allele frequency (MAF) were listed in Table 2. For Montesanto *et al*. 2018, the actual P_{HWE} value was not presented but mentioned all variants with $P_{HWE} > 0.05$. In addition, Jin *et al*. 2020 only presented HWE and MAF of the two datasets (rs1800592: MAF = 0.499, $P_{HWE} > 0.999$ and rs659366: MAF = 0.437, $P_{HWE} = 0.640$).

3.2. Data analysis

Five studies were identified for the investigation of *UCP1* rs1800592, among which Rudofsky*et al*. 2007 was not included in the pooled effects analysis as its P_{HWE} in the control subjects was less than 0.05 [24]. Only 2 studies, Brondani*et al*. 2012 and Crispim*et al*. 2010, included the patients with type-1 DM [17, 26]. Hence, rs1800592 and rs659366 were further analyzed only with patients in type-2 DM. For *UCP1* rs1800592, a total of 1781 patients as cases (DR) and 3610 patients as control (DWR) was used for the meta-analysis in the allelic model, and the number of cases and controls in other models were 1212 and 2004, respectively. Similarly, for *UCP2* rs659366, the number of cases and controls in the allelic model

were 1318 and 3316, respectively, and 1318 patients as case and 3316 patients as control in other models. The pooled effect analysis of *UCP1*rs1800592 showed no significant association in type-2 DM patients for all five genetic models (Table 3 and Fig. 2). Similarly, for *UCP2* rs659366, the pooled effect analysis showed no statistically significant association with DR in type-2 DM patients for all five genetic models (Table 3 and Fig. 3). In the subgroup analyses, *UCP1* rs1800592 showed statistically significant association with PDR in type-2 DM patients for the allelic (A allele versus G allele: OR = 1.26, 95% C.I.: 1.02–1.56, $P = 0.035$), homozygous model (AA versus GG: OR = 1.60, 95% C.I.: 1.01–2.52, $P = 0.044$), but not for the heterozygous, dominant and recessive models. ($P > 0.05$; Table 4 and Fig. 4). However, for other subgroup analyses, no statistically significant association was found in the stage of DR and ethnicity (Supplementary figure).

3.3. Evaluation of publication bias and sensitivity analyses

The Egger's test, which was used to quantitatively measure the publication bias, showed no statistically significant publication bias ($P > 0.05$; Table 5). The results of the sensitivity analysis showed that the pooled OR lied within the 95% C.I. of the total pooled OR (Fig. 5).

4. Discussion

DR is one of the most common microvascular complications in DM patients. The hyperglycemia-induced ROS is considered as one of the initial and major pathways causing the damage to the endothelial cells. The UCPs is an anion carrier protein in mitochondrial inner membrane. UCPs function to reduce mitochondrial ROS, especially hyperglycemia-induced oxidative stress, and protect endothelial cells from oxidative stress by balancing the proton motive force across the mitochondrial inner membrane [23]. Therefore, UCPs could possibly participate in the development and pathogenesis of DR.

This meta-analysis verified the association of the reported UCPs variants with the susceptibility of DR. Our results showed that *UCP1* rs1800592 variant was not significantly associated with DR in type-2 DM patients in the pooled effects analysis (Table 3 and Fig. 2); yet, in the subgroup analysis, *UCP1* rs1800592 was significantly associated with PDR in type-2 DM patients in the allelic and homozygous models (Table 4 and Fig. 4). The patients carrying allele A of *UCP1* rs1800592 variant have 26% higher risk developing PDR than those carrying allele G. This might be explained by a previous study demonstrated that the carriers of rs1800592 GG genotype exhibited higher *UCP1* gene expression than those with AA genotype in the retina samples[17]. Conversely, *UCP1* expression was lower in carriers of GG genotype than those with AA genotype in intraperitoneal adipose cells, indicated the tissue-specific effect of rs1800592 on UCP1 expression activity [36]. Moreover, allele G of *UCP1* rs1800592 also showed elevated expression of MnSOD2 gene, which is another major scavenger for mitochondrial ROS [17, 37]. However, our discovery was resulted only from 2 studies. Further studies in more cohorts are needed to verify the association of this variant with PDR.

UCP2 is the most widely distributed uncoupling protein and most frequently studied in DM and DR, and itis associated with the increased oxidative stress and negatively regulates the insulin secretion [38, 39].

Total 4 *UCP2* variants, *UCP2* rs659366, *UCP2* rs660339 (p.A55V), *UCP2* 45-bp Ins/Del and *UCP2* rs1800849, were reported in the association analysis with DR; however, only *UCP2* rs659366 variant comprised enough studies for the meta-analysis, and other*UCP2*variants have not been further analyzed in this study. In this meta-analysis, we demonstrated that *UCP2* rs659366 variant showed no pooled association with DR in the type-2 DM patients (Table 3 and Fig. 3). *UCP2* rs659366 has been reported to be associated with type-2 DM [40]. The elevation of UCP2 expression could be induced by high glucose treatment in epithelial cell of human vein, and the A allele of *UCP2* rs659366 increases promoter activity as compared to the G allele, which can be exacerbated under hyperglycemic condition to exert a protective effect [41]. The negative association of UCP2 rs659366 variant with DR in this meta-analysis might indicated that *UCP2* gene variation may not be contributed to the development of DR. Nevertheless, it is of worth to note that, in the F-SNP database analyses, *UCP2* rs660339 is strongly linked with *UCP2* rs659366, and partially linked with *UCP2* 45-bp Ins/Del variant[26].One report showed that the haplotype of 3 different*UCP2* variants [Ins (45 bp Ins/Del), A (rs659366) and Ala (rs660339)] is associated with the decreased *UCP2* gene expression in human retina [42].This could be an independent risk factor for PDR in both type-1 and 2 DM patients[26]. Additional association studies are necessary in order to confirm the association of all 4 *UCP2* variants with DR in different ethnic groups.

We conducted the subgroup analyses on ethnicity in this meta-analysis. There was no significant association in different ethnic group, which could be due to the limited and sample sizes after stratification. Thus, the ethnicity-specific effects of these variants need to be determined with larger sample sizes in additional cohort studies.

There are several limitations in this meta-analysis. First, the number of studies for each UCPs variant was still limited. Second, the lack of original clinical information would be difficult to adjust the relevant variables, such as duration of diabetes, medications and other chronic diseases.

5. Conclusions

In summary, our meta-analysis revealed no significant pooled association of *UCP1* rs1800592 and *UCP2* rs659366 with DR in DM patients. Yet, A allele of UCP1 rs1800592 variant could be associated with the increased risk of PDR in type-2 DM patients, suggesting its potential as a genetic marker for PDR prediction in future population screening.

6. Declarations

Acknowledgments

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Author's contributions

Conceived and designed the study: X.L. and Z.W; Data acquisition: X.L. and Z.J.; Data analysis and Interpretation: X.L.; Drafting the manuscript: X.L.; Revising the manuscript critically: T.K.N, Z.W. and G.Z. Both authors made substantial contribution to this manuscript meeting authorship criteria, agreed to be accountable for all aspects of the work and have read and approved the final version.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

Not applicable.

Consent for publication

This work was consented for publication by the academic broad of Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1 to 5 xlsx are only available as a download in the Supplemental Files section.

Figures

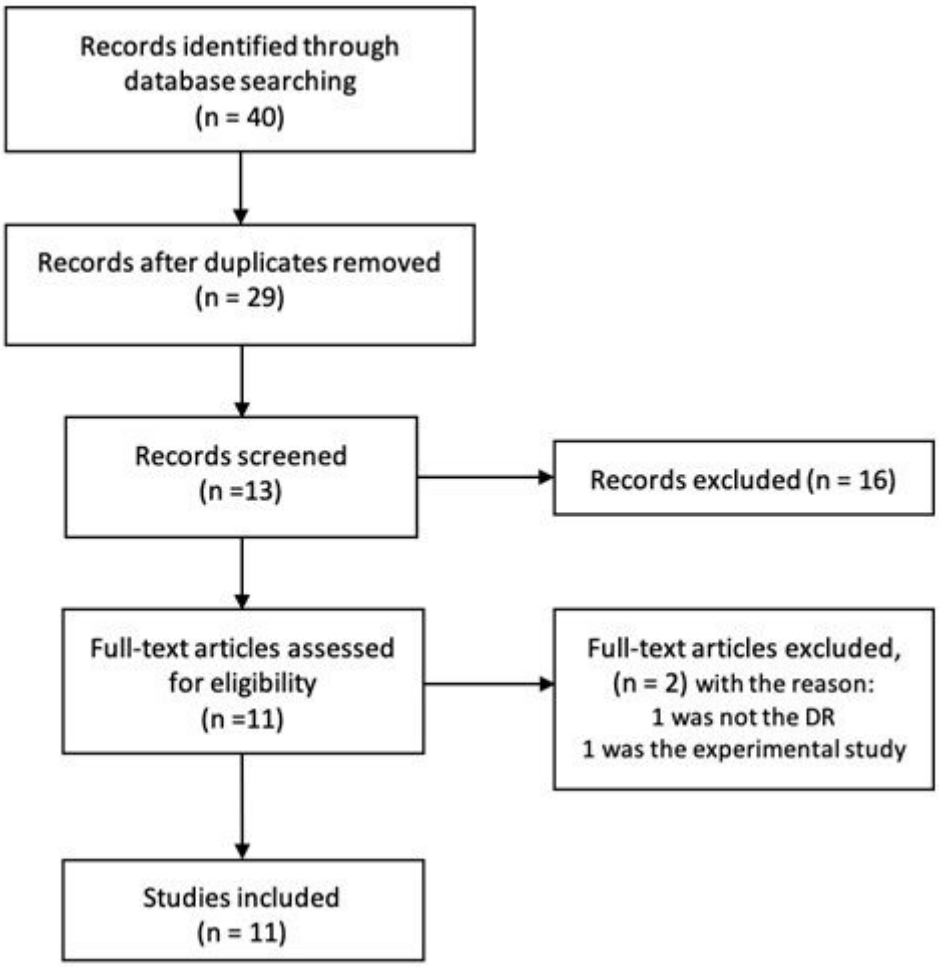

Figure 1

Flow chart of the inclusion and exclusion of the studies

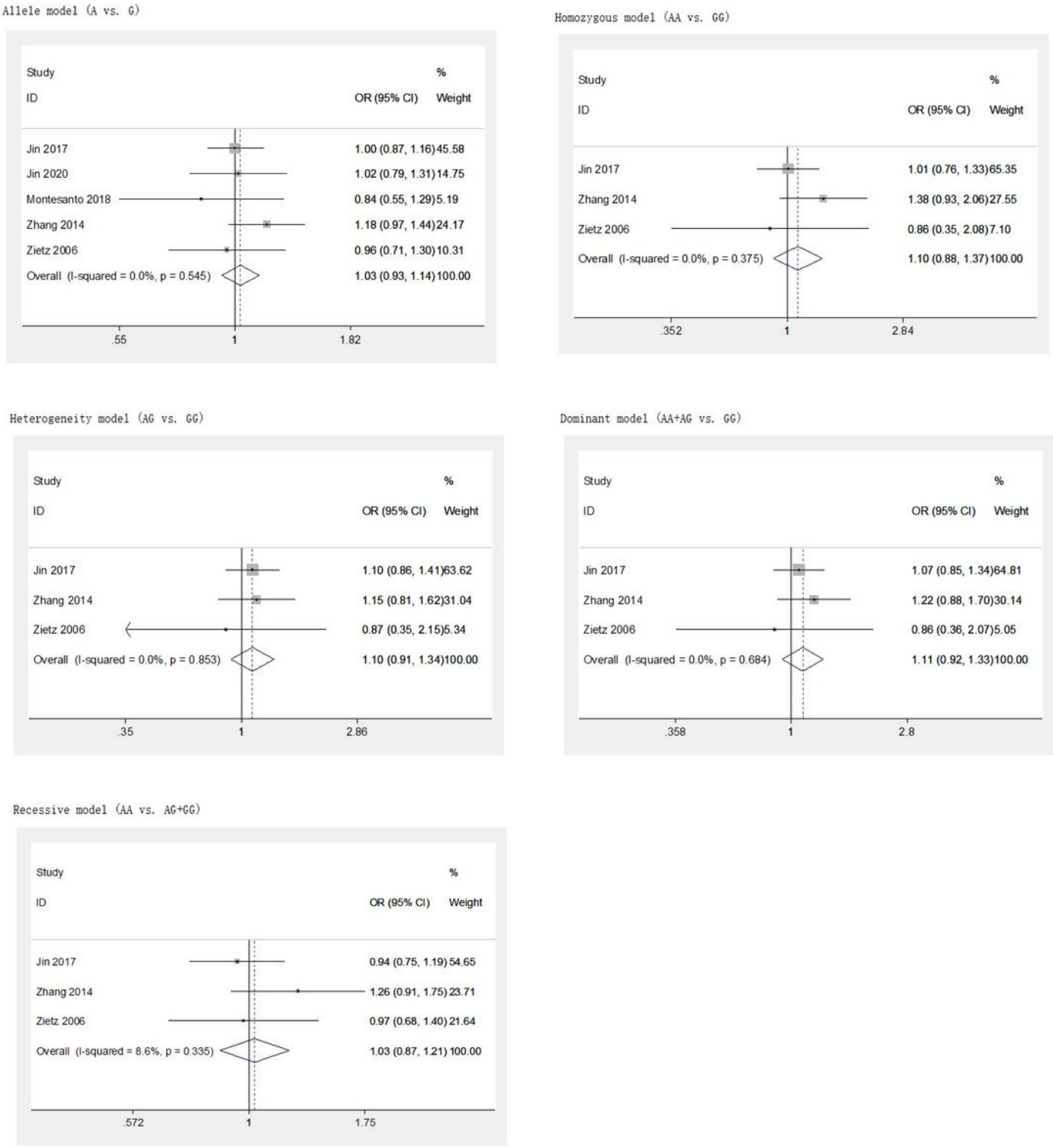

Figure 2

Forest plot for evaluating the association between the UCP1 rs1800592 and diabetic retinopathy in type 2 diabetic patients at five genetic models.

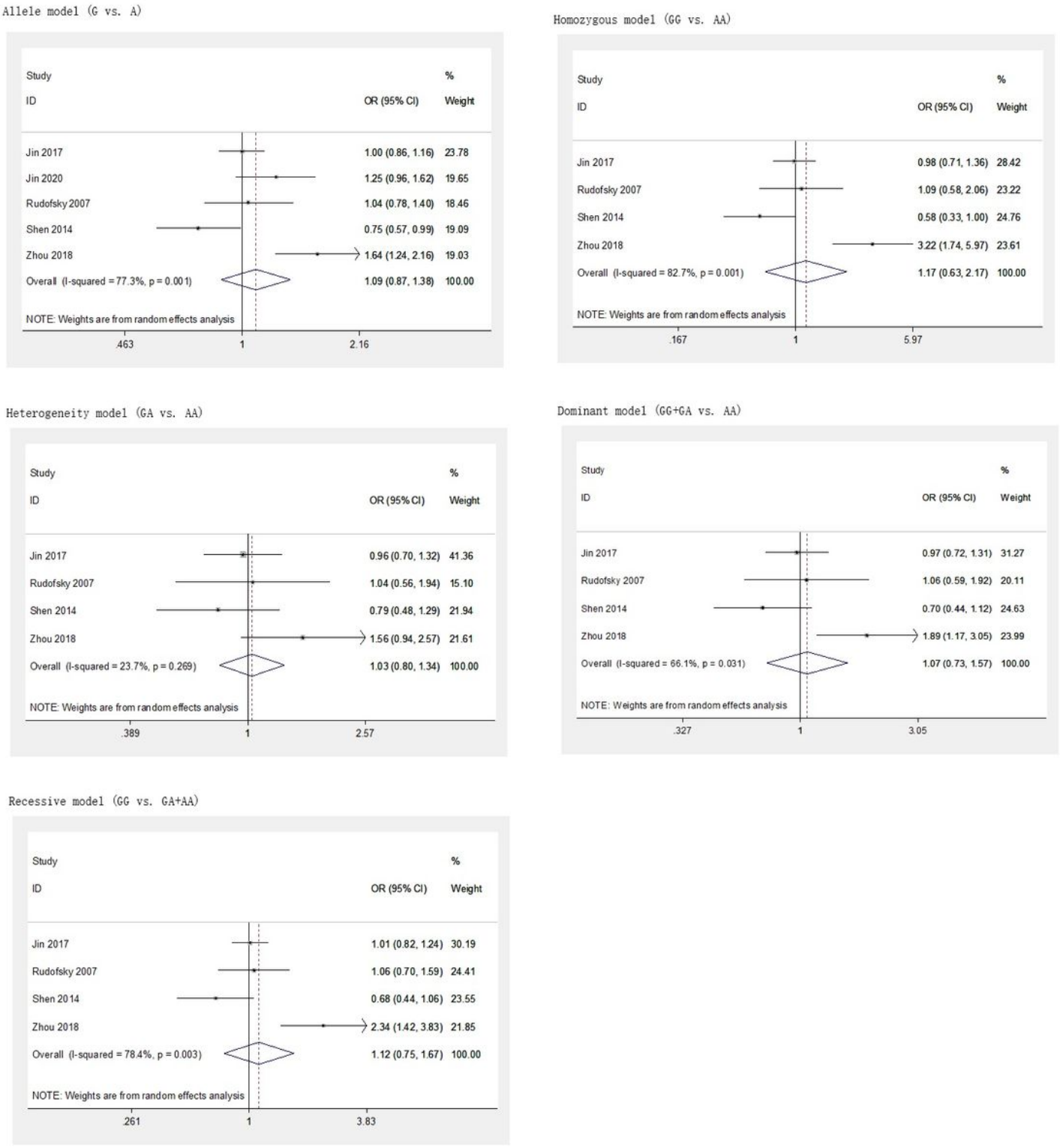

Figure 3

Forest plot for evaluating the association between the UCP2 rs659366 and diabetic retinopathy in type 2 diabetic patients at five genetic models.

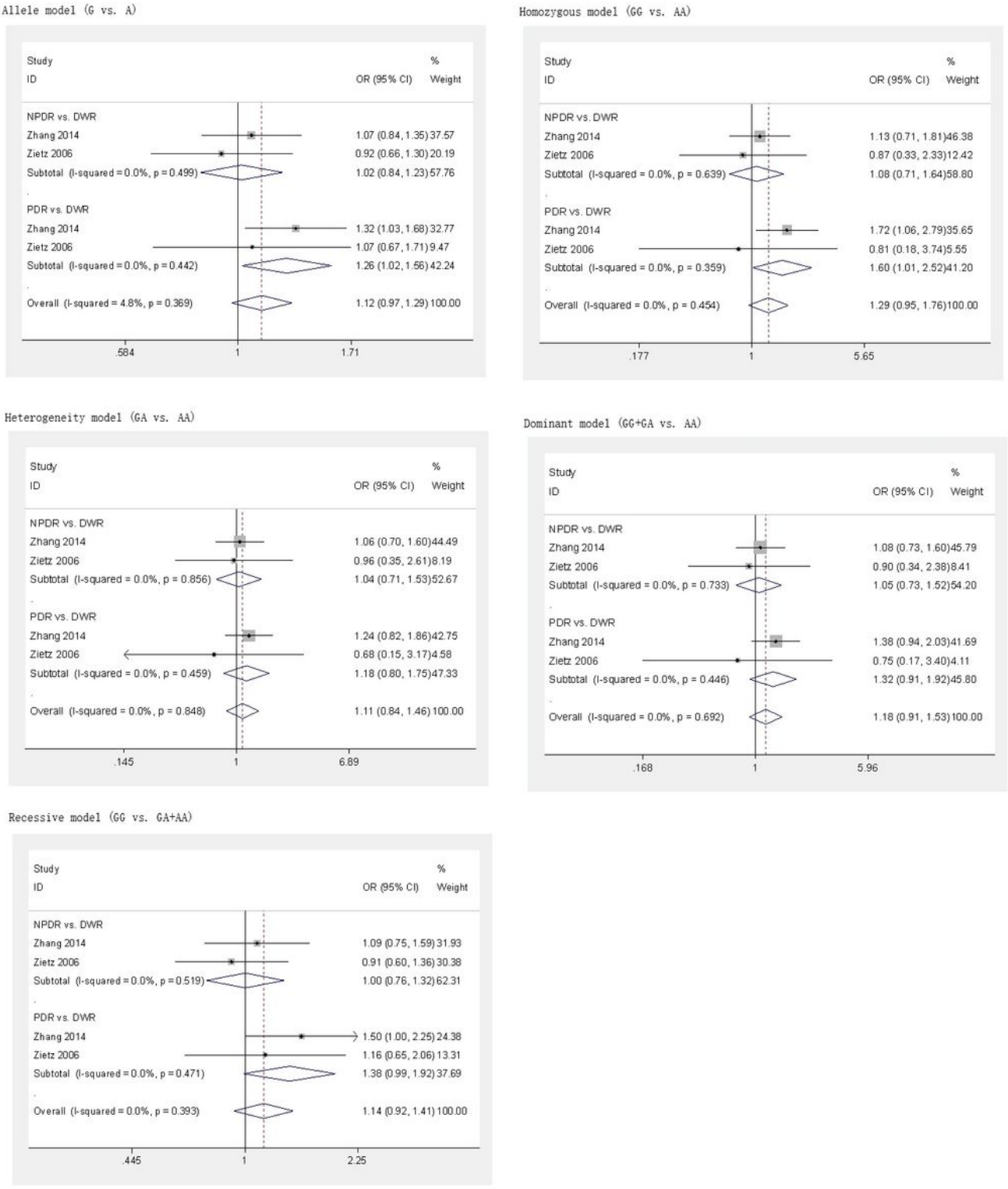

Figure 4

Forest plot for subgroup analysis between the UCP1 rs1800592 and diabetic retinopathy in type 2 diabetic patients by the stage of diabetic retinopathy at five genetic models.

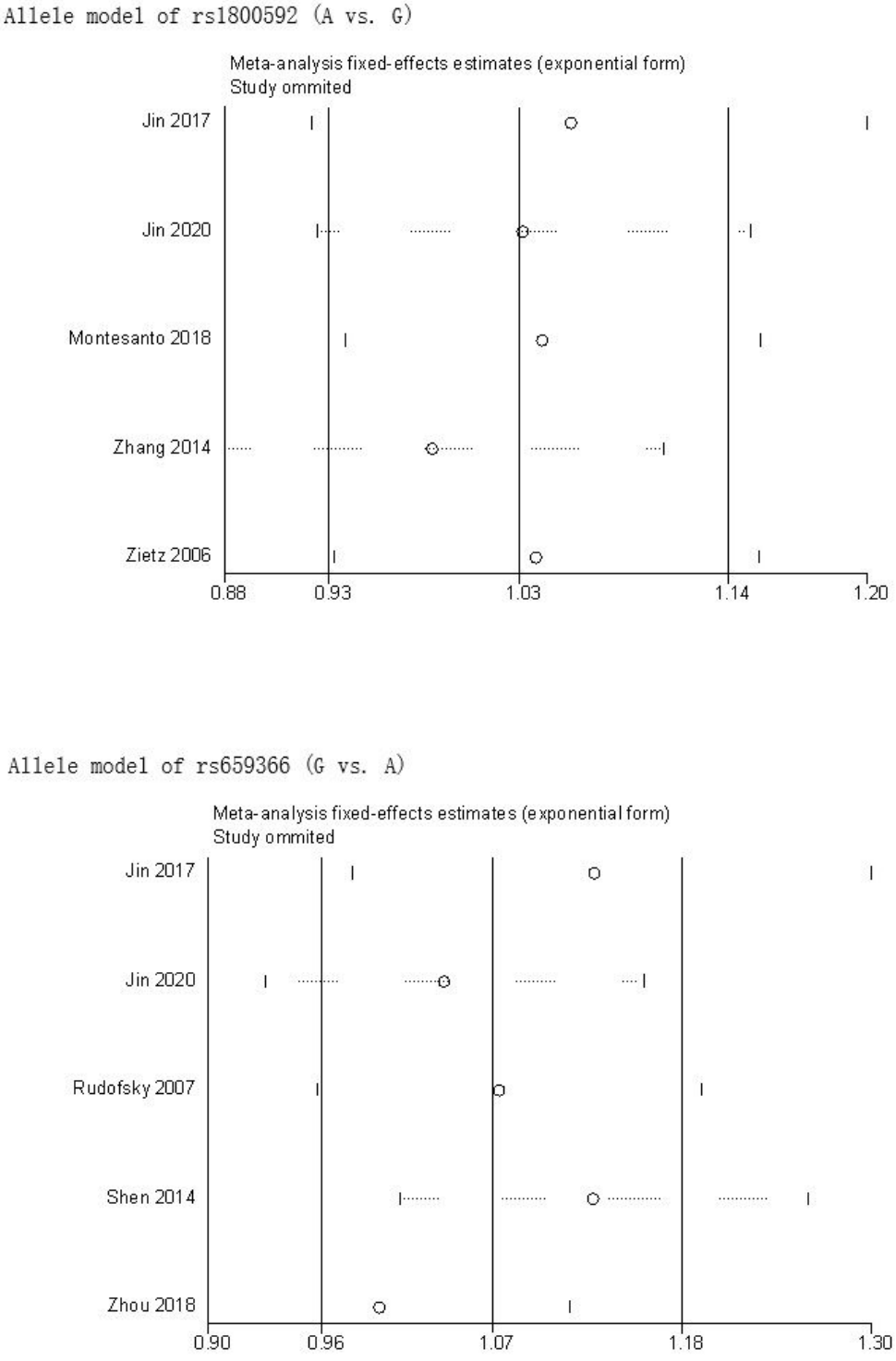

Figure 5

Sensitivity analysis of rs1800592 and rs659366 in allele model

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- Table4.xlsx
- Table5.xlsx
- Dataextraction.xlsx
- NEWCASTLEOTTAWAQUALITYASSESSMENTSCALEforCASECONTROL.pdf
- NOScasecontrol.xlsx
- PRISMAChecklist.doc
- Sensitivityanalysesforrs1800592.docx
- Sensitivityanalysesforrs659366.docx
- Supplementdocument.docx
- Supplementarytable.xlsx