Associations of Obesity with the Risk of Anal Fistula: A Mendelian Randomization Study

Zongxian Zhao  
Fuyang People's Hospital

Shiling Song  
Fuyang People's Hospital

Jun Zhang  
Fuyang People's Hospital

Keywords: obesity, anal fistula, mendelian randomization, risk, causal effect

 Posted Date: June 18th, 2024

 DOI: https://doi.org/10.21203/rs.3.rs-4455281/v1

 License: This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License

Additional Declarations: No competing interests reported.
Abstract

(1) Background: Obesity has become a worldwide pandemic, while anal fistulas represent a prevalent anorectal disorder that affects a significant number of individuals across the globe. However, the relationship between obesity and anal fistula remains unclear.

(2) Methods: We assess obesity comprehensively through multiple indicators including body mass index (BMI), body fat percentage (BFP), waist circumference (WC), and waist-hip ratio (WHR). In order to evaluate the causal effects of obesity on the risk of anal fistula, two-sample Mendelian randomization (MR) analysis was completed using five methods: inverse variance weighting (IVW), MR-Egger, weighted median, simple mode and weighted mode. IVW method was used as the main method.

(3) Results: IVW method found that there were positive effects of genetically determined BMI (OR: 1.001, 95%CI: 1.001-1.002, p = 0.022), BFP (OR: 1.001, 95%: 1.000-1.003, p = 0.035), WC (OR: 1.001, 95%CI: 1.000-1.003, p = 0.035), WHR (OR: 1.001, 95%CI: 1.000-1.003, p = 0.024) on the risk of anal fistula. The MR-Egger intercepts and MR-PRESSO method show no evidence for significant pleiotropy and heterogeneity.

(4) Conclusion: Our MR study supports a causal role of obesity in increasing the risk of anal fistula. We emphasize that obese patients with anal fistula underscore the urgent need for attention to weight control.

1. Introduction

Anal fistula is identified as ‘a pathological connection between the anal canal and perianal skin’, which can cause severe pain, perianal swelling, bleeding, and purulent discharge. Anal fistula is one of the prevalent ailments observed by anorectal surgeons. The incidence rate of anal fistula is 12.3 cases per 100,000 male population and 5.6 cases per 100,000 female population. At present, the preferred treatment methods of anal fistula are operation, including line hanging method, ligation of the intersphincteric fistula tract (Lift), anal fistula plug, fibrin glue injection, flap moving repair, etc. The pathogenesis of anal fistula is still unclear, which may be related to increased androgen, immune factors, intestinal flora disorders, smoking, special anatomical structure and improper care. In addition, it is technically challenging that the surgery of anal fistula has a recurrence rate of approximately 50% and with the risk of incontinence. The meta-analysis has revealed statistically significant increases in risk of anal fistula recurrence with prior anal surgery, high trans-sphincteric fistula, undetected internal opening, and presence of horseshoe extensions, seton placement surgery and multiple fistula tracts. Other studies have shown that smoking and obesity are associated with the recurrence of anal fistula after surgery. In conclusion, the cause of anal fistula is complex and lack of powerful etiology studies, which still needs further research.

Recently, obesity has emerged as a global pandemic, with over 600 million individuals classified as clinically obese. The prevalence of obesity is closely linked with cardiovascular and cerebrovascular diseases, neurodegenerative disorders, multiple cancers, endocrine and metabolic dysfunctions, exerting a substantial impact on the global population's health. Currently, a multitude of clinical indicators exist for the evaluation and diagnosis of obesity, encompassing body mass index (BMI), body fat percentage (BFP), waist circumference (WC), and waist-hip ratio (WHR) etc. These measures provide comprehensive assessments of obesity from diverse perspectives. Whether obesity is a risk factor for anal fistula is still controversial. D Wang and G Yang conducted a case-control study and illustrated that obesity was a significant risk factor for anal fistula. S Dong and B Chen completed an observational study and found that obesity (BMI) was not associated with the occurrence of anal fistula after perianal abscess surgery. However, obesity is subject to various confounding factors, including socioeconomic status, dietary patterns, and daily lifestyle habits. These confounding factors can exert an influence on the final analysis outcomes, or even yield diametrically opposing results. Moreover, given the limited scope of previous studies that evaluated obesity using only one or a few indicators, there is a dearth of comprehensive and direct research on the association between anal fistula and obesity.

The genetic epidemiological approach of mendelian randomization (MR) is used for rigorous and scientific causal inference. In MR studies, genetic variations are commonly employed as instrumental variables (IVs), typically represented by single nucleotide polymorphisms (SNPs). The distribution of genetic variation is stochastic and occurs prior to disease occurrence and potential confounding factors, enabling MR studies to avoid confounding biases and reverse causation. Therefore, this study represents the first attempt to investigate the causal relationship between obesity (expose) and anal fistula (outcome) directly using a Mendelian randomization (MR) approach by using multiple evaluation indicators (BMI, BFP, WC, WHR etc).

2. Materials and Methods

2.1 Study Design

The research study the causal impact of obesity on anal fistula through tow-sample MR analysis. The study design is depicted in Fig. 1. In MR analysis, three prerequisites must be fulfilled. 1. The instrumental variables (usually SNPs) must be strongly associated with the exposure factor (BMI, BFP, WC, WHR). 2. SNPs must be found to have no correlation with any potential confounding factors. 3. SNPs were only associated with the risk of outcome (anal fistula) though exposure factor (BMI, BFP, WC, WHR). The genetic data in this study came from public genome-wide association study (GWAS) data. And this study was constructed in accordance with the suggestions from the Strengthening the Reporting of Observational Studies in Epidemiology-Mendelian randomization (STROBE-MR) guidelines (Supplementary table 5 Checklist). All data we used were obtained from published studies that had received participant content and ethical approval. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations.

2.2 Data sources

The analysis was carried out using published statistics from publicly available genome-wide association studies (GWAS) data. The GWAS database collected extensive and comprehensive genetic information from participants, encompassing genome-wide genotyping, genetic variation, and a diverse array of health-
related outcomes\textsuperscript{20}. Multiple obesity datasets (BMI, BFP, WC, and WHR adjusted for BMI) and anal fistula datasets were included in this study (Table 1). The European Bioinformatics Institute (EBI) is one of the important molecular biological information websites in the world. Summary statistic data for BMI (532,396 subjects and 11,973,091 SNPs), WC (407,661 subjects and 10,783,687 SNPs), WHR adjusted for BMI (458,349 samples and 4,238,887 SNPs) were acquired from the EBI of European population. UK Biobank (UKB) is a large-scale biomedical database and research resource containing de-identified genetic, lifestyle and health information and biological samples from half a million UK participants. The dataset (ukb-a-264) comprises 331,117 European subjects and their corresponding BFP, with a total of 10,894,596 SNPs included from UKB. The outcomes datasets (ukb-b-6721) consisted of a total of 1,003 individuals with anal fistula were included as cases, while 462,007 individuals without anal fistula were selected as controls. The exposure and outcome samples all were from European population for the similarity of the genetic variant. In addition, the different consortiums of ukb-a-264 and ukb-b-6721 ensure the credibility of MR results.

### Table 1
Details of the exposure group and outcome group datasets.

<table>
<thead>
<tr>
<th>Datasets</th>
<th>Trait</th>
<th>Sample size</th>
<th>Number of variants</th>
<th>Year</th>
<th>Population</th>
<th>Consortium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure group datasets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ebi-a-GCST90029007</td>
<td>BMI</td>
<td>5,322,396</td>
<td>11,973,091</td>
<td>2018</td>
<td>European</td>
<td>EBI</td>
</tr>
<tr>
<td>ukb-a-264</td>
<td>BFP</td>
<td>331,117</td>
<td>10,894,596</td>
<td>2017</td>
<td>European</td>
<td>Neale Lab</td>
</tr>
<tr>
<td>ebi-a-GCST90014020</td>
<td>WC</td>
<td>407,661</td>
<td>10,783,687</td>
<td>2021</td>
<td>European</td>
<td>EBI</td>
</tr>
<tr>
<td>ebi-a-GCST90025996</td>
<td>WHR adjusted for BMI</td>
<td>458,349</td>
<td>4,238,887</td>
<td>2021</td>
<td>European</td>
<td>EBI</td>
</tr>
<tr>
<td>Outcome group datasets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ukb-b-6721</td>
<td>Anal fistula</td>
<td>463,010</td>
<td>9,851,867</td>
<td>2018</td>
<td>European</td>
<td>MRC-IEU</td>
</tr>
</tbody>
</table>

BMI: body mass index; BFP: body fat percentage; WC: waist circumference, WHR: waist-hip ratio; EBI: European Bioinformatics Institute. MRC-IEU: The MRC Integrative Epidemiology Unit.

#### 2.3 Selection and Validation of SNPs

Independent SNPs associated obesity (BMI, BFP, WC, WHR) were identified according to four criteria. First, since SNPs must be strongly associated with exposure factors, only SNPs that had reached a genome-wide significance level ($p < 5 \times 10^{-8}$) were selected. Second, in order to avoid the effects of potential bias, SNPs with $F$-statistics > 10 were selected, where $F$ can be calculated by following formula: $F = R^2 / (1 - R^2) \times (N - k - 1) / k$. In this formula, $N$ was the sample size and $k$ was the number of SNPs. Third, genetic variants in close genomic proximity tend to be co-inherited, a phenomenon known as linkage disequilibrium. The closer the SNPs are in terms of linkage disequilibrium, the more likely they are to have similar functions. To avoid the impact of linkage disequilibrium, SNPs with $R^2 > 0.001$ and width of linkage disequilibrium > 1 Mb. $R^2$ can be calculated by the formula: $R^2 = 2 \times \beta^2 \times EAF \times (1 - EAF)$, where $\beta$ was the estimated effect size of SNPs and EAF indicated effect allele frequency. Finally, the selected SNPs were uploaded on phenoScanner website (www.phenoscanner.medschl.cam.ac.uk) and the SNPs with potential confounders were removed.

#### 2.4 MR methods

We conducted five two-sample MR analysis methods, including inverse-variance weight (IVW), MR-Egger, weighted median, simple mode and weighted mode to evaluate the causal links between obesity and anal fistula. The results were reserved for three decimal places to present the accuracy. We used the IVM method as the main analysis to evaluate the effect estimates, which can combine the ratio estimates of all selected SNPs based on a fixed-effects model. In order to increase the credibility of the results of MR, MR pleiotropy residual sum and outlier (MR-PRESSO) analysis was completed for verifying the positive results.

#### 2.5 Sensitivity analysis

The horizontal pleiotropy must be evaluated, which is the basic prerequisite for the results of MR (the exclusion restriction assumption). And the intercept term from MR-Egger regression was used. The intercept $p > 0.05$ shows that there is no pleiotropy. Sensitivity analysis was performed to verify and adjust the validity and stability of the results, which included heterogeneity test (Cochrane's Q statistics, funnel plots, forest plots), and leave-one-out test. Finally, MR pleiotropy residual sum and outlier (MR-PRESSO) was performed to evaluate and calibrate horizontal pleiotropic outliers of IVW model. The MR-PRESSO global test was completed and $p > 0.05$ was considered no horizontal pleiotropic outliers. All statistical analysis were performed using the “TwoSampleMR” packages in R 4.2.1. The results were visualized by “ggplot2” packages in R and GraphPad Prism 8.

### 3. Result

#### 3.1 Selected IVs of MR

Each IV was deemed valid with an $F$-statistic > 10 and $p < 5 \times 10^{-8}$. The flow chart illustrating the process of SNP selection is depicted in Fig. 1. Finally, a total of 161 BMI related SNPs (Supplementary table 1), 92 BFP related SNPs (Supplementary table 2), 104 WC related SNP2 (Supplementary table 3) and 78 WHR associated SNPs (Supplementary table 4) were identified, respectively.

#### 3.2 Results of MR
In the primary MR analysis employing the IVM method, obesity demonstrated a positively associated with anal fistula incidence (Table 2). In detail, for each 1-SD increase in BMI, the incidence of anal fistula increased by 1.001 times (95% CI, 1.001–1.002, \( p < 0.05 \)). Likewise, for 1-SD increase in BFP, the incidence of anal fistula increased by 1.001 times (95% CI, 1.000–1.003, \( p < 0.05 \)). Furthermore, for a one-unit increase in waist circumference (cm), the incidence of anal fistula increased by 1.001 times (95% CI, 1.000–1.003, \( p < 0.05 \)). Similarly, for a one-percent increase in WHR adjust for BMI, incidence of anal fistula increased by 1.001 times (95% CI, 1.000–1.003, \( p < 0.05 \)). In total, the MR results indicated that obesity was significantly associated with anal fistula risk, respectively (Fig. 2A). Moreover, the causal estimates obtained through the MR-PRESSO method remained consistent both before and after outlier correction, underscoring the reliability of the MR analysis results (Table 3, Fig. 2B).

### Table 2

<table>
<thead>
<tr>
<th>Exposure</th>
<th>BMI</th>
<th>BFP</th>
<th>WC</th>
<th>WHR adjusted for BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>Beta (95%CI)</td>
<td></td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>MR-Egger</td>
<td>1.001 (0.999–1.004)</td>
<td>0.001 (0.999–1.002)</td>
<td>0.001 (0.995–1.002)</td>
<td>0.001 (0.999–1.007)</td>
</tr>
<tr>
<td>Weighted median</td>
<td>1.001 (0.999–1.002)</td>
<td>0.001 (1.001–1.002)</td>
<td>0.001 (1.001–1.004)</td>
<td>0.001 (1.001–1.003)</td>
</tr>
<tr>
<td>IVW</td>
<td>1.001 (0.997–1.004)</td>
<td>0.000 (1.000–1.002)</td>
<td>0.000 (1.000–1.003)</td>
<td>0.000 (1.000–1.003)</td>
</tr>
<tr>
<td>Simple mode</td>
<td>1.000 (0.999–1.003)</td>
<td>0.000 (1.001–1.002)</td>
<td>0.000 (1.001–1.004)</td>
<td>0.000 (1.001–1.003)</td>
</tr>
<tr>
<td>Weighted mode</td>
<td>1.001 (0.999–1.003)</td>
<td>0.000 (1.000–1.002)</td>
<td>0.000 (1.000–1.003)</td>
<td>0.000 (1.000–1.003)</td>
</tr>
</tbody>
</table>


### Table 3

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Raw Estimates</th>
<th>Outlier Corrected Estimates</th>
<th>Distortion Test</th>
<th>Causal Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nSNPs</td>
<td>Beta (95%CI)</td>
<td>P-Value</td>
<td>nSNPs</td>
</tr>
<tr>
<td>BMI</td>
<td>161</td>
<td>0.001 (1.001-1.002)</td>
<td>0.019</td>
<td>NA</td>
</tr>
<tr>
<td>BFP</td>
<td>92</td>
<td>0.002 (1.002-1.003)</td>
<td>0.006</td>
<td>NA</td>
</tr>
<tr>
<td>WC</td>
<td>104</td>
<td>0.001 (1.001-1.003)</td>
<td>0.038</td>
<td>NA</td>
</tr>
<tr>
<td>WHR adjusted for BMI</td>
<td>78</td>
<td>0.001 (1.001-1.003)</td>
<td>0.027</td>
<td>NA</td>
</tr>
</tbody>
</table>

MR-PRESSO: mendelian randomization pleiotropy residual sum and outlier, nSNPs: number of single nucleotide polymorphisms, BMI: body mass index, BFP: body fat percentage, WC: waist circumference, WHR: waist-hip ratio, OR: odds ratio.

### 3.4 Sensitivity Analysis

We completed the pleiotropy test for the MR analysis results by the MR-Egger intercepts and MR-PRESSO methods. As shown in Table 4, the MR-Egger intercepts show no evidence for significant directional pleiotropy for the MR results (\( p > 0.05 \)). Furthermore, MR-PRESSO global test showed that there was no pleiotropy (\( p > 0.05 \)). According to the heterogeneity test, both the MR-Egger and inverse variance weighted methods indicated no significant heterogeneity in the MR results. Additionally, scatter plots, forest plots, and funnel plots for the analysis are displayed in Supplementary Figs. 1A-D, 2A-D, and 3A-D, respectively. The leave-one-out analysis demonstrated that no individual SNP significantly influenced the overall causal estimate (Supplementary Figs. 4A-D). Furthermore, in the MR analysis between WC and anal fistula, it’s worth noting that discrepancies were observed between the results obtained from the simple mode method and the other four MR methods (Supplementary Fig. 1C), highlighting the need for further in-depth investigation.
4. Discussion

In this study, we conducted an MR analysis to investigate, for the first time, the potential causal links between obesity and the risk of anal fistula. Our results demonstrated that obesity has a positive causal effect on the risk of anal fistula in the European population. Sensitivity analysis confirmed that our findings were robust, showing no evidence of heterogeneity or pleiotropy, indicating an unconfounded effect of obesity on the risk of anal fistula. Consequently, this study underscores the urgent need for global attention to obesity control in patients with anal fistula.

As economies rapidly develop, dietary changes, and insufficient exercise have led to a global epidemic of obesity, emerging as a significant health risk factor. Previous observational studies have shown that overweight and obese individuals are more prone to anal fistulas than those of normal weight \( \text{OR}_{\text{obesity}} = 1.35, 95\% \text{CI: } 1.00-1.82, \ p = 0.047 \) \( \text{OR}_{\text{obesity}} = 3.44, 95\% \text{ CI: } 2.26-5.26, \ p < 0.001 \) \cite{31,32}. As BMI levels increase, so does the risk of suffering from anal fistula. Likewise, a retrospective case-control study showed that obesity (BMI > 25.0 kg/m\(^2\)) could increase an individual's risk of developing anal fistula \cite{16}. Furthermore, a retrospective study revealed that obese individuals (BMI > 30 kg/m\(^2\)) with anal fistula were significantly more prone to recurrence compared to non-obese patients (28% vs. 14%, \( p < 0.01 \)). And the results suggested a potential association between obesity and anal fistula \cite{22}. The aforementioned research aligns with our findings, reinforcing the reliability and validity of our results. However, most previous studies were retrospective, case-control, or observational, and could not account for the effects of unmeasured confounding factors inherent in observational studies. It is widely known that obesity is influenced by various confounding factors, including socioeconomic status, dietary habits, and daily lifestyle. Some studies suggest that these confounding factors (regular ingestion of spicy and greasy foodstuffs, diabetes, lacking of sports activities, prolonged sitting on the toilet etc) are high-risk factors for the onset of anal fistula \cite{16}. This study employed two-sample MR analysis to investigate the causal impact of obesity on anal fistula. And the results of MR analysis are relatively immune to above confounding factors because of the random assignment of alleles during meiosis \cite{18,22}. Furthermore, this study is the first to investigate the potential causal associations between obesity and fistula anal using MR methods. This approach significantly contributes to the expansion of existing literature concerning the causal relationship between obesity and the incidence of anal fistula.

This study analyzed the causal relationship between obesity and anal fistula from a genetic perspective. Several possible mechanisms have been proposed to explain the effect of obesity on anal fistula. Overweight and obesity are considered to be linked with acute infections, including respiratory, skin, and urinary tract infections, particularly those that are posttraumatic, ICU-acquired, pregnancy-related, and postpartum \cite{24-26}. And anal fistula is an infectious disease, usually present with a recurrent abscess \cite{6,27}. In addition, obesity has been shown to have substantial effects on the immune system, likely due to the structural and functional similarities between immune cells and adipocytes, both of which can produce mediators such as cytokines, chemokines, and adipokines \cite{28,29}. In obesity, visceral adipose tissue produces an excess of cytokines, including tumor necrosis factor \( \alpha \) (TNF\( \alpha \)) and interleukins 6 and 1\( \beta \) (IL-6 and IL-1\( \beta \)), which can impair the immune response during infectious stimuli \cite{30}. Furthermore, overweight or obesity can lead to hyperleptinemia and immune imbalance, resulting in increased production of IL-6, IL-12, and TNF\( \alpha \). This promotes neutrophil recruitment, macrophage polarization, and a heightened risk of infectious diseases \cite{31,32}. Besides, a high BMI has been found to be inversely related to the number of gamma delta T cells, which play a crucial role in wound repair. Their dysfunction can lead to delays in wound healing and an increased risk of infectious complications \cite{33}. One the other hand, obesity is associated with glucose and lipid metabolic abnormalities \cite{34}. Hyperglycemia and hyperlipidemia can increase the risk of perianal infectious diseases, delay wound healing, and ultimately lead to the increasing risk of perianal abscesses and anal fistulas \cite{35}.

This study has several limitations. Firstly, not all MR analysis methods yielded valid causal relationships. However, most methods produced similar results. Since all instrumental variables (IVs) passed the heterogeneity and pleiotropy tests, we selected the results of the inverse-variance weighted (IVW) method, which had the highest test efficacy, as the primary reference. Secondly, in the MR analysis between waist circumference (WC) and anal fistula, the results from the simple model method and the IVW method were not identical, indicating possible horizontal pleiotropy among the IVs used. However, the MR-PRESSO test did not detect any effect of horizontal pleiotropy on the results obtained by the IVW method. Thirdly, the datasets used in this study included only patients of European ancestry, limiting the generalizability of the findings to other demographics, such as patients of African or Asian ancestry. Future research is needed to validate the applicability of these results to other populations and ethnicities.
Conclusion

In summary, we performed a two-sample MR design to provide genetic evidence supporting causal associations between obesity (BMI, BFP, WC, WHR) and anal fistula. And our results illustrated that obesity may be associated with risk of anal fistula. Those findings underscored the urgent need for global attention towards obesity control in the prevention of anal fistula.

Declarations

Funding

This work was supported by the Health Commission of Fuyang City, Anhui, China (No. FY2021-18 to Zhao ZX), Health Commission of Anhui Province (No. AHWJ2023BAa20164 to Zhao ZX).

Institutional Review Board Statement

This study used summary data published by multiple GWAS.

Informed Consent Statement

Patient informed consents were obtained by corresponding studies.

Data Availability Statement

Code and extracted data are available on request for Zhao ZX. All the data analyzed can be found in already published studies, and no new original data were generated or analyzed in this study.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contribution

Z.Z. and S.S. wrote the main manuscript text and J.Z. completed the study design. All authors reviewed the manuscript.

References


**Figures**
Figure 1

The flowchart of the study design and selected single nucleotide polymorphisms (SNPs). MR: mendelian randomization.

A MR results

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>BFP</td>
<td></td>
<td>0.023</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td>0.035</td>
</tr>
<tr>
<td>WHR adjust for BMI</td>
<td></td>
<td>0.024</td>
</tr>
</tbody>
</table>

B MR-PRESSO result

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>BFP</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td>0.038</td>
</tr>
<tr>
<td>WHR adjust for BMI</td>
<td></td>
<td>0.027</td>
</tr>
</tbody>
</table>

Figure 2

Forest plots for the mendelian randomization (MR) analysis on the causal effect of obesity on anal fistula risk. (A) MR analysis from the inverse variance weighted (IVW) method of obesity with anal fistula risk. (B) Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) method for testing. OR: odd ratio; CI: confidence interval; BMI: body mass index; BFP: body fat percentage; WC: waist circumference; WHR: waist-hip ratio.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• SupplementaryFigureS1.tif
• SupplementaryFigureS2.tif
• SupplementaryFigureS3.tif
• SupplementaryFigureS4.tif
• SupplementaryTable1.csv
• SupplementaryTable2.csv
• SupplementaryTable3.csv
• SupplementaryTable4.csv
• SupplementaryTable5Checklist.doc