1 **Causal Associations of Sjögren's Syndrome with Sex**

2 **Hormones: Case-control and Mendelian Randomization Study**

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21 **Abstract**

22 **Objective:** To identify the association between sex hormones and

23 Sjögren's Syndrome (SS).

24 **Methods:** A case-control study was conducted from January 2018 to

25 January 2024 at Nanjing Drum Tower Hospital to investigate the

26 relationship between sex hormones and SS. Two-sample Mendelian

27 randomization (MR) was then performed to identify the causal

28 association by using the public genome-wide association study (GWAS)

29 data from the UK biobank and the FinnGen consortium.

30 **Results:** In the case-control study, a total of 93 cases diagnosed with SS

31 were compared to 90 SS-like non-SS controls in the population of women

32 after natural postmenopausal age. A strong direct relationship was

33 observed between hypo-estradiol (hypoE2) and SS (aOR, 2.195; 95%CI:

34 1.156-4.165; *p* = 0.016). Regarding the adjusted estimates, each 1 unit

35 increase in E2 level was related to a 1.6% decrease (1-aOR) in the odds

36 of SS (aOR, 0.984; 95% CI, 0.971-0.997; *p* = 0.018). However, MR

37 analysis revealed no significant associations were observed for the

38 effects of E2 on SS susceptibility. In turn, a heightened risk of SS was

39 associated with decreased E2 levels in females, as indicated by

40 inverse-variance weighted (IVW) (OR, 0.954; 95% CI, 0.917-0.992; *p* = 41 0.019).

42 **Conclusion:** A strong direct relationship was observed between hypoE2

43 and SS in the population of women after natural postmenopausal age.

44 However, this relationship may be due to the direct effect of SS on the

45 genetic variation at low E2 levels.

46 **Keywords:** Sex hormones; Sjögren's Syndrome; case-control study;

47 mendelian randomization; genome-wide association study

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65 **Introduction**

66 Sjögren's Syndrome (SS) is a systemic autoimmune disorder

67 characterized by the lymphocyte infiltration in exocrine glands [ 1, 2].

68 Although the exact pathogenesis of SS remains unknown, 69 epidemiological features of SS such as a higher prevalence in women, 70 onset usually around menopause, and often triggered by significant

71 stressful events, suggest an important role of hormones in the

72 development of SS [3-6].

73 Previous studies have shown that reduced production of ovarian

74 estrogens (E) and adrenal pro-hormone dehydroepiand rosterone (DHEA)

75 are closely related to the onset of SS, especially in postmenopausal

76 women [6-10]. However, the findings of different studies are controversial, 77 and the specific association between E2 and SS remains unclear. Based

78 on the previous findings, we conducted a matched case-control study, 79 and hypothesized that there is an association between low E2 levels and

80 an increased risk of SS. To validate the results, the Mendelian

81 randomization (MR) method was further used to analyze the exact causal

82 relationship between sex hormones (testosterone, E2, and sex

83 hormone-binding globulin (SHBG)) and SS.

84

85 **Materials and methods**

86 A matched case-control study was performed. The study protocol

87 was approved by the Ethics Committee of Nanjing Drum Tower Hospital

88 (No. 2022-529-04). Our study was conducted in accordance with the

89 Strengthening the Reporting of Observational Studies in Epidemiology

90 (STROBE) guidelines [ 11]. The checklist is presented in Supplementary

91 Table S1.

92 **Selection of Cases and Controls**

93 A total of 189 cases were randomly selected from Nanjing Drum

94 Tower Hospital between January 2018 and January 2024. Of these, 6

95 cases with missing data on key variables were excluded, leaving 183

96 valid samples for subsequent analysis. All cases consisted of

97 postmenopausal women. The case group (n = 93) consisted of patients

98 newly diagnosed with SS who visited our Rheumatology Outpatient Clinic, 99 while the control group (n = 90) included sex-, age-, and race-matched

100 patients who visited our department with SS-like symptoms but were not

101 diagnosed with SS. In both groups, blood samples were collected during

102 the patients' first visit to our department, before any treatment was 103 administered. The timing of menopause in all cases fell within the natural

104 age range for menopause based on a large population-based

105 epidemiological survey [ 12]. The classification of SS was based on the

106 2002 American-European Consensus Group (AECG) criteria or 2016

107 American College of Rheumatology/European League Against

108 Rheumatism classification criteria for primary Sjögren's syndrome, with

109 all cases categorized under ICD-10 M35.0 [13, 14]. Individuals with other

110 autoimmune disorders, premature ovarian failure, estrogen replacement

111 therapy, polycystic ovarian syndrome, and hysterectomy without bilateral

112 oophorectomy were all excluded from the controls/cases.

113 **Measurement of Exposure and Outcomes**

114 For the exposure, laboratory values including neutrophilic

115 granulocyte (NE), hemoglobin (HB), platelet (PLT), complement3 (C3)

116 and complement4 (C4), immunoglobulin G (IgG), fasting blood glucose

117 (FBG), thyroxine levels, estradiol (E2), testosterone, SHBG, follicle

118 stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL)

119 were all recorded. The cut-off value of E2 levels < 89.76 poml/L was

120 classified as hypo-estradiol (hypoE2) by ROC analysis. Age, menopausal

121 age, body mass index (BMI), smoking, tea/coffee addiction, medical

122 history of hypertension and diabetes and thyroxine levels were

123 considered possible confounding factors. All samples for sex hormone

124 were obtained from the patients' early morning fasting blood. For the

125 outcome, the main clinical manifestations of the patients in the case

126 group were collected on the basis of EULAR Sjögren's syndrome disease

127 activity index (ESSDAI) score by means of questionnaires. All of the

128 laboratory results were tested by the Clinical Laboratory Center of

129 Nanjing Drum Tower Hospital Affiliated to Medical College of Nanjing

130 University according to the instructions of the corresponding kit.

131 **Mendelian randomization analyses**

132 Supplementary Figure S1 presents a brief summary of the

133 bidirectional MR design that investigated the relationship between sex

134 hormones and SS. Two MR analyses were conducted using summary

135 statistics from a genome-wide association study (GWAS) to explore this

136 relationship. In the forward MR analyses, sex hormones were considered

137 as the exposure and SS as the outcome, whereas in the reverse MR

138 analyses, SS was considered as the exposure and sex hormones as the

139 outcome. MR analyses use publicly available summary statistics and do

140 not require ethical approval. This part followed the Strengthening the

141 Reporting of Observational Studies in Epidemiology–Mendelian

142 Randomization reporting guidelines (Supplementary Table S2), available

143 on the *JAMA Network* at

144 <https://jamanetwork.com/journals/jama/fullarticle/2785494)> [ 15].

145 **Instrumental Variable Selection for MR Analyses**

146 For each exposure factor, SNPs were filtered based on the three

147 primary assumptions of MR. Initially, SNPs were included if they reached

148 genome-wide significance (*p* < 5 × 10-8). Due to the insufficient number of

149 SNPs for estradiol analysis, more lenient thresholds were opted for (*p* < 5

150 × 10-7). All the *F*-values are greater than 10 (Supplementary Table S3).

151 Subsequently, only variants with the lowest p-values were retained as

152 independent instruments, considering linkage disequilibrium (LD) with an

153 r2 threshold (r2 > 0.1 in the European 1000 Genome Reference Panel).

154 Finally, we calculated F-statistics to assess the strength of the

155 instrumental variables. We recommend a threshold of F-statistics >10 for

156 MR analysis.

157 **Sources of Data and Selection of Instrumental Variables for Sex**

158 **Hormones**

159 Sex hormones, including testosterone, E2, and SHBG, were

160 obtained from the summary statistics. The summary statistics for female

161 (N = 230,454) and male (N = 194,553) testosterone were obtained from a

162 previous extensive GWAS that used genotype and phenotype data from

163 the UK Biobank [ 12]. Estradiol summary statistics were sourced from

164 another GWAS that used data from the UK Biobank [ 16], encompassing

165 163,985 samples in women and 147,690 in men. Ruth et al. provided

166 data on SHBG, which involved 214,989 samples in women and 185,221

167 in men [ 17]. Supplementary Table S4 contains comprehensive details of

168 the summary data for all the GWAS.

169 **Data Sources and Selection of Instrumental Variables for SS**

170 Summary-level statistics for SS were obtained from the FinnGen

171 consortium in 2023, which included 392,423 samples (2495 cases and

172 389,928 controls). The complete GWAS dataset for SS is publicly

173 available through the FinnGen research project

174 (<https://www.finngen.fi/en>), which aims to identify novel therapeutic

175 targets by assessing genotype-phenotype correlations. The diagnosis of

176 Sjögren's syndrome, unspecified, was established using International

177 Classification of Diseases codes, specifically ICD-10 M35.0.

178 **Statistical analysis**

179 In the case-control study, normal data were presented as the means

180 ± standard deviations, skewed data were presented as the medians

181 (interquartile ranges), and categorical data were presented as the

182 absolute values. The independent-samples T test and the Mann-Whitney

183 U test were used to compare the continuous parameters between groups, 184 while the chi-square test was used to compare the categorical data. The

185 logistic regression analysis was conducted to predict the relationship

186 between sex hormones levels and the risk of SS. *P* < 0.05 was

187 considered significant in the two-tailed tests. Data analysis was

188 conducted by using *SPSS* software (Version 26.0). In the MR study, the

189 inverse-variance weighted (IVW) method was uesed to explore potential

190 bidirectional causal links between sex hormones and SS, with additional

191 checks using MR-Egger, Weighted Median, and MR-Pleiotropy Residual

192 Sum, and Outlier (MR-PRESSO) to address potential biases like invalid

193 instruments and pleiotropy. A *p*-value exceeding 0.05 indicates the

194 absence of horizontal pleiotropy [ 18]. Heterogeneity was assessed using

195 Cochrane's Q test. We applied the IVW random effects method [ 19] to

196 estimate the main effect. MR-PRESSO identified outliers influencing

197 heterogeneity [20], which were subsequently excluded. A meta-analysis

198 synthesized MR results across sexes for sex-stratified hormone levels

199 and SS using *R* software (Version 4.30), with the Two-Sample MR and

200 Meta packages utilized for the respective analyses.

201 **Results**

202 **The relationship between Sex Hormones and SS**

203 The characteristics of the 93 patients included in the case group are

204 shown in Table 1.

205 The distributions of all the study variables in the cases and controls

206 are presented in Table 2. SS cases showed significantly lower mean total

207 E2 levels than controls (84.41 pmol/L vs 91.57 pmol/L). Similarly, the rate

208 of hypoE2 was significantly higher in the SS group (62.37% vs 46.67%).

209 The crude odds ratio (cOR) and adjusted odds ratio (aOR) between SS

210 and both E2 levels and hypoE2 are shown in Table 3. According to the

211 results of univariate logistic regression analyses, each 1 unit increase in

212 E2 levels corresponded to a 1.2% decrease (1-cOR) in the risk of SS

213 (cOR, 0.988; 95% CI, 0.975-1.000; *p* = 0.047) and hypoE2 seemed to act

214 as a risk factor against SS (cOR, 1.894; 95% CI, 1.050-3.415; *p* = 0.034).

215 Regarding the adjusted estimates, each 1 unit increase in E2 level was

216 related to a 1.6% decrease (1-aOR) in the odds of SS (aOR, 0.984; 95%

217 CI, 0.971-0.997; *p* = 0.018) . Consistent with the findings, a strong direct

218 relationship between hypoE2 and SS was observed in the adjusted

219 analysis, with an aOR value of 2.195 (95%CI, 1.156-4.165; *p* = 0.016).

220 However, no significant relationship was found for other hormone levels

221 at the onset of SS (*p* > 0.05), which suggests a weak association

222 between SS and other hormone levels.

223 **The Casual Effect of Sex Hormones on SS**

224 The impact of each hormone on susceptibility to SS was examined

225 individually. However, no significant association was observed, as shown

226 in Figure 1. To further validate these findings, scatter plots, the

227 leave-one-out test, funnel plot, and forest plot were employed, offering

228 additional confirmation of the results (see Supplementary Figures

229 S2-S7).

230 **The Causal Influence of SS on Sex Hormones**

231 The Figure 2 showed that a heightened risk of SS was associated

232 with decreased E2 levels in females, as indicated by the IVW method

233 (OR, 0.954; 95% CI, 0.917-0.992; *p* = 0.019) . However, this effect was

234 not observed in males. In relation to testosterone levels, SS was

235 identified as a risk factor for testosterone in both sexes (OR, 0.993; 95%

236 CI, 0.987-1.000; *p* = 0.047). However, no significant difference was

237 observed in the sex-stratified testosterone levels. Additional confirmation

238 of the results was obtained through the use of scatter plots, the

239 leave-one-out test, funnel plot, and forest plot (Supplementary Figures

240 S8-S13).

241 **Assessment of Heterogeneity, Pleiotropy, and Sensitivity Analysis**

242 In addition, we conducted sensitivity analyses to support a causal

243 relationship between sex hormones and SS. The Cochran's Q test did not

244 show any detectable heterogeneity of effects across the instrumental

245 variables (Table 4). Furthermore, the *F* statistics for all instrumental

246 variables exceeded 10, indicating the absence of weaknesses in the

247 selected instruments. No signs of horizontal pleiotropy were detected as

248 the intercept of MR-Egger did not significantly deviate from zero.

249 Additionally, the MR-PRESSO analysis did not identify any potential

250 instrumental outliers. The leave-one-out results suggest that the causal

251 effect was not solely influenced by a single instrumental variable.

252

253 **Discussion**

254 SS is an autoimmune disease that primarily affects the exocrine

255 glands, particularly the salivary and lacrimal glands. Follicular cells of the

256 external glands are damaged and destroyed in SS, resulting in reduced

257 secretion of saliva and tear fluid [21]. A number of clinical studies and

258 animal studies have suggested that decreased level of sex hormones

259 may increase the risk of SS [22-25]. In this part of case-control study, we

260 found a strong direct relationship between low E2 exposure and SS in

261 menopausal women which is consistent with the previous studies.

262 Furthermore, each unit increase in E2 was associated with a 1.4%

263 reduction in the odds of SS as shown in our results.

264 Estrogens exert their influence on specific intracellular estrogen

265 receptor subtypes (ERs) found within every cell of the immune system

266 [26-28], especially in modulating the development and function of

267 lymphocytes [29, 30]. Estrogens may facilitate protective effects that

268 counteract harmful changes following inflammatory responses. For

269 example, human lymphocyte cultures have shown a reduction in

270 CD4+/CD8+ T-cell-subset ratios after estrogen treatment [31]. Estrogen

271 can directly affect the subsets of B lymphocytes subsets. Studies in

272 animals have shown that estrogens can increase the population of bone

273 marrow progenitor B cells by protecting them from apoptosis [29, 32] and

274 by improving the survival of B cells [29]. It has been suggested that

275 estrogens have a protective effect on secretory glandular acinar cells by

276 shielding them from apoptosis. Additionally, testosterone can be

277 converted to dihydrotestosterone (DHT) in exocrine glands. DHT has

278 anti-apoptotic properties, which protect acinar cells against apoptosis

279 [ 10]. In menopausal women, a lack of local intracellular DHT may

280 increase susceptibility to SS when estrogen levels are low [33]. The

281 above findings had prompted a strong interest in hormone replacement

282 therapy (HRT) as a prospective treatment for SS [34-36].

283 Due to the natural limitations of case-control study, we further

284 analysed by MR, and revealed no significant associations were observed

285 for the effects of E2 on SS susceptibility. In turn, a heightened risk of SS

286 was associated with decreased E2 levels in females, which is also

287 consistent with the findings of a number of studies. In a nested

288 case-control study, the modified composite estrogen scores (mCES)

289 demonstrated no significant association with SS in adjusted models, 290 considering 546 SS cases and 1637 age-matched controls [24]. In both

291 human and mouse studies, it has been observed that the number of X

292 chromosomes, rather than sex-steroid hormones, is associated with an

293 increased risk or susceptibility to develop autoimmunity, particularly

294 rheumatic diseases like SS. This observation suggests that the number

295 of X chromosomes plays a crucial role in the development of

296 autoimmunity [37]. Research findings suggest that the differences in the 297 immune system between sexes can be attributed to inherent composition.

298 Women generally exhibit a higher count of T lymphocytes CD4/CD8, B

299 lymphocytes, and plasma cells (PC), whereas men demonstrate a higher

300 proportion of Natural Killer cells (NK), CD14, and CD16 monocytes [38].

301 Genetic mechanisms linked to sex chromosomes have been identified as

302 possible factors contributing to sex differences in immune responses

303 across different age groups [39, 40]. Individuals with Klinefelter syndrome

304 (47,XXY) exhibit a susceptibility to lupus similar to that of females (46, XX)

305 [41]. Conversely, females with Turner syndrome (45, X0) appear to have

306 a protective effect [42]. Therefore, the dosage of the X chromosome

307 seems to be involved in the development of lupus pathology, scleroderma, 308 and SS. Certain immune-related genes can escape X chromosome

309 inactivation (XCI) to varying degrees, resulting in bi-allelic expression in 310 immune cells. This ultimately increases the likelihood of developing 311 immune-related disorders [37].

312 The efficacy of hormone therapy in SS has also varied significantly in

313 the results of a number of clinical trial studies. For instance, numerous

314 studies conducted on post-menopausal women have yielded conflicting

315 results regarding the efficacy of estrogen use in managing SS symptoms.

316 While some studies found that estrogen replacement therapy have no

317 significant effect on osmolarity, tear volume, breakup time, or ocular

318 symptoms [43, 44], others have shown that therapy can alleviate ocular

319 symptoms in this population [45-47]. Notably, one study revealed that 3

320 months of estrogen replacement therapy in post-menopausal women

321 without SS symptoms led to the development of dry eyes, with

322 symptomatic patients showing no improvement after the same duration of

323 therapy [48]. Therefore, there are further observations concerning the

324 interplay between estrogen treatment and SS signs and symptoms.

325 Regarding androgens, individuals with SS exhibit lower serum levels of

326 DHEA, dihydrotestosterone (DHT), and dehydroepiand rosterone sulfate

327 (DHEA-S) compared to healthy controls [6]. For example, in a case study,

328 the use of testosterone cream applied to the eyelids was suggested to

329 effectively reduce SS symptoms and improve the lipid layer breakup time

330 and its thickness to normal levels [49]. Similarly, another case study

331 indicated that systemic androgen and testosterone therapy in

332 post-menopausal women resulted in a reduction in dry eyes [50].

333 However, a study found that DHEA showed no evidence of efficacy in SS.

334 Due to the lack of evidence for its efficacy, patients with SS should avoid

335 using unregulated DHEA supplements, as the long-term adverse

336 consequences of exposure to this hormone are unknown [51]. Taken

337 together, our results suggest that early sex hormone replacement

338 therapy for normal menopausal women may not be helpful in the

339 prevention of SS; moreover, previous observational studies may have

340 overlooked the direct effect of SS on genetic variation in estrogen itself.

341 Our study has several limitations. First, our collection of sex

342 hormone levels only once before SS diagnosis and lack of follow-up, 343 causality still need to be clarified by designing longitudinal prospective

344 studies or retrospective nested cohorts. Second, the GWAS datasets

345 used in this study were not stratified by age. And the accuracy of our

346 findings may be affected by the age-specific nature of SS. The impact of

347 the genetic variation may be underestimated. Then, the limited sample

348 size in the case-control cohort, consisting of only 93 SS cases and 90

349 controls, may reduce the statistical power of our analysis and limit the

350 generalizability of the findings. Last, the observational study used a

351 Chinese postmenopausal female cohort, while the MR analysis relied on

352 European GWAS summary data. Due to genetic differences across

353 populations, this cross-ancestry approach may impact the validity of the

354 causal inferences.

355

356 **Conclusion**

357 A strong direct relationship was observed between hypoE2 and SS

358 in the population of women after natural postmenopausal age. However, 359 this relationship may be due to the direct effect of SS on genetic variation

360 at low E2.

361

362 **Ethics statement**

363 The case-control study conformed to approved guidelines, and all

364 experimental protocols were approved by the Ethics Committee of

365 Nanjing Drum Tower Hospital (No. 2022-529-04). Our study complied

366 with the Declaration of Helsinki, and all subjects provided written

367 informed consent. In the MR study, ethical review and approval were not

368 deemed necessary for this study involving human subjects, in

369 accordance with local laws and institutional regulations. Additionally, 370 written informed consent for participation was not required for this study, 371 aligning with national laws and institutional guidelines.

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373 We would like to extend our appreciation to the UK Biobank and the

374 FinnGen consortium for generously sharing publicly available

375 summarized data derived from GWAS studies, which proved invaluable

376 for our research.

377

378 **Author contributions**

379 JZ and SL conceptualized and designed the study; JZ and QC

380 conducted the statistical analysis; A-NW, M-GP, ZL and W-WW collected

381 the clinical specimens; JZ and QS organized the data, drafted the

382 manuscript, and revised it; QC and SL edited the manuscript and

383 supervised the study. All authors contributed to the article and approved

384 its final version.

385

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387 This study had no funding.

388

389 **Conflict of Interest**

390 The authors affirm that there are no competing interests that could

391 be construed as influencing the impartiality of this study.

392

393 **Data availability**

394 For the data of Mendelian randomization: The data that support the

395 findings of this study are openly available in the UK Biobank and the

396 FinnGen research project (<https://www.finngen.fi/en>). For the data of

397 case-control: The data that support the findings of this study are available

398 on request from the corresponding author, SL, upon reasonable request.

399

400 **Supplementary material**

401 The Supplementary Material for this article can be found online.

402

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