



## Estrogen receptors are linked to angiotensin-converting enzyme 2 (ACE2), ADAM metallopeptidase domain 17 (ADAM-17), and transmembrane protease serine 2 (TMPRSS2) expression in the human atrium: insights into COVID-19

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Premenopausal women have a reduced incidence of cardiovascular disease (CVD) compared to postmenopausal women or age-matched men, suggesting a cardioprotective role for estrogen [1]. Although estrogen replacement maintains cardiac structure and function in ovariectomized rodent models, clinical trials of estrogen-based hormone therapy have yielded inconsistent results with regard to improving heart function in older women. Overall, it is critical to further elucidate the functional roles of estrogen, especially its individual receptors, in the heart to develop more effective and specific hormone therapy for postmenopausal women.

Estrogen interacts with the renin-angiotensin system (RAS), one of the most critical pathways in CVD, by inhibiting or downregulating renin, angiotensin-converting enzyme (ACE), and angiotensin II (Ang II) type 1 receptor (AT<sub>1</sub>-R). However, the effects on cardiac ACE2 expression involve both increases and decreases depending on the species and experimental model studied. The identification of the ACE2 enzyme receptor, which acts with host transmembrane serine protease 2 TMPRSS2 [2], as the primary

means of cellular entry by the novel  $\beta$ -coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) justifies the importance of examining the potential contributory function of sex hormones in COVID-19 pathogenesis [3, 4].

Newer lessons from cancer biology have uncovered a critical role of estrogen receptor alpha (ER $\alpha$ ) and ER $\beta$  in regulating oncogenic TMPRSS2-ERG fusion [5]. Both ER $\alpha$  and the recently identified G protein-coupled estrogen receptor (GPER) are expressed in the heart of both humans and rodents, without sex differences [6]. To determine links among estrogen receptors, ACE2 expression, the protein-coding gene ADAM Metallopeptidase Domain 17 [4], and TMPRSS2 [4] in the human heart, we analyzed expression levels of these genes by real-time PCR in the right atrial appendage tissue of patients undergoing heart surgery for the correction of left heart valvular disease, resistant atrial fibrillation or ischemic heart disease ( $n = 34$ , mean age  $65 \pm 7$  years of age, 79% men). According to our results, right atrial appendage ACE2 mRNA correlated positively with ER $\alpha$  ( $p = 0.004$ , Fig. 1A) but not GPER (Fig. 1D) or ER $\beta$  (data not shown) expression. This result is consistent with the findings that estrogen increases ACE2 gene expression in the human atrium, which is inhibited by an antagonist of ER $\alpha$  but not ER $\beta$  [7].

As a key counterregulator of the RAS, ACE2 has beneficial effects in the cardiovascular system by converting Ang II to angiotensin-(1–7), which protects the heart against various stresses through its Mas receptor. RAS inhibitors augment ACE2 levels to protect organs from Ang II overload, which may benefit COVID-19 patients with CVDs [8]. Moreover, the positive correlation between ER $\alpha$  and ACE2 mRNA in human atrial tissue might explain the sex differences observed in the clinical course and morbidity of COVID-19. However, as ACE2 also serves as the main route of entry for SARS-CoV-2 into human host cells,

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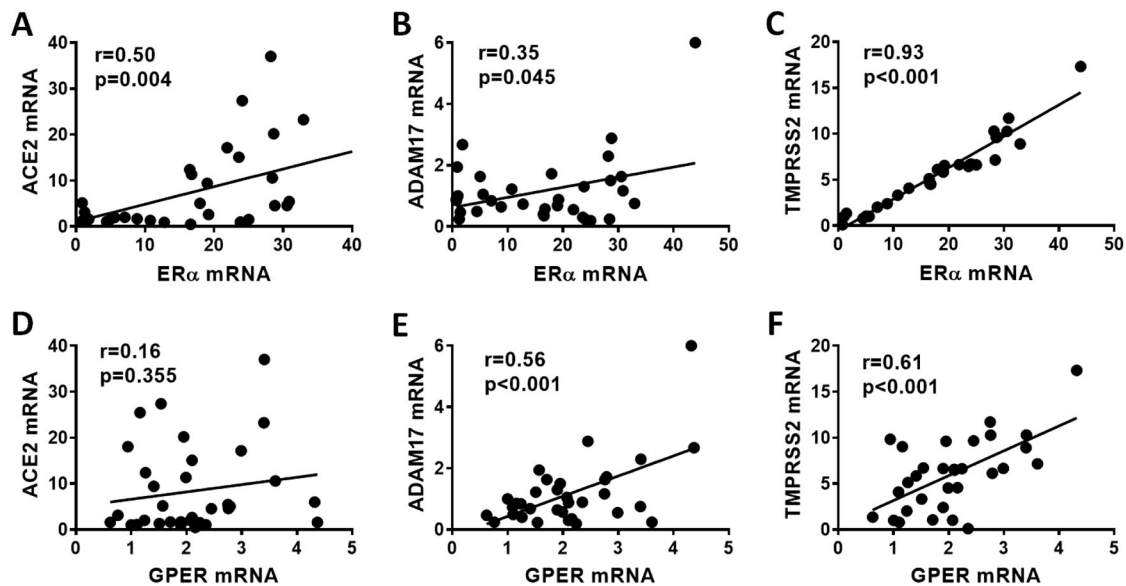
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**Fig. 1** Correlations among mRNA levels of estrogen receptors (ERs) and ACE2/ADAM17/TMPRSS2 in the human atrium. Right atrial appendage tissues were collected from 34 patients undergoing heart surgery for the correction of valvular heart disease, resistant atrial fibrillation or ischemic heart disease (mean age  $65 \pm 7$  years of age,

79% men). The study was approved by the Wake Forest University Health Sciences (IRB 22619). mRNA levels in the atrium were determined by real-time PCR and corrected by the housekeeping gene GAPDH. Correlations between mRNA levels were determined using GraphPad Prism 8 software

elevated ACE2 expression might facilitate viral entrance. In view of this dual role of tissue ACE2, the sexual dimorphic pattern of SARS-CoV-2 infection severity might involve multiple mechanisms, including differences in immune system responses between women and men. Further investigations of the exact roles of ACE2 and its interaction with estrogen in COVID-19 patients with CVDs are needed to address the potential benefit of sex-specific therapeutic interventions to protect against disease progression.

In addition, we discovered that the mRNA levels of both ERα (Fig. 1B, C) and GPER (Fig. 1E, F), but not ERβ (data not shown), correlated positively with ADAM17 and TMPRSS2 in human right atrial appendage tissues. Indeed, the TMPRSS2 gene transcript showed the highest level of association with ERα, as illustrated in panels C and F of the figure. To our knowledge, this is the first report demonstrating strong associations between estrogen receptors and the expression levels of ADAM-17 and TMPRSS2 in human cardiac tissue, providing new insight into the relationship between estrogen and the heart.

These results from atrial appendage tissue suggest that estrogen, primarily through its receptors ERα and GPER, affects cardiac ACE2 levels and activity by regulating ACE2 shedding via ADAM-17 and TMPRSS2. Interestingly, the linear correlation of ACE2 gene transcripts with ERα and GPER mRNAs appears to be associated with higher values of ACE2 gene expression. This new finding may assist in explaining the discordant results reported in the literature regarding the impact of SARS-CoV-2

infection on plasma ACE2 activity [9]. We realize the limitations of our reported data. Due to a small sample size, we used tissue from patients with a variety of cardiac pathologies and treatments, which might have influenced the expression levels of estrogen receptors, ACE2, ADAM-17, and TMPRSS2. Future studies focused on specific cardiac conditions will advance our understanding of the potential relationships among estrogen receptors and ACE2, ADAM-17, and TMPRSS2. Moreover, tissue samples from both sexes were included in this study. Although estrogen receptors are similarly expressed in the heart of males and females and estradiol protects the heart against various stresses in both male and female animals [6, 10], future studies with sex separation are needed to elucidate how estrogen regulates cardiac ACE2 in both sexes. As only mRNA levels were determined in this study due to the small amount of tissue obtained from each patient, protein levels and enzymatic activities of ACE2, ADAM-17, and TMPRSS2 should be further examined to elucidate how activation of ERα and GPER affects ACE2 shedding and therefore participates in CVD and COVID-19. Nevertheless, the novel demonstration of associations among cardiac ACE2, ADAM-17, and TMPRSS2 gene transcripts with ERα and GPER mRNA levels reveals for the first time a functional modulatory mechanism of estrogen receptors for the structural elements that may regulate ACE2.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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