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Genetic Associations With Plasma Angiotensin Converting Enzyme 2 Concentration

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Potential Relevance to COVID-19 Risk

Male sex has emerged as a major independent risk factor for severe disease and death from severe acute respiratory syndrome coronavirus–related coronavirus disease 2019 (COVID-19).[1] Severe acute respiratory syndrome coronavirus 2 infects cells via the angiotensin converting enzyme 2 (ACE2) receptor, a widely distributed cell-surface receptor.[2] In 2 cohorts of patients with heart failure (BIOSTAT-CHF [Biology Study to Tailored Treatment in Chronic Heart Failure]), we recently showed that age-adjusted plasma concentrations of ACE2 are higher in men than in women.[3]

To explore whether there are sex-specific genotype effects on plasma ACE2 concentrations, we undertook sex-specific and combined genome-wide associations studies of plasma ACE2, measured using the Olink platform (which provides results expressed in the form of relative quantification without units), in the combined index and validation cohorts of BIOSTAT-CHF (2420 men and 1022 women, mean age 70.9±11.6 years).[3] The BIOSTAT-CHF study was approved by institutional review boards at participating centers. Further details on the cohorts are available in Sama et al.[3] Mean plasma ACE2 concentrations were 5.41 (SD 0.74) in men and 5.13 (0.75) in women ($P=2.6\times10^{-26}$). Genotyping was performed using the Affymetrix Axiom Genome-Wide UKB WCSG Genotyping Array and variants imputed to 1000G Phase 1 v3. Further details on imputation and quality control are available on request. We tested variants with a minor allele frequency >1% and an imputation quality score >0.5 and adjusted the analyses for BIOSTAT-CHF cohort, age, and the first 5 principal components.

We identified 3 loci associated with plasma ACE2 concentrations at genome-wide significance in men, but none in women (Figure A). No additional genome-wide significance loci were identified in the combined analysis.

The strongest association was with an X-chromosome locus (lead single-nucleotide polymorphism rs12551879, minor allele frequency 27.2%, beta 0.13±0.02, $P=5.93\times10^{-15}$), which includes the *ACE2* gene (Figure B). The strongest association in women was rs4646131 ($P=1.58\times10^{-3}$) with a $R^2=0.16$ and D′=0.86 with the lead single-nucleotide polymorphism in men (Figure B). The lead variant (rs71076692, minor allele frequency 48.4%, beta 0.14±0.02, $P=1.49\times10^{-9}$) at the second locus in men on chromosome 12 (Figure C) is an insertion-deletion (indel) variant located proximate to *HNF1α* that encodes a transcription factor known to regulate *ACE2* expression.[4] The lead variant (chr21:39834295, minor allele frequency 24.8%, beta 0.13±0.03, $P=2.87\times10^{-9}$) at the third locus in men on chromosome 21 (Figure D) is also an indel located within the *ERG* gene which encodes a transcription factor involved in vascular development and remodeling. Fusion of *ERG* with the nearby gene *TMPRSS2*, encoding a transmembrane serine protease involved in ACE2 cleavage and entry of severe acute respiratory syndrome coronavirus 2 into the cell, is a frequent event in prostate cancer.[2,5]

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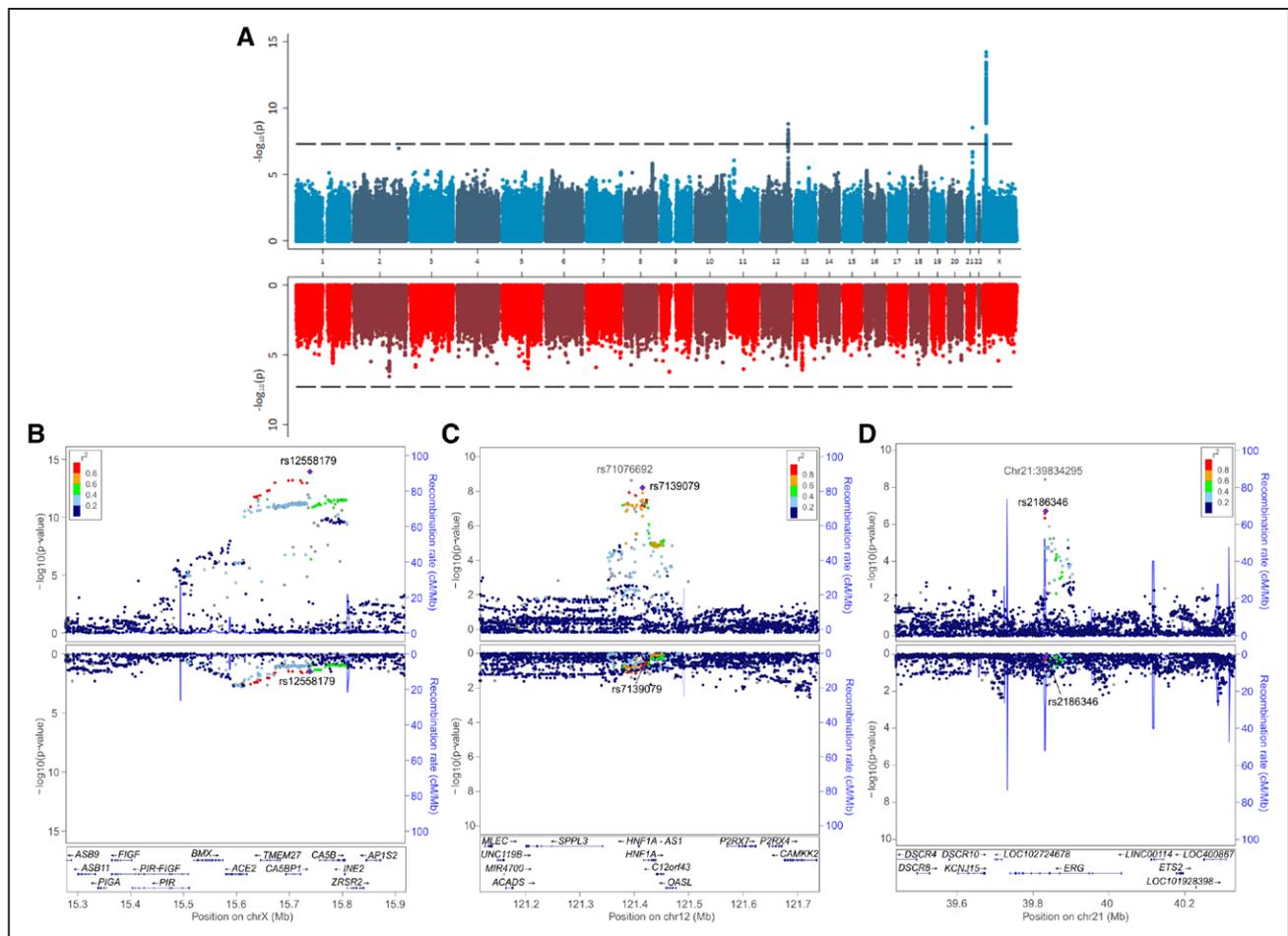**Leong L. Ng, MD**
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Figure. Association of genetic variants with plasma ACE2 concentration.

A, Miami plot showing genome-wide association analysis of genetic variants with plasma ACE2 concentration. Chromosomal location of the variants is shown on the x axis and $-\log_{10}$ (P value) of the association on the y axis for men (blue, **Upper**) and women (red, **Lower**). Dashed reference line shows the genome-wide significance threshold of $P=5\times10^{-8}$. **B** through **D**, Regional plots showing association with plasma ACE2 concentration of genetic variants on the X-chromosome (chrX) around the *ACE2* gene (**B**), on chromosome 12 (chr12) around the *HNF1α* gene (**C**), and on chromosome 21 (chr21) around the *ERG* gene (**D**). Each plot shows associations for men in the upper half and women in the lower half. The top signals for chromosome 12 (rs71076692) and for chromosome 21 (chr21:39834295) are both indels. Therefore, proxy single-nucleotide polymorphisms (rs7139079 and rs2186346, respectively) in high-linkage disequilibrium with these lead variants were used to generate the linkage disequilibrium relationships with other variants for signals on chromosomes 12 and 21.

A sex interaction analysis showed a significant interaction for the third locus on chromosome 21 (P=0.001) but not for the loci on chromosomes X (P=0.25) or 12 (P=0.15). Because of the smaller sample size in women, we estimated the power to detect the effects seen in men. The power to detect the smallest effect size seen in men (chromosome 21) was 53.9% at 5×10^{-8} and 93.5% at 5×10^{-5}. The 3 loci explained 4.91% of variation in plasma ACE2 concentration in men and 1.14% in women. The associations were independent of other clinical or demographic factors or medication.

The lack of association signals in women at the 3 identified loci may reflect smaller sample size and lower power; however, the >4-fold difference in variation in ACE2 concentration explained by these loci between men and women supports an hypothesized sex-specific genotype effect on plasma ACE2 concentration. The association of X-chromosome variants is probably mediated by *cis*-effects on *ACE2* transcription. Although

there are strong candidate genes that may explain the associations at the other 2 loci (see earlier), causal genes underlying genome-wide association study loci are often neither the nearest nor the strongest putative candidate genes and further investigation of their involvement is necessary.

Plasma ACE2 arises from shedding of the receptor from cells through the effect of several enzymes including TMPRSS2 and ADAM17.[2] Any association between ACE2 levels and severity of COVID-19 and the extent to which plasma ACE2 concentration correlates with expression in tissues are both unclear. The genotype effects observed for plasma ACE2 concentration could reflect a similar effect on tissue expression or on receptor shredding, at least in some sites. In turn, this could possibly influence susceptibility to the severity of infection by severe acute respiratory syndrome coronavirus 2.

Our analyses were performed in relatively elderly subjects of primarily European Caucasian ancestry with heart

failure.[3] These results require replication in other cohorts. As suitable cohorts collected during the COVID-19 pandemic become available, our findings provide an opportunity to determine whether these genetic markers associated with ACE2 concentrations in BIOSTAT-CHF are also associated with differences in outcomes from COVID-19, especially in men. If this is the case, genotyping may help inform future personalized prevention strategies against COVID-19. More broadly, a primarily protective role for ACE2 has been proposed in several cardiovascular diseases, and our findings provide new genetic instruments to investigate its precise involvement.

ARTICLE INFORMATION

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Disclosures

None.

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