Increased Hepatic ACE2 Expression in NAFL and Diabetes—A Risk for COVID-19 Patients?

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The current pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced disease (coronavirus disease 2019 [COVID-19]) is a major global threat. Although most infected patients have a mild course, a relevant proportion of approximately 20% develop a severe and often lethal condition. Recent findings demonstrate that COVID-19 is a multiorgan disease. In addition to the characteristic pneumonia, it affects heart, kidney, pancreas, the coagulation system, and liver. Major risk factors for severe COVID-19 with multiorgan complications include male sex, older age (>65 years), cardiovascular and chronic lung disease, and especially diabetes (1,2). However, it is still unclear why these conditions predispose to a severe course of COVID-19.

To enter the target cell, SARS-CoV-2 requires the binding of its spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor (3). The entry is additionally facilitated by subsequent priming and cleavage of the spike protein through proteases such as transmembrane serine protease 2 (TPMRSS2) and furin (3). While the ACE2 receptor is mainly expressed in heart, lungs, endothelial cells, kidney, and the gastrointestinal tract, expression has also been detected in cholangiocytes and in lower amounts in hepatocytes (4). Of note, hepatic ACE2 expression was found to be increased upon chronic liver damage in rodents as well as in humans.

Nonalcoholic fatty liver (NAFL) is the most prevalent liver disorder in people with type 2 diabetes and is present in more than two-thirds of these patients. To our knowledge, no testing has been done so far to determine whether liver fat accumulation or diabetes affects ACE2 expression. As increased presence of ACE2 likely promotes the entry of SARS-CoV-2, this could be one underlying mechanism for liver damage and subsequent lethality in patients with diabetes. We therefore investigated how NAFL and diabetes impact ACE2 expression in the human liver.

We investigated hepatic ACE2 mRNA expression by real-time PCR in surgical liver samples of normal, nondiseased tissue from a cohort of 165 individuals (62 women/103 men, mean ± SD age 63.5 ± 11.7 years, BMI 25.2 ± 4.1 kg/m^2) of European descent, including 31 subjects with type 2 diabetes. The local ethics committee at the University of Tübingen approved the protocol (239/2013BO1), and all patients provided informed written consent.

Hepatic ACE2 mRNA expression was significantly higher in males compared with females ($P = 0.024$) (Fig. 1*A*) and higher in older patients ($P = 0.011$, $r = 0.197$) (Fig. 1*B*). Obesity had no link to hepatic ACE2 expression ($P = 0.24$, $r = 0.092$) (Fig. 1*C*). We next addressed the impact of liver fat. In our cohort of patients with a wide range of liver fat content, we detected increasing ACE2 expression with increasing fat accumulation ($P = 0.002$, $r = 0.239$) (Fig. 1*D*). This relation remained significant even after adjustment for age, sex, and BMI ($P = 0.0028$).

Patients with diabetes had significantly higher ACE2 expression than those without the disease ($P = 0.0085$) (Fig. 1*E*). In the 73 patients with available fasting blood samples, neither fasting glucose nor insulin sensitivity (assessed by HOMA of insulin resistance) was associated with hepatic ACE2 expression ($P = 0.67$, $r = 0.158$, and $P = 0.94$, $r = 0.009$, respectively) (Fig. 1*F*).

We next addressed the proteases, TMPRSS2 and furin, that facilitate ACE2-dependent cell entry of SARS-CoV-2. Both were present in our liver specimens, and TMPRSS2 expression was approximately 10-fold lower than ACE2. However, TMPRSS2 had similar associations with sex ($P = 0.048$) and diabetes

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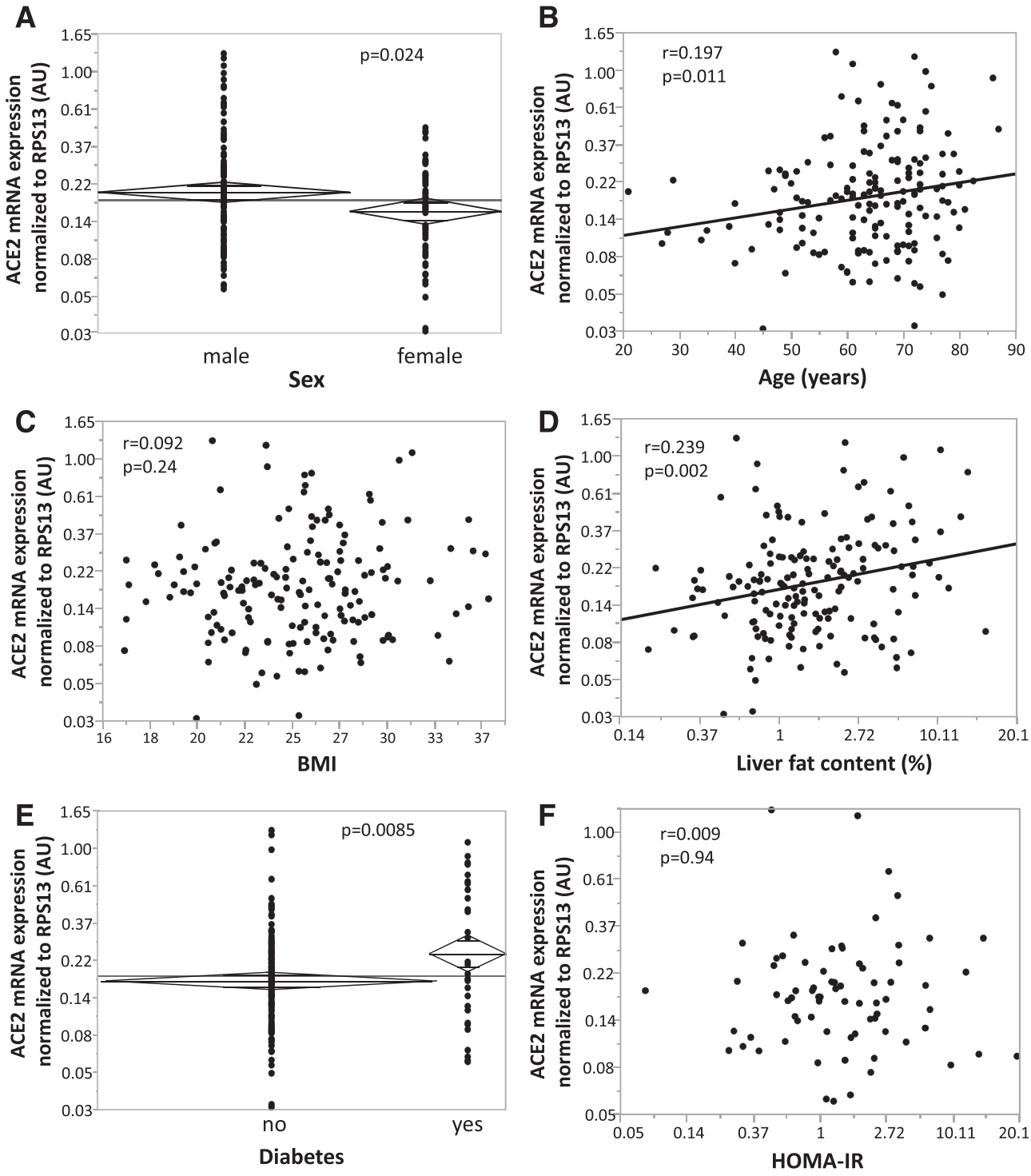

Figure 1—Correlations between ACE2 mRNA expression normalized to RPS13 in human liver tissue samples ($n = 165$) and different determinants. ACE2 mRNA expression is higher in male than in female subjects (*A*) and correlates positively with increasing age (*B*), but shows no correlation with BMI (kg/m^2) (*C*). Elevated liver fat content is positively correlated with ACE2 mRNA expression (*D*), and subjects with diabetes had higher ACE2 mRNA expression (*E*), whereas HOMA of insulin resistance (HOMA-IR), assessed in 73 subjects, showed no correlation with ACE2 expression (*F*). For statistical analyses, nonnormally distributed parameters were log transformed.

($P = 0.035$), as well as a positive correlation with liver fat content ($P = 0.0023$, $r = 0.234$) but not with age ($P = 0.48$). In contrast, furin expression was approximately 10-fold higher than ACE2, but we detected no significant associations with sex, diabetes, age, BMI, or liver fat content.

We report upregulated expression of ACE2 in the livers of patients with diabetes, male sex, older age, or NAFL, i.e., situations that predispose to an adverse course of COVID-19. The greater availability of ACE2 and its major cofactor TMPRSS2 most likely fosters viral penetration into cells. Hence, we presumably identified one novel mechanism that contributes to the susceptibility for hepatic complications in these patients.

In line with this, SARS-CoV-2 was detected in some, but not all, autopsy liver samples of COVID-19 patients (5). However, it is still not clear whether SARS-CoV-2 infection affects hepatocytes or cholangiocytes. Our current results could be the basis for further investigations in COVID-19 patients to test how diabetes and liver fat accumulation contributes to hepatic viral infection and subsequent severity of and mortality from the disease. Since NAFL is often asymptomatic and remains undetected, it could underlie some severe cases of COVID-19 in patients without known risk factors. Based on the results obtained from our study, NAFL could play an important role in the development of severe liver damage during infection with SARS-CoV-2 in an older population with diabetes.

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References

1. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. JAMA 2020;323:2052–2059

2. Williamson EJ, Walker AJ, Bhaskaran K, et al. OpenSAFELY: factors associated with COVID-19 death in 17 million patients. Nature 2020;584:430–436

3. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281–292.e6

4. Chai X, Hu L, Zhang Y, et al. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. 4 February 2020 [preprint]. bioRxiv 2020.02.03.931766

5. Puelles VG, Lütgehetmann M, Lindenmeyer MT, et al. Multiorgan and renal tropism of SARS-CoV-2. N Engl J Med 2020;383:590–592