Title page

Comparative evaluation of nasal and small intestine expression of ACE2, TMPRSS2 and ACE1 and in children and in adults

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Abstract

Background: Clinical severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection seems to be lower in children compared to that in adults. Defining the pathophysiological mechanisms of such disease patterns maybe relevant for development of effective public health strategies. It has been hypothesised that the lower severity of SARS-CoV-2 infection in children could be due to the differential expression of angiotensin-converting enzyme 2 (ACE2), which serves as a virus receptor.

Objective: To evaluate the expression of ACE2, ACE1, and TMPRSS2 genes at the level of the two most relevant entry sites for SARS-CoV-2, the upper respiratory tract and small intestine, in healthy children and adult subjects.

Methods: This prospective study included healthy individuals of both sexes, aged 1-10 years in the paediatric population (n=30) and 20-80 years in the adult population (n=30). The participants were consecutively evaluated at two tertiary centres for paediatrics, gastroenterology, and otolaryngology. Expression of ACE2, ACE1, and TMPRSS2 genes in samples collected from the upper respiratory tract and small intestine.

Results: We found no difference in ACE2, ACE1, and TMPRSS2 expression in the nasal epithelium between children and adult subjects. ACE2 expression was more abundant in the small intestine of children compared to that in adults. ACE1 expression was higher in the small intestine of adults compared to that in children. Intestinal TMPRSS2 expression was similar in the two study populations.

Conclusions: The general lower severity of SARS-CoV-2 infection in children does not seem to be related to a lower expression of ACE2 and/or TMPRSS2 in the respiratory tract or in the gastrointestinal tract. Other co-factors may confer protection against SARS-CoV-2 in children. The exploration of such factors is of pivotal importance for development of innovative protective strategies against SARS-CoV-2.

Introduction

The frequency of positive laboratory tests and common symptoms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection seems to be lower in children compared to that in adults[1]. Defining the pathophysiological mechanisms of such disease patterns maybe relevant for development of effective public health strategies. It has been hypothesised that the lower risk among children could be influenced by differential expression of angiotensin-converting enzyme 2 (ACE2), which serves as the receptor used bySARS-CoV-2 for host entry[2], but data on a possible age-dependent ACE2 expression pattern are conflicting[3,4]. Additionally, data on possible age-related patterns of other cellular components involved in SARS-CoV-2 infection, such as transmembrane serine protease-2 (TMPRSS2), in the respiratory and gastrointestinal tracts remain elusive.

Methods

Given the role of ACE2 and TMPRSS2 in SARS-CoV-2 infection, we conducted a prospective study to comparatively evaluate the expression of these genes at two most relevant entry sites for SARS-CoV-2, the upper respiratory tract and the small intestine[5], in healthy children and adult subjects. ACE1 expression was also evaluated at both sites as a positive regulator of the renin-angiotensin system (RAS), which promotes angiotensin II (AngII) conversion[6]. Individuals of both sexes, aged 1-10 years in the paediatric population and 20-80 years in the adult population, were consecutively evaluated at two tertiary centres for paediatrics, gastroenterology, and otolaryngology for suspected respiratory or gastrointestinal disorders. Only subjects with negative results for any clinical, laboratory, and endoscopic procedures were included in the study. We excluded all individuals with a positive history of immunodeficiencies, allergies, metabolic and genetic disorders, tumours, cystic fibrosis, malformations, cardiovascular diseases, hypertension, inflammatory bowel diseases, food allergies and intolerances, celiac disease, infections, or drug usage in the previous 12 weeks. The study was approved by the Ethics Committee of the University Federico II of Naples, Italy. Written informed consent was obtained from the adult participants and from the parents/tutors of minors.

Nasal epithelial samples were collected using a cytology brush (EndoscanPlus, Medico, Melbourne, Australia), whereas small intestinal epithelial biopsies were collected by esophagogastroduodenoscopy (EGDS). All samples were immediately placed in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) and stored at -80°C until analysis. Total RNA was extracted

with the TRIzol reagent (Invitrogen, Thermo Scientific, Waltham, MA, USA). All samples were quantified using theNanoDrop 2000c spectrophotometer (Thermo Scientific) and RNA quality and integrity were assessed with the Experion RNA Standard Sense kit (Bio-Rad, Hercules, CA, USA). cDNA was synthesised with random primers using the SensiFASTcDNA Synthesis Kit (Bioline) on theCFX96 RealTime System instrument (Bio-Rad**,** Hercules, CA, USA). Quantitative real-time PCR (qRT-PCR) analysis was performed using the SensiFAST SYBR Hi-ROX Kit (Bioline) on the7900HT Fast Real-Time PCR System (Applied Biosystems) with the following primer pairs:

ACE1 (NM_000789.4) 5'- CAGAACACCACTATCAAGCG -3' and 5'- GTCTTCATATTTCCGGGACG -3';

ACE2 (NM_021804.3) 5'- GCAGACCAAAGCATCAAAGTG -3' and 5'- GGTTTCAAATTAGCCACTCGC -3';

TMPRSS2 (NM_005656.4) 5'- AGCCTCTGACTTTCAACGAC -3' and 5'- TCAATGAGAAGCACCTTGGC -3';

HPRT (NM_000194.3) 5'- GACCAGTCAACAGGGGACAT -3' and 5'- GTGTCAATTATATCTTCCACAATCAAG -3'

Data analysis was performed using the comparative threshold cycle (CT) method and expressed as 2^-delta CT. Gene expression was normalised against the expression of the reference gene hypoxanthine phosphoribosyltransferase 1 (HPRT). For statistical analysis, two-sided tests were used and $P \leq 0.05$ was considered as statistically significant.

Results

From May 2020 to July 2020, 38 children and 35 adult subjects were evaluated for this study. Eight children and five adults were excluded because of the presence of at least one exclusion criterion. Thus, 30 children and 30 adult subjects were finally considered for enrolment. All subjects were evaluated under stable clinical conditions. A sample of the nasal epithelium was collected by nasal brushing from 15 children (9 males, median age of 1 year, range 1-3 years) and 15 adults (6 males, median age of 29 years, range 20-55 years). Small intestinal epithelial samples were collected during the EGDS procedure from 15 children (7 males, median age of 8.5 years, range 1-10 years) and from 15 adults (9 males, median age of 59 years, range 22-80 years).

We found no significant difference in ACE2 and TMPRSS2 expression in the nasal epithelium between children and adult subjects (Fig. 1a). A similar pattern was observed for the ACE1 gene. In contrast, ACE1 mRNA levels were higher in the small intestine of adult subjects compared to those in children (Fig. 1b). In contrast, ACE2 expression was more abundant in the small intestine of

children compared to that of adult subjects (Fig. 1b). Intestinal TMPRSS2 expression was similar in the two study populations (Fig. 1b).

Discussion

It has been hypothesised that differential expression of ACE2 is responsible for the general lower severity of SARS-CoV-2 infection in children, but data are conflicting[3,4,7,8]. In this study, we did not find significant differences in ACE2 and TMPRSS2 expression in the nasal epithelium between children and adult subjects. In agreement with previous data[9-11], we found that ACE2 expression in the intestine was approximately 100 times higher than that in the respiratory epithelium. However, its expression was more abundant in the small intestine of children compared to that in adult subjects. In contrast, intestinal TMPRSS2 expression was similar in the two study populations. Altogether, these data suggest that the lower severity of SARS-CoV-2 infection may not be due to a different expression of ACE2 and/or TMPRSS2 in the upper respiratory tract. It has been hypothesised that other factors may confer protection against SARS-CoV-2 in children, including cross-reactive humoral and T-cell immunity between common coronaviruses and SARS-CoV-2, protective Th2 immunity, and lower production of inflammatory cytokines[12]. Additionally, novel co-factors have been proposed to be involved in the modulation of SARS-CoV-2 infection that maybe responsible for such a different disease pattern[13,14]. Further, considering the pivotal role of ACE2 in modulation of inflammation, it is reasonable to suggest that higher levels of intestinal ACE2 activity in children may be effective in limiting the severity of SARS-CoV-2 infection in this age group, as previously hypothesised[15]. On the contrary, the concomitant higher ACE1 expression in the intestinal tract observed in adult subjects may facilitate tissue inflammation. In fact, the combination of higher ACE1 with lower ACE2 expression in adults may facilitate angiotensin II (AngII)-mediated vasoconstriction, inflammation and fibrosis, thereby aggravating the severity of the SARS-CoV-2 infection[15].

In conclusion, despite the relatively low number of observations, our data obtained in a well-characterised population of healthy subjects suggest the importance of elucidation of other co-factors besides ACE2 and TMPRSS2 which modulate the age-dependent severity of SARS-CoV-2 infection. These co-factors may become innovative targets of intervention for prevention and treatment of SARS-CoV-2 infection.

Authorship

RBC, FA, and GC designed the study, coordinated the research team, and wrote the first draft of this report. CS, AG, EM, EC, NG, LC and VG were responsible for the study subjects and evaluated their health status. FA, LP, MC, GC, CB and IZ conducted the laboratory experiments. FA and LP performed the statistical analysis and data interpretation. All of the authors revised and approved the final version of this article.

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Conflict of Interest Statement

The authors have no other conflict of interests that are directly relevant to the content of this manuscript, which remains their sole responsibility.

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Figure Legend

Figure 1. qPCR analysis of Angiotensin I Converting Enzyme (ACE1), Angiotensin II Converting Enzyme (ACE2) and Transmembrane Serine Protease 2 (TMPRSS2) genes in children and adult healthy subjects.

Comparative expression of ACE1, ACE2 and TMPRSS2 genes in nasal epithelium (**a**) and in small intestine epithelium **(b)** in children and adult healthy subjects. Genes expression were normalized against Hypoxanthine Phosphoribosyltransferase 1 (HPRT) expression levels as reference gene. Data are expressed as median±SD, the X in the bars indicates mean values.

Significant differences in gene expression are indicated with relative *p*-value (*p<0.01, **p<0.005).

Figure 1