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Review

Age-related differences in SARS-CoV-2 binding factors: An explanation for reduced susceptibility to severe COVID-19 among children?

Thomas Abrehart [a,*], Randy Suryadinata [b,c], Conor McCafferty [a,d], Jonathan Jacobson [c,h], Vera Ignjatovic [a,d], Phil Robinson [a,b,c], Nigel W. Crawford [a,c], Paul Monagle [a,d,e,f], Kanta Subbarao [g,h], Catherine Satzke [a,c,h,1], Danielle Wurzel [a,b,c,i,1]

[a] Department of Paediatrics, the University of Melbourne, Parkville, Australia
[b] Department of Respiratory Medicine, Royal Children's Hospital, Melbourne, Victoria, Australia
[c] Infection and Immunity, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia
[d] Haematology, Murdoch Children's Research Institute, Melbourne, Australia
[e] Department of Haematology, Royal Children's Hospital, Parkville, Australia
[f] Kids Cancer Centre, Sydney Children's Hospital, Randwick, Australia
[g] WHO Collaborating Centre for Reference and Research on Influenza, the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia
[h] Department of Microbiology and Immunology, the University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia
[i] Melbourne School of Population and Global Health, the University of Melbourne, Melbourne, Victoria, Australia

Educational Aims

The reader will be able to appreciate:

- The mechanism of SARS-CoV-2 cellular entry.
- The age-dependent differences in expression of key host SARS-CoV-2 binding factors (ACE2, TMPRSS2, furin).
- The aetiology of age-related differences in COVID-19 severity remains unknown.
- The importance of identifying the mechanisms underpinning paediatric resistance to severe COVID-19 as this will inform future therapies to reduce mortality, particularly in the elderly.

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ABSTRACT

Context: In contrast with other respiratory viruses, children infected with SARS-CoV-2 are largely spared from severe COVID-19.
Objectives: To critically assess age-related differences in three host proteins involved in SARS-CoV-2 cellular entry: angiotensin-converting enzyme 2 (ACE2), transmembrane serine protease 2 (TMPRSS2) and furin.
Methods: We systematically searched Medline, Embase, and PubMed databases for relevant publications. Studies were eligible if they evaluated ACE2, TMPRSS2 or furin expression, methylation, or protein level in children.
Results: Sixteen papers were included. Age-dependent differences in membrane-bound and soluble ACE2 were shown in several studies, with *ACE2* expression increasing with age. TMPRSS2 and furin are key proteases involved in SARS-CoV-2 spike protein cleavage. *TMPRSS2* expression is increased by circulating androgens and is thus low in pre-pubertal children. Furin has not currently been well researched.
Limitations: High levels of study heterogeneity.
Conclusions: Low expression of key host proteins may partially explain the reduced incidence of severe COVID-19 among children, although further research is needed.

INTRODUCTION

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to affect millions

* Corresponding authors at: Department of Paediatrics, the University of Melbourne Grattan Street, Parkville, Victoria 3052, Australia.
E-mail address: tabrehart@student.unimelb.edu.au (T. Abrehart).
[1] These authors contributed equally.

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of people worldwide. Curative treatments are not yet available, and despite global vaccination efforts, COVID-19 mortality rates remain high. As of 10 September 2021, there have been an estimated 4,602,822 deaths worldwide [1]. Severe COVID-19 disease is much less common amongst children [2–4]. This pattern strikingly contrasts that of other respiratory viruses, where the prevalence and severity are often higher in children [5]. Epidemiological studies have shown increasing age to be the strongest risk factor for severe COVID-19 disease, however the reasons for this remain poorly understood [6].

Until recently, the SARS-CoV-2 B.1.617.2 (Delta) variant had quickly become the dominant variant across the globe. This variant demonstrates increased transmissibility and reduced sensitivity to antibody neutralisation across all age groups [7,8]. However, the B.1.1.529 (Omicron) variant has recently emerged, and early data suggests it may be twice as infectious as the Delta variant [9]. A recent study also found it to have significantly higher binding affinity for human ACE2 than the Delta variant [10]. Evidence from the USA and South Africa shows increasing numbers of paediatric hospitalisations over recent months, which may be explained by the lack of a licenced COVID-19 vaccine for children younger than 12 years of age, coupled with higher infection rates with the Omicron variant [11,12]. Despite increasing hospitalisations, severe morbidity and mortality in children remains rare even with the Omicron variant [11].

A potential driver for age-related differences in disease susceptibility are the SARS-CoV-2 binding factors implicated in viral attachment and fusion-mediated internalisation, as shown in Fig. 1. SARS-CoV-2 entry is mediated by the coronavirus surface spike (S) protein, which attaches to target cells via ACE2. Heparan sulfate helps recruit SARS-CoV-2 to the cell surface, and is an essential cofactor for ACE2 binding [13]. Successful binding of ACE2 allows the S-protein to undergo proteolytic cleavage by TMPRSS2 and furin, thus allowing membrane fusion and viral internalisation [14]. SARS-CoV-2 entry is also possible via cathepsin-mediated endocytosis, although this pathway is less efficient [15,16].

Despite increasing literature characterising SARS-CoV-2 tropism, the aetiology of age-related differences in COVID-19 severity remains unclear. Understanding the precise mechanisms underpinning such differences may inform future therapeutic targets to reduce mortality, particularly amongst older patients. Age-specific differences in *ACE2* gene methylation, membrane-bound receptor expression and soluble ACE2 concentration in serum have been proposed as potential explanations. Likewise, since *TMPRSS2* has androgen receptor elements which are upregulated by high circulating androgen levels, its expression may be reduced in pre-pubertal children, thus limiting viral entry. Here, we critically review the literature to inform the hypothesis that age-related differences in SARS-CoV-2 binding factors are responsible

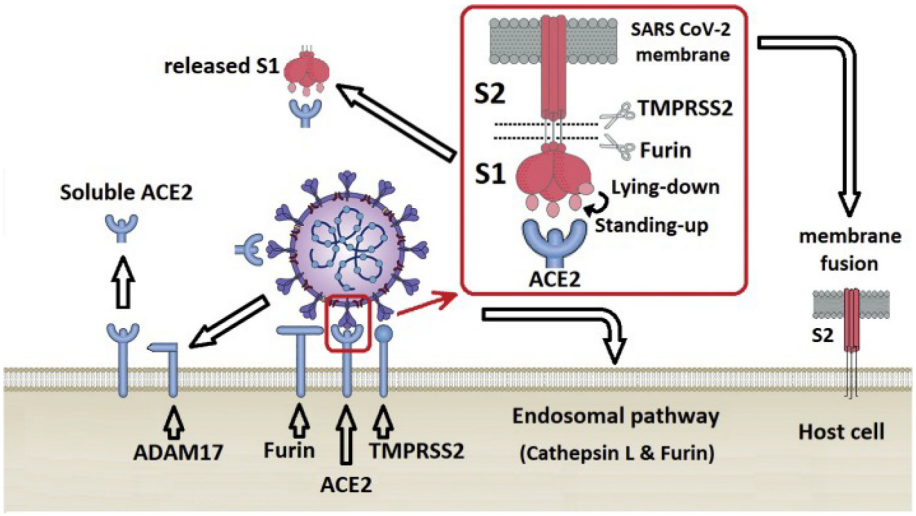

Fig. 1. Schematic representation of the host cell receptors and proteases involved in SARS-CoV-2 cellular entry via the membrane-fusion pathway, as originally published in Oz et al. [15].

Table 1
Inclusion and exclusion criteria.

Category	Exclusion criteria	Inclusion criteria
Study type	Conference abstract/paper/review or practice guideline	Clinical studies, clinical trials, in-vitro studies, epidemiological studies, retrospective studies, prospective studies, cohort studies
Publication language	Not English	English
Year of publication	Prior to 2020	2020 – current
Subject tissue	Non-human	Human
Data characteristics	Articles that did not evaluate ACE2, TMPRSS2 or furin expression, methylation, or protein level in children in the context of SARS-CoV-2, as well as articles that did not report the number of patients/children/public databases used	Articles that reported data on the expression, methylation, or protein level of ACE2, TMPRSS2 or furin in children in the context of SARS-CoV-2. Articles selected also needed to report the number of patients/children (or named public databases used to access RNA-seq data)

for the difference in disease severity observed between children and adults.

MATERIALS AND METHODS

This review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [17]. Following an initial screening phase, eligible studies were included in a qualitative analysis.

References were identified through searches of Medline, Embase, and PubMed, conducted between 21 June 2021 and 26 July 2021; a partial updated search was also conducted on 9 August 2021. Keyword search terms were "COVID-19", "SARS-CoV-2", "cell-surface receptor", "ACE2", "TMPRSS2", "furin", "heparan sulfate" and "children", with articles from Jan 2020 onwards considered. Additional articles were also identified through web searching and reference chaining. Due to the evolving nature of COVID-19 research, non-peer reviewed articles were included in the review. Only papers published in English were included Table 1.

Duplicates were removed using Endnote X8. Subsequently, a single reviewer screened the title and abstract of each paper to remove studies unrelated to the review topic. Potentially admissible articles then underwent full-text analysis. The final reference list for review was generated based on originality and relevance.

RESULTS

1085 results were identified from the database searches, with 817 being unique. Following abstract and title screening to remove studies unrelated to the review topic, 58 studies were retained. An additional 12 reports were obtained through website searching and reference chaining. Following full-text analysis and application of the inclusion/exclusion criteria, a total of 16 studies were included in the detailed review, 3 of which had not been peer-reviewed Fig. 2.

DISCUSSION

Children are largely protected from severe COVID-19 disease, however the specific mechanisms underpinning this remain poorly understood. Several factors have been postulated, including differences in the innate immune response, viral binding factors on epithelial surfaces (e.g., ACE2), and cross-immunity from exposure to seasonal coronaviruses. Elucidating the specific factors that play a role in the age-specific response to SARS-CoV-2 may help to inform future therapies to reduce morbidity and mortality associated with COVID-19. This review focuses specifically on viral binding factors. Herein, we critically assess age-dependent differences in binding factors implicated in SARS-CoV-2 cellular entry, and postulate on their role in COVID-19 severity.

ACE2 expression levels

ACE2 is the primary receptor for SARS-CoV-2 cellular entry [34]. This receptor is widely expressed across multiple organs, including on epithelial cells of the nasopharynx, heart, lungs, kidney, intestine, central nervous system, and blood vessels [35]. Recent single-cell RNA-sequence (scRNA-seq) analysis revealed *ACE2* gene expression in multiple epithelial cell types across the respiratory tract, most notably in nasal epithelial cells. *ACE2* is also expressed in alveolar epithelial type II cells (ATII), the primary site of distal lung infection [36]. ACE2 is a critical mediator within the renin-angiotensin-aldosterone system, and thus is an important regulator of inflammation [37]. A key salutary function of ACE2 is the conversion of angiotensin II to angiotensin 1 to 7. This conversion limits the detrimental effects of angiotensin II, which include increased inflammation, thrombosis, and vasoconstriction [38]. However, ACE2 receptors are significantly downregulated and shed via ADAM17 (A disintegrin and metalloprotease 17) following SARS-CoV-2 cellular entry, consistent with markedly increased plasma angiotensin II levels in COVID-19 patients. As such, the

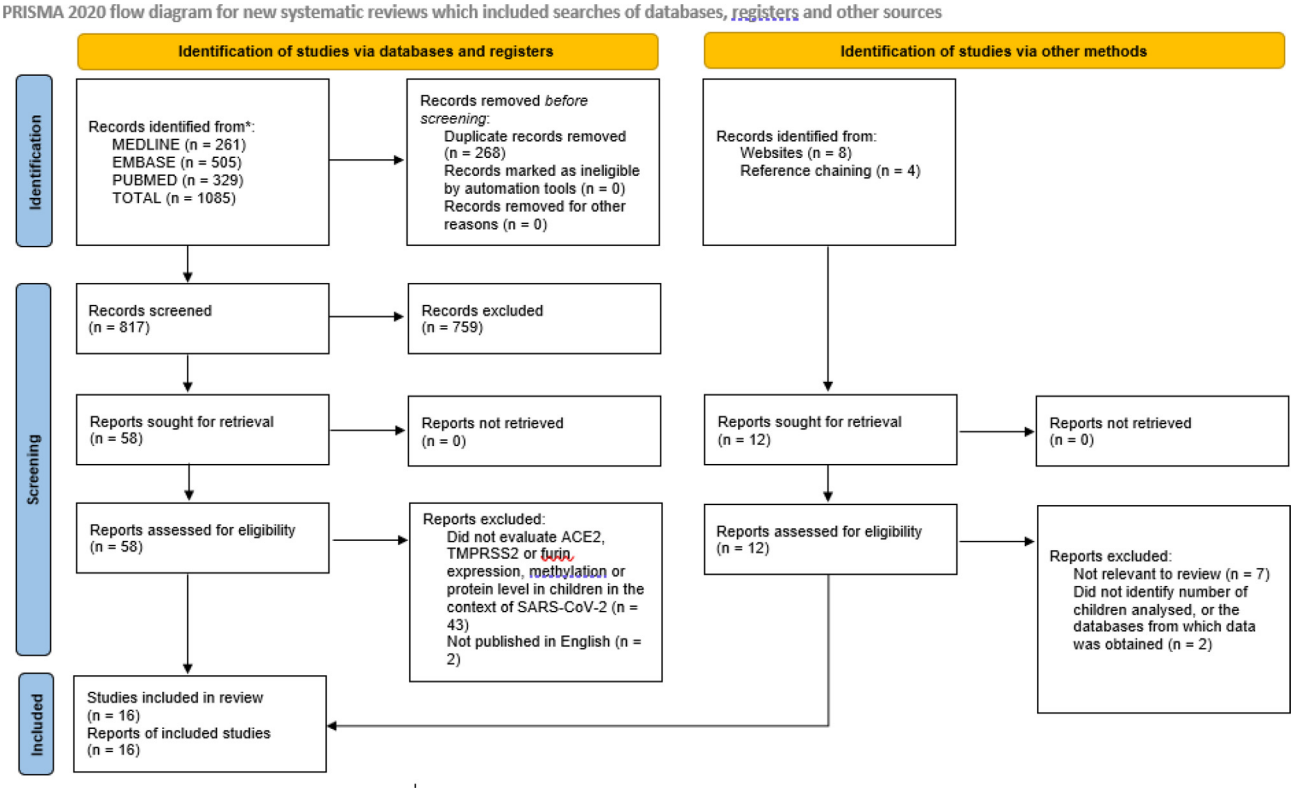

Fig. 2. PRISMA flow chart of article identification, retrieval, and inclusion.

beneficial effects of ACE2 are lost locally, resulting in worsened inflammation and disease [38,39]. This is supported by ACE2 knockout mice, who demonstrate a pathology analogous to acute respiratory distress syndrome [40]. Furthermore, several studies have found increased shedding of ACE2 facilitates viral replication, vascular permeability, and local inflammation, and thus an exacerbation of disease [41–43]. Therefore, not only does increased ACE2 expression increase disease susceptibility through its function as the primary receptor for SARS-CoV-2, it also contributes to increased disease severity through increased ADAM17-mediated shedding.

Nasal epithelial cells are the primary target cells for SARS-CoV-2 infection and replication [36]. Multiple studies included in this review demonstrated lower *ACE2* expression in nasal epithelial cells in neonates and children compared with adults [18,19,32]. Using several covariant-adjusted models, Bunyavanich et al. [18] found that this age-dependent relationship was independent of sex and asthma. Hence, reduced nasal *ACE2* expression may confer a lower risk of children acquiring SARS-CoV-2 infection than adults [25]. However, recent epidemiological studies have demonstrated similar infection rates across paediatric and adult cohorts, contradicting the notion of reduced receptor expression leading to lower viral infection [2,44].

Whilst the nasal epithelium is the primary site of infection, COVID-19 mortality and morbidity largely stem from lower respiratory infection. ACE2 is found on the apical surface of ATII cells and is colocalised with TMPRSS2 [45]. Significant intraindividual and interindividual heterogeneity of *ACE2* expression exists within distal lung epithelium, which may account for the varied conclusions reported in Table 2. Several studies have demonstrated an age-dependent increase in *ACE2* expression in distal lung epithelium. Muus et al. [21] in a meta-analysis of 107 scRNA-seq studies found *ACE2* and *TMPRSS2* gene expression in ATII cells was higher in adults compared with children [21]. Bickler et al. [23] also found a marked increase in *ACE2* expression in the elderly using genome-wide RNA-sequence profiles.

In contrast, several studies have not observed a difference in *ACE2* expression between children and adults, both in nasal epithelium and distal lung epithelium [24,26,27,45]. Zhang et al. [27] found ACE2-positive cells were generally decreased in the elderly in bronchial tissue samples, compared with children. However, ACE2-positive cells were largely distributed in the lower respiratory tract in the elderly, which contrasts the pattern of predominantly nasal expression in children. An increased proportion of distal ACE2-positive cells may increase sensitivity to SARS-CoV-2 infection in the distal airways and contribute to more severe infection via increased ACE2 shedding. Additionally, Zhu et al. [24] found no significant difference in expression of *ACE2* or *TMPRSS2* between children and adults, despite paediatric cells being less permissive to viral replication. Instead, they suggest that the innate immune response of children, specifically type I and III interferon responses, may contribute to the reduced severity for this group. Most studies included in this review acquired data from RNA-sequencing database, and as such do not necessarily reflect protein levels. Ortiz et al. [45] used a combination of scRNA-seq and immunohistochemistry and found ACE2 protein abundance did not correlate with age in nasal and distal lung epithelium.

Thus, the question remains whether *ACE2* expression and protein levels differ between paediatric and adult cohorts? A recent study by Wang et al. [46] demonstrated significant discordance between mRNA and protein levels of ACE2 across many tissue types, suggesting some degree of post-transcriptional or translational modification. There is also substantial intraindividual and interindividual heterogeneity, and many of the studies have limited sample sizes. It would be useful for future studies to combine techniques to compare expression and protein levels, such as RNA sequencing and immunohistochemistry. Future studies should also be adequately powered to enable appropriate sub-analyses according to age and sex. Whilst imperfect, on the balance of current evidence *ACE2* expression alone does not appear to explain reduced paediatric susceptibility to severe COVID-19. It appears that nasal ACE2 receptor levels may contribute minimally to risk of infection, whereas alveolar ACE2 receptor levels, in combination with other factors involved in SARS-CoV-2 immunity, play a role in determining disease severity.

ACE2 gene methylation

Differences in *ACE2* methylation have also been suggested as a potential factor in the age-related difference in susceptibility to severe COVID-19. *ACE2* hypomethylation has been demonstrated among African American children in the USA, a cohort of children with significantly higher rates of severe COVID-19 than Caucasian children. Corley et al. [47] also found that DNA hypomethylation near the transcription start site of the *ACE2* gene was associated with increasing age, however only tested adults [30]. Cardenas et al. [30] suggests that *ACE2* hypomethylation may promote increased transcription and expression of ACE2 protein, thus leading to increased SARS-CoV-2 infectivity and severity via a greater abundance of ACE2 receptors. However, given the discordance between ACE2 expression and protein levels, this cannot be assumed. As such, future methylation studies should concurrently assess both gene expression and protein levels, and should include children.

Soluble ACE2

Soluble ACE2 receptors also play an important role in SARS-CoV-2 pathogenesis. The soluble form of ACE2 circulates in small amounts in the blood and is capable of binding the SARS-CoV-2 S-protein on circulating viral particles [48]. *In vitro* studies show that soluble ACE2 can neutralise SARS-CoV-2 when fused to the Fc portion of immunoglobulin [49]. However, attempts to utilise human recombinant soluble ACE2 to inhibit SARS-CoV-2 infection have required concentrations much higher than is physiological in plasma [50,51]. At physiological levels, soluble ACE2 can facilitate SARS-CoV-2 entry through receptor-mediated endocytosis [52]. A recent study found that the administration of human recombinant soluble ACE2 at a physiological concentration initially increased viral loads from tracheal aspirates and nasopharyngeal swabs [53]. However, despite increased viral loads, the patient demonstrated a concurrent reduction in inflammatory cytokines, and thus the clinical relevance of the increase in viral load remains unclear.

Swärd et al. [28] analysed serum ACE2 concentrations from children and young adults, finding an age-dependent increase in soluble ACE2 concentrations. Additionally, soluble ACE2 was higher in men, who are also at a greater risk of severe infection. These data are supported by Pavel et al. [29], who compared serum ACE2 of infants and toddlers against that of adults. Increased ACE2 protein levels were observed in adult serum compared with infants and toddlers, as well as in males versus females. These study results are in contrast to work by Sharif-Askari et al. [32], who found no difference in plasma ACE2 levels between children and adults. A notable limitation of Sharif-Askari et al. [32] however was the assessment of ACE2 levels on peripheral blood mononuclear cells, which may not be directly comparable to serum levels.

Further studies are needed to investigate the role serum ACE2 plays during active SARS-CoV-2 infection, as well as to accurately characterise the role of ADAM17 and ACE2 shedding in disease pathogenesis. Current studies have not assessed serum ACE2 levels in elderly populations, the cohort most at risk of severe disease. Hence, while it remains plausible that higher serum ACE2 levels

Table 2
Primary information extracted from the final selected articles.

First Author and Publication Year	Patients	Number	Population Sample	Sample Type	SARS-CoV-2 Receptor Expression	Main Study Conclusions
Bunyavanich et al. 2020 [18]	Age < 10 years 10–17 years 18–24 years ≥ 25 years	45 185 46 29	4 to 60 years old 48.9% male 49.8% asthma	Nasal epithelium	ACE2	Expression of ACE2 in the nasal epithelium (primary site of infection) was lower in children compared with adults, using covariate-adjusted models
Heinonen et al. 2020 [19]	Newborns (mean 36 weeks gestation) Adults (30–60 years)	28 10	17 term, 11 preterm % male not specified Non-diseased	Nasal epithelium	ACE2 and TMPRSS2	Nasal epithelium expression of ACE2 and TMPRSS2 was lower in newborns compared with adults
Wang et al. 2020 [20]	30 weeks gestation 3 years 30 years	3 3 3	Newborn to 33 years old 67% male Non-diseased	Lung tissue specimens (small airway)	ACE2 and TMPRSS2	ACE2 and TMPRSS2 expression was lower in newborns and children, compared with adults
Muus et al. 2020 [21] Not yet peer-re-viewed	Children Adults	107 scRNA-seq and snRNA-seq studies (22 lung, 85 other diverse tissues)	Newborn to 80 years old 60% male Non-diseased	Lung tissue specimens & other diverse tissue specimens	ACE2 and TMPRSS2	ACE2 and TMPRSS2 co-expression in ATII cells was reduced in children compared with adults
Inde et al. 2021 [22]	Children ≤ 18 years) Adults (18–75 years)	6 94	9 to 75 years old % male not specified Non-diseased	Lung tissue specimens	ACE2 and TMPRSS2	ACE2 expression in distal lung epithelium cells increased with age. However, there was significant intraindividual and interindividual heterogeneity
Bickler et al. 2021 [23]	Children (≤10 years) Elderly (≥80 years)	14 33	1 to 96 years old 83% male Non-diseased	Human dermal fibroblasts (GSE 113,957)	ACE2	ACE2 expression showed a marked increase in the 80 + age group
Zhu et al. 2021 [24] Not yet peer-re-viewed	Children (2–7 years) Adults (21–35 years)	8 5	2 to 35 years old 46% male Non-diseased	Nasal epithelial cells	ACE2 and TMPRSS2	Whilst paediatric cells were less permissive to viral replication, there was no significant difference in expression of ACE2 or TMPRSS2
Ortiz et al. 2020 [25]	Children (0•5-9 years Adults (19–71 years)	7 22	0.5 to 71 years old 55% male 52% chronic comorbid condition (asthma, cystic fibrosis, CVD, COPD, DM, smoking)	Nasal biopsies, lung donors	ACE2	ACE2 was found on the apical surface of a subset of ATII cells and colocalised with TMPRSS2. The ACE2 protein was not reduced in children when compared with adults
Koch et al. 2021 [26] Not yet peer-re-viewed	Children Adults	7 healthy 36 SARS-CoV-2 24 RSV 9 IV 13 healthy 16 SARS-CoV-2	0.1 to 42 years old 42% male 19% pre-existing respiratory condition	Nasal epithelial cells	ACE2	No difference in ACE2 or TMPRSS2 expression was observed between children and adults. No increase in ACE2 and TMPRSS2 expression was observed during SARS-CoV-2 or other active viral infections
Zhang et al. 2021 [27]	Children (0–16 years) Adults (16–80 years)	173 126	0.2 to 80 years old 52% male 20% chronic comorbid condition (COPD, smoking, lung carcinoma, congenital pulmonary cyst)	Nasopharyngeal swabs and lung tissue specimens	ACE2	Compared with children, ACE2-positive cells generally decreased in the elderly and were mainly distributed in the lower pulmonary tract. Lung progenitor cells were also decreased in adults

(*continued on next page*)

Table 2 (*continued*)

First Author and Publication Year	Patients	Number	Population Sample	Sample Type	SARS-CoV-2 Receptor Expression	Main Study Conclusions
Swärd et al. 2020 [28]	Children (<18 years) Adults (>18 years)	824 241	6 to 25 years old 54% male 11% chronic comorbid condition (unspecified)	Serum ACE2	Soluble ACE2	Subjects with a higher risk of severe SARS-CoV-2 infection had higher soluble ACE2 (adults > children, and men > women)
Pavel et al. 2021 [29]	Children Adults	19 healthy 29 atopic dermatitis 17 healthy 55 atopic dermatitis	1.6 to 44 years old 53% male 70% atopic dermatitis	Serum ACE2	Soluble ACE2	Significantly higher ACE2 protein expression in adult serum compared with infants and toddlers
Cardenas et al. 2020 [30]	Children	547 (11.8–15.4 years)	11.8 to 15.4 years old 50.6% male 16.3% African American Disease status not reported	Nasal epithelial cells	*ACE2* DNA methylation	*ACE2* gene hypomethylation in cells from the nasal epithelium in African American children (greater rates of severe COVID-19 demonstrated amongst this group). Authors suggest this may lead to increased SARS-CoV-2 infectivity and severity via a greater abundance of ACE2 receptors
Schuler et al. 2020 [31]	Infants (<2 years) Children (3–17 years) Adults (54–69 years)	7 9 4	0.1 to 69 years old Male and female donors, % not specified Non-diseased	Lung tissue	TMPRSS2	Adults have higher TMPRSS2 expression and protein levels as opposed to either paediatric group. No significant difference observed between infants and children
Sharif-Askari et al. 2020 [32]	Children Adults	4 datasets for children groups (healthy and asthmatics) 15 datasets with different comorbidities	60% chronic disease (asthma, COPD, sarcoidosis, pulmonary fibrosis, smoker)	Blood, upper and lower respiratory tract tissue, and saliva	ACE2 and TMPRSS2	Age-dependent differential expression of ACE2 and TMPRSS2 in nasal and bronchial airways. No difference was observed in the serum expression levels of ACE2 and TMPRSS2 between children and adults
Tao et al. 2021 [33]	Children Adults	10 8	0.1 to 70 years old 33% male Non-diseased	Lung tissue	ACE2, TMPRSS2, furin	No significant difference in expression of ACE2, TMPRSS2 or furin in ATII cells. However, the number of AT2 cells expressing TMPRSS2 and furin was significantly higher in the lungs of adults compared with children.

ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane serine protease 2; scRNA-seq, single cell RNA sequencing; snRNA-seq, single nucleus RNA sequencing; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; RSV, respiratory syncytial virus; IV, influenza virus.

may predispose adults to higher viral loads and severe disease, additional research is needed prior to this conclusion being drawn.

TMPRSS2 and the role of circulating androgens

Transmembrane serine protease 2 (TMPRSS2) is a canonical protease which cleaves the SARS-CoV-2 spike protein to facilitate binding to the epithelial surface and is an important mediator of viral entry. The *TMPRSS2* gene, located on chromosome 21, has several androgen receptor elements which regulate gene expression [54]. Since prepubertal children produce significantly lower sex-steroid hormones, it is plausible that their *TMPRSS2* expression is lower, which may consequently impede viral entry. This notion is supported by *in vivo* data that demonstrated increased *TMPRSS2* expression in ATII cells following the administration of exogenous androgen in mice [55]. Further, males are significantly more likely to develop severe infection than females [56]. Additionally, androgen receptor gene variants that lead to increased androgen sensitivity (implicated in diseases such as androgenetic alopecia and prostate cancer) are associated with worse COVID-19 disease severity [57].

Several studies have demonstrated age-dependent expression of *TMPRSS2*, both in nasal and alveolar tissue samples [21,31,33]. Muus et al. [21] showed increased co-expression of *ACE2* and *TMPRSS2* in adult alveolar epithelial cells. Although Tao et al. [33] found no difference in *TMPRSS2* expression at a single-cell resolution, they found an increased proportion of adult ATII cells expressed *TMPRSS2* compared with cells from children. In the context of increased co-expression of two key binding factors, these findings suggest adult alveolar cells may be more permissible to viral entry than children. Furthermore, Schuler et al. [31] combined RNA quantification and immunofluorescence techniques to analyse the expression and protein levels of TMPRSS2, finding both to be significantly increased in adults compared with children. In contrast, several other studies have found no difference in *TMPRSS2* expression between adults and children [24,26,33]. Koch et al. [26] assessed the nasal mucosa following SARS-CoV-2 infection and found that *TMPRSS2* expression was not associated with age. Furthermore, they observed that the immune response at the site of primary infection is similar between children and adults, suggesting that factors beyond the nasal mucosa are responsible for the observed differences in disease severity.

Despite the findings of Koch et al. [26], it is biologically plausible that age-related differences in TMPRSS2 exist, particularly in distal lung tissue. The developmental regulation of TMPRSS2, a key protease implicated in SARS-CoV-2 entry, provides a rationale that may explain the reduced incidence of severe COVID-19 disease amongst paediatric patients. Furthermore, age-related differences in TMPRSS2 provide an opportunity to explore TMPRSS2 inhibitors as potential therapies for SARS-CoV-2. This concept is supported by data from Hoffman et al. [14], who recently showed that small-molecule TMPRSS2 inhibitors partially block SARS-CoV-2 entry [14]. Future research should focus on validating these findings by measuring TMPRSS2 in distal lung tissue, both at baseline and during SARS-CoV-2 infection. Analogous to ACE2 studies, future research should also include protein quantification techniques to support differences observed in gene expression.

Other receptors and future direction

There are limited studies to date on other potential SARS-CoV-2 receptors, such as furin. Furin is an endoprotease which can cleave the SARS-CoV-2 S-protein, enabling membrane fusion and viral internalisation [14]. The only study included in this review involving furin, performed by Tao et al. [33], did not detect an age-dependent difference in the average expression level of furin in ATII cells at a single-cell resolution. However, the percentage of furin-expressing ATII cells was higher in adult lungs compared with children. The biological significance of these findings is not currently well understood and small sample numbers limit the conclusions drawn from this study. Further, age-dependent differences in heparan sulfate, a necessary co-factor for SARS-CoV-2 binding, have not been investigated. This is despite a recent study which showed unfractionated heparin, non-anticoagulant heparin, and heparin lyases potently blocked SARS-CoV-2 spike protein binding and subsequent infection [58]. This is thought to be explained by the homologous structure of heparin and heparan sulfate, which allows exogenous heparin to bind and disrupt the SARS-CoV-2 receptor binding domain [59]. Additionally, animal models have found age-specific differences in heparan sulfate on vascular epithelium [60,61]. Together, these findings highlight the importance of performing further research to assess the role of heparan sulfate as a potential therapeutic target for SARS-CoV-2.

A limitation of this review is that most of the RNA-sequencing data was acquired from publicly available databases. As a result, researchers often did not assess protein levels to provide tissue validation of their findings. Many of the studies were underpowered, particularly those that extracted RNA expression data at a single-cell resolution. Only studies published in English were included. Finally, most publications to date focus on two key binding factors; ACE2, TMPRSS2. There is significant scope to explore other novel factors implicated in SARS-CoV-2 binding, such as furin and heparan sulfate.

CONCLUSION

Increasing age is the strongest predictor of disease severity in COVID-19. Whilst the exact mechanisms underpinning this observation are not fully understood, there is mechanistic plausibility that viral binding factors, such as ACE2 and TMPRSS2, play an important role. The current literature reporting on age-dependent differences in viral binding factors is inconclusive. However, it remains possible that a complex interaction between host factors and one or more SARS-CoV-2 binding factors exists. Future studies elucidating this interaction will provide important insights into future preventative strategies and therapeutic interventions in both paediatric and adult populations.

DIRECTIONS FOR FUTURE RESEARCH

- Many studies included in this review assessed RNA-sequencing data which was acquired from publicly available databases. As a result, researchers often did not assess protein levels to provide tissue validation of their findings.
- Given the significant discordance demonstrated between mRNA and protein levels of ACE2, it is highly recommended that future research combines sequencing and protein quantification techniques.
- There is also significant scope to further investigate age-dependent differences in heparan sulfate, a necessary co-factor for SARS-CoV-2 binding. This is particularly pertinent given a recent study which found that unfractionated heparin, non-anticoagulant heparin, and heparin lyases potently block SARS-CoV-2 spike protein binding and subsequent infection.
- Additionally, future studies should assess dynamic changes in SARS-CoV-2 binding factors during active infection. This is important given the shedding of ACE2 that occurs following viral entry.

- Finally, future studies should focus on assessing differences in binding factors in distal lung tissue, given the clinical manifestations of COVID-19 largely stem from alveolar epithelial type II cellular infection.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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