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Covid-19 and adolescent acute kidney injury: Renal recovery with combined enalapril and estrogen therapy

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ABSTRACT

Covid-19 in adolescence with multisystem inflammatory injury (MIS-C) is a newly described condition sharing key features with Kawasaki disease and toxic shock syndrome. A May 2020 United Nations WHO brief covering findings from North America and Europe drew notice to this acute post-viral illness characterized by severe, diffuse hyperinflammation leading to multiorgan failure. While females diagnosed with Covid-19 generally have more favorable outcomes than males, this protection is negated by a low estrogen state. This case reports on acute kidney injury/MIS-C with amenorrhea from ovarian insufficiency in childhood, itself an uncommon presentation of idiopathic hypogonadism. Three exon variants were previously identified in a healthy, phenotypically normal 46,XX adolescent who subsequently underwent whole genome sequencing (WGS). She had only two spontaneous menses with a provisional diagnosis of premature ovarian insufficiency made by age 15. Against this background, Covid-19 infection necessitated hospital admission where progressively reduced renal function was a prime component of MIS-C. Combined angiotensin-converting enzyme inhibitor plus transdermal estrogen replacement therapy resulted in normalized estimated glomerular filtration rate (eGFR) from baseline 43 to 68 ml/min/1.73 m^2, post-treatment. Serum cystatin-C also improved during this interval from 1.69 to 1.19 mg/L. Among 7 Covid-19 high risk intron variants identified was rs3131294 (6p21), near NOTCH4. Another finding at rs8068318 (17q23) was associated with creatine level and eGFR. This is the first work to explore Covid-19 and associated kidney injury as a component of MIS-C at the intersection of rare multigene variants and functional ovarian loss. The context of transition from adolescence to adulthood is also considered, where successful recovery of renal function was achieved with combined enalapril and supplemental estrogen.

1. Introduction

Secondary amenorrhea from ovarian insufficiency before age 40yrs is uncommon and is exceptionally rare in adolescence [1]. When present, renal disease complicates management of hypoestrogenism as the reduced kidney function will impact elimination half-life of hormone replacement [2,3]. When the Coronavirus disease 2019 (Covid-19) damage sequence extends to multisystem inflammatory syndrome, direct renal cytopathic effect, tubular injury from cytokine storm, or immune-mediated glomerulonephritis are acutely relevant [4]. In this condition, enalapril and other ACE-inhibitors conserve kidney tissue by lowering intraglomerular pressure [5] and estrogen can also play a beneficial role. In practice, renal status is usually monitored via estimated glomerular filtration rate (eGFR) derived from serum creatinine clearance, but this is less reliable in underweight conditions where serum cystatin C is preferred [6]. Here an unusual alignment of *RUNX2, SALL1,* and *SAMD9* variants is presented in the context of additional intron mutations, where pediatric Covid-19 illness and renal injury emerged in the setting of early ovarian insufficiency.

2. Methods & results

Accompanied by her mother, an 18 yr old nonsmoking Caucasian female attended for second opinion to discuss secondary amenorrhea, chronically low serum estrogen, elevated serum FSH and creatinine and prior hospitalization for Covid-19. There was no history of proteinuria or

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electrolyte imbalance. Menarche was at age 11 and normal ovarian, uterine, and cervical structures were confirmed on abdominal ultrasound and/or pelvic computed tomography. However, menses had completely ceased by age 13 and serum FSH was consistently above 100mIU/ml thereafter. Serum anti-Mullerian hormone was also undetectable. She was evaluated by a reproductive endocrinologist where clomiphene challenge test showed bilateral follicular response. However, by age 15 no specific explanation for 'ovarian failure' was offered.

In late 2020, the patient—then age 17½—was evaluated for fever, headache, fatigue, nausea and emesis. Pregnancy test and Covid-19 screen were negative; oral vancomycin was initiated for *C. difficile* which was diagnosed incidentally. When temperature increased to 39.7 °C two days later, a second Covid-19 test was positive. Now underweight, the patient was admitted to hospital where supportive care was provided and vancomycin was adjusted to i.v. administration. Three days later, her body mass index was 16.8 kg/m^2 as fever, diarrhea, and vomiting continued. Proteinuria was accompanied by elevated serum creatinine (3.5 mg/dL). The diagnosis was revised to Covid-19-associated multisystem inflammatory syndrome in children (MIS-C) and she was transferred to the intensive care unit (ICU). Next, vancomycin was halted while remdesivir, cefepime, dexamethasone, and intravenous immunoglobulin therapy were started. Erythrocyte sedimentation rate, C-reactive protein, and D-dimer level - all of which had been markedly abnormal - gradually normalized in ICU. The patient was discharged home after 15 days with local outpatient renal clinic consult and comprehensive genetics panel set for follow-up.

Her kidney function was assessed in local outpatient nephrology unit by eGFR formulae based on serum creatinine (Cr) alone [7].

Cr eGFR = $142 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.200} \times 0.9938^{age} \times 1.012$;

serum cystatin C alone [8],

Cystatin C eGFR = $133 \times \min(S_{cys}/0.8, 1)^{-0.499} \times \max(S_{cys}/0.8, 1)^{-1.328} \times 0.996^{age} \times 0.932$;

and both [9],

Cr + cystatin C eGFR = $135 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.200} \times \min(S_{cys}/0.8, 1)^{-0.323}$.

x $\max(S_{cys}/0.8, 1)^{-0.778} \times 0.9961^{age} \times 0.963$.

where:

eGFR = estimated glomerular filtration rate; S_{cr} = serum creatinine (mg/dL); S_{cys} = serum cystatin C (mg/L); $\kappa = 0.7$ (for females); $\alpha = -0.241$ (for females); $\min(S_{cr}/\kappa, 1) = \min S_{cr}/\kappa$ or 1.0; $\max(S_{cr}/\kappa, 1)$ is the maximum of S_{cr}/κ or 1.0; age (yrs).

Precautionary enalapril 2.5 mg/d was prescribed while renal biopsy was scheduled; the steep rise in cystatin C was sufficiently alarming (see Fig. 1) that the patient and her family were advised about renal transplant options.

Exon sequencing had identified variants Q253H in *SALL1* and R824Q in *SAMD9* plus a previously unreported multiexon 3′ terminal duplication of *RUNX2,* as described earlier [10] although these mutations were considered unrelated to ovary. On renal biopsy, six of 19 fields showed extensive/total fibrosis and two were partially scarred with foot process effacement and mild interstitial chronic inflammation, all consistent with focal segmental glomerulosclerosis (see Fig. 2).

Whole genome sequencing analysis (Nebula Genomics; San Francisco USA) began with an emphasis on regions with known association with Covid-19 and renal function. Here, seven 'critical Covid-19 illness' intron variants were confirmed: rs73064425 (3p21), rs143334143 (6p21), rs2109069 (19p13), rs10735079 (12q24), rs3131294 (6p21, near NOTCH4), rs9380142 (6p22), and rs2236757 (21q22). Population variant frequencies (VF) for these seven ranged from 12 to 90 %. In addition, two intron variants associated with serum creatinine elevation were identified: rs8068318 (17q23) and rs3127573 (6q25), VF = 27 % and 13 %, respectively.

Transdermal estradiol (phase-in at 25mcg, with increase to 100mcg)

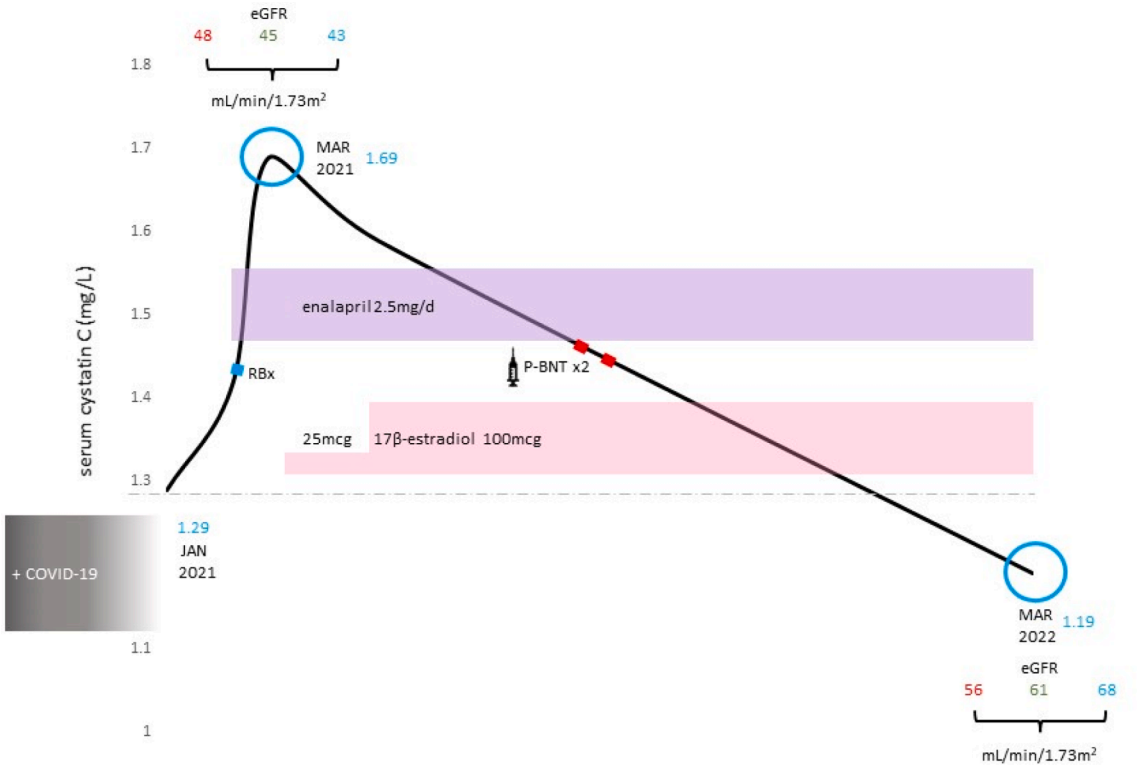

Fig. 1. Post Covid-19 serum cystatin-C pattern, demonstrating onset of acute kidney injury within 6–8 weeks of infection (grey bar). Maximum cystatin-C (upper circle) corresponds to peak renal impairment, which responded to daily enalapril (purple bar) and tapered transdermal estrogen replacement (pink bar). Estimated glomerular filtration rate (eGFR) calculations were derived from serum creatinine (red), and serum cystatin C (blue) are shown. Calculations using both eGFR markers (green) are included. Renal biopsy = blue square; Pfizer/BioNTech coronavirus vaccination series = red square x2.

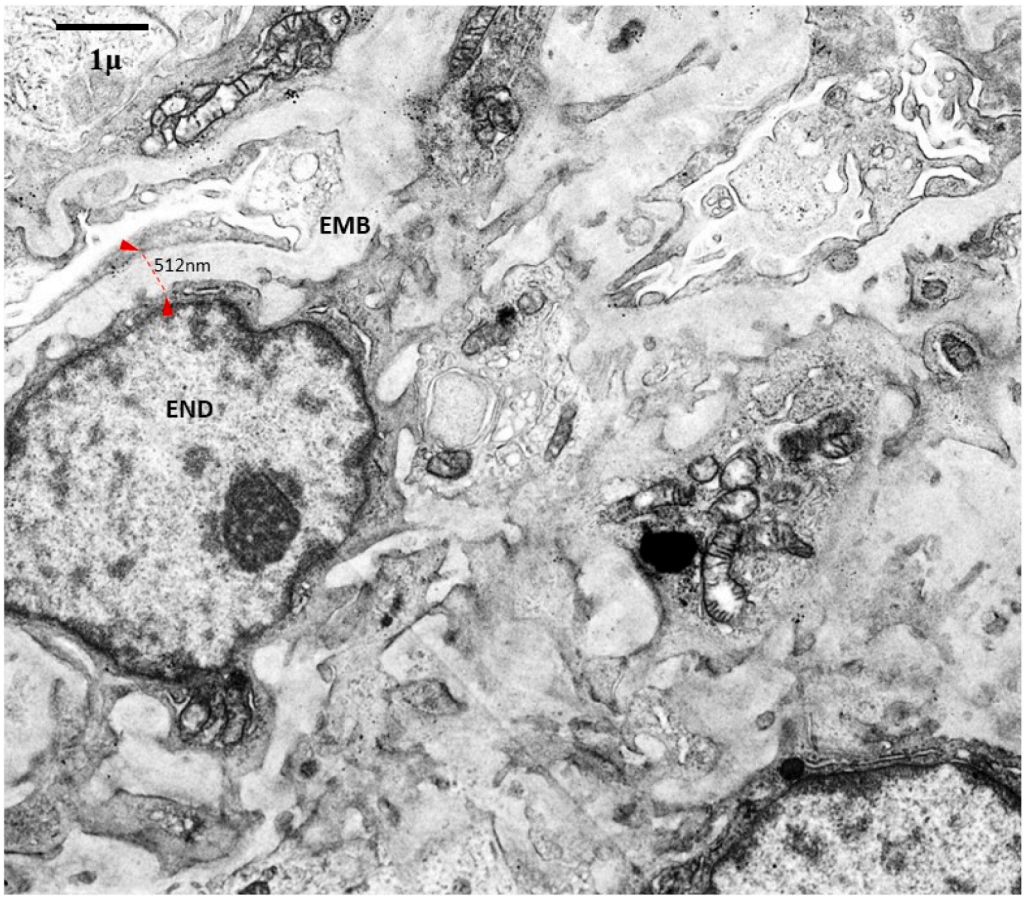

Fig. 2. High-voltage (80 kV) transmission electron microscopy view (x1500) of renal ultrastructure (female, age 17½ yrs) during post-Covid-19 multisystem inflammatory syndrome in children including acute kidney injury. END = endothelial cell, EBM = basement membrane thickness, 512 nm (red).

was initiated within one month of angiotensin-converting enzyme inhibitor therapy, as steady declines in serum creatinine & cystatin C showed improvement in kidney function. As with the enalapril, supplemental estrogen was well tolerated. Management is coordinated across nephrology, endocrine, and genetics clinics with regular checkups and monthly complete blood counts to guide the need for repeat renal biopsy, or other interventions.

3. Conclusions

Concurrence of genetic variants, secondary amenorrhea from ovarian insufficiency, Covid-19, and MIS-C with renal injury presents an unusual opportunity to describe how these factors can merge clinically. As the understanding of MIS-C and Covid-19 remains limited, data on *RUNX2, SALL1* and *SAMD9* mutations in this setting remain sparse. *SAMD9* has been placed among 'hub genes' from protein-protein network analysis during Covid-19 [11] but thus far no research has linked either *RUNX2* or *SALL1* with Coronavirus or MIS-C. Of note, the same R824Q variant in *SAMD9* was identified in a boy age 15 months with adrenal insufficiency, ambiguous genitalia, dysmorphic features and global developmental delay [12]. Myelodysplasia and monosomy 7 were seen on bone marrow biopsy, but his *SAMD9* variant occurred in the absence of any *RUNX2* or *SALL1* mutation.

Why might this *SAMD9* variant fail to produce the expected gain-of-function effect in our patient via heightened action of the gene's growth repressor product? Here the finding of multiple intron variants associated with clinical outcomes may be relevant, and forms the basis of additional research. The answer may also be that in isolation, an altered SAMD9 protein can be resolved by monosomy 7 or secondary somatic nonsense/frameshift loss-of-function change. This adaptive reversion rectifies a solitary SAMD9 disruption [13], but the co-presence of *RUNX2* and *SALL1* variants here cannot be discounted. Indeed, this convergence of all three variants together appears to have enabled a milder phenotype. While dysfunction at *RUNX2, SALL1,* or *SAMD9* could be unrelated to ovarian insufficiency, simultaneous changes across these 'master regulators' support an autosomal influence on overall gonadal function [14]. Regulatory sequence disruptions due to multiple intron variants found here, particularly those with known clinical correlations to relevant disease processes, must also be considered.

Females with Covid-19 generally have better prognoses than males—a protective effect attributed to estrogen—but this advantage disappears after menopause [15]. Recent research has found an androgen-sensitive transmembrane serine protease facilitates SARS-CoV-2 access to cells, so women with relative androgen surplus might be at increased risk of Covid-19 infection by this pathway. Because our patient was effectively post-menopausal for at least one year before her Coronavirus infection, the usual benefits of functional ovaries could not be realized.

The hyperinflammatory renal presentation of MIS-C is congruent with features of Kawasaki disease and toxic shock syndrome, particularly high fever and multisystem compromise (*e.g.*, cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic, or neurologic). CDC national surveillance data on Covid-19 patients age $\leq$ 24yrs find only 2.5 % of this group require hospitalization, and fewer still (0.8 %) need ICU support.

The current problem highlighted different ways to measure renal function, so we incorporated eGFR calculations using all three age-adjusted formulaic standards. Cystatin C is present in essentially all human tissues as an amino acid product of nucleated cells, and is a highly sensitive marker for preclinical loss of renal function. The age-related rise in serum cystatin C is also a good predictor for health

outcomes such as cardiovascular death and diminishing cognitive function. Interestingly, osteoblast response to cystatin C has been examined in a murine model with special focus on bone matrix mineralization and bone growth. Activity of RUNX2 was boosted in cystatin C-treated cells, suggesting that cystatin C drives osteoblast differentiation, mineralization, and bone formation. Moreover, renal failure itself has been shown to cause enhanced RUNX2 expression via dampened microRNA-93 levels. MicroRNA-93 inhibits the Wnt/β-catenin pathway, targeting Transcription factor 4 (TCF4) with improved renal function by reducing vascular calcification.

While nephrons—like oocytes—are not believed to retain any regenerative capacity in adult mammals, *in situ* regrowth of nephrons has been observed in an animal model and specific expression of SALL1 was observed among markers promoting this process. The role of ACE-inhibitors in managing renal disease is well established and estradiol can also provide protection in acute injury. This case validates application of both agents in tandem, and ovarian and renal findings described here should extend the characterization of how these specific variants impact quality of life.

Authors' contributions

ESS developed the research and organized initial drafts; ESS, SHW, and APHW reviewed the literature; SHW and APHW supervised the project and editorial aspects. All authors read and approved the final manuscript.

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.

Data availability

Data will be made available on request.

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