Peer Review File

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Reviewer comments

Reviewer A

This is an important and very relevant topic to be discussed because of the relative and evident lack of the systematized data. This review can be of great help for the practitioners in the field of pediatric endocrinology, nephrology and for the researchers on hypertension in general. Here are the observations to be considered by the authors:

Comment 1: Line 32-33 and 42-45: Low renin and aldosterone levels causing hypertension by excessive water reabsorption as monogenic form is contradictory to high renin / aldosterone levels causing the same... Maybe it should be written in another manner. It seems that both conditions cause the same effect, but it doesn't appear how or why. In the abstract it is not clear if these types of HT are related to age/ gender."

Reply 1: Abstract, page 2, lines 39-41 and 42-45: Reviewer A outlines one of the key points of our manuscript which is that not only high renin but also suppressed renin levels are an indicator of hypertension. This is has already been stated in the abstract: "While general practitioners and nephrologists are well aware of the causes and the long-term consequences of elevated renin and aldosterone levels, the opposite situation with low renin and/or low aldosterone levels is frequently underappreciated." Reviewer A is correct that both conditions (low renin and high renin) can result in hypertension. We respectfully disagree with reviewer that no mechanism ("…but it doesn't appear how or why…") is provided. The mechanism for the low renin is provided in lines 42-45: "The low renin and aldosterone levels may be indicators of inherited (especially when associated with hypokalemia), monogenic forms of hypertension stimulating excessive tubular sodium and water absorption which subsequently results in plasma volume expansion and hypertension." Therefore, we would prefer not to change the abstract.

Changes in the text: not applicable.

Comment 2: "Line 79-81 - statement without reference."

Reply 2: We added references 15 and 16.

Changes in the text: This sentence corresponds now to page 3, lines 65- 69: "Primary

hypertension is polygenic and results from complex interaction among different genes and environmental factors (15). On the other hand, secondary hypertension can be caused by a large number of different conditions that can be either acquired secondary to medication or related to renovascular, endocrine, oncologic, or neurological problems (16) (Table 1)."

Comment 3: Line 127 - statement without reference, in this particular case it should be of interest to provide reference

Reply 3: References 42 and 16 were added.

Changes in the text: This sentence corresponds now to page 7, lines 143-154 "It may seem difficult to decide which child to work up for hypertension. Although the prevalence of inherited hypertension is unknown and thought to be rare, there are increasing numbers of reports about patients with inherited hypertension (42) (Table 2). With this manuscript, we attempt to increase the sensitivity and suspicion of the general provider. The level of scrutiny may be low as children with inherited forms of hypertension may initially present without severe hypertension and the associated electrolyte disorders may be absent. Work-up of hypertension starts with a good history and physical exam. The history should focus on prematurity, pain, medications, snoring (as obstructive sleep apnea also contributes to hypertension), and very important the family history. The family history should not only include kidney disease, hypertension, and need for a kidney transplant but also myocardial infarctions, aortic aneurysms, and strokes (16)."

Comment 4: Line 134-136 - statement already present, in other form, in the first paragraph (123-125). Also, I suggest avoiding: "please keep in mind"

Reply 4: We deleted the repetitive statement and changed the wording.

Changes in the text: Page 7, lines 156-159: "Laboratory work up should include a urinalysis, chemistry panel including electrolytes, BUN, and creatinine, lipid profile (fasting), HgbA1c, liver enzymes, TSH, T4, cortisol (morning level), renin, aldosterone, and CBC (11, 20). The hyperkalemia linked with Gordon syndrome is not always present (29)"

Comment 5: Line 136-147 - provide references for the mentioned entities.

Reply 5: References 43-48 were added.

Changes in the text: This sentence corresponds now to page 7, lines 159-171: "The hyperkalemia linked with Gordon syndrome is not always present (43). Hypokalemia is only

diagnosed in 50% of Glucocorticoid-Remediable Aldosteronism (GRA) (44) and is not persistently diagnosed in all patients with Apparent Mineralocorticoid Excess (AME) (45) or Liddle's syndrome (46). In most of the inherited forms of hypertension mild metabolic alkalosis is detected except for Gordon syndrome which usually is associated with metabolic acidosis. Urine electrolytes are not helpful in the diagnosis. Perhaps the most useful tools in the detection of inherited forms of hypertension are the renin and aldosterone levels (47). Preferably, these parameters should be obtained prior to therapy, in particular when an ACE inhibitor or ARB is used. A helpful tool to assess for increased aldosterone activity is the plasma aldosterone (ng/dl)-to-plasma renin activity (ng/ml/h) ratio, which is an indicator for excess aldosterone even in the context of a normal plasma aldosterone level. An aldosterone-to-renin ratio above 30 is consistent with primary aldosteronism (PA) (48)."

Comment 6: Line 152 - The renin-angiotensin-aldosterone system (RAAS) - maybe this section should be the first one after introduction and after that work on hypertension (only suggestion)?

Reply 6: We rewrote this whole section focusing on the opposite roles of ACE and ACE2 in hypertension and other physiologic processes. We touched upon the possible role of imbalance between ACE and ACE2 in COVID-19 infections to make this section more current. We also moved this section to start before the Work up for Hypertension section as suggested.

Changes in the text: This sentence corresponds now to page 5-6, lines Line 100-141: "The RAAS system has been extensively studied for its role in maintaining fluid homeostasis and blood pressure control (23). Upregulation of renin and aldosterone activity due to renal scarring, frequent UTIs, and chronic kidney disease is a major cause for hypertension with kidney disease (24). In addition to fluid balance and blood pressure control, RAAS plays an important role in chronic kidney disease progression (25), cardiovascular remodeling (26), activation of the thirst center and sympathetic nervous system (27), obesity and metabolic syndrome , inflammation and immune activation (18), lung (28) and liver fibrosis (29). Renin is an aspartyl-protease produced by the juxtaglomerular cells and the collecting duct. It converts angiotensinogen, a 14 amino acid peptide produced mainly in the liver, into angiotensin I (Ang-I), a 10 amino acid peptide (30). Renin secretion is regulated by mechanisms that reflect changes in blood volume and subsequently glomerular filtration (31). This includes kidney baroreflex and Chloride (Cl^-) delivery at the level of the macula densa in addition to vasoactive molecules such as nitric oxide, adenosine, and prostaglandins (32). Angiotensin I (Ang-I) has several ensuing fates that

depends on the type of angiotensin converting enzymes present. This has been thoroughly reviewed by Silva et al in the paper discussing the interaction of RAAS with coronavirus (33). In the classical pathway, Ang-I is cleaved by Angiotensin Converting Enzyme (ACE), a dicarboxypeptidase located on the surface of endothelial cells mainly in the pulmonary and intestinal capillaries (34), into an 8 amino acid peptide Angiotensin II (Ang-II). Ang-II is the effector molecule of the classical pathway. It has opposing effects depending the receptor it binds. Activation of Angiotensin Receptor Type 1 (AT1R) will overall lead to increase in blood pressure, sodium retention, inflammation, remodeling, and fibrosis mediated by vasoconstriction, stimulation of aldosterone production and anti-diuretic hormone (ADH) production, and activation of sympathetic nervous system (SNS) tone (26). On the other hand, activation of the Angiotensin Receptor type 2 (AT2R) has an opposite effect that results in lowering blood pressure and increased sodium excretion. However, AT2R expression is significant during fetal development and early neonatal life (31). In the alternative pathway, Ang-I is cleaved by ACE2, a monocarboxypeptidase integral membrane protein (35). ACE2 is cleaved by ADAM17, a metalloprotease, and released into the blood stream. It cleaves Ang-I to generate Ang-(1-7), a heptapeptide, that preferentially binds Mas receptor (28). Although the downstream molecular pathways of Mas receptor activation are not fully understood, most of the experimental studies showed an overall counter Ang-II effect including vasodilation, decreased aldosterone and anti-diuretic hormone production, decreased inflammation and reactive oxygen species production (36, 37). Due to COVID-19 pandemic, ACE2 has gained attention in the scientific community (38). ACE2 acts as viral receptor for COVID-19. The binding of the virus leads to ACE2 entry into the cell rendering the enzyme unavailable to counter the ACE effect (39). The imbalance between Ang-(1-7) and Ang II is associated with disease severity and seems to play a role in ARDS commonly seen in severe COVID-19 infection (40, 41)."

Comment 7: Line 184-185 - is it possible to give incidence / prevalence estimation of these conditions? You have already stated that it is unknown, but it seems that it is not that rare at all...

Reply 7: Unfortunately, it is hard to give an estimation of prevalence or incidence for such conditions due to discovery of new mutations and variable penetrance. What we know is that although still considered uncommon, the prevalence/incidence of such conditions is higher than previously thought.

Changes in the text: not applicable.

Comment 8: Line 475 and 494 - "This is another" - attention to the style (repeated in two paragraphs).

Reply 8: Thank you for your remark. We changed the wording in 494.

Changes in the text: This sentence corresponds now to page 22, lines Line 498-499: "This type of familial hyperaldosteronism is caused by autosomal dominant germline mutations in *CACNA1H*."

Comment 9: Tables - the format of the tables is not standard. I suggest reconfiguring all the tables. (Two horizontal lines at the top that mark only the first row and one in the bottom only, the last one and nothing in the middle. Also, no vertical lines).

Reply 9: We deleted tables 1 and 2. We reconfigured table 3 (this is now table 1). Please see below.

Changes in the text:

Table 1. Causes of secondary hypertension.

Age	***Primary or Secondary***	***Causes for secondary hypertension***
Birth to 1 year	Secondary (99%)	**Neonatal:** Maternal hypertension, maternal substance abuse, antenat steroids, maternal obesity, and maternal diabetes mellitus, low birthweigh hypoxia, prematurity **Medications:** steroids, indomethacin, vasopressors, bronchodilator theophylline, caffeine, vitamin intoxication, pancuronium, erythropoietin **Neurologic:** seizures, increased intracranial pressure, intracrani hemorrhage, pain **Renal:** Renal parenchymal disease, congenital nephrotic syndrom ARPKD, renovascular defect, cortical necrosis **Pulmonary:** Bronchopulmonary dysplasia **Endocrine:** Congenital adrenal hyperplasia, hyperthyroidisn hyperaldosteronism, adrenal hemorrhage

		Neoplasia: Wilms tumor, Neuroblastoma, mesoblastic nephroma **Cardiac:** Coarctation of aorta, patent ductus arteriosus
Age 1-12 years	Secondary (70-85%) Primary (15-30%)	**Medications:** steroids, NSAIDs, vasopressors, bronchodilators, vitamin intoxication, pancuronium, erythropoietin, herbal medication decongestants, oral contraception, cyclosporine, tacrolimus, ADH medications **Neurologic:** seizures, increased intracranial pressure, intracrani hemorrhage, pain **Renal:** acute kidney injury, tuberous sclerosis, multicystic-dysplast kidneys, obstructive uropathy, reflux uropathy, renal hypoplasi interstitial nephritis, pyelonephritis, cortical necrosis, hypercalcemia **Renovascular disease:** renal artery stenosis, renal venou thrombosis, midaortic syndrome, vascular compression **Endocrine:** hyperthyroidism, hyperaldosteronism, congenital adren hyperplasia **Neoplasia:** Wilms tumor, pheochromocytoma **Cardiac:** Coarctation of aorta
Age 12-18 years	Primary (85-95%) Secondary (5-15%)	Same causes as in the 1-12 year group

Comment 10: Table 2- is this table necessary? Maybe it could be provided better as a figure (one column only) or just stated in the body text.

Reply 10: We deleted tables 1 and 2.

Changes in the text: not applicable.

Comment 11: Table 3 - if possible, put the table in standard format and add the references of frequencies (primary or secondary).

Reply 11: We placed table 3 (now table 1) in standard format.

Changes in the text: Please see table 1 provided in reply to comment 9.

Comment 12: Table 4 - if possible, add the onset of the hypertension and/or hydro-electrolyte disturbance (infancy, school age, adolescence, adulthood, or similar) and estimated frequency of each condition. Inheritance is AD for almost all conditions, with one exception for AME, thus it could be mentioned in legend beneath the table and eliminate the column (suggestion).

Reply 12: We removed the "Inheritance" column and added "Onset" column as suggested. As mentioned before, it is hard to give an estimation of prevalence or incidence for such conditions due to discovery of new mutations and variable penetrance. Please see table 4 (now table 2) at the end of this document.

Changes in the text:

Table 2. Summary of the different causes of monogenic hypertension.

Monogenic HTN (Low Renin)	**Aldosterone Level**	**Genetic defect**	**Mechanism**	**Onset**
Liddle Syndrome	low	*SCNN1B* *SCNN1G*	Increased ENaC expression and activity Decreased ENaC degradation	Early adolescenc young adulthood
Gordon Syndrome (PHAII)	normal to high	*WNK-1* *WNK-4* *KLHL3* *CUL3*	Increased NCC activity Increased ROMK channel internalization	Infancy, ea childhood, adolescenc early adulthood
Apparent Mineralocorticoid excess (AME)	low	*HSD11B2*	Inactivation of 11HD2 Increased cortisone binding to MR	Infancy
Hypertension exacerbated by pregnancy (Geller Syndrome)	low	*NR3C2*	Activation of MR	Young adu
Glucocorticoid-remediable aldosteronism (FH-1)	high	*CYP11B1/CYP11B2* cross over	ACTH mediated-aldosterone	Late childhood,

			production	early adolescents
FH-2	high	*CLCN2*	Depolarization of granulosa cells	Adult
FH-3	high	*KCNJ5*	Depolarization of granulosa cells	Early childhood
FH-4	high	*CACNA1H*	Increased calcium influx in granulosa cells	Mid childhood

Note that all the listed conditions are autosomal dominant except for AME which is autosomal recessive. Ag different penetrance of the same mutation. ENaC: amiloride sensitive- epithelial (Na^+) channel, NCC: sodiu type II, ROMK: renal outer medullary potassium (K^+) channel, *WNK*: with no lysine serine/threonine protei gene, 11HD2: 11β-hydroxysteroid dehydrogenase type 2 enzyme, FH: familial hyperaldosteronism, *CYP11B* gene, *CLCN2*: voltage gated chloride channel gene, *KCNJ5*: inward rectifying K channel gene

Comment 13: Figures - the lack of abbreviations' legend and the title of each figure. It would be better if all figures and tables were presented in the continuity of the text, and when first mentioned, and not at the end of the manuscript, for better understanding and following.

Reply 13: We added titles and abbreviations for each figure. As for the placement of the figures and tables within the text, this is usually done by the journal editorial body based on their standard format.

Changes in the text: not applicable.

Comment 14: Figure 2 - is it possible to provide better resolution of the image? Not all the letters are visible.

Reply 14: We provided a higher resolution picture. Please see attached (Figure 2 PNG).

Changes in the text: not applicable.

Reviewer B

Mashmoushi and Wolf aimed to revise different forms of hypertension characterized by low

renin/low aldosterone and low renin/high aldosterone levels, how to diagnose these forms of hypertension, and how to treat them.

General comments: This review is well written and contains relevant data. I have only some suggestions to improve readability.

Comment 1: The introduction is very long and without a clear focus on the subject of the review. I recommend the authors to focus on the aims of the review and to summarize general information regarding hypertension.

Reply 1: We shortened the introduction to provide a more focused overview on hyporeninemic hypertension. We focused on the role and implications of low renin in hypertension.

Changes in the text: Introduction, pages 3-4, lines 65-87: Primary hypertension is considered polygenic in etiology and results from complex interaction among different genes and environmental factors (15). On the other hand, secondary hypertension can be caused by a large number of different conditions that can be either acquired secondary to medication or related to renovascular, endocrine, oncologic, or neurological problems (Table 1). There is a subcategory of secondary hypertension characterized by low renin and referred to as hyporeninemic hypertension or low-renin hypertension (16) (Table 2). These forms of hypertension are frequently caused by pathogenic variants in a single gene and referred to as monogenic hypertension (15, 17). Most of these genes are involved in the renal and adrenal regulation of blood pressure and intravascular volume. The net result of these mutations leads to either one or more of the following mechanisms: (1) excessive sodium reabsorption at the level of the nephrons, (2) inappropriate response to normal/low mineralocorticoid levels, and (3) increased mineralocorticoid synthesis. All three mechanisms result in volume excess and inability to get rid of excess sodium (18). Recent advancement in genetic studies revealed that this form of hypertension is under- diagnosed in patients with hypertension (19, 20). The suppressed levels of renin and/or aldosterone are underappreciated as indicators for monogenic hypertension. Moreover, the aldosterone-to-renin ratio provides an indicator of aldosterone excess even in patients with normal aldosterone levels (21). The identification of hyporeninemic hypertension followed by the correct diagnosis of the specific condition are crucial for the appropriate treatment. In this article, we outline the different conditions, their genetic causes, how to diagnose them, and how to treat the specific conditions.

Comment 2: The authors should add a brief Methods section informing the narrative characteristic of the review as well as the database evaluated and the terms used for search.

Reply 2: We included a brief method section as suggested by our reviewer. We elaborated on the terms that we used for our online search. We also included the focus of our literature review on pediatric patients and latest suggested pathophysiologic mechanisms.

Changes in the text: Methods, pages 4-5, lines 90-98: We searched publications in PubMed online (www.pubmed.org) using title and abstract keywords "hyporeninemic hypertension", "low renin hypertension", "Liddle Syndrome", "Gordon Syndrome", "Apparent Mineralocorticoid Excess", "Geller Syndrome", "hypertension exacerbated by pregnancy", "Glucocorticoid Remediable Aldosteronism", "Familial Hyperaldosteronism", "monogenic hypertension", "inherited hypertension", "genetic hypertension". We focused our search on pediatric population. For pathophysiology we focused our search on the past 5 years. For the different syndromes, we referred to the original studies that provided early characterization of the disease."

Comment 3: Tables 1 and 2 are unnecessary. I suggest the authors to remove both and to reduce the text about other secondary causes of hypertension rather than the forms related to the review.

Reply 3: We removed Table 1 and Table 2.

Comment 4: On the other hand, the subheading The Renin Angiotensin Aldosterone System (RAAS) is incomplete. Some aspects of the actual view about the RAAS should be included to this review. I recommend the authors to read recent review articles on this issue as, for instance, https://doi.org/10.1007/s00467-020-04759-1.

Reply 4: We thank the reviewer for the suggestion to elaborate on this section and also for providing a very informative reference on the Renin Angiotensin Aldosterone System. We redrafted this section and we provide now an updated and more detailed overview about the RAAS.

Changes in the text: The renin-angiotensin-aldosterone system (RAAS), page 6-8, lines 135-172: The RAAS system has been extensively studied for its role in maintaining fluid homeostasis and blood pressure control (24). Upregulation of renin and aldosterone activity due to renal scarring, frequent UTIs, and chronic kidney disease is a major cause for hypertension with kidney disease and stricter blood pressure control slows down the progression of kidney disease (22, 25). In addition to fluid balance and blood pressure control,

RAAS plays an important role in chronic kidney disease progression (26), cardiovascular remodeling (27), activation of the thirst center and sympathetic nervous system (28), obesity and metabolic syndrome, inflammation and immune activation (17), lung (29) and liver fibrosis (30). Renin is an aspartyl-protease produced by the juxtaglomerular cells in response to lower circulating blood volume and subsequently glomerular filtration (31). This includes the renal baroreflex and reduced Chloride (Cl -) delivery at the level of the macula densa in addition to vasoactive molecules such as nitric oxide, adenosine, and prostaglandins (32). Renin converts angiotensinogen, a 14 amino acid peptide produced mainly in the liver, into angiotensin I (Ang-I), a 10 amino acid peptide (33). Two different signaling mechanisms, the (i) classical and the (ii) alternative RAAS pathways, can utilize angiotensin I in different ways which has recently been extensively reviewed (34). The well-known classical pathway converts angiotensin I to an 8 amino acid peptide named angiotensin II (Ang-II) via the angiotensin converting enzyme (ACE), a dicarboxypeptidase located mainly in the lungs (35). Ang-II is the effector molecule of the classical pathway. Ang-II binding of the angiotensin receptor type 1 (AT1R) leads to increase in blood pressure, sodium retention, inflammation, remodeling, and fibrosis mediated by vasoconstriction, stimulation of aldosterone production and anti-diuretic hormone production, and activation of the sympathetic nervous system (27). On the other hand, if Ang-II binds to the angiotensin receptor type 2 (AT2R) opposite effects compared to AT1R stimulation are seen with lowering blood pressure and increased sodium excretion. However, AT2R expression is mostly significant during fetal development and early neonatal life (31). In the alternative RAAS pathway, angiotensin I is cleaved by ACE2 to generate Ang 1-9 and Ang 1-7, a heptapeptide (36). Ang 1-7 preferentially binds to the Mas receptor (29). While the downstream molecular pathways of Mas receptor activation are not fully understood, most of the experimental studies showed an overall counter Ang-II effect including vasodilation, decreased aldosterone and anti-diuretic hormone production, decreased inflammation and reactive oxygen species production (37, 38). Due to COVID-19 pandemic, ACE2 has gained attention in the scientific community because it acts as viral receptor for COVID-19 (39). The binding of the virus leads to ACE2 entry into the cell rendering the enzyme unavailable to counter the ACE effect (40). The imbalance between Ang-(1-7) and Ang II is associated with disease severity and seems to play a role in ARDS commonly seen in severe COVID-19 infection (41, 42).