3-1-2019

Dr Conn Lives on: Insights Into Screening and Genetics of Primary Aldosteronism

Debbie L. Stein

Jerry Yee
Henry Ford Health, JYEE1@hfhs.org

Recommended Citation
Stein DL, and Yee J. Dr. Conn Lives on: Insights Into Screening and Genetics of Primary Aldosteronism. Adv Chronic Kidney Dis 2019; 26(2):81-84.

ACKD Advances in Chronic Kidney Disease

A Journal of the National Kidney Foundation®

Vol 26, No 2, March 2019

EDITORIAL

Dr. Conn Lives on: Insights Into Screening and Genetics of Primary Aldosteronism

Check for updates

This issue of *Advances in Chronic Kidney Disease* features an old standard, hypertension. This topic is reviewed periodically. This time, Dr. Kausik Umanath has taken up the gauntlet to press forward our collective mission to diagnose and treat this most fundamental medical problem early, accurately, and efficiently. In this issue, the variegation of the pathophysiology of hypertension will be revealed along with its treatments. In Dr. Umanath's Guest Editorial, you will see a glimpse of what is contained within, but first, a story.

Several years ago, after a gratifyingly well-cooked lunch, I explored the shops in Dexter, MI. I ventured into the Mule Skinner Boot Shop. After initial salutations, proprietor Billy Conn asked me what I did for a living. I told him that I, Jerry Yee, was a nephrologist. He parried and said that he knew what "that" was, but his father, Jerome W. Conn, had been an endocrinologist. I exclaimed, "Wow! Your father first-described an aldosteronoma at the University of Michigan." Impressed with my riposte and validation of his father's clinical acumen, Mr. Conn waxed lyrically about the virtues of his father-physician who had also described the mitigation of insulin resistance through weight loss and acclimatization of man to warm climates, later attributed to aldosterone-mediated effects. Before we parted ways, I made a purchase and vowed to return to the shop.

The pathophysiology of Conn's original description of a female patient who had been plagued for 7 years with lower extremity weakness and cramping hands, while also exhibiting diabetes insipidus (viz., kaliopenic nephropathy), tetany, hypernatremia, hypokalemic metabolic alkalosis, and non-edematous hypertension, stemmed from hyperproduction of the mineralocorticoid aldosterone that heretofore will be labeled "electrocortin".[1] Subsequent investigation led to the diagnosis of aldosterone hypersecretion, identified by urine biochemical analysis, from a 4-cm adrenal tumor as the instigator of this surgically remediable form of resistant hypertension. Since Conn's seminal observations and investigations, the knowledge base of primary aldosteronism (PA) has expanded exponentially. However, in practice, early diagnosis of aldosteronism in resistant hypertensive patients, when surgical treatment may be curative, remains vexingly uncommon. In our brief treatise, as an ode to Jerome Conn, Debbie L. Stein and I enumerate some barriers to the diagnosis of aldosteronism and report some of the more recent biomolecular findings regarding this disorder that produces difficult-to-treat high blood pressure.

PA is the most common cause of secondary hypertension and affects 3–20% of hypertensive patients.[2] PA is more likely present in patients with severe or resistant hypertension. PA is treatable and potentially curable. Consequently, PA is an important secondary form of hypertension that should be excluded. Patients with PA have an increased risk of cardiovascular disease, atrial fibrillation, chronic kidney disease, and cardiovascular death compared with individuals with essential hypertension and equivalent blood pressure elevations.[3-6]

PA should be suspected in the following clinical scenarios: new-onset hypertension that is frequently difficult to control, recent exacerbation of hypertension in a patient with previously well-controlled blood pressure, history of leg cramps, or unprovoked hypokalemia. However, despite declarations in numerous textbooks regarding PA as a hypokalemic hypertensive disorder, less than 50% of patients have hypokalemia. In actuality, normokalemia is common, and when present, should not deter evaluations for PA, detection of an incidental adrenal adenoma, or probing the family history for aldosteronism.

Classically, PA is diagnosed by initially acquiring plasma renin activity (PRA) and plasma aldosterone concentrations (PACs). If plasma renin is suppressed (usually <1 ng/ml/hour) and aldosterone levels are at least >15 ng/dL, with an aldosterone-to-renin ratio (ARR) > 20–30, further workup is warranted. Subsequent confirmatory testing was typically called for before

1548-5595/$36.00
https://doi.org/10.1053/j.ackd.2019.03.018

proceeding to more invasive testing. However, recent guidance from the Endocrine Society no longer recommends confirmatory testing in patients with spontaneous hypokalemia, plasma renin concentrations below detection levels, and PACs >20 ng/dL.[7] Patients meeting these criteria may proceed directly to imaging studies. ARR screening is optimally conducted in patients ingesting an unrestricted sodium diet and those who are normokalemic. As required, potassium should be repleted until serum potassium concentrations are within the normal range. Mineralocorticoid-receptor antagonists should be withdrawn for no less than 4 weeks before ARR screenings.

The ARR can be confidently interpreted in many cases despite the effects of onboard medications and/or other suboptimal conditions of testing. Notably, the "washout" of all antihypertensive medications that potentially interfere with interpretation of ARRs is only feasible in patients with mild hypertension. Some medications that have minimal obfuscating effects on the ARR include sustained-release verapamil, hydralazine, prazosin, doxazosin, and terazosin. Once a biochemical diagnosis is established, all patients should undergo an initial adrenal computed tomography imaging study as an initial step before proceeding to confirmatory adrenal vein sampling. The latter is considered if and only if adrenal surgery is a feasible treatment option.

DIRECT RENIN CONCENTRATION VS PLASMA RENIN ACTIVITY

There are an increased number of laboratories measuring direct renin concentration (DRC) as an alternative to PRA. The DRC assay provides several advantages compared with PRA. Specifically, DRCs are less labor- and time-intensive procedures and have shorter turnaround times, better reproducibility, and greater ease of standardization.[8] The calculation of the ratio of aldosterone-to-direct renin concentration (ADRR) may be employed as a screening test for PA instead of the ARR. The ADRR is also termed the plasma aldosterone–to–direct renin concentration ratio (PAC/DRC). Numerous conversion factors have been proposed in an effort to generate clinically meaningful and stable cutoffs between assays. Nevertheless, it is uncertain whether an interchangeable relationship exists, particularly because PRAs and DRCs are biologically distinct entities.[7-9]

The correlation between DRC and PRA is generally good, except when PRAs are <1 ng/ml/hour, which corresponds to the most relevant PA clinical screening scenario.[7] In a recently introduced and already commonly used automated DRC assay, the conversion factor is 12. Numerous factors affect PAC, DRC, PRA activity, and calculated ratios. However, these effects are generally similar for DRC and PRA, aside from a few clinical situations, including pregnancy and estrogen administration. Administration of oral contraceptives increases angiotensinogen with subsequently low PRA/DRCs. In congestive hepatopathy, there is low angiotensinogen production, with consequently high DRCs and low PRAs.[9] Briefly, exert caution when interpreting these values in particular clinical circumstances.

The diagnostic efficiency of PAC/DRC has varied in studies, and no general consensus exists concerning the impact of antihypertensives on this ratio. A recent meta-analysis included 9 studies involving 974 participants and revealed an overall sensitivity of 0.89 (95% confidence interval [CI], 0.84 to 0.93), specificity of 0.96 (95% CI, 0.95 to 0.98), area under the curve of 0.985, and diagnostic odds ratio for ADRR of 324.[10] The associated meta–regression analysis revealed that the antihypertensive status affects the ADRR and may account for the heterogeneity ($P = 0.03$). Principally speaking, antihypertensive drug therapy in PA may interfere with the interpretation of ADRRs. In a subgroup analysis of patients who discontinued antihypertensive medications, the ADRR had a sensitivity of 0.99 (95% CI, 0.95 to 1.00) and specificity of 0.98 (95% CI, 0.96 to 0.99).[9] This study established the ADRR as a reliable screening assay for aldosteronism.

Another study from Poland included 62 patients with suspicion for PA and 35 healthy volunteers.[9] All participants had PACs, DRCs, and PRAs measured after a night's rest and again after walking for 2 hours. Correlations of ARR with ADRR in the supine position were $r = 0.9162$ and $r^2 = 0.8165$ ($P < 0.01$). Corresponding values in the upright position were $r = 0.7765$ and $r^2 = 0.9153$ ($P < 0.01$). Cutoff values for ARRs and ADRRs ≥100 represented the highest specificity (99%) for the diagnosis of PA. However, quite acceptable specificity and sensitivity of ≥80% and 100%, respectively, were recorded at ratios ≥30.

Another population-based study was conducted in Canada with the goal of establishing an ARR cutoff during the transition from PRA to DRC assays.[8] In Calgary, DRC testing replaced the PRA for routine testing in 2014; 4301 participants who received testing between January 2012 and November 2015 were included. During the initial portion of the study using PRAs and locally validated cutoff values, 4.9% of screenees were classified as highly probable cases of PA, 10.4% probable, and 28.9% possible. ADRRs were subsequently determined. A highly probable PA case corresponded to a cutoff level of >100 pmol L^{-1} mIU^{-1} L^{-1} with hypokalemia. A probable case corresponded to a cutoff level of >100 pmol L^{-1} mIU^{-1} L^{-1} and a possible case to >35 pmol L^{-1} mIU^{-1} L^{-1}. In contrast, cutoffs derived using a conversion factor resulted in significantly higher cutoffs and the potential for missed cases. Based on their local experience, the authors suggested that it may be preferable to use large-population data to establish cutoff values rather than simply applying conventional direct-method comparisons. This data-driven approach would lead to fewer false-negative results, i.e., greater sensitivity.[8]

In summary, the use of DRCs and ADRRs as opposed to classical PRAs and ARRs remains problematic because standardized conversion methodologies do not work well when renin is suppressed—the clinical scenario most relevant during screening for PA. As feasible, health care providers evaluating patients for possible PA should

rely on local experience and continue to order PRAs and ARRs until issues surrounding DRCs and ADRRs are clarified.

GENETIC ASSOCIATIONS OF PRIMARY ALDOSTERONISM

Somatic and germline mutations have been identified in several genes in aldosterone-producing adenomas (APAs) and familial hyperaldosteronism. Somatic mutations are present in approximately 50% of patients with aldosterone-producing adenomas. These mutations include *KCNJ5*, *ATP1A1*, *ATP2B3*, and *CACNA1D*. These gene alterations have accordingly provided insights into mechanisms of dysregulated aldosterone production.[11] Most of the mutations activate the intracellular signaling pathway that normally regulates aldosterone production. As a common final pathway, all the described gene mutations, germline or somatic, increase intracellular calcium concentrations and trigger increased aldosterone production and cellular proliferation.

The first of these somatic mutations was identified in 2011 during whole exome sequencing of surgically resected APAs. Genetic sequences from the tumor were aligned with native DNA from the same patient to identify somatic DNA changes that induced transformation of normal adrenal tissue into an APA.[12] Somatic mutation of a single gene *KCNJ5*, which encodes an inwardly rectifying potassium channel Kir 3.4, was established in 8 of 22 APAs examined.[13] A genomic mutation in a family previously reported to have a familial form of aldosteronism distinct from glucocorticoid-remediable aldosteronism (GRA) was also identified.

Three of the loci of the *KCNJ5* mutations (i.e., G151 R, L168 R, and T158 A) were localized to the region encoding the selectivity filter of this potassium channel. These mutations promoted sodium conductance through this normally sodium-resistant channel. The increased sodium conductance and ensuing cell depolarization enhanced intracellular calcium entry, a potent signal for aldosterone production and cell proliferation.[14] *KCNJ5* mutations are more common in females and younger patients. This mutation is more likely associated with higher PACs and propensity for resistant hypertension.[15]

Further investigations, using a similar approach have led to the identification of somatic gene mutations of *ATP1A1* (encodes a Na^+/K^+ ATPase alpha-1 subunit) and *ATP2B3* (encodes Ca^{2+}-ATPase isoform 3) in, respectively, 5.2 and 1.6% of 308 APAs studied.[16] These mutations are more commonly found in male patients with APAs. Notably, these mutations are also associated with increased PACs and lower potassium concentrations than individuals with mutation-negative adenomas.

Within resected APAs, somatic mutations have been identified in *CACNA1D*, which encodes the alpha-1 subunit of CaV1.3, a voltage-dependent L-type Ca channel.[17] *CACNA1D* subunit mutations induce a shift of voltage-dependent gating to more negative voltages, thereby suppressing calcium-current inactivation, i.e., increasing currents. *CACNA1D* mutations have also been shown to be present in two children with a novel syndrome of PA and epilepsy.[18] *CACNA1D* mutation has been shown to be present in older, male patients. The tumors are typically smaller and are often not visualized by conventional imaging techniques. Among black patients with APAs, a large proportion (89%) has an aldosterone-driving mutation.[11] In a study of 73 tumors from 79 black patients with PA, 65 tumors (89%) harbored an aldosterone-driving mutation. The most prevalent genetic alteration by next-generation sequencing was *CACNA1D* (42%), trailed closely by *KCNJ5* mutations (34%). *ATP1A1* (8%) and *ATP2B3* mutations (4%) were less frequent.[11]

In conclusion, although we acknowledge that somatic mutations are present in at least 50% of APAs, genetic sequencing of APAs is still a research tool, and its clinical use is yet unknown. Despite that we have discerned the various somatic mutations more likely to occur in different genders and racial groups, optimized protocols by which these observations can be reasonably applied to personalized medicine are enigmatic. Currently, treatment does not differ whether a somatic mutation is present or not.

It is now more than 6 decades since Conn's original description of PA. Our knowledge base has been enhanced by molecular science, and screening for PA is improved. Plus, we have more antihypertensive medication choices, although mineralocorticoid-receptor antagonism in PA remains *medicamentum electio*. We must endeavor to diagnose this disorder earlier to effect surgical cures, where applicable, and prevent cardiovascular complications. After all, it was Dr. Conn's tenet that up to 20% of individuals with essential hypertension might actually have PA.

> Reason tells us that we must acquiesce in a vague kind of way to the statement that every fact has a potential usefulness. In the meanwhile, it is regarded as background, available to all who choose to use it. Are there any of us capable of evaluating which fact, when eventually correlated with others, will have the greatest impact upon the lives of men? The answer is "No."[19]
>
> —Jerome ("Jerry") W. Conn.

Debbie L. Stein, MD
Professor of Medicine
University of Pennsylvania
Renal Division
Philadelphia, PA

Jerry Yee, MD, FNKF
Editor-in-Chief
Henry Ford Hospital
Division of Nephrology and Hypertension
Detroit, MI

Professor of Clinical Medicine
Wayne State University
Detroit, MI

Financial Disclosure: The authors declare that they have no relevant financial interests.

REFERENCES

1. Conn JW. Primary aldosteronism, a new clinical syndrome. *J Lab Clin Med.* 1955;45(1):3-17.
2. Williams TA, Reincke M. Management of endocrine disease: diagnosis and management of primary aldosteronism: the Endocrine Society guideline 2016 revisited. *Eur J Endocrinol.* 2018;179(1):R19-R29.
3. Stowasser M, Sharman J, Leano R, et al. Evidence for abnormal left ventricular structure and function in normotensive individuals with familial hyperaldosteronism type I. *J Clin Endocrinol Metab.* 2005;90(9):5070-5076.
4. Rossi GP, Cesari M, Cuspidi C, et al. Long-term control of arterial hypertension and regression of left ventricular hypertrophy with treatment of primary aldosteronism. *Hypertension.* 2013;62(1):62-69.
5. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of atrial fibrillation and mineralocorticoid receptor activity in patients with medically and surgically treated primary aldosteronism. *JAMA Cardiol.* 2018;3(8):768-774.
6. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Renal outcomes in medically and surgically treated primary aldosteronism. *Hypertension.* 2018;72(3):658-666.
7. Funder JW, Carey RM, Mantero F, et al. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2016;101(5):1889-1916.
8. Leung AA, Orton DJ, Chin A, Sadrzadeh H, Kline GA. Novel approach to establishing an aldosterone: renin ratio cutoff for primary aldosteronism. *Hypertension.* 2017;69(3):450-456.
9. Glinicki P, Jeske W, Bednarek–Papierska L, et al. The ratios of aldosterone/plasma renin activity (arr) versus aldosterone/direct renin concentration (adrr). *J Renin Angiotensin Aldosterone Syst.* 2015;16(4):1298-1305.
10. Li X, Goswami R, Yang S, Li Q. Aldosterone/direct renin concentration ratio as a screening test for primary aldosteronism: a meta–analysis. *J Renin Angiotensin Aldosterone Syst.* 2016;17(3):1470320316657450.
11. Nanba K, Omata K, Gomez-Sanchez CE, et al. Genetic characteristics of aldosterone–producing adenomas in blacks. *Hypertension.* 2019;73(4):885-892.
12. Choi M, Scholl UI, Yue P, et al. K^+ channel mutations in adrenal aldosterone–producing adenomas and hereditary hypertension. *Science.* 2011;331(6018):768-772.
13. Geller DS, Zhang J, Wisgerhof MV, Shackleton C, Kashgarian M, Lifton RP. A novel form of human mendelian hypertension featuring nonglucocorticoid-remediable aldosteronism. *J Clin Endocrinol Metab.* 2008;93(8):3117-3123.
14. Geller DS. New developments in the genetics of hypertension: what should clinicians know? *Curr Cardiol Rep.* 2015;17(12):122.
15. Boulkroun S, Beuschlein F, Rossi GP, et al. Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension.* 2012;59(3):592-598.
16. Beuschlein F, Boulkroun S, Osswald A, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet.* 2013;45(4):440-444. 444e1–e2.
17. Azizan EA, Poulsen H, Tuluc P, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet.* 2013;45(9):1055-1060.
18. Scholl UI, Goh G, Stolting G, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet.* 2013;45(9):1050-1054.
19. Daughaday WH. Jerome W. Conn, In: *Biographical Memoirs.* vol 71. Washington, DC: National Academy Press; 1997:9.