Primary Aldosteronism: New Answers, New Questions

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Abstract

There have been 2, and possibly 3, major questions for primary aldosteronism (PA) answered at least in principle over the past 5 years. The first is that of somatic mutations underlying the majority of aldosterone producing adenomas. The second is the extension of our knowledge of the genetics of familial hypertension, and the third the role of renal intercalated cells in sodium homeostasis. New questions for the next 5 years include a single accepted confirmatory/exclusion test; standardisation of assays and cut-offs; alternatives to universal adrenal venous sampling; reclassification of 'low renin hypertension'; recognition of the extent of 'occult' PA; inclusion of low-dose mineralocorticoid receptor antagonist in first-line therapy for hypertension; and finally, possible resolution of the aldosterone/inappropriate sodium status enigma at the heart of the cardiovascular damage in PA.

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At the outset we need to draw a line in the sand, to define new. The Endocrine Society guidelines on primary aldosteronism were published in 2008 [1], and those of the Japanese Endocrine Society in 2009 [2]; both are in the process of revision, and both too far back as a starting point. I have thus arbitrarily chosen new answers to include those from 2011–2015, inclusive, with (rather more) new questions that need to be addressed in the 5 following years, 2016–2020. The new questions section will be the longer: there are, indeed, a range of issues pre-2011 that remain to be addressed, as well as those from the last 5 years.

Somatic Mutations in APA

The most exciting finding over the past 5 years has been that of the characterisation of somatic (i.e., confined to the adrenal) mutations in over 50% of aldosterone producing adenomas (APA). The initial, prismatic report by Choi et al. [3] from the Lifton laboratory found somatic mutations in KCNJ5, encoding the Kir3.4 potassium channel, in 8 of 22 APA from Europe and the USA. In this series, the mutation-bearing APA were larger, more common in females, found at a younger age than wild-type APA, and apparently more florid, reflected in higher aldosterone/renin ratios (ARR). Shortly thereafter, in a much larger collaborative study from the ENS@T Group in Europe, these findings were confirmed in large part and extended [4] in over 300 patients. The percentage of mutation bearing tumours was similar. The female predominance was similarly pronounced, and they were similarly more florid on the basis of higher plasma aldosterone concentrations (PAC). Points of difference were in the proportion of the 2 mutations found: in the Choi study of 8 cases, 2 were G151R and 6 L168R; in the ENSAT study the numbers were reversed to 76 G151R vs. 53 L168R. Secondly, in the larger study, no difference in size was found between mutation-bearing and wild-type APA.

Subsequently, the area has been rapidly extended in several ways. First, and successively, other genes have been found in APA – for sodium and calcium ATPases (ATP1A1, ATP2B3) in 2 different series [5,6], and in the calcium channels CACNA1D [7] and CTNNB1 [8]. Although the proportions of these mutations vary between laboratories (e.g., ATPases: [5]), those in KCNJ5 remain the most common to date. In a very recent series [8], the breakdown was KCNJ5 37, CACNA1D: 10, ATP1A1: 8, ATP2B3: 3, and CTNNB1: 2. Perhaps of interest in terms of the genesis of APA, CACNA1D mutations were found in 2 hyperplastic adrenals without an adenoma. Whereas the KCNJ5 mutation-bearing APA are more prevalent

in females, the opposite appears to be the case for the others as a whole. Although for the less common variants numbers are smaller, they are also on average less florid in phenotype.

The second extension has been to document the variety of KCNJ5 mutations in addition to the 2 initially described [3,4], the extent to which the mutation increases intracellular calcium concentrations, and their phenotype: to date more than half a dozen different loci have been reported [9–16]. Third, a series of relatively small studies, in most cases, have extended the countries of origin of the original cohorts (Europe, America) to Japan and China [17–22]. In both the latter the percentage of mutation-bearing APA is commonly reported to be much higher than in European, American, and Australian cohorts with APA. This has led to speculation about ethnic differences: a more likely reason for the higher percentage is selection bias, until proven otherwise.

Familial Hyperaldosteronism

Familial hyperaldosteronism type 1 (FH-I: also known as glucocorticoid remediable aldosteronism, or glucocorticoid suppressible hypertension) was first described in 1966 [23], and its mode of inheritance first described in 1992 [24]. Not surprisingly, the advances in familial hyperaldosteronism over the past 5 years have been in FH-II, and more clearly on a genetic basis, FH-III. For FH-II – primary aldosteronism in 2 first-degree relatives – an association with a locus at 7p22 has been found in some families, but the gene(s) responsible not identified [25,26]. In a large recent study [27], Mulatero and his colleagues put the prevalence at 6% of primary aldosteronism, which is likely to be a very conservative figure, for several reasons – smaller families, increased intergenerational spacing, mobility – and finally occult PA, to be canvassed in the 'new questions' section.

FH-III was first described by Geller et al. in 2008, in a family of 3 with children aged 4 and 7 years requiring adrenalectomy for fulminant hyperaldosteronism [28]. The defect in this family was established as a germ-line KCNJ5 mutation (T158A) and published together with the first description of the somatic mutations [3]. Subsequently a number of other KCNJ5 mutations have been reported: the mutation (G151E) leading to the most marked in vitro derangement is perhaps counter-intuitively matched by a relatively mild phenotype, presumed caused by increased in vivo mortality of the affected cells [29]. Subsequently patients with germ-line mutations in CACNA1D have been described, with an array of developmental neurological abnormalities plus primary aldosteronism [30]. More recently, families with juvenile hypertension and primary aldosteronism have been reported with germline inherited or spontaneous mutations in CACNA1H. Whether these should be termed FH-IV is debatable, particularly given the finding of spontaneous CACNA1H mutation in 2 of the 5 families [31].

Intercalated Cells

In addition to their ground-breaking discovery of KCNJ5 somatic mutations in APA, the Lifton laboratory subsequently published a breathtakingly elegant study documenting a hitherto undescribed additional pathway of distal tubular sodium retention. Current orthodoxy locates mineralocorticoid receptors (MR) in principal cells in the distal tubule, where they are rendered aldosterone selective ('protected') via the action of the enzyme 11βHSD2. The paper by Shibata et al. [32] demonstrates the presence of MR but not 11βHSD2 in intercalated cells. In the resting sodium replete state, these MR are phosphorylated on serine 843, and thus rendered inactive. When, however, levels of angiotensin rise in response to volume depletion or sodium deficiency, angiotensin acts through a WNK4 signalling pathway to dephosphorylate MR, allowing them to be activated, by either aldosterone or cortisol, consistent with the absence of 11βHSD2. What the ramifications of this discovery are for the pathophysiology of primary aldosteronism are not clear. They provide a satisfying explanation for the difference between the MR–/– and the aldosterone synthase –/– mouse. The former cannot survive a low sodium diet; the latter shows a fall in blood pressure, unsurprisingly, but survives – presumably due to the high angiotensin levels acting in intercalated cells to dephosphorylate MR S^{843}, which is then activated by corticosterone to retain sodium [33]. Given that we are yet to understand the pathogenetic mechanisms of blood pressure elevation and tissue damage in primary aldosteronism, studies on angiotensinogen –/– mice might also be worth a moments' reflection: in response to sodium deprivation –/– and wild-type mice elevate their PAC to indistinguishable levels [34].

Net of these findings, there are no new answers over the past 5 years: other advances have been incremental rather than paradigm shifts. Many of these in fact represent new questions, in that they constitute a point of departure, preliminary studies that need to be repeated, extended and able to be generalised before being admitted to the canon of 'new answers', hopefully by 2020. If this is the case, then by that time we may see real progress in the management of primary aldosteronism.

New Answers: Basic Studies

First, we may see major advances in the pathophysiology of both APA and BAH; there is, for example, tantalizing preliminary evidence for the drivers of bilateral adrenal hyperplasia. At present, over 50% of patients with an APA can be shown to have a mutation in any one of 5 different genes: anything is possible, but it seems very unlikely that all of the remainder are truly 'wild-type'. We know how the presently detected somatic mutations increase aldosterone secretion, but not the steps in adenoma formation. Zona fasciculata cells appear to harbour the KCNJ5 mutation, but APA with the other somatic mutations are of mixed cell type: what does this imply, for adenoma formation, for oversecretion of aldosterone?

With the advent of specific antisera able to discriminate human CYP11B1 from CYP11B2 the histology of the adrenal cortex has been established with a precision not previously possible on the basis of cell morphology on haematoxylin and eosin stained sections [35]. Counterintuitively, CYP11B2 expression has been reported as essentially absent in some APA [36], but present in aldosterone producing cell clusters in normal adrenals and those from patients with primary aldosteronism. Very recently, in normal adrenals from renal transplantation donors, next generation sequencing revealed that in 8 of 23 cases the APCC harboured aldosterone driver mutations, absent from the surrounding zona glomerulosa and with a pattern of mutation (6 CACNA1D, 2 ATP1A1) different from that found to date in mutation-bearing APA [37]. This is a prismatic finding, and bodes well for major advances over the next 5 years in terms of the preconditions for pathophysiological aldosterone excess.

Of one thing we can be certain, that whether or not there are answers to these questions in 5 years time it will not be for want of trying.

New Answers: Clinical Questions

1. Confirmatory testing

In the clinical area things may unfortunately move much more slowly: of all the possible clinical advances this essay will consider 5 in detail. The first, simplest but perhaps the least tractable is the existence of 5 or more confirmatory/exclusion tests used to winnow 'true' PA from within the group of hypertensives who screen positive for PA on the basis of an elevated aldosterone to renin ratio (ARR). Direct comparisons between tests are few, often post-hoc, on relatively small groups of subjects. It is, of course, possible that no one test is superior to another – or any other – of the 5, in terms of specificity and selectivity, possible but unlikely. Currently what maintains the diversity may be lack of evidence that a particular test is inferior, but is more likely to be habit-formed by local history and usage, familiarity and – often underestimated – the desire not to compromise a series by changing horses in mid-stream.

Among the confirmatory/exclusion tests the fludrocortisone saline suppression test (FST) is sometimes (disputedly) referred to as 'the gold standard'. In terms of hospitalisation (not mandatory), time, compliance, adequacy of sodium and potassium intake and cost it is clearly the most complex. To their credit the Brisbane group recently and very successfully compared their customary FST with a seated saline suppression test (SSST), distinct from the customary recumbent oral or intravenous saline suppression tests [38]. In the preliminary study they reported a 23/24 patient congruence between the FST and the SSST, compared with much lower levels between the FST and recumbent saline suppression tests. There is in progress a larger study: if this recapitulates the preliminary study, there would appear to be a very strong case for its widespread adoption, and discontinuation of the current range of testing, on the basis of accuracy, convenience, compliance and cost. It would, however, be unwise to hold your breath until this happens.

2. Assays, 'normal' levels, and cut-offs

A second, not dissimilar area of variation is in the measurement of renin and aldosterone, the overly ample 'normal ranges' for plasma aldosterone, with the result – at least in part – of variation in cut-offs for a positive ARR and elevated plasma aldosterone levels. Immunoassays of aldosterone are notoriously unreliable; renin activity or concentration are at least one step backwards from angiotensin II. The goal therefore in terms of measurement is to measure aldosterone by mass spectrometry, and similarly plasma angiotensin – or a validated surrogate. This means that proposed aldosterone to angiotensin ratio measurement needs to be centralised into tertiary medical institutions, rather than the ARR being done at present in a wide-ranging variety of laboratories. Importantly, the normal ranges of plasma aldosterone concentration need to be revisited, with due regard to ethnic variation and differences in sodium and potassium intake. The published normal PAC at the Mayo Clinic has been 4–21 ng/dl, and that in Ancona 3–31 ng/dl: both ranges are unlikely to reflect ethnic differences, nor differences in dietary intake.

3. Avoiding AVS

A third area in which answers are needed is that of alternatives to adrenal venous sampling (AVS) as a means to lateralise (or not) the source of the hyperaldosteronism. In patients with hypokalaemia, immeasurably low renin and high aldosterone, some centres omit confirmatory/exclusion testing, and move directly to AVS. In others, similar young patients (< 35–40) with a unilateral adenoma and a contralateral normal adrenal on imaging have proceeded to surgery without AVS, on the basis of the unlikelihood of adrenal incidentaloma at that age. There have been several post hoc studies on adrenal venous and peripheral levels of 18-hydroxy- and 18-oxocortisol in patients previously lateralised on AVS, as possible predictors of a distinction between high levels (APA) and low levels (BAH). In 2 such studies [39,40] there were highly significant differences, with the latter claiming ~60% ability to discriminate on the basis of peripheral levels, so that only ~40% of patients would require AVS. Given the cut-offs widely but not universally used in Japan, these values may or may not be generalisable elsewhere. Finally, if APA cells shed into the bloodstream can be confirmed as harbouring one of the somatic mutations described above, and imaging is consistent with a clearly unilateral adenoma, it would appear appropriate to omit AVS.

4. Low Renin Hypertension?

The fourth area is that of whether or not the current cut-offs, and subsequent workup, miss a major group of patients with primary aldosteronism. This question was first addressed over 35 years ago [41], and answered positively. Helber and colleagues took 3 groups – 35 healthy normotensive controls, 100 'essential hypertensives' and 16 patients with proven primary aldosteronism (12 APA, 4 BAH), maintained them on a fixed high sodium intake, and measured urinary aldosterone levels, plasma electrolytes and blood pressure. What they found was unequivocal, despite which it has been largely ignored. All but 2 (of the 4 BAH) the proven primary aldosteronism patients had urinary aldosterone levels above the normal range. Among the 100 'essential hypertensives', those with normal aldosterone levels (group A, n = 64) had average urinary aldosterone levels of 2.7 ± 1.4 µg/day, compared with 10.0 ± 3.0 µg/day in group B (n = 36) who had levels above the normal range. In group A the mean plasma [K^+] was 4.26 meq/l; in group B 3.81 meq/l. Tellingly, when all patients were put on a trial of spironolactone, in group A mean blood pressure fell by 9 mm Hg, compared with 21 mm Hg in Group B. On these findings it seems that at least 36% of hypertensives in this cohort had occult primary aldosteronism, given that those with established PA had already been so diagnosed. This, of course, was in stark opposition to received wisdom at that time, that primary aldosteronism was a rare (< 1% of all hypertension) and relatively benign form of elevated blood pressure.

Over 20 years later a study on the role of MR, rather than aldosterone per se, was published [42]. In this study 397 essential hypertensives, carefully screened and normal in terms of everything except their starting BP (average value: 154/100 mm Hg) were enrolled in a titration-to-effect trial of eplerenone as monotherapy. For the first 4 weeks all subjects received 50 mg/d eplerenone, at the end of which period 44% reached goal BP (diastolic < 90 mm Hg) with a marked fall in BP (16/14); they continued on 50 mg and the non-responders (BP fall 6/3) went onto 100 mg/day for weeks 5–8. At 8 weeks 66/156 of the non-

responders to 50 mg/day showed a mean fall in BP of 20/15 mm Hg, compared with a fall of 5/3 mm Hg in those who did not reach goal BP on this dose. The responders to 100 mg/day continued on that dose, and the 156 non-responders were given 200 mg/day for weeks 8–12. On this dose 75 subjects reached goal DBP, with a mean fall of 17/15: the remaining 81 subjects (~20% of the total group) were unresponsive (2/1) to mineralocorticoid receptor antagonist administration.

The interpretation of these data was that in ~80% of essential hypertensives the elevation in blood pressure is dependent at least in part on MR activation. Given the screening process for entry into the study (mean plasma [K^+] was 4.27 meq/l, cf. 4.26 meq/l in the Helber et al. study cited above) it is difficult to ascribe MR activation to aldosterone. On the other hand, normal levels of cortisol occupy the majority of MR in 11βHSD2 protected tissues as an aldosterone antagonist under normal circumstances; in the context of tissue damage, however, as occurs in hypertensive vasculature, cortisol becomes an MR agonist [43]. That the effect of eplerenone is not due to a diuretic action is shown by the finding that at no dose did the obligate changes in plasma [K^+] differ between responders and non-responders, underlined by the title of the paper – "Distinguishing the Blood Pressure and Electrolyte Effects of Eplerenone".

Fast-forward a decade to a study from Israel, by Ori and colleagues [44]. From 39 consecutive patients referred with 'low renin hypertension' they took 24 who screened positive on ARR but had a plasma aldosterone level below their cut-off (14.5 ng/l); they then compared them with 24 patients with confirmed primary aldosteronism, of whom 7 had an APA. The patients were put on spironolactone (n = 46) or eplerenone (n = 2) for 3 years, and assessed at 1 and 3 years against baseline. At one year antagonist dose averaged 33 mg/day, and at 3 years 29 mg/day. Despite these modest doses the authors reported highly significant results. The requirement for other antihypertensives fell from 2.6 to 1.5, on average; SBP from 149 ± 14 to 126 ± 12; DBP from 88 ± 10 to 78 ± 7 mm Hg; and left ventricular mass index from 143 ± 25 to 118 ± 20, all at one year. These changes persisted, and for some became more marked, at 3 years.

What is striking is that absolutely no difference between the 2 groups of 24 was seen. The authors' conclusion is that "in patients with PA/low renin hypertension, long-term regression of left ventricular hypertension may be achieved with low-dose mineralocorticoid receptor blockers". True, but in the opinion of the present author, there are 2 other salient conclusions that can be drawn from the study. The first is that perhaps ~60% (24/39) patients with so-called low renin hypertension may in fact have occult primary aldosteronism. Secondly, and the reason why such patients were consigned to the LRH category, is that for them the 14.5 ng/dl cut-off for aldosterone used in this study was pitched too high. What this underlines is that whatever the PAC, if the renin levels are sufficiently low to yield an indicative ARR, the patient should undergo confirmatory/exclusion testing.

5. ACTH: the silent confounder?

The fifth area addresses a cognate question, but deserves a separate section. In 2011 Gouli et al. [45] published a paper in which they detailed a further variation of the fludrocortisone saline suppression test (FST) – the dexamethasone-enhanced fludrocortisone saline suppression test (FDST). In 80 normotensives with normal adrenals on imaging who underwent an FDST, the upper limit (97.5%) of normal PAC was set at 3.0 ng/dl, with 2 mg dexamethasone given at midnight before the final day of study. When the same test was administered to 130 consecutive hypertensives, 29% showed PAC values above the upper limit found in control, non-hypertensive subjects. The interpretation offered of this study is that 'normal' levels of PAC are to a surprising extent driven by ACTH, and that as a consequence, a diagnosis of autonomous aldosterone secretion, at lower levels, is thus lost in the noise. The inevitable conclusion from this interpretation is that autonomous aldosterone secretion is at least in part the driver of ≥ 30% of so-called essential hypertension.

An additional, potential and mechanistically puzzling role for ACTH in PA is that of a driver, rather than a confounder, and was very recently published from the same group [16]. A group of essential hypertensives – that is, negative on ARR and FDST – were given ultra-low dose ACTH and their aldosterone and cortisol responses compared with control normotensive subjects. The hypertensives fell into 2 remarkably tight groups, on the basis of a briskly elevated PAC in response to ACTH: hyper-responders showed a marked rise whereas non-responders had PAC levels indistinguishable from control, and which rose to levels < 20% of those in hyper-responders; plasma cortisol levels rose indistinguishably in all groups. Subjects were then subjected to a treadmill stress test: the hyper-responders to ultra-low dose ACTH showed a major increase in PAC, in contrast with a much lesser (~20%) change in controls and non-responders: in all 3 groups cortisol levels were indistinguishable, and did not rise.

The interpretation of these studies is that hyper-responders (27% of 'non-PA' patients on ARR/FDST criteria) have an adrenal glomerulosa which is extremely sensitive to very small increases in ACTH levels, such as might be seen in episodic minor stress, and that a concatenation of such episodes might thus produce a net daily production of aldosterone above normal.

Between them, these studies have the potential to redefine primary aldosteronism. They include some unusual features – persuading control subjects to undergo imaging and then a 4-day FDST; remarkably low variations in plasma aldosterone and cortisol levels in the ultra low-ACTH test; a renin level not low enough to exclude a proportion of the hyper-responders on the basis of ARR, given that their mean PAC was 2.8 ng/dl, which means a reasonable number would have PAC > 3 ng/dl, the FDST cut-off, as previously noted [45].

What this means is that these potential roles of ACTH need to be further studied, not by slavish repetition of the above studies, but by a slightly different approach. Persuading normal subjects to undergo an FDST is a very tall order: ideally, then, the baseline PAC values might be set using a dexamethasone-enhanced seated saline suppression test (DSSST), followed by its application to a cohort of hypertensives. If the results – as expected – are similar to those of Gouli et al. [45], then hypertensives with a PAC below the upper limit of normal might undergo an ultra-low dose ACTH test, to seek to reproduce the findings in Markou et al. [16] cited above.

6. Low dose MR antagonist as part of first-line therapy

The sixth, and last, clinical question is the public health issue inherent in primary aldosteronism. The risk profile of PA is considerably higher than in age-, sex- and BP-matched essential hypertension [46]. The prevalence of hypertension in adults is commonly quoted as ~40% in Japan, and 29% in the USA. On current population figures, plus a figure of PA being 10% of hypertension, this means almost 5 million subjects with PA in Japan, and over 10 million in the USA. In no country at present are sufficient numbers of hypertensives screened to identify 1% of

patients with PA; in 99+% of such subjects, the condition remains occult over their lifetime, truly the tip of the iceberg.

In addition, no country has the capacity, or the available resources, to manage the additional 99+% of patients by the pathways currently recommended [47]. Once an ARR is positive, the patient is then committed to confirmatory/exclusion testing, imaging, AVS and possibly surgery. This is a time-consuming, costly exercise; in addition to cost, there are, for example, very few interventional radiologists able to safely and reproducibly perform AVS.

The question then is what to do? The answer proffered is to include a low dose mineralocorticoid receptor antagonist into first-line treatment for all hypertensives with reasonable renal function. It is safe and efficacious in truly essential hypertension [38], selective in resistant hypertension [48], and game changing in PA. The model is that of vaccination of 12 year old girls against human papilloma virus to prevent carcinoma of the cervix. We do not immunise half that population, and then wait 40 years to compare the outcomes. Primary aldosteronism is far more common in women than cervical cancer, but far less immediately bleak; it deserves at least a similar degree of intervention to protect those at risk.

7. New answers: pathophysiology: passé or phoenix?

One final note, to revert from the clinical back to basic science. In the excitement of molecular genetics, we may be excused for jettisoning pathophysiology, though at our peril. In the mountains of New Guinea during the monsoon season the average daily sodium intake is 2–3 meq; in response aldosterone levels are sky-high, blood pressure low normal and there is absolutely no cardiovascular damage. For the latter what is needed are aldosterone levels inappropriate for salt status; we do not know how salt acts under these circumstances. One possible way is to elicit the protracted over-secretion of endogenous ouabain-like (EO) molecules, as has been previously canvassed in full [49]. EO rises, like aldosterone in response to ACTH, angiotensin II (but via AT_2R) and – in sharp contrast to aldosterone – to sodium loading. EO acts on membrane-located Na^+/K^+ATPase to cause vasoconstriction and resultant tissue damage. If this is the case, then the deleterious cardiovascular effects of autonomous aldosterone secretion may not primarily lie in its (unlikely) ability to be directly cardiotoxic – for example, in cardiomyocytes unprotected by 11βHSD2 – but its ability to over-reabsorb sodium in the renal tubules, and to raise EO levels as the proximate cause of cardiovascular damage.

If this were shown to be the case it would bring considerable satisfaction not just to nephrologists, who have always stoutly maintained the primacy of the kidney, but also to clinicians and physiologists. For clinicians, it would validate using ENaC inhibitors (amiloride, triamterine) routinely in conjunction with low dose mineralocorticoid receptor antagonists; for physiologists, it would mean the answer to a long-vexing conundrum, in demonstrating the pathophysiologic role of a pathway which presumably evolved to produce a pressure diuresis after sodium loading, when the requisite lowering of aldosterone levels cannot be accomplished.

If this conundrum were answered over the next 5 years it would represent a triumph for interactive medicine, systems biology – or whatever is currently modish name for physiology.

Conflict of Interest

The author declares no conflict of interest.

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