# Platt versus Pickering: what molecular insight to primary hyperaldosteronism tells us about hypertension

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DECLARATIONS

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## Summary

Recent genome-wide analyses have found 50 loci associated with variation in blood pressure but failed to advance understanding of the molecular basis of hypertension. Whether hypertension is not after all due to multiple common variants or is simply an order of magnitude more complex than previously suspected remains unsettled – in part because only a minority of subjects in the analyses had true hypertension. A better starting point than normotensive subjects for explaining hypertension may be the most common distinct cause of hypertension, primary hyperaldosteronism (PHA). The findings that 40% of patients with an aldosterone-producing adenoma (APA) of the adrenal have somatic gain-of-function mutations in a single gene, *KCNJ5*, and that this gene is, less frequently, mutated in inherited cases of PHA, potentially transform the understanding and management of hypertension. Firstly, they illustrate how hypertension could be due to a multiplicity of uncommon variants. Mutations that present with abnormal electrolytes and anatomy are the easiest to detect but are likely the tip of the iceberg. Secondly, we found a genotype:phenotype pattern, with *KCNJ5* mutations inducing larger APAs in the cortisol-secreting zona fasciculata in young women. Smaller APAs without *KCNJ5* mutations usually present in older men with resistant hypertension, having been overlooked earlier because of their size. This reflects their compact zona glomerulosa cells. Routine measurement of plasma renin in hypertension and a new positron emission tomography/computerized tomography allow prompt diagnosis and management of PHA before resistant hypertension ensues. Wider recognition of distinct phenotypes should permit earlier, specific treatment and reduce life-time risk of complications.

## Genetic analysis of complex disorders

The advent of genome-wide association studies (GWAS) has brought bitter-sweet results for complex disorders like hypertension. On the one hand, we can now finger – with the sort of statistical certainty previously reserved for forensic DNA fingerprinting – dozens of sites in the genome that are contributing to inherited variation in blood pressure. On the other, the highest risk from any site is 1 mmHg of blood pressure – in most cases rather less; in no case can we be absolutely certain which specific gene is responsible, far less have any idea of where or how in the gene a variant is influencing blood pressure; and the

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assumption that hypertension is no more than the sum of its parts remains untested and unproven.[1] In some diseases, even small relative risks may be the clue that Pharma needs to identify a biochemical pathway worth targeting with novel drugs, and it will be the response to such drugs that helps eventually elucidate disease mechanisms. In hypertension, the glut of existing successful drugs reduces the chances of speculative development, and a 1 mmHg relative risk offers an unpromising signal for mechanistic studies to work with.

There is now an interesting discussion as to whether GWAS has employed insufficient data (single nucleotide polymorphisms [SNPs] or subjects), under-interpreted the data or used the wrong data.[2–4] Whichever turns out to be the case, the potential positive aspect of this outcome from the GWAS experience is the renaissance of clinical research and acumen. These will be required in the search for distinct 'extreme' phenotypes in whom genetic analysis might reveal rare variants explaining most or all of the phenotype. GWAS was undertaken using the million SNPs whose minor allele is present in 5% or more of the population. These SNPs are old, and being in so-called 'linkage disequilibrium' with other SNPs in the vicinity, can be used as signposts to the presence of disease susceptibility alleles. By contrast, with individuals differing from each other by an average of three million SNPs, with 200 novel SNPs discovered each time a new exome (coding region) is sequenced, the rare SNPs outnumber many-fold those used for GWAS. Many are now documented by the '1000 genomes project'; but on our exome sequencing we have found about 5% of SNPs to be novel, with almost 2% changing amino acid coding. While the majority of these SNPs are probably of no functional consequence, the dispute among geneticists is what portion of common disease is due to rare variants – defined as those present in <1% of the population.[2–4] In hypertension, the question is whether each family with hypertension is a different phenocopy, with its own private mutation; or hypertension is a 1000-piece jigsaw, with each patient a different permutation among the many million created by interactions between alleles of individually low relative risk.

This question will be recognized as a 21st century re-statement of the famous Pickering versus Platt debate of the 1950s, with Platt regarding hypertension as a distinct condition, and Pickering as one extreme of a continuous distribution.[5] If blood pressure is set by the number of low-risk susceptibility variants (or absence of protection variants), then there can be no qualitative distinction between hypertension and normotension. But if hypertension is commonly due to high-risk rare variants with each family having a genetically private phenocopy, then the use of tens of thousands of normotensive subjects for GWAS may have contributed to this being even less useful than in other complex disorders.

Until now, the view has been that – unless there was some heterozygote advantage, such as protection against common infections – powerfully deleterious mutations would be rapidly selected against, so that high relative-risk variants are likely to be rare and relatively new in the population. Some support for this is offered by the growing evidence that a higher proportion of patients with clearly monogenic syndromes have de novo mutations than might be expected from their accumulation over generations. If this is true, then a corollary will be that organ-specific disease due to somatic mutations are stochastically even more likely than inherited cases, because the number of cell divisions during organogenesis and subsequent repair, is much larger than the number of germ cell divisions. A recent exciting discovery in the field of hypertension illustrates this point, and shows how a mutation can have everything to do with hypertension and nothing with normal blood pressure variation.

## KCNJ5 mutation in the adrenal – dawn of a new era

The commonest identifiable cause of hypertension, present in >10% of all patients, is primary hyperaldosteronism (PHA), with approximately half the patients having the curable form, namely a unilateral aldosterone-producing adenomas (APAs) of the adrenal. Since the original description of PHA, by Conn in 1956, the biochemical basis – and hence tests for initial diagnosis – have been clear, namely an elevated aldosterone secretion despite a suppressed plasma renin. Usually PHA is sporadic, but rare

germline syndromes are known. One is caused by a chimaera between the neighbouring genes – CYP11B1 and CYP11B2 – which respectively encode the final enzymes in the cortisol and aldosterone synthetic pathways.[6] In 2008, a new Mendelian syndrome was reported causing hypertension in which the children of one family had the biochemical features of primary PHA, and bilaterally large adrenal glands.[7] Then in 2011, Choi *et al*.[8] used exomic sequencing to find that two out of four APAs of the adrenal had somatic mutations of a *K*+ channel, KCNJ5, that sequencing of 16 further APAs revealed another six with KCNJ5 mutations, that these mutations removed the selectivity of the channel for *K*+ – allowing *Na*+ entry and hence cell depolarization – and, finally, that the probands of the 2008 report had a germline mutation of KCNJ5 which also caused depolarization. Whether the depolarization causes increased aldosterone secretion, cell division or both, was unclear. But despite the inclinations of the accompanying editorial in Science to mute the excitement of these discoveries, by suggesting that Choi's APAs were atypically large compared with those removed from most patients with PHA, the implications are writ large.[9]

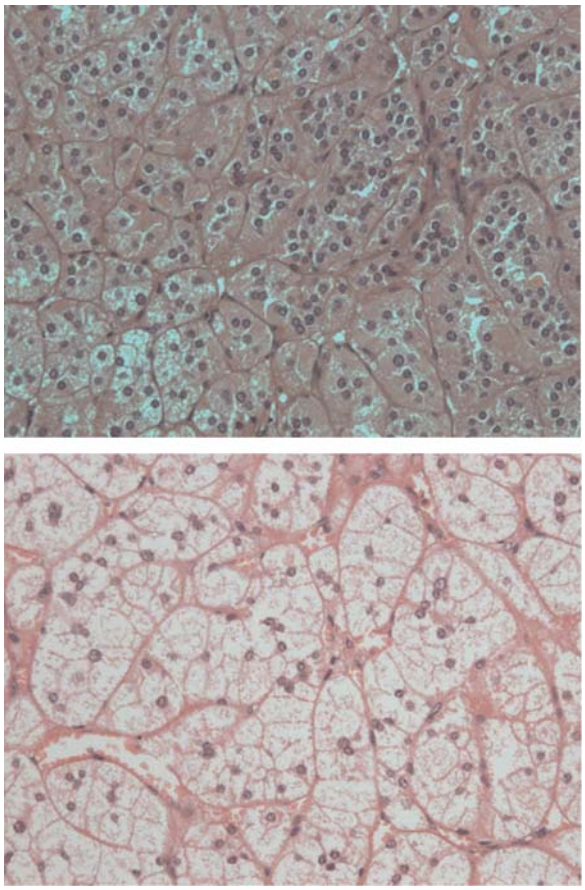
At one level, the interest from Choi's discovery is in the implications for diagnosis and management of the most common curable cause of hypertension. But the wider, more speculative implications are for the overall contributions of somatic and germline mutations to hypertension, and to complex disorders in general. PHA is of particular interest as a distinct cause of hypertension because in some patients it arises from an anatomically distinct abnormality that can be removed.[10] And its classical (if frequently absent) feature of hypokalaemia facilitates diagnosis in a patient group having routine electrolyte tests. But, as with the rare monogenic syndromes causing hypertension, it is unlikely that the only syndrome with common somatic mutations is one whose phenotype trips off the autoanalyser. To date the mutations have been reported only in APAs – but only APAs have been studied as it is rare to remove adrenals without them. Yet at least half of PHA is due to bilateral disease, sometimes with radiologically visible nodules but often not. From Choi's initial report, it seemed that KCNJ5 mutations would be associated with either adenoma formation (the APAs) or gross hyperplasia (the Mendelian syndrome). Subsequently, however, it has been reported that germline mutation can cause PHA without radiologically abnormal adrenals.[11] Our own exome sequencing suggests that mutations in APA are multiple, but does not resolve how many arise before APA development. So now for the moment we have a paradigm where a long recognized subset of hypertension, PHA, may be due to common, single mutations and more rarely to germline mutation in the same gene: the proportion that might be expected from the stochastic argument above. The lack of a distinct anatomical abnormality in some patients with germline KCNJ5 mutation further suggests that somatic mutations might be commoner than APA frequency.

## Clinical implications

Since the germline mutations are rare, and somatic mutations cannot currently be diagnosed without the surgical specimen – a bit late for influencing the decision whether to operate! – their discovery might at first sight seem of great academic but little practical import. This would be wrong. PHA is estimated to be present in at least 10% of patients with hypertension, and up to 25% of those with resistant hypertension – usually older patients with blood pressure above target despite treatment with three or more drugs.[12,13] Since apparent resistant hypertension can include patients who simply do not adhere to therapy, even 25% may be an underestimate. Although rigorous evaluations are lacking for postoperative outcomes after adrenalectomy for PHA, it is probably less common for the older patients presenting with resistant hypertension to be cured than the younger patient diagnosed at an earlier stage in their hypertension. Until now, there has been no consideration of whether these two ends of the spectrum might be different diseases, and of the relevance this would have to diagnosis and management.

Shortly before Choi's report, we had performed a microarray analysis comparing eight APAs with the adjacent 'normal' adrenal. Because of the clinical heterogeneity among PHA patients, we chose a range of APAs to span this spectrum. It became apparent, from unsupervised cluster analysis of

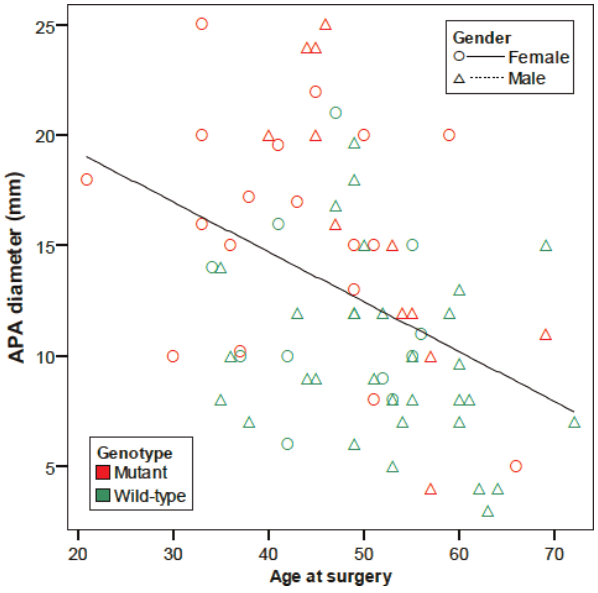
**Figure 1**
**Comparison of zona glomerulosa-like (upper panel) and zona fasciculata-like (lower panel) aldosterone-producing adenomas. These were subsequently found to lack or possess, respectively, a somatic mutation in KCNJ5. In 46 aldosterone-producing adenomas, the 20 with mutations had 15% of compact, zona glomerulosa -like, cells, compared with 40% of cells in the 26 APAs with no mutation ($P = 0.001$)**



the transcriptomes, that we had indeed selected among our eight two outlying patients at opposite ends of the transcriptional spectrum. Histological review of all the APAs, and grading by their expression of the steroidogenic enzyme CYP17A1, showed that the two extreme APAs appeared to arise, respectively, from the zona fasciculata (ZF) and zona glomerulosa (ZG) of the adrenal cortex. This was confirmed by reviewing all 46 pairs of APA and adjacent adrenal which we had collected and frozen immediately after adrenalectomy. While many of the APAs had the classical hybrid picture of mixed ZG- and ZF-type cells, there were a few which had all or none of each cell type (Figure 1). There was a highly significant correlation between the CYP17A1 expression and percentage of ZF cells, estimated by an adrenal pathologist blind to the biochemical data; CYP17A1 encodes the enzyme which converts aldosterone precursors to cortisol precursors, and is normally considered absent from ZG.[14]

So Choi's paper suggested two immediate questions. Most pressing was whether the high rate of mutation was reproducible in larger collections of APAs, of a size more typical of those diagnosed in everyday practice. But even more interesting to us was whether the APAs with mutations are different from those without – the 'wild-type' APAs. The answer is as clear a 'yes' as one could hope from a complex piece of biology. First, we found that in our unselected series of 46 APAs, the prevalence of KCNJ5 mutation was even higher than in Choi's initial series of 22, at 20/46 (43%), with a further novel mutation – I157DEL – in addition to the two somatic, L168R and G151R and germline T158A.[15,16] Secondly, when we examined the demographics of the patients, and characteristics of the APAs, it was apparent that the mutant and wild-type phenotypes differ by age, gender, size, biochemistry and histology, and appear to arise respectively from ZF and ZG. Mutant APAs were three times larger, with >3-fold higher concentration of CYP17A1 (ZF-enzyme). And most strikingly, the proportion of ZG-like cells in the wild-type APAs was almost three times higher than in the mutant. In order to replicate the clear inference from this data that the mutant and wild-type APAs are a different disorder, we genotyped a further 27 APAs from collaborators in Australia. As illustrated in Figure 2, there are two overlapping patterns. The mutant APAs are larger, and present mainly in younger women; the wild-type APAs are smaller, and present mainly in older men. We did not have expression data from the Australian APAs, but their clinical work-up showed that the wild-type and mutant APAs differed in their aldosterone response to change of posture, interpreted as presence or absence, respectively, of angiotensin responsiveness.[17–19] This is a feature of ZG cells, so consistent with our histological and biochemical findings in the

**Figure 2**
**Genotype:phenotype relation of aldosterone-producing adenomas. The tumours with KCNJ5 mutations were larger and clustered in younger women; the wild-type tumours were smaller and clustered in older men. Age ($\beta = -0.32$, $P = 0.004$) and genotype ($\beta = -0.42$, $P < 0.001$) were independent predictors of size (data from Azizan *et al.*[15])**




46 core Cambridge APAs. The ZF nature of some APAs explains why hybrid steroids – those needing the enzyme products of both the CYP11B1 and CYP11B2 for synthesis – might be a marker for APA rather than bilateral adrenal hyperplasia, though we would predict only for the KCNJ5-mutant APAs.[20]

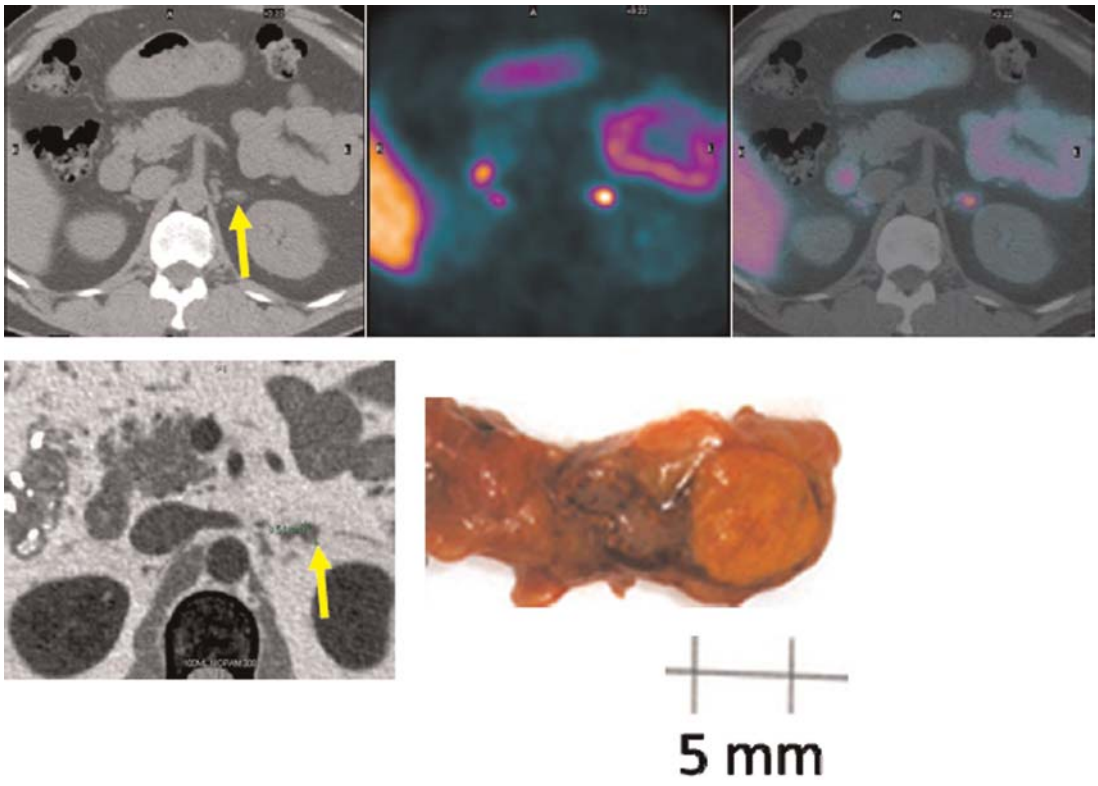
The clinical importance of these genotype:phenotype findings is this. The younger patients with classical 1–2 cm APAs, more often women than men, have mainly mutant APAs, seemingly of ZF origin. The older patients, who are most likely to present with drug-resistant hypertension, have mainly wild-type APA, <1 cm in diameter, arising in ZG. The implication is that we either overlook or ignore the wild-type APAs because of their smaller size. Yet the smaller size, we can now see, is not a reflection of lower potency or importance of the tumour. The opposite is the case, with the small size reflecting the smaller size of ZG – relative to ZF – cells.

It is possible that there is genetic or ethnic variation in the effect of the somatic KCNJ5 mutations since neither the American or European series found the size difference in the Anglo-Australian collaboration.[8,21] While the possibility cannot yet be excluded that the ZF-appearance of mutant APAs is due to a phenotypic change in a ZG-cell, it now seems more likely that the depolarization by KCNJ5 mutation of ZF cells induces CYP11B2 expression. In support of this is the distribution of the related channel KCNJ3. Both these genes need to be co-expressed in order to induce a $K+$ current. Whereas immunohistochemistry shows KCNJ5 to be predominantly ZG, with some ZF staining, the reverse is true of KCNJ3. So the mutations in KCNJ5 are more likely to depolarize ZF- than ZG-cells.

## How clinical practice should change

So what is to be done? If 10–25% of resistant hypertension is the consequence of curable APAs being missed, we need to be more alert to the small adenomas. Figure 3 illustrates one of our patients whose PHA came to light because of our practice of screening all patients with a plasma renin, and recognition in particular that a renin mass in single figures in younger patients requires explanation – and, usually, an adrenal scan. This patient was normokalaemic except when challenged with a thiazide diuretic.[22] The scan needs to have thin, 1–2 mm cuts in order to have a chance of detecting 5 mm microadenomas. The next challenge is to show that aldosterone secretion is lateralized to one adrenal. Adrenal vein sampling (AVS) does not always succeed even if both adrenal veins are cannulated – in this patient, there was less than the diagnostic four-fold difference in aldosterone/cortisol ratio between sides on two occasions. So we treated him medically for four years until we could use our 11C-metomidate positron emission tomography computed tomography (PET CT).[23] This has been validated as a non-invasive alternative to AVS, of particular value when AVS is technically unsuccessful. However, further research is evaluating our preliminary evidence that it detects small APAs which escape detection by AVS or even show as a distinct adenoma on CT/magnetic resonance imaging (MRI). This potential is exemplified by the patient in question, since only on

**Figure 3**
**11C-metomidate positron emission tomography computed tomography of wild-type male with microadenoma. The initial magnetic resonance imaging, showing a possible adenoma, and operative specimen are shown below**




the PET CT was it clear that there was a distinct adenoma, and that it was functional and should be removed.

## Implications for hypertensive patients without APAs

Not infrequently the main adenoma is associated with microscopic nodules which may stain irregularly but densely with a specific anti-CYP11B2 antiserum. Alternatively, the ZG may simply show a several-fold increase from the 2–3 layers of cells in a Na+-replete normal subject. Neither of these changes will create an abnormal CT or MRI scan – or PET CT with the current tracer, but maybe by using one of the new selective aldosterone synthase inhibitors as tracer. Somatic mutation – of KCNJ5, or other genes which exomic sequencing should reveal – is unlikely to be limited to macroadenomas. Micronodular hyperplasia is often assumed to be bilateral, although unilateral cases are described.[24,25] But on reflection, cases are as likely as not to be unilateral if due to mutational events which occur after the first adrenal stem cell has divided left and right.

Whether it is attractive to contemplate a large increase in adrenal surgery is a moot point. As a physician and pharmacologist, my delight at seeing cure is tempered by a preference for a tablet to the scalpel. The current incentive to seek cure is driven by the inadequacies of chronic mineralocorticoid receptor blockade. Spironolactone, even at low doses, causes insidious onset of gynaecomastia in men; it is no longer licensed in the UK for the treatment of hypertension, because of an excess of thyroid cancers in

rodents; and the long-term consequences of the large increases in renin and aldosterone are unknown.[26] The alternative, eplerenone, is relatively ineffective in PHA.[27] The combination of eplerenone with amiloride is one option, which seems to achieve good blood pressure control in most patients, but has not been formerly tested. A better option may be the advent of selective aldosterone synthase inhibitors.[28–30] And the discovery of common somatic mutations in the adrenal of patients with PHA may also encourage development of entirely novel classes targeting the mutated gene product. Persuading either Pharma or doctors that another class of antihypertensive drugs is required for hypertension may be an uphill struggle. But this is largely because of the mistaken perception, from apples and pears meta-analyses, that all hypertension is much the same – a murky mixture of minute and unfathomable causes, which responds equally well to any of the numerous available treatments.[31–33] How different if hypertension is a mixture of distinct molecular disorders. The challenge is to find the 5% of patients who would benefit from receiving an aldosterone synthase inhibitor before they have developed resistant hypertension and the target organ damage that results from the hypertension and, possibly, hyperaldosteronism.[29,34] Whether the diagnostic fingerprint will be a circulating piece of the genome, a urinary proteome, or some other omic novelty, it will surely be economic to apply our exploding knowledge of the adrenal to the prevention of heart disease and stroke in patients whose blood pressure is anything but normal.

## Conclusions

The discovery of a somatic mutation in APAs has within a year had ramifications for our treatment and understanding of hypertension. The underdiagnosis of the commonest curable cause of hypertension can now be addressed and reversed. Whether such patients are exceptions or a paradigm remains unanswered. However we should no longer assume that hypertension is a mere continuation of the normal blood pressure distribution, or that most patients' disease is too complex for an understanding of pathogenesis to influence treatment.

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