# The role of Aldosterone and PTH in Human Primary Aldosteronism and Vascular Calcification

# Addendum

Valorisation

Academic research is the most important repository of cutting-edge knowledge. A joint community of scientists share discoveries, being frontier of and expand knowledge that benefits society. The social impact of research is obvious when it provides a product. The findings presented within this thesis do not offer an immediate commercialized solution to cardiovascular problems but provide detailed pieces of a larger puzzle that might result in an explanation for the cause and consequences of cardiovascular disease.

In the era where CVD represent 31% of global deaths[1] I would like to cite Paul Dudley White, the 'Father of US Cardiology' who loved to say "Heart disease's death, before 80, is our fault, not God's or Nature's will". This somewhat bold statement does connect however to hypertension, a condition that is a major risk factor for CVD and which can effectively be prevented by proper management.

Because of its high prevalence and poor rate of blood pressure control, arterial hypertension (HT) is the major cause of mortality and early disability worldwide [2]. Most forms of arterial hypertension include primary aldosteronism (PA), secondary aldosteronism (due to renin-producing tumors, renovascular stenoses, and high-renin essential hypertension), hypertension associated with overweight-obesity[3,4], and drug-resistant forms[5]. All forms of arterial hypertension recognize aldosterone as main determinant of blood pressure. PA, albeit often overlooked, is the most common form of arterial hypertension, implicated in 5-13% of hypertensives[6–8]. Lack of mechanistic knowledge has impeded the development of effective preventive strategies and timely diagnostic strategies[9]. This results in late, or even missed diagnoses with ensuing development of drug-resistant hypertension and cardiovascular complications. The key objective of my thesis was to gain further insight into the mechanisms by which aldosterone synthesis is regulated with an overall impact on the cardiovascular system.

Molecular studies performed in the last decade have highlighted that most of the familial and sporadic cases of PA involve mutations in the selectivity filter of the KCNJ5 gene[10], encoding for the Kir3.4 potassium channel. It has been shown that H295 cells overexpressing mutant Kir3.4 have altered channel function causing: i) permeability to $Na^+$; ii) cell depolarization; and iii) $Ca^{2+}$ influx[10]. Increased intracellular $Ca^{2+}$ levels were also discovered in other gene mutations controlling ZG cell cation homeostasis, such as ATP1A1, ATP2B3, CACNA1H and CACNA1D[11–13]. Our work embarked on the recent discovery of two Kir3.4 mutations (G151R and L168R) that render the KCNJ5 channel in HAC-15 cells specifically sensitive to inhibition by macrolides, and that these agents concentration-dependently blunt Aldosterone production. This is indicative of an altered physiology of this channelopathy underlying PA that can be corrected[14]. However, the mechanisms whereby macrolides and their derivatives work are unknown, and our work laid down the fundament for further personalized treatment of PA. Developing better investigative tools will

be a prerequisite for novel diagnostic tests. It is conceivable that these will have a positive economic and technological impact on society at large in partnership with industry.

Our team was the first to analyze the molecular signature of aldosterone-producing adenoma using a whole transcriptome analysis. This led to the discovery that increased levels of microRNA-23 and microRNA-34 via direct binding to the 3' UTR of the type 2 TWIK-related acid-sensitive $K^+$ channel (TASK-2) gene cause a consistent under-expression of this channel in APA15. These genetic variations in the promoter region of TASK2 gene could explain development of PA in 25% of APA cases. Our work explains some 25% of APA cases, however in most of the cases the mechanisms have still to be unraveled.

To further understand the mechanisms leading to the pathogenesis of PA we investigated the intertwined hormonal implications. Intriguingly, our observations show that estrogens have a novel role in the regulation of aldosterone synthesis via GPER activation. Aldosterone acts in an autocrine-paracrine manner in GPER overexpressing APA, thereby generating high local concentration of mineralcorticoid hormone. Thus, self-perpetuating hyperaldosteronism, whereas the renin angiotensin system is blunted. These findings might have implications for the vast population women with breast or ovarian cancer, who are being treated with ER modulators (SERM). Although there is no knowledge of the off-target effects of SERM it is conceivable that these GPER agonists increase aldosterone production, and thus subsequently increase BP. Our data reveal a yet unknown side effect of ER modulatory cancer treatment on the cardiovascular system.

Single patient cases can shed light on molecular pathways leading to cardiovascular disease. Analyzing a patient with concurrent aldosterone-producing adenoma and primary hyperparathyroidism (PHPT) due to a parathyroid- hormone-secreting adenoma revealed that hyperparathyroidism is a feature of primary aldosteronism and particularly of aldosterone-producing adenoma16. In addition to primary aldosteronism, PHPT is known to be related to hypertension, which is not surprising, given that parathyroid hormone stimulates aldosterone secretion[17], promotes vascular calcification and affects arterial stiffness[18]. Thus, a possible genetic link between PHPT, hypertension and the feasibility of genetic testing is mandatory and currently under further investigation.

Considering the vast impact of cardiovascular disease on health care systems, it is fundamental to investigate the most prevalent complication of CV disease, such as vascular calcification. Vascular calcification is predominantly present in chronic kidney disease due to the imbalance in calcium and phosphate homeostasis. Moreover, chronic kidney disease patients suffer from severe vitamin K deficiency, in part induced by phosphate lowering strategies. We

developed a novel animal model of kidney failure with additional vitamin K-deficiency, thereby mimicking the clinical situation. We showed that PB therapy in combination with vitamin K2 supplementation strongly attenuated VC, whereas the single treatments were not effective. Our in vivo preclinical study combining PB therapy with vitamin K2 supplementation shows that combination therapies might be superior compared to single therapies. This finding might have a great clinical impact since phosphate binder therapy is common standard therapy for end stage kidney patients.

Translational medicine projects like described in my thesis are known to be most challenging but also most rewarding. The reward comes from the drive to generate knowledge that will potentially provide solutions for better care. This project, dissecting the molecular effectors of hyperaldosteronism and use of animal models in combating vascular calcification is, therefore, relevant not only for hypertensive patients, but also for those affected by other common cardiovascular diseases. Hence, a better understanding of the mechanisms underlying the complex regulation of aldosterone synthesis represents a major scientific achievement, with far reaching implications for general cardiovascular health.

# References


1. Cardiovascular diseases (CVDs). *WHO* Available at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). (Accessed: 18th March 2020)
2. Kearney, P. M. *et al.* Global burden of hypertension: analysis of worldwide data. *Lancet* **365,** 217–223 (2005).
3. Bentley-Lewis, R. *et al.* Body Mass Index Predicts Aldosterone Production in Normotensive Adults on a High-Salt Diet. *J. Clin. Endocrinol. Metab.* **92,** 4472–4475 (2007).
4. Rossi, G. P. *et al.* Body Mass Index Predicts Plasma Aldosterone Concentrations in Overweight-Obese Primary Hypertensive Patients. *J. Clin. Endocrinol. Metab.* **93,** 2566–71 (2008).
5. Williams, B. *et al.* Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): A randomised, double-blind, crossover trial. *Lancet* **386,** 2059–2068 (2015).
6. Rossi, G. P. *et al.* A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J. Am. Coll. Cardiol.* **48,** 2293–2300 (2006).
7. Monticone, S. *et al.* Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. *J. Am. Coll. Cardiol.* **69,** 1811–1820 (2017).
8. Maiolino, G., Calò, L. A. & Rossi, G. P. The Time has Come for Systematic Screening for Primary Aldosteronism in All Hypertensives. *J. Am. Coll. Cardiol.* **69,** 1821–1823 (2017).
9. CONN, J. W. & LOUIS, L. H. Primary aldosteronism, a new clinical entity. *Ann. Intern. Med.* **44,** 1–15 (1956).
10. Choi, M. *et al.* K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science* **331,** 768–772 (2011).
11. Beuschlein, F. *et al.* Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat. Genet.* **45,** 440–4, 444–2 (2013).
12. Azizan, E. A. *et al.* Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat. Genet.* **45,** 1055–1060 (2013).
13. Scholl, U. I. *et al.* Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat. Genet.* **45,** 1050–1054 (2013).
14. Scholl, U. I. *et al.* Macrolides selectively inhibit mutant KCNJ5 potassium channels that cause aldosterone-producing adenoma. *J. Clin. Invest.* **127,** 2739–2750 (2017).
15. Lenzini, L. *et al.* Heterogeneity of aldosterone-producing adenomas revealed by a whole transcriptome analysis. *Hypertension* **50,** 1106–1113 (2007).
16. Maniero, C. *et al.* Primary hyperparathyroidism with concurrent primary aldosteronism. *Hypertension* **58,** 341–346 (2011).
17. Mazzocchi, G., Aragona, F., Malendowicz, L. K. & Nussdorfer, G. G. PTH and PTH-related peptide enhance steroid secretion from human adrenocortical cells. *Am. J. Physiol. Endocrinol. Metab.* **280,** E209-13 (2001).
18. Widimsky, J. *et al.* Vascular disturbances in primary aldosteronism: Clinical evidence. *Kidney and Blood Pressure Research* **35,** 529–533 (2013).
19. Lenzini, L. *et al.* PTH Modulation by Aldosterone and Angiotensin II is Blunted in Hyperaldosteronism and Rescued by Adrenalectomy. *J. Clin. Endocrinol. Metab.* **104,** 3726–3734 (2019).
20. Caroccia, B. *et al.* Aldosterone Stimulates Its Biosynthesis Via A Novel GPER Mediated Mechanism. *J. Clin. Endocrinol. Metab.* (2019). doi:10.1210/jc.2019-00043