The epithelial Na^+ channel: progressing from Liddle's syndrome to essential hypertension

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The amiloride-sensitive epithelial sodium channel (ENaC) is expressed in the distal part of the renal tubule where it constitutes the rate-limiting step of Na^+ reabsorption, under the control of aldosterone [1]. Activating mutations of the β [2] and γ [3] subunits of ENaC lead to increased Na^+ and water reabsorption and are at the origin of the rare form of autosomal dominant hypertension (HTN) described by Liddle in 1963 [4]. The classical description of Liddle's syndrome includes early severe HTN with hypokalemia, metabolic alkalosis and suppressed plasma renin activity (PRA) and aldosterone. These abnormalities are corrected by low Na^+ diet associated with amiloride, a specific blocker of ENaC, but not by spironolactone [4].

In view of the critical role of ENaC in the regulation of Na^+ reabsorption, it was hypothesized that more subtle genetic changes in ENaC subunits might be at the origin of less rare forms of HTN. To test this hypothesis, screening of the entire coding sequence of the β and γ subunits of ENaC was performed in a large database of essential hypertensives [5,6]. Unfortunately, variants leading to changes in the amino acid sequence of the ENaC subunits were seldom encountered in Caucasians and appeared unlikely to play a major role in the pathogenesis of essential HTN in this subset. By contrast, in patients of African descent, genetic variants of ENaC subunits were found to be much more frequent [5,7]. In particular, the polymorphism T594M of the β subunit of ENaC was associated with hypertension in Black patients from the London area [8], although this finding was not confirmed in US Blacks [9]. Sodium currents measured after expression of the mutated T594M ENaC in *Xenopus* oocytes were not increased compared to the wild type (5). However, Cui *et al.* [10] reported a loss of protein kinase C inhibition in lymphocytes from patients harbouring the T594M variant. Another variant frequently encountered in patients of African descent, G442V [5] was found to be associated with decreased urinary aldosterone/K^+ ratio compared to controls subjects, consistent with increased ENaC activity [7]. However, it was not associated with HTN within the same population and did not alter ENaC function in *Xenopus* oocytes [7]. In view of these conflicting data, the functionality of ENaC polymorphisms remains a matter of debate.

The 'story of ENaC' is reminiscent of many research works in the field of genetics of hypertension. Indeed, while the genetic abnormalities at the origin of several forms of Mendelian hypertension (glucocorticoid remediable aldosteronism, Liddle's syndrome, apparent excess of mineralocorticoids) have been successfully unravelled, the search for milder genetic abnormalities involved in the pathogenesis of essential hypertension has been disappointing [11].

One of the main pitfalls in the hunt for HTN genes is the major heterogeneity of essential hypertension. Rather than screening large databases of unselected essential hypertensive patients, it might prove more rewarding to identify subsets of patients with well-defined clinical and biochemical profiles, who are likely to share common genetic and physiopathological mechanisms, and/or to look for intermediary phenotypes suggestive of particular genetic influences [11]. Using this strategy, Nicod *et al.* [12] recently suggested the existence of a significant association of a biallelic polymorphism of *CYP11B2*, the gene encoding aldosterone synthase with increased aldosterone-to-renin ratio in Caucasian hypertensive patients.

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Similarly, in this issue of the *Journal*, Rayner *et al.* [13] describe a new variant of βENaC, R563Q, associated with low-renin, low-aldosterone hypertension (LRHT) in patients of African or mixed descent from South Africa. Indeed, in these ethnic groups, the variant was significantly more frequent in hypertensive patients with LRHT than in normotensives or normal–high renin hypertensives. Furthermore, nine of the 14 patients harbouring the variant had both suppressed PRA and plasma aldosterone and two additional R563Q patients showed suppressed aldosterone with only incomplete suppression of renin. Although direct evidence of ENaC activation in patients harbouring the variant is lacking, which is a clear limitation of the study, these intriguing data are in favour of a functional effect. The authors suggest that the R563Q substitution might prevent phosphorylation of the consensus site KSLR by protein kinase C, leading to inefficient ENaC downregulation, subsequent increased Na^+ and water reabsorption and suppression of the renin–angiotensin axis. The finding of the R563Q variant in one normotensive subject and the fact that the full-blown Liddle's phenotype, including hypokalemia, was only present in a minority of R563Q patients should not lead to rejection of this hypothesis, as such a phenotypic variability is well described, even within classical Liddle's kindreds [14,15].

Hypertension is particularly common in patients of African descent. These patients are known to have more frequently HTN of the low-renin type and to be more salt and diuretic-sensitive than Caucasian patients [16]. They differ also from Caucasians by lower plasma aldosterone [17,18] and lower urinary aldosterone/K^+ ratio [7], as well as by increased ENaC activity [19]. Furthermore, polymorphisms of ENaC are much more prevalent in patients of African descent than in Caucasian patients [5,7]. It is thus tempting to hypothesize that suppression of the renin–aldosterone axis in these patients is a corrective mechanism designed to maintain sodium balance in the presence of an increased Na^+ reabsorption, due to ENaC activation [16]. Increased Na^+ reabsorption could be the result of either ENaC variants, such as R563Q, or mutations of factors involved in the turnover of ENaC at the apical plasma membrane, such as the ubiquitine-protein ligase Nedd4 and the serum glucocorticoid-inducible kinase1 (Sgk1) [1].

Although provocative, the finding of a new variant of ENaC associated with LRHT is the beginning, rather than the end of the work. Definite evidence of its implication in LRHT would require more direct demonstration of an activating effect of ENaC, either *in vitro* or *in vivo*, and preferably both. Unfortunately, previous work has revealed the difficulties involved in establishing the functionality of ENaC variants [20]. While Liddle's mutations affecting the classical PY domain involved in internalization of the channel have been associated with clear increases in amiloride-sensitive Na^+ currents in *Xenopus* oocytes [21,22], this was not the case for point mutations of ENaC subunits located outside this critical region, until recently [23]. However, such negative results do not rule out an in-vivo effect of the variants studied, because (i) the cellular machinery of amphibian cells such as *Xenopus* oocytes is very different from that of mammalian cells of the distal tubule, and (ii) small changes in ENaC activity that may remain unnoticed by this technique could lead to significant Na^+ and water retention over time [20]. Another in-vitro approach also validated in Liddle's syndrome consists of measuring amiloride-sensitive Na^+ currents in immortalized B lymphocytes derived from subjects harbouring or not the variant [24]. Furthermore, the measure of nasal membrane potential differences before and after amiloride might allow the activity of the channel to be evaluated *in vivo* [25]. An increase in nasal potential differences was indeed shown in situations thought to be associated with ENaC activation, such as Liddle's syndrome [25] or African descent [19]. However, it should be acknowledged that none of these methods provides a straightforward demonstration of the functionality of ENaC variants, and that each of them has it own limitations, especially due to species and/or tissue differences [20].

Other validation criteria for this new candidate variant as a predisposing factor for LRHT include replication in other populations showing a significant prevalence of the variant, as well as demonstration of genotype–phenotype cosegregation. While cosegregation with HTN might be obscured by a host of genetic and environmental factors, cosegregation with the low renin, low aldosterone phenotype might prove easier to demonstrate. In this respect, it is interesting to note that the only normotensive patient harbouring the R563Q variant in this study had suppressed PRA and plasma aldosterone.

If confirmed, the association of the R563Q variant with LRHT implies that mutations located outside the critical PY domain of the β subunit of ENaC may be at the origin of an incomplete form of Liddle's syndrome. In the same sense, Hiltunen *et al.* [23] recently described a family with all the characteristics of Liddle's syndrome due to a mutation ($Asn^{530}Ser$) located outside the PY domain (i.e. in the extracellular loop of the γ subunit of ENaC). Furthermore, expression of the mutant γ subunit of ENaC in *Xenopus* oocytes demonstrated a significant increase in ENaC activity, compared to the wild-type. To make things more complicated, it is well known that patients belonging to Liddle's pedigrees harbouring mutations affecting the PY domain may present as essential hypertensives,

without any of the biological characteristics reported in classical forms of Liddle's syndrome, or even remain normotensive [14,15]. Thus, inactivation of the PY domain is probably neither necessary nor sufficient to develop the caricatural phenotype magistrally described by Grant Liddle in the 1960s [4]. The frontier between monogenic and polygenic hypertension, and between mutations and polymorphisms might thus be less clear than previously thought.

Although preliminary, the study presented by Rayner *et al.* [13] highlights the paramount importance of good characterization of hypertensive patients prior to genetic screening, and the significance of identifying intermediary phenotypes likely to reveal particular genetic influences. Such an approach, already successful in other multifactorial diseases such as diabetes (with the genetic characterization of MODY) and cancer, might prove equally useful in the genetic dissection of essential hypertension.

References

1 Snyder PM. The epithelial Na^+ channel: cell surface insertion and retrieval in Na^+ homeostasis and hypertension. *Endocr Rev* 2002; **23**:258–275.
2 Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, *et al.* Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* 1994; **79**:407–414.
3 Hansson JH, Nelson-Williams C, Suzuki H, Schild L, Shimkets R, Lu Y, *et al.* Hypertension caused by a truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. *Nature Genet* 1995; **11**:76–82.
4 Liddle GW, Bledsoe T, Coppage WS. A familial renal disorder simulating primary aldosteronism but with negligible aldosterone secretion. *Trans Assoc Am Physician* 1963; **76**:199–213.
5 Persu A, Barbry P, Bassilana F, Houot AM, Mengual R, Lazdunski M, *et al.* Genetic analysis of the beta subunit of the epithelial Na^+ channel in essential hypertension. *Hypertension* 1998; **32**:129–137.
6 Persu A, Coscoy S, Houot AM, Corvol P, Barbry P, Jeunemaitre X. Polymorphisms of the gamma subunit of the epithelial Na^+ channel in essential hypertension. *J Hypertens* 1999; **17**:639–645.
7 Ambrosius WT, Bloem LJ, Zhou L, Rebhun JF, Snyder PM, Wagner MA, *et al.* Genetic variants in the epithelial sodium channel in relation to aldosterone and potassium excretion and risk for hypertension. *Hypertension* 1999; **34**:631–637.
8 Baker EH, Dong YB, Sagnella GA, Rothwell M, Onipinla AK, Markandu ND, *et al.* Hypertension in a London Black population is associated with a mutation (T594M) in the β subunit of the epithelial sodium channel. *Lancet* 1998; **351**:1388–1392.
9 Su YR, Rutkowski MP, Klanke CA, Wu X, Cui Y, Pun RY. A novel variant of the beta-subunit of the amiloride-sensitive sodium channel in African Americans. *J Am Soc Nephrol* 1996; **7**:2543–2549.
10 Cui Y, Su YR, Rutkowski M, Reif M, Menon AG, Pun RY. Loss of protein kinase C inhibition in the beta-T594M variant of the amiloride-sensitive Na^+ channel. *Proc Natl Acad Sci USA* 1997; **94**:9962–9966.
11 Ferrari P. Genetics of the mineralocorticoid system in primary hypertension. *Curr Hypertens Rep* 2002; **4**:18–24.
12 Nicod J, Bruhin D, Auer L, Vogt B, Frey FJ, Ferrari P, *et al.* A biallelic gene polymorphism of CYP11B2 predicts increased aldosterone-to-renin ratio in hypertensive patients [Abstract]. *Joint Meeting of the Belgian Hypertension Committee and the Swiss Society of Hypertension*, 30 November to 1 December 2003; Lugano.
13 Rayner BL, Owen P, King JA, Soule SG, Vreede H, Opie LH, *et al.* A new mutation, R563Q, of the beta subunit of the epithelial sodium channel associated with low-renin, low-aldosterone hypertension. *J Hypertens* 2003; **21**:921–926.
14 Botero-Velez M, Curtis JJ, Warnock DG. Brief report: Liddle's syndrome revisited – a disorder of sodium reabsorption in the distal tubule. *N Engl J Med* 1994; **330**:178–181.
15 Jeunemaitre X, Bassilana F, Persu A, Dumont C, Champigny G, Lazdunski M, *et al.* Genotype–phenotype analysis of a new family with Liddle's syndrome. *J Hypertens* 1997; **15**:1091–1100.
16 Sagnella GA. Why is plasma renin activity lower in populations of African origin? *J Hum Hypertens* 2001; **15**:17–25.
17 Pratt JH, Jones JJ, Miller JZ, Wagner MA, Fineberg NS. Racial differences in aldosterone excretion and plasma aldosterone concentrations in children. *N Engl J Med* 1989; **321**:1152–1157.
18 Pratt JH, Manatunga AK, Bloem LJ, Li W. Racial differences in aldosterone excretion: a longitudinal study in children. *J Clin Endocrinol Metab* 1993; **77**:1512–1515.
19 Baker EH, Ireson NJ, Carney C, Markandu ND, MacGregor GA. Transepithelial sodium absorption is increased in people of African origin. *Hypertension* 2001; **38**:76–80.
20 Corvol P, Persu A, Gimenez-Roqueplo AP, Jeunemaitre X. Seven lessons from two candidate genes in human essential hypertension: angiotensinogen and epithelial sodium channel. *Hypertension* 1999; **33**:1324–1331.
21 Snyder PM, Price MP, McDonald FJ, Adams CM, Volk KA, Zeiher BG, *et al.* Mechanism by which Liddle's syndrome mutations increase activity of a human epithelial Na^+ channel. *Cell* 1995; **83**:969–978.
22 Schild L, Lu Y, Gautschi I, Schneeberger E, Lifton RP, Rossier BC. Identification of a PY motif in the epithelial Na channel subunits as a target sequence for mutations causing channel activation found in Liddle syndrome. *EMBO J* 1996; **15**:2381–2387.
23 Hiltunen TP, Hannila-Handelberg T, Petajaniemi N, Kantola I, Tikkanen I, Virtamo J, *et al.* Liddle's syndrome associated with a point mutation in the extracellular domain of the epithelial sodium channel gamma subunit. *J Hypertens* 2002; **20**:2383–2390.
24 Bubien JK, Watson B, Khan MA, Langloh AL, Fuller CM, Berdiev B, *et al.* Expression and regulation of normal and polymorphic epithelial sodium channel by human lymphocytes. *J Biol Chem* 2001; **276**:8557–8566.
25 Baker E, Jeunemaitre X, Portal AJ, Grimbert P, Markandu N, Persu A, *et al.* Abnormalities of nasal potential difference measurement in Liddle's syndrome. *J Clin Invest* 1998; **102**:10–14.