With-No-Lysine Kinases

The Discovery of a New Pathway in Hypertension Using Human Genetic Studies

Céline Delaloy, Juliette Hadchouel, Xavier Jeunemaitre

Arterial hypertension is a complex trait caused by many environmental and genetic factors playing together and in interaction. This complexity has limited the identification of susceptibility genes for this major cardiovascular risk factor to controversial loci and candidate genes.1 Conversely, search for major genes implicated in rare Mendelian forms of the disease has been particularly successful. Up to now, the genes identified correspond to products already known or suspected to play a role in blood pressure regulation. Examples include the CYP11B1 and CYP11B2 genes in glucocorticoid remediable aldosteronism, in which the role of genes involved in the synthesis of aldosterone could be expected. This is also the case of the genes encoding the subunits of the epithelial Na channel that was suspected to be responsible for Liddle’s syndrome, based on the excessive salt retention causing the disease, and on the knowledge of the molecular structure of the channel. The discovery of particular mutations affecting the proline-rich consensus motif in the C terminus of the β- and γ-subunits led to further exciting research on the regulation of the channel and on proteins, hormones, and local factors influencing sodium and potassium excretion in the distal tubule but did not lead to discovery of new genes primarily involved in human hypertension. However, the discovery of hypertension caused by mutations of the WNK kinases initiated by Lifton’s group and ours resulted in the identification of completely unsuspected genes.2

Familial hyperkalemic hypertension (FHHt) syndrome, also referred to as Gordon’s syndrome or pseudohyperaldosteronism type 2 (PHA2), is a rare inherited form of low-renin hypertension associated with hyperkalemia and hyperchloremic metabolic acidosis despite a normal glomerular filtration rate. The clinical and biological phenotypes that mirror Gitelman’s syndrome and the marked sensitivity to thiazide diuretics that correct hypertension and metabolic abnormalities suggested several hypotheses concerning its mechanism. Among them, the possible involvement of the thiazide-sensitive sodium-chloride cotransporter NCCT was ruled out by linkage and candidate gene analyses. As for other dozens of autosomal dominant traits, human genetics proved to be very successful in the identification of the causative genes. A first study showed evidence for 2 loci at chromosomes 1q31-q42 (called PHA2A locus) and 17p11-q21 (called PHA2B locus).3 The analysis of a large French pedigree allowed us to identify a new PHA2C locus at chromosome 12p13.3.4 This locus turned out to be the key that opened the door leading to the gene identification. Linkage to this locus led to the detection of a deletion suppressing 1 microsatellite marker in another FHHt kindred analyzed by Wilson et al.2 This particular type of mutation was confirmed by the identification of a similar deletion in the intron 1 of the With-No-Lysine(K) kinase type 1 (WNK1) gene in the French kindred. Search for a homologous gene in the 2 previously known loci eventually led to the characterization of mutations in the WNK4 gene, thus demonstrating the causative role of these 2 members of a novel family of serine/threonine kinases in FHHt and consequently in sodium and potassium homeostasis and blood pressure regulation.2 This seminal observation has promptly stimulated very active research. An interrogation of the PubMed database with “WNK*” as a key word shows a first original article in 2000, corresponding to the cloning of the rat WNK1 gene by Cobb’s group; 2 articles in 2001, including the identification of the WNK2 and WNK3 genes and the first mutations in the WNK1 and WNK4 genes; 4 articles in 2002; 13 articles in 2003, including the first evidence for the in vitro interaction between WNK4 and NCC, the characterization of multiple WNK1 isoforms, and the first WNK1 KO model; 13 articles in 2004, including the first in vitro arguments for a direct role of WNK4 on chloride conductance; and already 8 articles in 2005, including those published in this issue of Hypertension, the majority of all these original studies being published in top-ranked journals.5–7

The study published in this issue of the journal8 is of interest for 2 main reasons. First, it is the first study independent of the initial observation that identifies a mutation in the WNK4 gene associated with a typical form of the disease. The 2 affected members of the family showed strong hyperkalemia with metabolic acidosis and hyperchloremia, all features rapidly corrected by the daily administration of a thiazide. As also reported previously, the young boy displayed typical biological abnormalities but not yet hypertension because it can be observed for other monogenic forms of hypertension such as the apparent mineralocorticoid excess. However, it was not discussed if the affected subjects of this family had hypercalciuria, a feature that seems to be associated with mutations in the WNK4 but not the WNK1 gene.9 The nature and the location of the mutation are also of importance because they replicate those described in the original article. The predicted variation of charge caused by the...
change from an aspartate to a histidine residue, its location in the same highly conserved acidic amino acid motif C terminal to the first coiled-coil domain of the protein, corroborate the 3 mutations found previously in that motif. Another Japanese study described 3 missense mutations located in other parts of the gene that were associated with arterial hypertension but with no biological phenotype. However, it is likely that these amino acid changes are rare nonfunctional variants because we could detect them in a large panel of hypertensive and normotensive European individuals (X. Jeunemaitre, unpublished data, 2004). Thus, this study confirms that FHHt-causing WNK4 mutations are not classical non-sense or truncating mutations but rather mutations affecting 1 of the 2 negatively or positively charged segment located close to a coiled-coil domain. Coiled-coil domains being generally described as protein–protein interaction domains, this suggests modification of interaction with yet unknown partners through amino acid segments having complementary polarity. In that regard, it is interesting to note that in vitro studies show that WNKs oligomerize with the possibility that WNK1 is a tetramer. The WNK1 autoinhibitory domain seems also to be able to inhibit the autophosphorylation of WNK4, suggesting that these 2 kinases may belong to the same cascade. Finally, WNK1 and WNK4 contain 3 proline rich regions that could potentially interact with SH3 domains of other proteins.

The second reason corresponds to the confirmation, at least in the Xenopus oocyte system, that WNK4 interacts with the thiazide-sensitive NaCl cotransporter NCCT and the inward rectifier K channel ROMK. In that study, coinjection of the cRNA for wild-type WNK4 with NCCT caused almost a complete inhibition of the Na+ uptake induced by injection of NCCT alone and a decreased expression at the membrane of the transporter. As expected, these changes were not observed with the new mutation (564D) similarly to what was observed for the 562Q/E mutation. On the basis of these observations, it is logical to conclude that FHHt-causing mutations act as loss-of-function mutations, impairing the physiological inhibitory effect of WNK4 on NCCT, thus resulting in abnormally high sodium reabsorption through the thiazide-sensitive pathway. However, discordant results have been obtained with 2 other disease-causing mutations (559E/K and 561D)A) that have not been explained yet. Golbang et al. also confirm that WNK4 inhibits the K+ current generated by the injection of the ROMK channel cRNA into Xenopus oocytes. This effect is enhanced by the presence of the 566D/H mutation and is associated with the reduction of ROMK surface expression. This mechanism was demonstrated previously by Kahle et al, who further showed that it is mediated by clathrin-dependent endocytosis and is independent of WNK4 kinase activity. These authors proposed that WNK4 regulates both sodium and potassium homeostasis in a coordinated fashion but with opposite trends according to different pathophysiological stimuli such as hypovolemia or hyperkalemia. The effect of the WNK4 mutation on chloride transport was not studied here, even though it is probably crucial in the pathophysiology of the disease. It has been shown that WNK4 colocalizes with ZO-1, a protein expressed in tight junctions of the renal distal tubule, and that it phosphorylates claudins 1 to 4, which are the major tight-junction membrane proteins involved in the regulation of paracellular ion permeability. Thus, WNK4 is probably important in the regulation of the tight junction pores that selectively drive paracellular chloride reabsorption in the distal nephron.

Whether FHHt families are very rare or simply uncommon is unclear. Because of phenotypic heterogeneity, it is possible that a certain number of hypertensives with mild hyperkalemia remain undiagnosed, especially if they are treated with thiazide diuretics, one of the most commonly prescribed classes of antihypertensive drugs. Knowledge of the causative gene(s) should facilitate this evaluation. Preliminary results obtained in our laboratory do not indicate the presence of WNK4 FHHt-causing mutations in 1000 subjects with essential hypertension. However, it is important to consider that WNK1 and WNK4 are not the only genes responsible for FHHt. Another locus is suspected (1q31-42), and at least another is still unmapped. Thus, hopefully, some advances and hypotheses may arise from genetic studies that should help to further decipher the complex regulation of ion transport and blood pressure.

References

With-No-Lysine Kinases: The Discovery of a New Pathway in Hypertension Using Human Genetic Studies
Céline Delaloy, Juliette Hadchouel and Xavier Jeunemaître

Hypertension. 2005;46:263-264; originally published online July 5, 2005;
doi: 10.1161/01.HYP.0000174328.06691.e9
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/46/2/263

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/