Familial Hyperkalemic Hypertension

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Familial hyperkalemic hypertension (FHHt) syndrome (1,2), also known as Gordon syndrome (3) or pseudohypoaldosteronism type 2 (4), is a rare inherited form of low-renin hypertension associated with hyperkalemia and hyperchloremic metabolic acidosis in patients with a normal GFR (OMIM no. 145260). This monogenic form of arterial hypertension has excited new interest since the discovery of a new, unsuspected molecular pathway that is responsible for both the biochemical abnormalities and the increase in BP observed. Genetic analysis has led to the identification of mutations in two genes that belong to a new family of kinases, the WNK family. Although the physiologic functions of these kinases and the pathophysiology of FHHt are not completely solved, these results have opened up major new avenues toward understanding the regulation of ion handling in the aldosterone-sensitive nephron.

Chasing the Gene

Phenotypic and Genetic Heterogeneity

Since the first description of the disease by Paver and Pauline in 1964 (1), approximately 50 other cases and families have been reported (5,6). The original case was a 15-yr-old boy with severe hypertension (180/120 mmHg) and very high potassium levels (7.0 to 8.2 mmol/L). Detailed analyses using different diets and pharmacologic stimuli showed that the kidney was probably involved but that the renal tubule reacted normally to an acid load and to carbonic anhydrase inhibitor. Sensitivity to thiazides—which are widely used in hypertension—and with a low probability of this rare disease’s being recognized by most doctors may have led to an underestimation of its frequency.

The mode of inheritance of the disease is consistent with autosomal dominant transmission in most, if not all, of the pedigrees reported. However, we have identified two families in which the parents of the affected individuals were first cousins, suggesting possible autosomal recessive transmission. This would provide further evidence of the genetic heterogeneity of the disease. Indeed, three loci have already been implicated in this disease, and additional loci probably are responsible for the same apparent phenotype. Genomic analysis of kindreds with FHHt revealed no linkage with SLC12A3, encoding the thiazide-sensitive NaCl co-transporter (NCC) (12 and our own data). In 1997, Lifton’s group at Yale University demonstrated locus heterogeneity of the trait, with a multilocus logarithm of odds score of 8.1 for linkage to two loci: a 20- to 33-cM interval on chromosome 1q31-q42 (PHA2A locus) and a 21- to 43-cM linkage interval on chromosome 17p11-q21 (PHA2B locus) (13). Our analysis of a large French pedigree led to the identification of a new locus on chromosome 12p13.3 (14). We were able to exclude linkage with the previously identified loci and with SLC12A3 in three other French kindreds, demonstrating the involvement of at least one other gene in the disease (15). Thus, at least four different genes are responsible for FHHt, suggesting that this syndrome is actually a set of related disorders (Table 1). Two of these genes were identified recently (16).

Identification of the WNK1 and WNK4 Genes

A fruitful collaboration with Lifton’s group led to the identification of deletions and missense mutations in two genes encoding members of a novel family of serine/threonine kinases. These two genes, WNK1 and WNK4, were shown to be responsible for the disease in six families (16).

One large genomic deletion (41 kb) was found in an American family, and a smaller deletion (22 kb) was found in a French family. Both of the deletions that were found in these unrelated kindreds were located in the same part of the first intron of the WNK1 gene. No mutation was identified in the coding se-
sequence of the gene. WNK1 is located at the telomeric part of chromosome 12 (12p12.3). It contains 28 exons in a 156-kb segment. This segment is particularly large as a result, in part, of the large size (60 kb) of intron 1 (NM_018979.1). Several WNK1 isoforms have been identified (see below). An analysis of WNK1 transcript levels in leukocytes from affected and unaffected members of one of these FHHt families revealed that the intronic deletion was associated with five times higher levels of its expression (16).

The identification of mutations in WNK1 and knowledge of the existence of a putative PHA2 locus on chromosomes 1 and 17 led to the identification of WNK4, located on 17q21-q22. This gene contains 18 exons in a 16-kb segment (NM_032387). WNK1 and WNK4 have similar sequences and intron-exon organizations, but the WNK4 mutations that were found in several FHHt kindreds differ considerably from the WNK1 mutations identified. The WNK4 mutations are missense mutations that affect short (approximately 10 amino acids) sequences that are highly conserved in the WNK family, immediately downstream from the first and second coiled-coil domains (Figure 1). They result in the substitution of a charged residue in these negatively and positively charged sequences (16,17). As coiled-coil domains are generally thought to be involved in protein–protein interactions, the mutations may affect the interaction of WNK4 with its partners.

**WNK Kinase Structure**

WNK (with no lysine [K] [18]) proteins form a small family of serine/threonine kinases that were identified by Xu et al. (19). They lack a conserved lysine that usually is found in subdomain II of the catalytic domain that is critical for ATP binding to the catalytic site and strictly conserved in all other serine/threonine kinases that have been identified to date. In WNK, this lysine is replaced by a cysteine, and the catalytic lysine is located in subdomain I. This new subfamily of protein kinases has been found only in multicellular organisms. Four members of the family have been identified in humans: WNK1, WNK2,
WNK3, and WNK4, located on 12p13.3, 9q22.31, Xp11.22, and 17p11-q21, respectively (16,20,21).

New insight recently has been provided into the structure and function of these kinases. The structure of the kinase domain of WNK1 has been resolved at a resolution of 1.8 A (22). This structure has confirmed that the lysine residue that is responsible for kinase activity is located in strand β2 rather than in β3 as in other protein kinases and the precise conformation of the activation loop and has identified residues that contribute to substrate specificity. These findings should facilitate the design of WNK1 inhibitors. In addition to the catalytic domain located near the N-terminus, WNK1 and WNK4 contain an autoinhibitory domain, two predicted coiled-coil domains, and three proline-rich regions that may interact with the SH3 domains of other proteins, all of which are strongly conserved in WNK (23). Several in vitro studies have shown that WNK oligomerize and that WNK1 may act as a tetramer (24).

WNK1 phosphorylates itself and a generic substrate (19,25). WNK1 activation requires the autophosphorylation of at least one serine residue, Ser-382, within the WNK1 activation loop, and is affected by extracellular ion concentration (19). Changes in NaCl concentration and other osmotic challenges activate the kinase in kidney epithelial cells and in a variety of cell lines, suggesting that WNK1 acts as an osmotic sensor (26). The autoinhibitory sequence is 55 residues long and is located just after the kinase domain. It regulates the kinase activity of WNK1 and probably also its interactions with substrates and/or partners. The WNK1 autoinhibitory domain was shown recently to inhibit the autophosphorylation of WNK4, suggesting that these two kinases may belong to the same cascade (26).

WNK1 probably has many functions. It has been shown to be involved in the extracellular signal-regulated kinase 5 mitogen-activated protein kinase pathway, suggesting that it may be regulated by the growth factors and stress stimuli that control this cascade (26). WNK1 has also been demonstrated to be one of the numerous substrates of Akt/protein kinase B, a kinase involved in the metabolic and mitogenic functions of insulin (27). WNK1 interacts with and phosphorylates synaptotagmin 2 (Syt2) (28), whereas WNK4 does not. Synaptotagmins are involved in the regulation of membrane trafficking and vesicle fusion via a calcium-sensing mechanism (29). Both binding and phosphorylation are enhanced by Ca^{2+} binding to Syt2. As the phosphorylation of Syt2 inhibits the interaction of this protein with phospholipid vesicles, the binding of Syt2 to the membrane requires higher Ca^{2+} concentrations after phosphorylation by WNK1. Thus, WNK1 is thought to regulate the function of Syt2, depending on cellular Ca^{2+} concentration. Like Syt2, WNK1 is present in the cerebellum (C. Delaloy et al., unpublished observations) and in neuroendocrine cells, in which WNK1 and Syt2 co-localize with secretory granules (28). Synaptotagmins are involved in exocytosis, endocytosis, and regulating the membrane insertion of transporters and channels. WNK1 phosphorylation therefore may affect these processes.

Much less is known about the determinants of WNK4 kinase activity. Comparisons of the WNK1 and WNK4 kinase domains showed differences in structure and function. Min et al. (22) looked at differences that might account for substrate specificity. They generated a homology-based structural model of WNK4, using the WNK1 coordinates. They identified two residues, at positions 318 and 448, that differed between the two enzymes and that seemed to mediate stable binding between WNK1 and Syt2. This may account for the poor phosphorylation of synaptotagmin 2 by WNK4 in their experiments. Wang et al. (25), using GST fusion constructs, confirmed the capacity of WNK1 constructs to autophosphorylate and to phosphorylate the generic substrate histone. Conversely, the WNK4 kinase domain displayed no kinase activity, either in vitro or in HEK 293 cells, suggesting that as-yet-unidentified factors are required for WNK4 kinase activation. In these experiments, the WNK4 autoinhibitory domain inhibited WNK1 kinase activity, consistent with the interaction demonstrated in experiments with Xenopus oocytes. Wilson et al. (30) demonstrated the co-immunoprecipitation of NCC and WNK4 in HEK 293 cells, but it is not known whether this interaction is direct or indirect. The partners of WNK4 therefore remain to be identified.

**Possible Physiological Functions of WNK1 and WNK4**

WNK1 and WNK4 are produced in many different tissues. Northern blot analysis has shown that WNK1 is predominantly expressed in the kidney, heart, and skeletal muscle in humans, rats, and mice (16,19,20,31–33). Immunostaining in mice and humans has shown that WNK1 is present in the kidney and in various reabsorptive epithelia (31). WNK1 expression is either intracytoplasmic or restricted to the basolateral membrane, depending on the tissue. WNK4 is mostly produced in the kidney, where it is specifically present in the cytoplasm and tight junctions of the distal convoluted tubule (DCT) and cortical collecting duct (CCD) (16). WNK4 transcripts and protein are also present in several epithelial tissues, particularly in tight junctions (34). Only one isoform of WNK4 has been identified, whereas multiple isoforms are produced from the WNK1 gene as a result of the existence of three promoters, two polyadenylation sites, and three alternatively spliced exons (32) (Figure 2). The first two proximal promoters, P1 and P2, are located upstream from and within exon 1, respectively, and generate ubiquitous isoforms that contain the entire kinase domain. A third promoter, rP, is located in intron 4 and controls the production of a kidney-specific isoform, with transcription initiated from a specific exon (exon 4a). This isoform lacks most of the kinase domain and is produced in large amounts in the DCT and connecting tubule (CNT). The consequences of the identified intronic deletions on the pattern of production of the various WNK1 isoforms are unknown. Their characterization is crucial if we are to understand the precise mechanism of the disease.

It is interesting that all epithelia that express WNK1 and WNK4 are involved in chloride transport. In *Xenopus laevis* oocytes, the activities of the basolateral isoform of the Na^+--K^+--2Cl^- co-transporter (BSC2/NKCC1) and of the apical Cl^-/HCO_3^- exchanger CFEX are reduced by WNK4 (34). Furthermore, in MDCK cells, WNK4 reduces transepithelial resistance by increasing chloride permeability but does not alter the flux of uncharged solutes (35,36). No such effect was observed in a
mutant with an inactivated kinase. The linear current-voltage curve and the pharmacologic properties of these effects indicated that they were attributable to the paracellular pathway. Yamauchi et al. (35) showed that WNK4 phosphorylates claudins 1 to 4. These proteins are the major tight-junction membrane proteins involved in regulating paracellular ion permeability. As no effect on tight-junction structure was observed on electron microscopy, these findings suggest that WNK4 is involved in regulating the tight-junction pores that selectively drive paracellular chloride reabsorption in the distal nephron (36).

Further studies in Xenopus laevis oocytes showed that the regulatory role of WNK4 is not restricted to chloride transport. Instead, it extends to the regulation of a wide range of transport systems expressed in the distal nephron (Figure 3). The injection of WNK4 into Xenopus oocytes decreases membrane expression of the distal Na-Cl co-transporter NCC (31) and of the renal apical K⁺/H⁺ channel ROMK (37) and increases that of the renal epithelial calcium channel (ECaC) (38). WNK4 seems to regulate NCC and ECaC membrane expression in a kinase-dependent manner. In contrast, ROMK inhibition is mediated by clathrin-dependent endocytosis and is independent of WNK4 kinase activity. However, two groups have studied the WNK4-mediated inhibition of NCC and have obtained conflicting results concerning the requirement for the WNK4 kinase domain. Wilson et al. (30) showed that an inactivating mutation in the kinase domain abolished the inhibitory effect observed in Xenopus oocytes. Yang et al. (39) showed that the C-terminal part of WNK4 was required for this inhibition, whereas the kinase domain was not.

These results highlight the role of WNK4 in coordinating transcellular and paracellular NaCl reabsorption and K⁺ secretion in the distal nephron (37). The regulation of WNK4 in individuals with hypovolemia would reduce ROMK activity without affecting NCC, increasing salt reabsorption while preventing excessive K⁺ loss. Conversely, in individuals with hyperkalemia, WNK4 regulation would inhibit NCC without affecting ROMK, thereby maximizing K⁺ secretion without altering salt reabsorption. The differences in the effects of WNK4 in conditions of hyperkalemia and hypovolemia prob-

Figure 2. (A) Structure of the human WNK1 gene. The two proximal promoters (pP1, pP2), the renal promoter (rP), and the two alternative polyadenylation sites (pA1 and pA2) are shown. ■, alternatively spliced exons (9, 11, and 12); □, kidney isoform-specific exon (exon 4a). The large intronic deletions found in the American (41-kb del) and French (22-kb del) kindreds are represented by triangles. (B) Structure and expression of WNK1 isoforms. The pP1 and pP2 promoters, located upstream of and in exon 1, respectively, give rise to long isoforms that contain the entire kinase domain. P1 isoforms are ubiquitously expressed, with a stronger expression in the skeletal muscle and the heart, as shown in the upper tissue Northern blot. P2 isoforms are ubiquitously expressed. The rP promoter gives rise to a shorter isoform, lacking the major part of the kinase domain and specifically and strongly expressed in the kidney, as shown in the lower tissue Northern blot, probed with the exon specific to this isoform (exon 4a).
ably involve other factors. One of these factors may be the kinase-deficient kidney-specific WNK1 isoform, which has been shown (40) to be upregulated by aldosterone in vitro and may regulate the interaction between WNK1 and WNK4 (see below). Finally, the regulation of ECaC (38) by WNK4 may make it possible to regulate the balances of sodium and calcium independently. An inverse relationship has been found between the rates of reabsorption of these two cations in the distal tubule. This might account for the hypercalciuria observed in FHHt, as a result of WNK4 mutations (see Genotype–Phenotype Relationships section). It might also explain the increase in nasal sodium current and sweat conductivity that was observed recently in a large family with WNK4-related FHHt (41).

The physiologic functions of WNK1 are less well understood. Several studies in vitro have shown that it may act as an osmotic sensor in various cells (24). In kidney, it has been difficult to obtain a clear overview of the functions of WNK1 because of weak, diffuse expression of the long WNK1 isoform and strong expression of the kinase-defective short isoform in the distal tubule. In a mouse CCD cell line that stably expresses a functional mineralocorticoid receptor, physiologic concentrations of aldosterone have been shown to upregulate aldosterone in vitro and may regulate the interaction between WNK1 and WNK4 (see below). Finally, the regulation of ECaC (38) by WNK4 may make it possible to regulate the balances of sodium and calcium independently. An inverse relationship has been found between the rates of reabsorption of these two cations in the distal tubule. This might account for the hypercalciuria observed in FHHt, as a result of WNK4 mutations (see Genotype–Phenotype Relationships section). It might also explain the increase in nasal sodium current and sweat conductivity that was observed recently in a large family with WNK4-related FHHt (41).

The second mechanism of action of WNK1 in the distal nephron seems to be mediated through its interaction with WNK4. Indeed, unlike WNK4, WNK1 was found to have no effect on NCC activity in the Xenopus laevis oocyte expression system (43). However, if WNK4 and WNK1 are coexpressed, WNK1 prevents the WNK4-mediated inhibition of NCC. This inhibition requires an interaction between the kinase domain of WNK1 and WNK4 and seems to be mediated by the C-terminal part of WNK4 (39). These results therefore suggest that a WNK1 protein that lacks the kinase domain, such as the kinase-deficient kidney-specific isoform, would not be able to regulate the WNK4-mediated inhibition of NCC activity. The precise mechanism of interaction between the two kinases is unclear as conflicting results have been obtained. It is also important to state that there is no clear evidence yet that WNK1 activity itself is regulated by aldosterone (24). Thus, we do not know yet whether the production or activity of WNK1 is affected by means other than the induction of changes in NaCl transport.

Is the physiologic effect of WNK1 on BP mediated exclusively by the kidney? Zambrowicz et al. (44) generated a knockout mutant of WNK1 by gene trapping in embryonic stem (ES) cells. Embryos that were homozygous for the mutation died in utero, during the first 13 days of development, whereas het-
erozygous adults showed a decrease in BP (10 mmHg lower than that in wild-type animals). However, no changes in electrolyte concentrations were found, even in animals that were fed a low-salt diet, suggesting that the decrease in BP was not due to ionic disturbances in the kidney. These data show that WNK1 is essential for embryonic development and is a key regulator of BP as the inactivation of a single copy of the gene led to a decrease in BP.

Pathophysiology of FHHt Revisited

The mechanism of FHHt has been debated for many years. The high sensitivity of both hypertension and metabolic disorders to thiazide diuretics and the low plasma renin levels have been interpreted as reflecting excessive sodium reabsorption via the thiazide-sensitive sodium-chloride co-transporter NCC in the DCT (45). This would decrease the rate of sodium delivery to the CNT and the CCD, thereby decreasing sodium flux through the ENaC, in turn impairing potassium excretion. Schambelan et al. (4) proposed an alternative hypothesis, suggesting that the syndrome may result from abnormally high levels of chloride reabsorption. This “chloride shunt” would favor sodium reabsorption via ENaC, decreasing the lumen-negative transtubular electrical potential driving potassium secretion. In both models, low-renin hypertension and hyperkalemic metabolic acidosis result primarily from positive sodium balance with secondarily decreased potassium secretion. These models have to be revisited according to the recent knowledge of causal mutations at the WNK4 and WNK1 genes.

FHHt Caused by WNK4 Mutations

In Xenopus laevis oocytes, WNK4 expression decreases NCC production and expression at the membrane (Figure 3). No such changes are observed in the same system with a WNK4 mouse cRNA harboring the Q562E disease-causing mutation (30,43). Similar results were more recently obtained with a novel mutation (S64D->H) that causes increased cell surface expression of NCC but reduced expression of ROMK (17). On the basis of these observations, it has been suggested that the mutations that are responsible for FHHt may be loss-of-function mutations, impairing the physiologic inhibition of NCC by WNK4, resulting in abnormally high levels of sodium chloride reabsorption through the thiazide-sensitive pathway. However, other disease-causing mutations (E559K and D561A) have been shown to have as strong an effect on NCC activity as wild-type WNK4 (43). This suggests that the mechanism of the disease may not be the same in all WNK4-linked FHHt kindreds, despite the existence of phenotypic similarities. However, this seems unlikely as all of these missense mutations lie in a short, highly conserved sequence and affect the polarity of this negatively charged segment in a similar manner.

The consequences of the Q562E and E559K mutations for the WNK4-mediated clathrin-dependent reduction of ROMK in the plasma membrane have also been studied in the Xenopus model. Both mutations further increased the inhibition of K⁺ current and surface expression of ROMK. Similarly, the E559K, D561A, and Q562E mutations had a markedly greater effect on paracellular chloride permeability than wild-type WNK4 (35,36). It was further demonstrated that wild-type WNK4 induced phosphorylation of the tight-junction proteins claudins 1 to 4 and that mutant WNK4 D564A further increased claudin phosphorylation (35).

These observations suggest that mutations in WNK4 would behave as loss-of-function mutations for NCC but gain-of-function mutations for ROMK and claudins. WNK4 seems to have multiple mechanisms of action, some of which are kinase activity dependent and some of which are kinase independent. Mutant WNK4 probably increases sodium chloride reabsor-
tion through NCC by weakening the physiologic inhibition of NCC. Increased ROMK internalization by WNK4 (wild-type or mutated) should lead to decreased potassium secretion. Finally, by increasing the chloride paracellular pathway, WNK4 further increases interstitial chloride concentration. These three effects concern major elements involved in sodium reabsorption in the distal nephron, which plays a key role in controlling sodium reabsorption and potassium secretion (46). They are also consistent with the clinical and biologic phenotype observed in patients with FHHt: high BP, hyperkalemia, and hyperchlor- emia, with high sensitivity to thiazide diuretics.

Although these data run along similar lines, it should be stressed that the levels of proof provided by the various experimental studies are not equivalent. The demonstration in relevant distal epithelial renal cell lines, by two independent groups, that all FHHt mutations studied abnormally increase the chloride-selective paracellular pathway is consistent with the co-localization of WNK4 with the tight-junction protein zona occludens-1 in the early DCT (16) and with the chloride shunt hypothesis proposed by Schambelan more than 20 yr ago (4). However, although the information gleaned from the Xenopus model should not be dismissed, that not all of the FHHt mutations tested failed to decrease NCC levels at the oocyte surface, is a matter of concern. Another recent study also suggested that the overproduction of WNK4 in renal epithelial cells affects the apical localization of NCC (47). However, this inhibitory effect did not differ between wild-type and mutant (D561A) WNK4. Our understanding therefore remains incomplete, and further studies of the WNK pathway are required. In that regard, results obtained in vivo are of utmost importance. Lifton’s group recently presented preliminary findings (48) showing that animals that were transgenic for mutant WNK4 had a higher thiazide-sensitive co-transporter protein abundance and a lower ROMK abundance at the membrane, thus supporting data obtained in oocytes.

**FHHt Caused by WNK1 Mutations**

As previously indicated, two large genomic deletions (41 and 22 kb, respectively) in the first intron of WNK1 have been identified as being responsible for FHHt in two unrelated kindreds, with no mutation detected in the coding sequence (16). An analysis of WNK1 transcript levels in leukocytes from affected and unaffected members of one of these families revealed that the intronic deletion was associated with a five-fold increase in the levels of a particular WNK1 isoform, the nature of which has yet to be determined.

What effect does the overproduction of WNK1 have in the renal tubule? If WNK4 and WNK1 are coexpressed in *Xenopus* oocytes, then WNK1 completely prevents the WNK4-mediated inhibition of NCC (43). Yang et al. (39) recently confirmed that WNK1 abolishes the effect of WNK4 on NCC activity but that a WNK1 construct lacking the kinase domain cannot block the effect of WNK4. Thus, the kinase-deficient kidney-specific isoform might not be able to inhibit WNK4, as it lacks the kinase domain (32). FHHt-causing WNK1 intrinsic mutations may increase production of the full-length WNK1 isoform that is normally produced in only small amounts. This in turn would abolish the inhibition of NCC by WNK4 and increase ENaC activity through SGK1, thereby increasing NaCl reabsorption (39,42). This attractive hypothesis requires confirmation *in vivo*.

The second major mechanism by which changes in WNK1 expression might affect ionic transport corresponds to its activity on ENaC, as shown by Xu et al. (42) and discussed above. Thus, the consequences of WNK1 mutations in the kidney would not be mediated solely through their consequences on WNK4 but also through a further increase in ENaC activity (Figure 3). With such an effect, an increased expression of the long WNK1 isoform would contribute to sodium reabsorption and hypertension in FHHt. An increased expression of the kidney-specific kinase-deficient isoform would lead to hypertension independent of SGK1. It remains to be determined *in vitro* and *in vivo* whether the FHHt-causative WNK1 intronic deletions lead to parallel or opposite changes in WNK1 transcripts expression. We also need to improve our understanding of the physiologically strong expression of the kinase-deficient WNK1 isoform in the DCT and CNT. Genetically modified mice that under- or overexpress WNK genes are currently being engineered by various groups and should help to unravel the complexity of WNK physiologic functions and FHHt pathophysiology.

**Genotype–Phenotype Relationships**

The identification of WNK1 and WNK4 has made it possible to study a few large FHHt pedigrees. Farfel and co-workers (49) observed that affected individuals in an Israeli family who bear the Q562E-WNK4 missense mutation displayed frank hypercalciuria in addition to hyperkalemic metabolic acidosis. This hypercalciuria was associated with a significant decrease in bone mineral density. In contrast, in our WNK1 pedigree, affected patients had hyperkalemic metabolic acidosis of similar severity to that observed in the Israeli family but similar calciuria to unaffected relatives (11). The association of an increase in urinary calcium excretion and an increase in sodium reabsorption in the distal nephron would not be unexpected given the inverse relationship between sodium and calcium transport. However, this correlation does not hold for WNK1 mutation. The discordance between the two families may be related to a specific interaction between WNK4 and the ECaC. However, Peng et al. (38) found no difference between wild-type WNK4 and disease-causing mutants. Thus, how these findings explain hypercalciuria is not yet clear. Alternatively, the phenotypic differences may be due to strong WNK1 expression in tissues and to unexpected central and/or cardiovascular effects. Thus, although the two FHHt forms probably share certain common mechanisms, the pathophysiology of the two defects is probably not strictly the same.

It is interesting that in both families, metabolic disorders preceded hypertension. In the French pedigree, hypertension did not develop until patients were in their 30s. The eight youngest patients of the 17 who bear the mutation were normotensive but displayed metabolic abnormalities of similar severity to those of the hypertensive individuals. Similar observations were reported for the Israeli pedigree (50). In this family, the mean time between detection of hyperkalemia and
appearance of hypertension was 13 yr. In both families, affected patients had lower plasma renin concentrations than unaffected relatives, but the extent of the decrease was similar in normotensive and hypertensive individuals. Low renin concentration indicates that excessive sodium reabsorption leading to volume overload is central to the disease, but that individuals can remain normotensive until mid-adulthood indicates that excessive sodium reabsorption is less characteristic than in other forms of monogenic hypertension, such as 11β hydroxysteroid dehydrogenase type 2 (11βHSD2) deficiency or even Liddle syndrome, and simply predisposes the patient to hypertension. It is interesting that hypertension resolved during two pregnancies in previously hypertensive women of the Israeli pedigree, whereas hyperkalemia and hypercalciuria persisted (51).

Future Directions
Characterization of the functions of WNK1 and WNK4 in the regulation of distal ion handling has opened up new and exciting areas of research that may revolutionize our understanding of aldosterone-responsive nephron physiology (46,52). However, the precise pathophysiologic mechanisms underlying FHHt remain unclear for both WNK1 and WNK4. The suggested effects on NCC and ROMK remain to be proved in vivo, as do the possible effects of aldosterone on WNK1 expression and activity. Furthermore, if WNK1 overexpression does indeed result in the abnormal activation of ENaC and WNK4 missense mutations lead to excessive sodium reabsorption via ENaC by increasing paracellular chloride reabsorption, then both WNK-related forms of hypertension share a common mechanism with Liddle’s syndrome. The observations of affected kindreds also raise challenging questions concerning the uncoupling of metabolic disorders and hypertension. Determining why patients with FHHt can remain normotensive until adulthood may well provide insight into the mechanisms underlying essential hypertension, the main clinical features of which emerge with aging.

In terms of genetics, one of the key findings of the past few years is the demonstration that several loci are responsible for FHHt. Two genes have already been implicated, and the involvement of another locus is suspected (1q31-42), with a fourth locus as yet unmapped (15). In addition to classical reverse genetics strategies, the identification of substrates and/or partners of WNK1 and WNK4 should help to identify the genes involved, possibly making it easier to decipher the complex regulation of ion transport in the distal nephron. Another question that is classically asked after the identification of genes that are responsible for a Mendelian trait concerns the possibility that more subtle mutations might be responsible for more common forms of the disease. Investigations of the genes that are responsible for monogenic forms of hypertension have produced disappointing results in analyses of essential hypertension. However, several lines of evidence implicate WNK genes in essential hypertension. Hypertensive individuals are often sensitive to thiazide diuretics, which are particularly effective in patients with FHHt. The locus that contains WNK4 lies within the largest genetic linkage region for BP variation observed in spontaneously hypertensive rats (53) and in the Framingham Heart Study population (54). This chromosomal region has also been linked to hypertension in a European study (55) but has not been reported in other genome-wide screens in large hypertensive populations (56). Comparative analysis of the complete coding sequence of WNK4 revealed no mutation in the SHRSP strain with respect to the normotensive WKY control (57). In humans, a number of genetic variants have been identified in populations of African and European origin (58). No association has yet been reported between WNK4 polymorphisms and hypertension, but definite negative arguments require confirmation by means of powerful and well-designed studies. In that regard, a comprehensive analysis of 19 WNK4 polymorphisms in 712 severely hypertensive British families recently showed a weak but significant association between one particular polymorphism located 3 kb from the WNK1 promoter and the severity of hypertension (59), coherent with the hypothesis that increased expression of WNK1 might contribute to susceptibility to essential hypertension.

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