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# Investigation of Mechanisms of Ubiquitination and Degradation of WNK Kinase Protein

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Summary

Mutations in WNK kinases cause the human hypertensive disease pseudohypoaldosteronism type II (PHAII), but the regulatory mechanisms of the WNK kinases are not well understood. Mutations in kelch-like 3 (KLHL3) and Cullin3 were also recently identified as causing PHAII. Therefore, new insights into the mechanisms of human hypertension can be gained by determining how these components interact and how they are involved in the pathogenesis of PHAII. The purpose of the present study was to determine the pathogenic role of PHAII-causing mutations in the WNK4, KLHL3, and Cullin3 genes. We found that KLHL3 interacted with Cullin3 and WNK4, induced WNK4 ubiquitination, and reduced the WNK4 protein level. When the expression levels of wild-type and mutant KLHL3 were similar, the R528H mutant was less able to reduce the endogenous protein level of WNK4 as compared to wild-type KLHL3. We also tested the effect of PHAII-causing mutations of WNK4 on WNK4-KLHL3 interaction and WNK4 ubiquitination. Although wild-type KLHL3 decreased WNK4, this decrease mediated by KLHL3 was blunted in all PHAII-causing WNK4 mutants. Thus, the reduced interaction of KLHL3 and WNK4 by PHAII-causing mutations in either protein reduced the ubiquitination of WNK4, resulting in an increased level of WNK4 protein. Transgenic mice overexpressing WNK4 showed PHAII phenotypes, and WNK4 protein was indeed increased in Wnk4(D561A/+) PHAII model mice. WNK4 is a target for KLHL3-mediated ubiquitination, and the impaired ubiquitination of WNK4 is a common mechanism of human hereditary hypertension. In summary, our study identified that WNK4 is a substrate of KLHL3 -Cullin3-mediated ubiquitination and that the impaired ubiquitination of WNK4 is a common mechanism of PHAII by WNK4, KLHL3, and Cullin3 PHAII-causing mutations.