Exome Sequencing to Identify Novel Genes in Hypertension

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Study Hypothesis

Previous genetic studies of patients with Mendelian forms of hypertension have identified causal genes involving renal electrolyte transport, highlighting new mechanisms for the regulation of blood pressure in humans. Pseudohypaldosteronism type II (PHAII), a rare genetic disorder causing hypertension, hyperkalemia, and metabolic acidosis, has been found, in some cases, to be caused by mutations in WNK1 or WNK4, but most cases remained unexplained at the time of the study. The authors proposed that exome sequencing and gene resequencing of a number of unrelated individuals with PHAII would uncover novel causal genes.

How Was the Hypothesis Tested?

Boyden et al recruited 52 kindreds with 126 patients with PHAII, of which just 7 kindreds were found to have WNK1 or WNK4 mutations. They performed exome sequencing of DNA samples of 11 unrelated PHAII index cases from 11 kindreds. They simultaneously performed genomewide genotyping of single nucleotide polymorphisms (SNPs) in all members of the kindreds, in order to perform linkage studies that would pinpoint the chromosomal regions in which causal mutations were most likely to lie. They combined the exome sequencing data with the linkage data to converge on 28 regions immediately surrounding the exon; only 2 of the mutations were distributed widely across the gene, the dominant mutations clustered in either “propeller” loop motifs or a BTB domain through which the protein interacts with substrates or CUL3, respectively. Thus, the investigators concluded that different mutations could result in either partial loss of function of the protein or a dominant negative effect.

Because it had previously been suggested that KLHL3 directly interacts with CUL3 in order to ubiquitinate substrates, the investigators reasoned that mutations in CUL3 might account for additional cases of PHAII. Resequencing of CUL3 identified novel single allelic mutations in 17 kindreds, all without KLHL3 mutations. Of note, all of these mutations occur in the vicinity of exon 9, most in the intronic regions immediately surrounding the exon; only 2 of the mutations had been detected in the original exome sequencing. Biochemical experiments demonstrated that these mutations uniformly result in the exclusion of exon 9 from the spliced CUL3 transcripts, resulting in a 57 amino acid deletion that presumably disrupts the ability of the CUL3-KLHL3 complex to engage in ubiquitination. Finally, the investigators established that both KLHL3 and CUL3 are expressed in the distal collecting tubules of mouse kidney, consistent with the electrolyte abnormalities observed in patients with PHAII.

Principal Findings

In their initial exome sequencing and linkage analyses, Boyden et al discovered 5 different mutations in KLHL3 in 3 kindreds, with all of the mutations cosegregating with PHAII-affected status. The KLHL3 protein is involved in the ubiquitination and degradation of target substrates. On resequencing KLHL3 in all of the index patients in the study with PHAII, they found novel gene mutations in 24 kindreds. Notably, affected individuals in 8 kindreds harbored 2 allelic mutations (whether homozygous or compound heterozygous), indicating a recessive mode of inheritance; affected individuals in 16 kindreds harbored only 1 allelic mutation, indicating a dominant mode of action. Whereas the recessive mutations were distributed widely across the gene, the dominant mutations clustered in either “propeller” loop motifs or a BTB domain through which the protein interacts with substrates or CUL3, respectively. Thus, the investigators concluded that different mutations could result in either partial loss of function of the protein or a dominant negative effect.

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yielded single or double allelic mutations in 1 of 4 genes: 2 known (\textit{WNK1} and \textit{WNK4}) and 2 novel (\textit{KLHL3} and \textit{CUL3}), thereby accounting for the vast majority of cases of the Mendelian disorder. Also notable was the finding of varied mutations with different modes of inheritance, some dominant and some recessive, with the genes being mutated in stereotyped ways (eg, all of the PHAII-associated \textit{CUL3} mutations specifically leading to the exclusion of exon 9 of the gene transcript). The investigators demonstrated that judicious use of a combination of techniques could unravel the genetic basis of a disease where 1 technique alone would have been insufficient. Finally, in their identification of 2 novel, interacting genes that can underlie PHAII, they uncovered a new molecular pathway regulating renal electrolyte transport and blood pressure in humans.

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Disclosures
None.
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