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Preparation for hypertension specialists

Genomics reveals the pathogenesis of hypertension.

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Abstract

Genomics is a discipline in genetics that applies recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes, the complete set of DNA within a single cell of an organism. Research into the genetics of hypertension has now expanded to genomics. Two approaches have dominated this field. One relies on large populations in which the phenotype, hypertension versus no hypertension, or hypertension-relevant phenotypes are compared. Genome-wide association (GWAS) analyses of (>1 million) common variants identify relevant loci and possible genes exerting small effects. Detailed studies on *APOL1* and *SH2B3* are opening entire new fields of research. Family-based Mendelian studies have identified rare variants that exert very large effects on blood pressure. Mechanistically, these studies have been a bonanza of new information. The approaches are complementary.

Genome-wide association studies

Essential (primary) hypertension results from many environmental and genetic factors. Candidate-gene, genome-wide linkage, and genome-wide association studies (GWAS) relying on single-nucleotide polymorphisms (SNPs) have all given insights into genes relevant for essential hypertension. Candidate gene studies have implicated aberrations in aldosterone signaling, catecholamine pathways, ion channel regulation, and inflammatory pathways. For GWAS, massive sample sizes have been used (>80,000 individuals) to find gene loci that exert only modest effects. Memorizing these genes and their variants seems counterproductive since thus far they collectively explain only about 2.5% of blood pressure variation. Nevertheless, ranking all the currently known findings could theoretically produce a genetic risk score indicating a 25% increase in the odds of developing essential hypertension. High-throughput massive parallel sequencing and the plummeting costs (currently about

$5000 for an entire human genome) suggest that practitioners will be confronted with this kind of information in the future.[1 2]

GWAS have also been performed to study structural and organ-related changes in essential hypertension. Examples include the evaluation of arterial stiffness and the propensity to develop hypertensive nephrosclerosis.[2] Mapping by admixture linkage disequilibrium recently led to an important finding in hypertension-related kidney disease in African Americans, demonstrating the role of the apolipoprotein L1 (*APOL1*) and non-muscle myosin heavy chain 9 (*MYH9*) genes. GWAS have also detected associations between kidney function and uromodulin (*UMOD*) and the shroom-related protein (*SHROOM3*) that may be involved in regulating cell shape in certain tissues.

Several genes have turned up in numerous GWAS studies on blood pressure, their effects are impressive, and their functions should interest hypertension specialists. Collaboration between the CHARGE consortium (n = 29,136) and the Global BPgen consortium (n = 34,433) produced interesting results. When ten CHARGE SNPs for each trait were included in a joint meta-analysis with the Global BPgen Consortium, four CHARGE loci attained genome-wide significance ($P < 5 \times 10^{-8}$) for systolic (ATP2B1, CYP17A1, PLEKHA7, SH2B3), six for diastolic blood pressure (ATP2B1, CACNB2, CSK-ULK3, SH2B3, TBX3-TBX5, ULK4) and one for hypertension (ATP2B1).[3] Particularly robust and interesting is the *SH2B3* gene that encodes SH2B3, also known as LNK, an intracellular adaptor protein. LNK regulates cytokine signals that control lympho-hematopoiesis. LNK also controls inflammatory CD8 T-cell proliferation. The role of T cells in hypertension, particularly blood pressure responses to angiotensin II, is well appreciated.[4] But probably the most impressive story arising from GWAS studies thus far is the discovery of APOL1 and its role in focal sclerosing glomerulosclerosis (FSGS) in African Americans.[5]

Epigenetics is the study of heritable changes in gene expression or cellular phenotypes caused by mechanisms other than changes in the underlying DNA sequence. Several molecular processes, including nucleic acid methylation, histone modification, nucleosome positioning, transcriptional control with DNA-binding proteins and noncoding RNAs, and translation control with microRNAs and RNA-binding proteins orchestrate epigenetic changes. Mammalian Sir2 (SIRT1, a NAD^+-dependent deacetylase), previously shown to extend the lifespan of lower organisms, is a promising target molecule to influence some aspects of hypertension, particularly as related to aging.[6] An NHLBI working group is pursuing the role of epigenetics in essential hypertension.[7]

Mendelian hypertension

Pheochromocytoma can be caused by Mendelian-inherited gene mutations.[8] In Europe, up to a third of pheochromocytomas represent Mendelian syndromes. Knowledge of these syndromes is essential for all hypertension specialists. An activating germ-line mutation in the *RET* proto-oncogene is responsible for an autosomal-dominant syndrome, multiple endocrine neoplasia type 2 (MEN 2). *RET* encodes a transmembrane-receptor tyrosine kinase involved in the regulation of cell proliferation and apoptosis. Von Hippel-Lindau (*VHL*) encodes a protein that regulates the activity of hypoxia-inducible factor-alpha. Loss of VHL protein function predisposes *VHL* carriers to both benign and malignant tumors in multiple organs including renal cell cancer and pheochromocytoma. Von Recklinghausen's disease is an autosomal-dominant disorder caused by inactivating mutations of the tumor-suppressor gene, *NF 1* encoding neurofibromin, a GTPase-activating protein involved in the RAS signaling cascade and mTOR kinase pathway. Succinate dehydrogenase enzyme complex consists of four subunits encoded by *SDHA, SDHB, SDHC,* and *SDHD.* For correct function of the SDHA subunit, a cofactor of flavin-adenine dinucleotide is necessary, *SDHAF2.* All five

genes are associated with pheochromocytoma. Finally, the tumor-suppressor gene, *TMEM-127* encoding a transmembrane protein, has recently been identified as a new pheochromocytoma gene.

Somatic *KCNJ5* mutations explain a subset of aldosterone-producing adenomas, the most frequent secondary cause of arterial hypertension.[9,10] *KCNJ5* encodes the inwardly rectifying K^+ channel Kir3.4 that exists both as homotetramers and heterotetramers with Kir3.1. Mutations result in loss of channel selectivity, with increased sodium conductance leading to membrane depolarization. In zona glomerulosa cells that produce aldosterone, membrane depolarization leads to opening of voltage-activated Ca^{2+} channels with activation of the calcium-signaling pathway. A similar heterozygous-inherited germinal *KCNJ5* mutation, T158A, located in the same conserved region, was described in a kindred with Mendelian hyperaldosteronism. The regulation of aldosterone secretion is exerted to a significant degree by activation of membrane potassium and calcium channels or pumps. Thus, it is not surprising that the known causes of disorders of aldosterone secretion in APA have been channelopathies, which activate mechanisms that increase aldosterone synthesis.[11]

Students of hypertension should be keenly aware of Mendelian syndromes causing hypertension (Figure) that are associated with low plasma renin activity (PRA). Familial hyperaldosteronism type I (FH-I), also known as glucocorticoid-remediable aldosteronism (GRA), was the first form of Mendelian hypertension to be recognized as a single-gene hypertensive disorder.[12] The genetic defect features a hybrid or chimeric gene on chromosome 8q consisting of the regulatory region of the 11β-hydroxylase gene, *CYP11B1,* coupled with the structural region of the aldosterone synthase gene, *CYP11B2.* The mode of inheritance is autosomal dominant with complete penetrance. The chimeric gene results in increased aldosterone production, as well as aberrant metabolites.

Familial hyperaldosteronism type II (FH-II) is clinically indistinguishable from sporadic forms of primary hyperaldosteronism due to bilateral adrenal hyperplasia. The genetic abnormality has been mapped to chromosome 7p22. In familial hyperaldosteronism type I (FH-I), glucocorticoids ameliorate aldosterone overproduction, since ACTH drives the chimeric gene. In contrast, hypertension in FH-II is unresponsive to glucocorticoids; however, spironolactone is effective. Congenital adrenal hyperplasia is inherited in an autosomal recessive manner. When 21-hydroxylase (*CYP21A2*) is deficient patients waste salt and are normotensive. However, in 11β-hydroxylase (*CYP11B1*) and 17α-hydroxylase (*CYP17*) deficiencies, production of deoxycorticosterone (DOC) is increased, leading to hypertension.

Investigators described a mutation in the gene encoding the mineralocorticoid receptor (MCR), a leucine-for-serine substitution at codon 810, which lies in the hormone-binding domain of the MCR. The mutation causes the receptor to be constitutively active and changes the MRC specificity such that the steroid hormones lacking a 21-hydroxyl group that are normally antagonistic (progesterone and cortisone), act as agonists. In the kidney, 11β-hydroxysteroid dehydrogenase-2 (11β-HSD-2) converts cortisol to cortisone, which does not activate the renal MCR. This mechanism protects the collecting tubules from inappropriate activation of the MCR by circulating cortisol. The autosomal recessive syndrome of apparent mineralocorticoid excess (AME) causes severe hypertension through mutations in the 11β-HSD-2 gene, which render the enzyme ineffective. This state-of-affairs allows cortisol, which circulates at much higher concentrations than aldosterone, to saturate the MCR and induce hypertension and hypokalemia.

Liddle's syndrome is an autosomal dominant disorder causing hyperactivity of the amiloride-sensitive sodium channel (ENaC) in the cortical collecting tubule.[13] Genetic studies showed that mutations of the beta or gamma subunits of ENaC (chromosome 16p) cause deletions of proline-rich regions. These regions regulate ENaC activity because they facilitate

binding of Nedd4, a regulatory repressor that promotes channel degradation. The inability of beta and gamma subunits to bind Nedd4 results in constitutive expression of sodium channels at the apical surface of principal cells, leading to increased rates of sodium reabsorption, volume expansion, and hypertension.

Mutations of the serine-threonine (with no lysine) kinases, WNK1 and WNK4, cause pseudohypoaldosteronism type II (also called Gordon's syndrome). Wild-type WNK1 and WNK4 inhibit the thiazide- sensitive Na–Cl co-transporter in the distal tubule.[14] Mutations of these proteins are associated with gain of function and increased co-transporter activity, excessive chloride and sodium reabsorption, and volume expansion. Hyperkalemia, another hallmark of this syndrome, might be a function of diminished sodium delivery to the cortical collecting tubule. Sodium reabsorption provides the driving force for potassium excretion, which is mediated by the renal outer medullary potassium channel ROMK). Alternatively, the same mutations in WNK4 that increase Na-Cl co-transporter activity could inhibit ROMK.

Interestingly, other genetic causes of pseudohypoaldosteronism type II exist. Investigators recently conducted exome sequencing to identify mutations in kelch-like 3 (*KLHL3*) or cullin 3 (*CUL3*) in pseudohypoaldosteronism type II patients from 41 unrelated families. KLHL3 mutations were either recessive or dominant, whereas CUL3 mutations are dominant and predominantly de novo.[15] Disease features in these families were reversed by thiazide diuretics, which inhibit the Na-Cl cotransporter in the distal nephron of the kidney. KLHL3 and CUL3 are expressed in this location, suggesting a mechanistic link between KLHL3 and CUL3 mutations, increased Na-Cl reabsorption, and disease pathogenesis. The findings demonstrate the utility of exome sequencing in disease gene identification despite the combined complexities of locus heterogeneity, mixed models of transmission and frequent de novo mutation, and establish a fundamental role for *KLHL3* and *CUL3* in blood pressure, potassium, and pH homeostasis.

Recently a form of Mendelian metabolic syndrome was described. Investigators identified three large families with coinheritance of early-onset coronary artery disease, central obesity, hypertension, and diabetes.[16] A founder mutation was identified in DYRK1B, substituting cysteine for arginine at position 102 in the highly conserved kinase-like domain. A hyperactivated DYRK1B promoted the expression of the key gluconeogenic enzyme glucose-6-phosphatase. These exciting findings indicate a role for DYRK1B in adipogenesis and glucose homeostasis and associate its altered function with an inherited form of the metabolic syndrome.

Autosomal-dominant hypertension with brachydactyly is particularly interesting since the phenotype does not exhibit salt sensitivity and since the renin-angiotensin-aldosterone system exhibits normal values.[17] Since a complex genetic rearrangement was associated with this syndrome that included deletions, reinsertions, and inversions, we had postulated an epigenetic explanation for this syndrome.[18] The advent of genome-wide sequencing at affordable prices, has led us to the discovery of mutations in a gene within the linkage interval that is likely responsible for both phenotypes. We have found not precisely identical but closely related mutations in five other families with this syndrome (unpublished observations). The mutation leads to a direct increase in peripheral vascular resistance throughout the entire vascular tree.

The Mendelian forms of hypertension are generally not difficult to diagnose. A careful family history is of course helpful. All, except for autosomal-dominant hypertension with brachydactyly, feature low PRA. In GRA, aldosterone levels are quite elevated but decrease with replacement prednisone. In patients with MCR mutations and AME, PRA and aldosterone levels are both low. Licorice gluttony should be in the differential diagnosis. In Liddle's syndrome, aldosterone levels are also low. Thus, spironolactone is helpful while blocking ENaC is effective. In persons with WNK1 and WNK4 mutations aldosterone values

are normal to elevated. Furthermore, the mild metabolic acidosis and hyperkalemia should be a useful clue, as opposed to mild metabolic alkalosis and hypokalemia, as featured by other Mendelian conditions. Autosomal-dominant hypertension with brachydactyly can be diagnosed with a handshake. The brachydactyly is a type E brachydactyly, which is not uncommon. Since essential hypertension is also common, we have worked up several families with autosomal-dominant type E brachydactyly that did not have the syndrome described above. But the effort has led us to discover other genetic findings, including a long noncoding RNA that leads to brachydactyly.[19]

One difficult question to answer is: why have the GWAS studies not identified the gene loci of Mendelian syndromes, since the genes responsible for these conditions are clearly highly related to hypertension? The effects of alleles in many genes contribute to common complex diseases such as hypertension. Whether risk alleles comprise a small number of common variants or many rare independent mutations at trait loci is largely unknown. However, this possibility has been carefully explored. Investigators have screened members of the Framingham Heart Study (FHS) for variation in three genes, namely SLC12A3 (NCCT), SLC12A1 (NKCC2), and KCNJ1 (ROMK) that cause rare recessive diseases featuring large reductions in blood pressure.[20] Comparative genomics, genetics and biochemistry were used to identify subjects with mutations proven or inferred to be functional. These mutations were all heterozygous and rare. They produced clinically significant blood pressure reduction and likely protected from the development of hypertension. The findings implicated many rare alleles that alter renal salt handling in blood pressure variation in the general population, and identified alleles with health benefit that were nonetheless under purifying selection. The observations have implications for the genetic architecture of hypertension. Furthermore, the presence of these variants in the general population could confound GWAS searches for hypertension genes.

Perspective

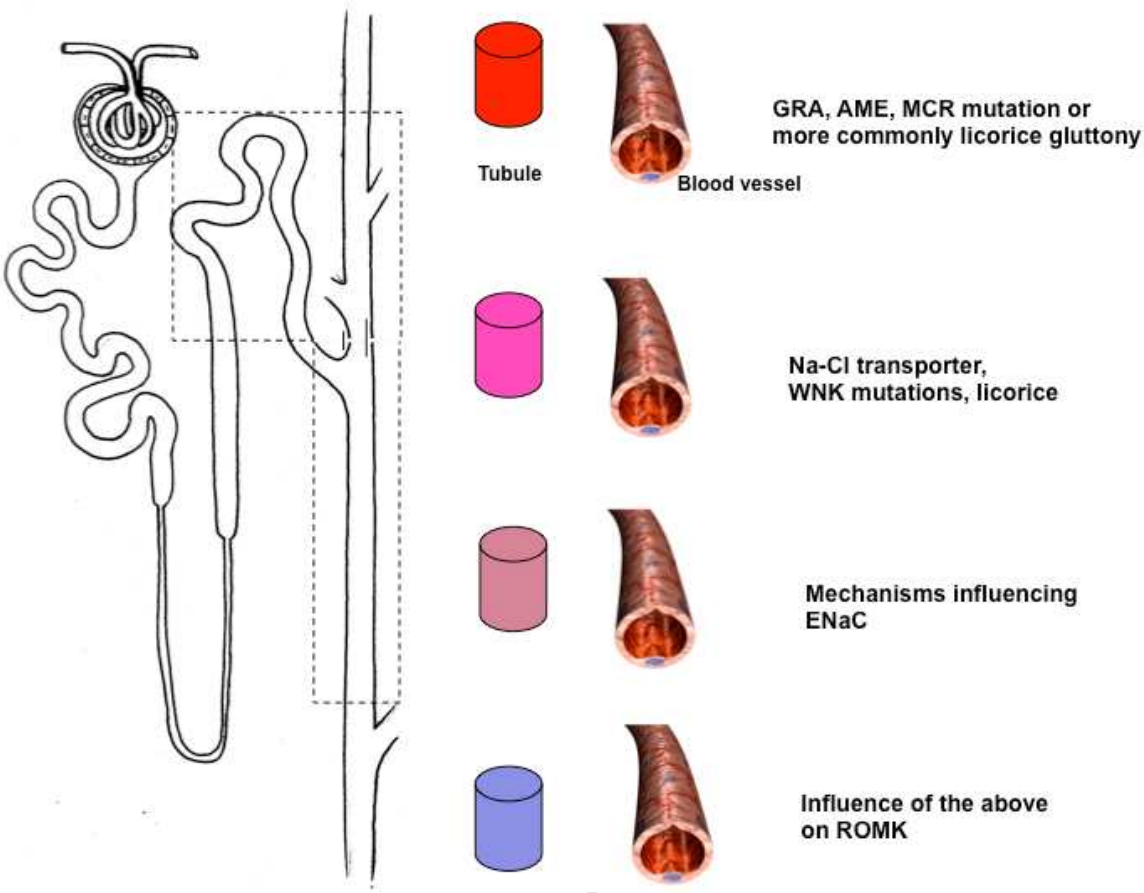
Genomics has come to hypertension-related research. In the last 15 years, the costs of total-genome sequencing have decreased from 200 million dollars to 5000 dollars, within the range of clinical investigators. Furthermore, ancillary technology and availability of powerful bioinformatics has made hypertension-related research not only tremendously exciting but also within the grasp of clinical investigators and interested clinicians. Important is the continuous participation of clinicians in this process. There is little reason to be intimidated by the technical aspects; help is readily at hand. Important is the issue that hypertension specialists stay tuned to the latest development. This research will steadily reduce the numbers of patients with “essential” hypertension and continually increase the patients with “secondary” hypertension. These advances will make “personalized” medicine possible.

Figure legend.

Figure. Mendelian hypertension thus far influences salt transport across the nephron (tubule to blood vessel. Shown are the nephron areas (hatched) in the distal tubule and collection duct (all fine-tuning sites). GRA, AME, and MCR are represented across the entire area. Na-Cl co-transporter is mostly in the distal tubule. ENaC also expends a great area. ROMK, as influenced by WNK, is also universally expressed in the distal nephron.

Disclosures

None.



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