Hypertension Research (2011) 34, 1255–1256

www.nature.com/hr

COMMENTARY

Utilization of genetic information for the dissection of complex diseases or traits

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Hypertension Research (2011) **34,** 1255–1256; doi:10.1038/hr.2011.174; published online 20 October 2011

In their study published in this issue of *Hypertension Research*, Hirose *et al.* utilized a candidate gene approach and found in the Japanese Ohasama cohort that an 18-base pair deletion/insertion (D/I) polymorphism in the adrenomedullin 2/intermedin (*AM2/IMD*) gene was associated with high ambulatory blood pressure, kidney abnormalities (such as proteinuria, elevated serum creatinine concentrations and reduced estimated glomerular filtration rate (eGFR)) and cerebrovascular lesions (such as lacunar infarction and white matter hyperintensity).[1] The polymorphism is common, with a minor allele frequency of 22.5% observed in the Japanese population. Only the least common genotype, DD homozygosity (genotype frequency 5.5%), was associated with the risk of high ambulatory blood pressure, renal disorders and cerebrovascular lesions. Among the 1073 subjects enrolled in the Ohasama study, the 24-h systolic and diastolic blood pressures were 5.1 and 3.1 mm Hg, respectively, higher in the 59 AM2/IMD DD homozygotes than in the 1014 I allele carriers ($P \leqslant 0.006$). The odds ratios associated with the DD genotype were 2.7, 2.4 and 2.7 for chronic kidney disease (defined as the presence of urinary protein on a dipstick test or an eGFR of $<60\,\mathrm{ml\,min^{-1}}$ per 1.73 $\mathrm{m^2}$), lacunar infarction and white matter hyperintensity ($P \leqslant 0.01$), respectively. This novel finding is important in understanding the role of AM2/IMD in the pathophysiology of cardiovascular and renal regulation.

AM2/IMD has two distinct names because this peptide was discovered independently by two research groups, which named this protein AM2 and IMD.[2,3] AM2/IMD is a member of a peptide super family that consists of calcitonin, calcitonin gene-related peptide (CGRP), amylin, adrenomedullin (AM) and AM2/IMD and is a potent hormone in cardiovascular and renal regulation. AM2/IMD is less potent in activating the CGRP receptor than CGRP is and is less potent in activating the AM1 and AM2 receptors than AM is.[4] Nonetheless, AM2/IMD might still be important in cardiovascular and renal regulation because of its multiple functions mediated via the CGRP and AM receptors and because of its specific distribution in the pituitary and kidneys. The studied polymorphism leads to the absence of the AM2/IMD_{1-53} peptide, which is one of the three functional mature peptides. The function of AM2/IMD_{1-53} in cardiovascular and renal regulation remains to be elucidated. However, absence of this peptide over a lifetime may be clinically relevant in the pathophysiology of cardiovascular and renal diseases and warrants further investigation and confirmation by other studies.

The novel finding about the AM2/IMD D/I polymorphism highlights not only the pathophysiological role of AM2/IMD but also the revival of the candidate gene approach for complex diseases or traits. The Ohasama study is one of the best-conducted population cohort studies, has been productive over the past decade, and significantly improves our knowledge of blood pressure monitoring and the genetics of cardiovascular and renal abnormalities.[5] The enriched phenotypes of blood pressure and related measurements are among the reasons why candidate gene studies using the Ohasama cohort are so informative. The Ohasama study is well-known for its home and ambulatory blood pressure monitoring[6] and the large quantity of phenotypic measurements relevant to the cardiovascular and renal systems.[5,7] All of these features contribute to the success of genetic studies using the Ohasama cohort. Such studies have demonstrated that even in the GWAS (genome-wide association studies) era, the candidate gene approach can still be meaningful and useful. The use of the candidate gene approach has decreased in the past few years, and in some researchers' minds, is obsolete and, therefore, should be discarded or banned. Indeed, candidate gene studies have long encountered difficulty in being published in high-impact journals. However, in the post-GWAS era, many newly discovered genes and genetic variants are becoming available for study. Accordingly, the candidate gene approach will again become common. Editors and reviewers should not only change their minds but also open their eyes so that well-conducted genetic studies using the candidate gene approach become available in high-impact journals.

A well-conducted candidate gene study should meet several requirements. First, the sample size should be sufficiently large. Under-powered studies have high probabilities of false-positive and false-negative findings. There is no definitive rule on the size of a study. However, the sample size can be empirically recommended based on the allele frequency of a genetic variant. If the allele frequency is 10% or more (and thus not rare), such as for the AM2/IMD D/I polymorphism in the Ohasama study, several thousand subjects would be sufficient for the analysis of associated diseases or traits. However, if the allele frequency is less than 10%, more subjects might be needed. Although larger is generally better, this is not necessarily true. Increasing the sample

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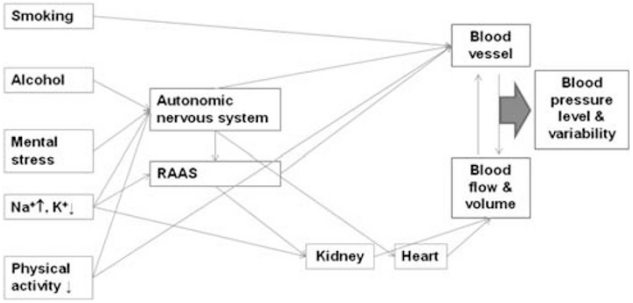

Figure 1 Complexity of the blood pressure regulatory system. Lifestyle risk factors, major regulatory systems, organs involved in blood pressure regulation and the basis of blood pressure are presented. RAAS denotes the renin–angiotensin–aldosterone system. A full color version of this figure is available at the *Hypertension Research* journal online.

size would not only increase the cost but also potentially decrease the quality of the phenotypic and genotypic data.

Second, phenotypic precision is important to the success of genetic studies focused on quantitative phenotypes of common diseases or traits, such as hypertension. Hypertension is a disease of complex pathophysiology (Figure 1). Any pathological change in one aspect of the blood pressure regulatory system can be compensated for by changes in other aspects in the body's attempt to maintain a stable blood pressure.[8,9] Blood pressure has been measured noninvasively using a mercury sphygmomanometer for more than a century. Using this technique, the scientific community has been successful in the detection and treatment of hypertension and in the protection against the damage of target organs and the prevention of cardiovascular events. However, blood pressure changes over time. Ambulatory blood pressure monitoring is not subject to the white-coat effect and observer bias, provides data about the circadian rhythm of blood pressure, and significantly improves the precision of blood pressure measurements. The observations of the Ohasama cohort provide additional evidence. Genetic differences were observed with respect to ambulatory but not casual blood pressure and were larger for the daytime blood pressure than for the night-time blood pressure.[1] Complex diseases or traits, such as blood pressure, require complex and precise phenotypic measurements.

Third, genetic associations should be prospectively investigated (not retrospectively, as is typical in the case–control design). Prospective observational studies have been successful in scrutinizing conventional risk factors. However, high-throughput technologies, such as GWAS, rush genetic association studies, examining an extremely large number of genes or genetic variants in an extremely large number of study subjects. Cross-sectional studies compare the lifetime risk of the study subjects and are theoretically powerful tools for the study of associations. However, the selective survival of individuals with favorable genotypes can decrease the size of possible associations and even change the direction of associations. Long-term prospective studies with the complete follow-up of patients and diseases are preferable. In this respect, the Ohasama study was apparently inadequately powered to explore prospective associations observed in the cross-sectional study.

A high-quality candidate gene study certainly requires more than the above three essentials. The candidate gene approach also has its limitations. Nonetheless, with the advances in genetic and molecular technologies, the hypothesis-driven candidate gene approach is the method to properly utilize genetic information for the dissection of complex diseases or traits, such as blood pressure.

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