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Aldosterone synthase (CYP11B2) polymorphisms and cardiovascular function ☆

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Abstract

In addition to regulating renal sodium resorption and, thus, intravascular volume, aldosterone may have direct effects on the cardiovascular system. We previously identified a polymorphism (-344C/T) in the promoter of the aldosterone synthase (CYP11B2) gene that affects binding of the SF-1 transcription factor and thus might influence gene expression. We found that, whereas this polymorphism has inconsistent associations with levels of aldosterone secretion and blood pressure, the -344C allele is strongly associated with increased left ventricular size and decreased baroreflex sensitivity in healthy individuals. These physiological parameters are cardiovascular risk factors. Indeed, preliminary studies suggest that the -344C allele is also associated with increased risk of myocardial infarction in high risk dyslipidemic males.

1. Introduction

Hypertension is an important risk factor for coronary heat disease (CHD). Other cardiovascular physiological parameters that predict morbidity and mortality from CHD include left ventricular hypertrophy [1] and altered autonomic function, evidenced by chronotropic incompetence (failure to achieve an appropriate heart rate with exercise) [2] or decreased baroreflex sensitivity [3]. Genetic factors that affect these parameters might in turn be risk factors for CHD.

The renin–angiotensin–aldosterone system is an important regulator of blood pressure and polymorphisms in genes encoding components of this system, and have indeed been associated with physiological risk factors for CHD. The most consistent of these associations are with the angiotensinogen (AGT) gene, the T235 variant which is associated with essential hypertension [4] and with increased CHD risk [5]. A deletion polymorphism in the angiotensin converting enzyme (ACE) gene influences ACE levels [6] and, while having little effect on blood pressure, it has been associated with left ventricular hypertrophy in some but not other studies [7–9]. It has also been inconsistently associated with CHD risk [10].

Aldosterone is one of the main effectors of the renin-angiotensin system. Its best characterized effect is to increase sodium resorption in the distal nephron and thus increase intravascular volume. Aldosterone secretion is regulated largely at the level of expression of the final enzyme required for its biosynthesis, aldosterone synthase (CYP11B2) [11]. Genetic rearrangements involving this locus lead to inappropriate expression of CYP11B2, excessive aldosterone secretion and high blood pressure, a rare disorder termed glucocorticoid suppressible hyperaldosteronism [12,13].

In addition to causing hypertension, aldosterone is known to have effects on the cardiovascular system that are independent of blood pressure. In rats on a high salt diet, it causes myocardial fibrosis and cardiac

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hypertrophy at doses that do not affect blood pressure [14,15]. In the isolated perfused rat heart, it decreases coronary blood flow [16]. In dogs, it decreases baroreflex sensitivity [17]. Humans with primary aldosteronism are more prone to left ventricular hypertrophy than individuals with essential hypertension of equivalent severity [18]. These effects may all be mediated by mineralocorticoid receptors in the heart [19] and/or in the vascular endothelium.

Thus, it seemed plausible that other polymorphisms in CYP11B2 might affect gene expression or enzymatic activity and thus have effects on cardiovascular function. In this paper, we summarize our recent studies delineating these effects.

2. Results and discussion

2.1. The -344C/T polymorphism in CYP11B2

Several polymorphisms in and near CYP11B2 have been identified [20,21]. Of these, the best candidate to influence CYP11B2 expression is located in the 5′ flanking region of the gene, 344 nucleotides upstream from the start of translation within a binding site for the transcription factor, Steroidogenic Factor-1 (SF-1) [22]; this position may be either a C or T nucleotide (-344C and -344 T alleles) [21]. These alleles are present at approximately equal frequencies in Caucasian populations [21,23]. The SF-1 transcription factor is required for development of the adrenal glands and other steroidogenic tissues and for expression of all enzymes involved in adrenal steroid biosynthesis [24]. The homolog of the -351 to -343 site is required for full expression of the aldosterone synthase gene in cattle and rodents. The -344C allele binds SF-1 four times more strongly than the -344 T allele (unpublished observations). All of these considerations make it plausible that this polymorphism could influence CYP11B2 expression and, thus, aldosterone levels. However, this site is unnecessary for either basal or hormone-regulated expression of human CYP11B2 reporter constructs in human adrenocortical cells, apparently because the human gene contains an additional SF-1 site nearer to the start of transcription that is preferentially utilized [22]. Nonetheless this site might be required for full expression of CYP11B2 in the intact adrenal cortex. Because of the obvious difficulty in obtaining human adrenal glands for direct measurement of CYP11B2 expression, this possibility is best assessed by measuring serum aldosterone levels or urinary aldosterone excretion under controlled conditions. Indeed, the -344C allele is strongly associated with higher serum aldosterone levels and lower plasma renin activity in a large French study [25]. Such an association has not been seen in other studies [26], but this may reflect differences in the conditions under which aldosterone levels were measured.

2.2. Associations between the -344C/T polymorphism and left ventricular size, mass and function

We analyzed a Finnish population sample of 84 persons (44 women) aged 36 to 37 years that had been studied by M-mode and Doppler echocardiography [27]. The Finnish population is highly homogeneous ethnically [28], making it particularly suitable for association studies, and the study design eliminated age as a variable. Subjects were genotyped for the -344C/T and intron 2 polymorphisms in CYP11B2. Multiple regression analyses were used to test the hypothesis of a gene dosage effect. The genotype of the -344C/T polymorphism predicted statistically significant variations in left ventricular end-diastolic diameter ($\beta = 0.40$, $p < 0.0001$), end-systolic diameter ($\beta = 0.33$, $p = 0.0009$), and mass ($\beta = 0.17$, $p = 0.023$). In all cases, the -344C allele (which is associated with increased binding of SF-1) was associated with larger size or mass. These effects were independent of potentially confounding factors including sex, body size, blood pressure, physical activity, smoking and ethanol consumption. Genotype groups also differed in a measure of left ventricular diastolic function, the heart rate adjusted atrial filling fraction ($p = 0.018$). Increased dietary salt, which is known to predict increased left ventricular mass, had this effect only in association with the -344C allele ($p < 0.001$); i.e., the regression of left ventricular mass on salt intake was strong and statistically highly significant in the -344CC homozygous group, intermediate in the -344CT heterozygous group and nonexistent in the -344TT homozygous group.

To the best of our knowledge, CYP11B2 genotype is the only genetic factor identified thus far that affects heart size in young adults. Our analysis suggests that as much as 16% of the variation in left ventricular and end-diastolic diameter in our population sample was attributable to the CYP11B2 promoter polymorphism.

2.3. Associations between the -344C/T polymorphism and baroreflex sensitivity

Encouraged by our findings with heart size, we decided to study other cardiovascular parameters. Baroreflex sensitivity (BRS) is the compensatory heart rate deceleration that occurs when blood pressure is increased by drugs or by the Valsalva maneuvre. It varies widely between healthy individuals. This is not well explained by demographic variables, cardiovascular risk factors or life style, suggesting a genetic component to interindividual variation of BRS.

BRS, measured from the overshoot phase of the Valsalva maneuvre, and genetic polymorphisms, were

examined in a subset of 29 men and 37 women aged 36 to 37 years from the group in which heart size had been studied, and also in a random sample of 161 women and 154 men aged 41 to 61 years. In the younger population, BRS was strongly related to the CYP11B2 promoter genotype averaging 19.2 ± 9.9, 12.9 ± 5.1 and 9.7 ± 4.1 ms $mmHg^{-1}$ in the -344TT, CT and CC groups, respectively, ($P = 0.0003$). The association was statistically significant both in men ($P = 0.015$) and women ($P = 0.03$). There was no strong correlation with heart size, suggesting that the genetic effects on heart size and BRS were independent. In the older population, BRS (mean $\pm$ SD) differed significantly across CYP11B2 genotype groups in women (10.1 ± 4.5, 8.7 ± 3.8 and 7.1 ± 3.2 ms $mmHg^{-1}$ in genotypes -344TT, CT and CC, respectively, $P = 0.003$), but not in men.

Thus, the -344C/T polymorphism predicts interindividual variation in BRS. The associations are stronger in young subjects and may diminish earlier in aging men.

3.4. The -344C allele in CYP11B2 increases risk of coronary heart disease (CHD) in smokers

Considering that the -344C allele is associated with increased left ventricular size and decreased baroreflex sensitivity, and that both of these physiological changes are associated with increased morbidity and mortality from CHD, it was of interest to determine if the -344C allele constituted an independent risk factor for CHD. Therefore, we used a nested case-control design to test this hypothesis in 141 cases and 270 matched controls of the Helsinki Heart Study, a coronary primary prevention trial in dyslipidemic, middle-aged men. Whereas there was a non-significant trend of increasing risk of myocardial infarction (MI) with a number of copies of the -344C allele, this allele acted in a gene dosage-dependent manner to markedly increase MI risk conferred by classical risk factors. Whereas smoking conferred a relative MI risk of 2.86 compared with nonsmokers in the entire study population ($p = 0.001$), it carried a risk of 5.08 in -344CC homozygous smokers, relative to nonsmokers with the same genotype ($p = 0.002$), but a risk of only 1.30 in -344TT homozygotes, relative to nonsmokers with this genotype. Similarly, decreased high density lipoprotein cholesterol was a more potent risk factor in -344CC homozygotes, and less potent in -344TT homozygotes, relative to the entire study population. We conclude that the -344C allele of CYP11B2 is a risk factor for myocardial infarction in dyslipidemic middle-aged Finnish male smokers; further studies are required to determine if this conclusion can be extended to other populations.

It is remarkable that the -344C/T polymorphism has such strong associations with left ventricular size, baroreflex sensitivity and (in one population) cardiovascular risk whereas associations with levels of aldosterone secretion are inconsistent. Thus one must consider an explanation for our observations other than differences in circulating aldosterone levels. For example, CYP11B2 expression has been reported in human [29] and rodent [30] vascular endothelium and rodent heart [31], so that aldosterone might have autocrine or paracrine effects on the heart and vasculature. If the -344C allele increased expression of CYP11B2 in these tissues, it might increase local concentrations of aldosterone and thus have cardiovascular effects without significantly increasing circulating aldosterone levels. Alternatively, another polymorphism in genetic linkage disequilibrium with -344C/T may be responsible for the observed associations. Elucidation of the mechanism(s) involved may provide useful insights into the role of aldosterone in the cardiovascular system.

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References

[1] D. Levy, R.J. Garrison, D.D. Savage, W.B. Kannel, W.P. Castelli, Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study, N. Engl. J. Med. 322 (1990) 1561–1566.

[2] M.S. Lauer, F.J. Pashkow, M.G. Larson, D. Levy, Association of cigarette smoking with chronotropic incompetence and prognosis in the Framingham Heart Study, Circulation 96 (1997) 897–903.

[3] M.T. La Rovere, J.T. Bigger, F.I. Marcus, A. Mortara, P.J. Schwartz, Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction, Lancet 351 (1998) 478–484.

[4] X. Jeunemaitre, F. Soubrier, Y.V. Kotelevtsev, R.P. Lifton, C.S. Williams, A. Charru, S.C. Hunt, P.N. Hopkins, R.R. Williams, J.M. Lalouel, et al., Molecular basis of human hypertension: role of angiotensinogen, Cell 71 (1992) 169–180.

[5] T. Katsuya, G. Koike, T.W. Yee, N. Sharpe, R. Jackson, R. Norton, M. Horiuchi, R.E. Pratt, V.J. Dzau, S. MacMahon, Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease, Lancet 345 (1995) 1600–1603.

[6] B. Rigat, C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol, F. Soubrier, An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the var-

iance of serum enzyme levels, J. Clin. Invest. 86 (1990) 1343–1346.
[7] H. Schunkert, H.W. Hense, S.R. Holmer, M. Stender, S. Perz, U. Keil, B.H. Lorell, G.A. Riegger, Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy, N. Engl. J. Med. 330 (1994) 1634–1638.
[8] M. Kupari, M. Perola, P. Koskinen, J. Virolainen, P.J. Karhunen, Left ventricular size, mass, and function in relation to angiotensin-converting enzyme gene polymorphism in humans, Am. J. Physiol. 267 (1994) H110711.
[9] K. Lindpaintner, M.A. Lee, M.G. Larson, U.S. Rao, M.A. Pfeffer, J.M. Ordovas, E.J. Schaefer, A.F. Wilson, P.W. Wilson, R.S. Vasan, R.H. Myers, D. Levy, Absence of association of genetic linkage between the angiotensin-converting-enzyme gene and left ventricular mass, N. Engl. J. Med. 334 (1996) 1023–1028.
[10] F. Cambien, O. Poirier, L. Lecerf, A. Evans, J.P. Cambou, D. Arveiler, G. Luc, J.M. Bard, L. Bara, S. Ricard, Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction, Nature 359 (1992) 641–644.
[11] K.M. Curnow, M.T. Tusie-Luna, L. Pascoe, R. Natarajan, J.L. Gu, J.L. Nadler, P.C. White, The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex, Mol. Endocrinol. 5 (1991) 1513–1522.
[12] R.P. Lifton, R.G. Dluhy, M. Powers, G.M. Rich, M. Gutkin, F. Fallo, J.R. Gill Jr, L. Feld, A. Ganguly, J.C. Laidlaw, D.J. Murnaghan, C. Kaufman, J.R. Stockigt, S. Ulick, J.M. Lalouel, Hereditary hypertension caused by chamaeric gene duplications and ectopic expression of aldosterone synthase, Nat. Genet. 2 (1992) 66–74.
[13] L. Pascoe, K.M. Curnow, L. Slutsker, J.M. Connell, P.W. Speiser, M.I. New, P.C. White, Glucocorticoid-suppressible hyperaldosteronism results from hybrid genes created by unequal crossovers between CYP11B1 and CYP11B2, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 8327–8331.
[14] M. Young, M.J. Fullerton, R. Dilley, J.W. Funder, Mineralocorticoids, hypertension, and cardiac fibrosis, J. Clin. Invest. 93 (1994) 2578–2583.
[15] Weber KT, Brilla CG, Campbell SE, Guarda E, Zhou G, Sriram K, Myocardial fibrosis: role of angiotensin II and aldosterone, Basic Research in Cardiology 1993;107–124 (88 Suppl. 1).
[16] D. Moreau, J.M. Chardigny, L. Rochette, Effects of aldosterone and spironolactone on the isolated perfused rat heart, Pharmacology 53 (1996) 28–36.
[17] W. Wang, Chronic administration of aldosterone depresses baroreceptor reflex function in the dog, Hypertension 24 (1994) 571–575.
[18] T. Denolle, G. Chatellier, J. Julien, C. Battaglia, P. Luo, P.F. Plouin, Left ventricular mass and geometry before and after etiologic treatment in renovascular hypertension, aldosterone-producing adenoma, and pheochromocytoma, Am. J. Hypertens. 6 (1993) 907–913.
[19] M. Lombes, N. Alfaidy, E. Eugene, A. Lessana, N. Farman, J.P. Bonvalet, Prerequisite for cardiac aldosterone action. Mineralocorticoid receptor and 11 beta-hydroxysteroid dehydrogenase in the human heart, Circulation 92 (1995) 175–182.
[20] R.P. Lifton, R.G. Dluhy, M. Powers, G.M. Rich, S. Cook, S. Ulick, J.M. Lalouel, A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension, Nature 355 (1992) 262–265.
[21] P.C. White, L. Slutsker, Haplotype analysis of CYP11B2, Endocr. Res. 21 (1995) 437–442.
[22] C.D. Clyne, Y. Zhang, L. Slutsker, J.M. Mathis, P.C. White, W.E. Rainey, Angiotensin II and potassium regulate human CYP11B2 transcription through common cis elements, Mol. Endocrinol. 11 (1997) 638–649.
[23] M. Kupari, A. Hautanen, L. Lankinen, P. Koskinen, J. Virolainen, H. Nikkila, P.C. White, Associations between human aldosterone synthase (CYP11B2) gene polymorphisms and left ventricular size, mass and function, Circulation 97 (1998) 569–575.
[24] K.L. Parker, B.P. Schimmer, Steroidogenic factor 1: a key determinant of endocrine development and function, Endocr. Rev. 18 (1997) 361–377.
[25] A. Benetos, O. Poirier, T.T. Guyene, S. Gautier, L. Pojoga, F. Cambien, Genetic determination of plasma aldosterone levels, Hypertension 30 (1997) 493.
[26] A. Hautanen, L. Lankinen, M. Kupari, O.A. Janne, H. Adlercreutz, H. Nikkila, P.C. White, Associations between aldosterone synthase gene polymorphism and the adrenocortical function in males, J. Intern. Med. 244 (1998) 11–18.
[27] M. Kupari, P. Koskinen, J. Virolainen, Correlates of left ventricular mass in a population sample aged 36 to 37 years. Focus on lifestyle and salt intake, Circulation 89 (1994) 1041–1050.
[28] J. Hastbacka, A. de la Chapelle, I. Kaitila, P. Sistonen, A. Weaver, E.S. Lander, Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland, Nat. Genet. 2 (1992) 204–211.
[29] Y. Takeda, I. Miyamori, M. Yoneda, H. Hatakeyama, S. Inaba, K. Furukawa, H. Mabuchi, R. Takeda, Regulation of aldosterone synthase in human vascular endothelial cells by angiotensin II and adrenocorticotropin, J. Clin. Endocrinol. Metab. 81 (1996) 2797–2800.
[30] H. Hatakeyama, I. Miyamori, T. Fujita, Y. Takeda, R. Takeda, H. Yamamoto, Vascular aldosterone. Biosynthesis and a link to angiotensin II-induced hypertrophy of vascular smooth muscle cells, J. Biol. Chem. 269 (1994) 24,316–24,320.
[31] J.S. Silvestre, V. Robert, C. Heymes, B. Aupetit-Faisant, C. Mouas, J.M. Moalic, B. Swynghedauw, C. Delcayre, Myocardial production of aldosterone and corticosterone in the rat. Physiological regulation, J. Biol. Chem. 273 (1998) 4883–4891.