Vol. 150, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1988 Pages 219-224

FREQUENCY IN HYPERTENSIVES OF ALLELES FOR A RFLP ASSOCIATED WITH THE RENIN GENE

Brian J. Morris* and Lyn R. Griffiths†

Departments of Physiology* and Medicine†, The University of Sydney, N.S.W., 2006, Australia

Received November 25, 1987

SUMMARY: The genetic basis of primary hypertension is not known. Renin is important in blood pressure and volume control and a *Hin*dIII restriction fragment length polymorphism (RFLP) is present within the human renin gene locus. To examine whether there is a relationship between this RFLP and primary hypertension, DNA and renin analyses were performed on leukocytes and plasma from hypertensive and normotensive individuals. In hypertensives the frequencies of alleles for the *Hin*dIII RFLP were found to be 0.55 and 0.45, compared with 0.60 and 0.40 in the total population of 231 subjects examined, a difference that was not statistically significant. There also appeared to be no significant difference in renin activity in plasma for hypertensive patients of each genotype, nor in their pre- or post-treatment blood pressures. We thus conclude that, within the limits of the present study, the suspected genetic abnormalities associated with primary hypertension in man do not appear to be related to a *Hin*dIII RFLP in the renin gene.

Primary hypertension in man is considered to result from an inherited defect in one or several of the genetic factors that determine blood pressure [1,2]. Populations whose intake of sodium chloride is high are at increased risk of hypertension in the face of a reduced ability to excrete the excess [3,4]. A renal defect has therefore been suggested as the underlying cause of hypertension [5,6]. One of the most important factors in the regulation of extracellular sodium and volume balance is the renin-angiotensin system, whose activity is determined by the rate of regulated renin secretion into plasma from the kidney [6,7]. No direct association of renin with primary hypertension has been noted, although patients with primary hypertension have plasma renin values with an overall distribution that differs from normal. On this basis hypertensive patients appear to fall into three major subgroups, namely, low renin (~30%), normal renin (~50%), and high renin (~15%), and such renin profiling has been used in deciding treatment regimen [8]. Renin secretion, when abnormally high or low, may be merely responding appropriately to primary disturbances elsewhere, although the possibility of an intrinsic abberration should be considered. If so, one possibility might be an alteration in rate of synthesis arising from an allelic variation in the structure of the renin gene or of its associated regulatory DNA in certain patients.

The gene for human renin (REN) has been isolated on two overlapping clones (λHR3 and λHR5) and its sequence determined [9]. REN contains a *Hin*dIII restriction fragment length polymorphism (RFLP), with 9.0-kb and 6.2-kb alleles detectable by Southern blotting and whose population frequency has been reported as 0.66 and 0.34, respectively [10]. The clone λHR5, whose 14 kb insert encodes exons 2-9 of REN, can detect this RFLP.

0006-291X/88 $1.50

The aim of the present study was to examine whether an allelic polymorphism of the renin gene is associated with primary hypertension. This was performed by comparing the genotypes of the *Hin*dIII RFLP in hypertensive subjects with those found in the normal population.

METHODS

Study design: Blood samples of ~20 ml were drawn in the morning from the antecubetal fossa of 231 adult caucasian subjects, of whom 29 were either receiving antihypertensive medication at the time of sample collection or who were prescribed medication subsequently. The frequency of primary hypertension in the study group (12.6%) was that expected for any western adult population. Since the study was directed at molecular analysis of genomic DNA structure rather than physiological parameters, the only criterion for selection of patients was that they had been correctly diagnosed as having primary hypertension. Diagnosis was made by the patient's general practitioner according to conventional criteria, including a diastolic pressure of >90 mmHg or systolic pressure of >140 mmHg over three successive consultations spanning two months. Complete details of both pre- and post-treatment blood pressures were available for 24 of the 29 patients. DNA was isolated from all subjects and used for reninRFLP analysis and plasma renin levels were determined for all hypertensive patients. Results were tabulated according to renin genotype and the possibility of an association of a particular renin allele with hypertension was examined.

RFLP analysis: DNA was isolated from peripheral blood leukocytes of the hypertensive and normotensive subjects, and was used for Southern blot analysis as described by Cavenee *et al.* [11]. In brief, 15 μg of DNA was restricted with *Hin*dIII, electrophoresed on 1% agarose gels, transferred to Gene Screen Plus membranes and probed with ^{32}P-labelled λHR5 at 42°C in 50% formamide, 0.9 M NaCl, 50 mM sodium phosphate buffer, pH 6.8, 200 mg/ml salmon sperm DNA, 10% dextran sulfate, 3.5% SDS. All filters were washed twice in 2 x standard saline citrate (SSC) (1 x SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) at 22°C, once in 2 x SSC / 1% SDS at 65°C, and twice in 0.1 x SSC at 65°C. After drying in air the filters were exposed to Kodak XAR-5 film, with a Lightning Plus intensifying screen (Dupont), for 1-7 days at -80°C.

Renin assay: Renin enzymatic activity in plasma was determined, as described previously [12], by its initial velocity of generation of angiotensin I during incubation at 37°C, pH 7.4, with angiotensinogen added as 4 vol. plasma from a nephrectomized sheep, i.e., at a concentration exceeding the K_m by 10-fold. Quantification of angiotensin I was by radioimmunoassay and renin activity values were expressed as International Units / ml plasma, where one unit of the International Reference Preparation of Human Renin (code 68/356; National Institute for Biological Standards and Control, Holly Hill, London) was found to generate 75,800 ± 8,800 SD pmol angiotensin I·h^{-1}·ml^{-1} (n = 8).

RESULTS

The three possible patterns of hybridization of ^{32}P-labelled λHR5 probe to Southern blots of *Hin*dIII-restricted DNA from human subjects is shown in Fig. 1. Homozygotes for the *Hin*dIII RFLP have either a 9.0- or a 6.2-kb band with invariable 3.4- and 2.5-kb bands. Heterozygotes have both the 9.0- and 6.2-kb bands. The genotypes have been abbreviated in the figure to 'a a', 'b b' and 'a b', where, in the present paper, the 9.0-kb allele of the REN *Hin*dIII RFLP has been designated 'REN a' and the 6.2-kb allele 'REN b'. The frequencies of allele REN a and allele REN b in the hypertensive patients were 0.55 and 0.45, respectively. These values were not significantly different, by χ^2 analysis, from the frequencies of 0.60 and 0.40, respectively, in all subjects examined.

Clinical details for the hypertensive patients are shown in Table 1, grouped according to genotype: REN a a, REN b b, and REN a b. The two sets of blood pressure values (mmHg) shown in the table are, respectively, those that were determined at the time of diagnosis of hypertension and those at the

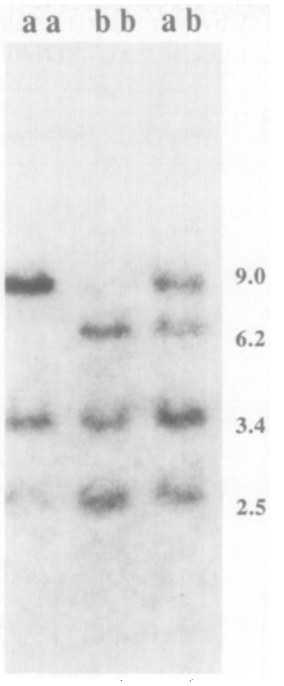

Fig. 1. The three patterns of hybridization on-Southern blots of human DNA hybridized to λHR5; these represent the three genotypes for the REN-associated RFLP at a *Hin*dIII site. The genotypes of the three subjects represented were designated 'a a', 'b b', and 'a b' for the 9.0-kb homozygote, the 6.2-kb homozygote, and the 9.0-kb / 6.2-kb heterozygote, respectively.

time when blood was collected for renin assay and DNA analysis. In Table 1, mean ± SE of blood pressures for the latter were determined using only values for patients that had been receiving anti-hypertensive medication. In two patients (AS and FD) hypertension was revealed at the time of collection of blood; these values were not included when determining mean ± SE for blood pressures at sampling, so that only blood pressures for treated patients were used in the calculation. All other information given in Table 1 pertains to that at the time of collection of the blood sample. Only three of the patients had evidence of possible renal disease, thought to be secondary to the hypertension, and in none of the patients studied had renin been determined at the time of diagnosis of hypertension.

There appeared to be no significant difference between the pretreatment blood pressure of hypertensive patients of each renin genotype, even after taking into account the unavailability of a few blood pressure values. Similarly, blood pressure values attained after treatment did not differ significantly between the groups.

Renin values for hypertensive patients of each genotype were found to be not significantly different from each other. Moreover, these values did not differ significantly from renin values in normotensive subjects, viz. 43 ± 8 SE μU/ml plasma (n = 12). As is usual for hypertensive patients the spread of renin values was broader than is seen in normotensive subjects, with a number of patients having low plasma renin and a few having high values. Renal disease was not detected in the latter patients. The few patients with high renin values contributed to the slight elevation in the mean for groups REN a a and REN a b.

Table 1. Blood pressures, renin activity in plasma, treatment, and other information for hypertensive patients belonging to each REN *Hin*dIII RFLP genotype

Patient	Age	Blood pressure (mmHg) Initial	At sampling	Yrs HT known	Medication	Renin (μU/ml)
REN a a						
EE	69	160/100	170/80	9	Betaloc^{B}	29
IT	79	210/112	145/80	20	Minipress^{A}, Moduretic^{D}	281
GT	35	155/90	150/90	3	Visken^{B}	39
ML*	66	n.k.	180/100	n.k.	Minipress^{A}	5
ML	36	160/100	130/95	10	Apresoline^{N}, Moduretic^{D}	49
CD#	37	180/100	n.k.	4	Tenormin^{B}	14
EH	71	150/95	140/80	10	Moduretic^{D}	142
HH	52	160/95	175/90	1	Tenormin^{B}, Enduron^{D}	5
JW	NK	140/100	190/100	4	None	-
JB	44	140/90	120/80	7	None	70
RY	53	150/100	140/80	1	Moduretic^{D}	26
BB	76	220/120	140/102	14	Aldomet^{F}, Chlotride^{D}, Span K^{P}	21
	56	166/100	149/88	8		62
	±5	±8 ±3	±6 ±3	±2		±25
REN a b						
EM	62	190/110	140/90	3	Tenormin^{B}, Chlotride^{D}	235
RC	49	180/120	150/95	1	Visken^{B}	96
TF	81	n.k.	n.k.	n.k.	Tenormin^{B}, Moduretic^{D}, Lanoxin^{H}	13
RF	36	153/95	130/95	2	Tenormin^{B}, Diazide^{D}	38
DD	79	170/90	130/80	16	Inderal^{B}	35
LS	77	170/100	150/90	6	Inderal^{B}	5
RW°	77	n.k.	140/80	>2	Tenormin^{B}, Moduretic^{D}	5
AW	49	150/100	135/85	1	Cordilox^{C}	111
	64	169/103	139/88	>4		68
	±6	±6 ±4	±3 ±2	±2		±28
REN b b						
AS	36	210/112	210/112	0	None (newly diagnosed)	30
FD	64	230/110	230/110	0	None (newly diagnosed)	8
BD	67	n.k.	140/85	>16	Aldomet^{F}	127
AA	86	150/85	120/80	>12	Visken^{B}, Moduretic^{D}, Lanoxin^{H}	29
TD	59	160/100	120/60	>6	Betaloc^{B}, Minipress^{A}, Moduretic^{D}	85
DT	58	210/115	150/90	20	Aldomet^{F}, Chlotride^{D}, Span K^{P}	70
RD	56	160/98	170/94	8	Cordilox^{C}	30
RC	48	160/110	135/85	3	Inderal^{B}, Dyazide^{D}	14
DH	47	155/85	140/95	4	Tenormin^{B}, Enduron^{D}	12
	58	179/101	139/84	>9		45
	±5	±11 ±4	±7 ±5	±2		±14

a = 9.0 kb allele; b = 6.2 kb allele. Medications taken for hypertension and other cardiovascular dysfunction are indicated as: A = α-adrenoceptor antagonist; B = β-adrenoceptor antagonist; C = calcium channel antagonist; D = diuretic; F = false transmitter; H = heart stimulant; N = depletor of transmitter in noradrenergic nerve fibres ; P = potassium supplement. The nature of the medications whose trade names are indicated above are: Aldomet = methyldopa; Apresoline = guanethidine; Betaloc = metoprolol; Chlotride = chlorothiazide; Cordilox = verapamil; Dyazide = hydrochlorothiazide + triamterine; Enduron = methylchlothiazide; Inderal = propranolol; Lanoxin = digoxin; Minipress = prazosin; Moduretic = amiloride + hydrochlorothiazide; Span K = KCl; Tenormin = atenolol; Visken = pindolol. * = albuminuria; # = haematuria; °= increased urinary creatinine and urea. n.k. = not known. Values below columns are mean ± standard error.

DISCUSSION

Within the limitations of the present study of 29 hypertensive patients, we were unable to demonstrate an association between an allelic variation associated with human renin genomic DNA, as revealed by a *Hin*dIII RFLP, and the occurrence of primary hypertension. The distribution of renin alleles did not differ significantly from those occurring in the normal population. Also, there appeared to be no

correlation of renin genotype with the severity of the hypertension or the level of renin activity in plasma. Although the number of hypertensive patients in the study was low, if a close association of the renin RFLP and hypertension existed, it should have been apparent in the subjects studied.

RFLPs are DNA sequence polymorphisms that result in differences in restriction enzyme cutting sites in different individuals. A number of allelic differences are likely to exist for the renin gene and some of these may be detectable as RFLPs [10,13]. Thus the fact that there was no apparent association of the particular *Hin*dIII RFLP with hypertension in the present study does not mean that an allelic difference associated with the renin gene is not responsible, at least in part, for hypertension. Possible differences in sequences in the renin gene or its regulatory DNA in different individuals requires extensive study to determine their nature, their location, and whether any given difference might alter the rate of transcription of the gene or cause a change in renin activity. Some of the putative regulatory regions associated with the renin gene, such as those in the 5'-flanking DNA, are currently being examined experimentally [14], but no detailed information has been published. Thus a considerable amount of work is yet to be done to decide whether or not allelic differences in the renin gene are pathophysiologically important. The present study may have helped exclude one of the possibilities, although re-examination of the hypothesis in a very much larger group of patients could perhaps reveal subtle differences not evident here.

Normal blood pressure is determined by many factors, so that genetic effects on blood pressure are polygenic [1]. These different genes will also contribute to the variation in blood pressure seen amongst hypertensive individuals, although the number of genes that are responsible for the primary abnormality in blood pressure are of course likely to be very much more restricted. NaCl appears to be a common environmental factor interacting with genotype [2]. Whether a defect in the renin gene, either alone or in combination with other genes concerned with cardiovascular function is responsible for hypertension awaits further detailed investigation.

ACKNOWLEDGEMENT

The present study was supported by a grant from the National Health and Medical Research Council of Australia. We are grateful to Michele Zwi for her help in gathering patient information.

REFERENCES

1. Rapp, J.H. (1983) In Hypertension: Physiopathology and Treatment (J. Genest, O. Kuchel, P. Hamet, and M. Cantin, Eds), pp. 582-598. McGraw-Hill, New York.
2. Sing, C.F., Boerwinkle, E., and Turner, S.T. (1986) Clin. Exp. Hypertension A8, 623-651.
3. Freis, E.D. (1976) Circulation 53, 589-595.
4. Meneely, G.R., and Battarbee, H.D. (1976) Am. J. Cardiol. 38, 768-785.
5. Coleman, T.G., Guyton, A.C., Young, D.B., DeClue, J.W., Norman, R.A., and Manning, R.D. (1975) Clin. Exp. Pharmacol. Physiol. 2, 571-581.
6. Hamlyn, J.M., and Blaustein, M.P. (1986) Am. J. Physiol. 251, F563-F575.
7. Guyton, A.C., Manning, R.D., Norman, R.A., Montani, J.-P., Lohmeier, T.E., and Hall, J.E. (1986) J. Hypertension 4, S49-S56.
8. Laragh, J.H., Letcher, R.L., and Pickering, T.G. (1979) J. Am. Med. Ass. 241, 151-156.

9. Hardman, J.A., Hort, Y.J., Catanzaro, D.F., Tellam, J.T., Baxter, J.D., Morris, B.J. and Shine, J. (1984) DNA 3, 457-468.
10. Frossard, P.M., Gonzalez, P.A., Fritz, L.C., Ponte, P.A, Fiddes, J.C., and Atlas, S.A. (1986) Nucl. Acids Res. 14, 4380.
11. Cavenee, W., Leader, R., Mohandas, T., Pearson, P., and White, R. (1984) Am. J. Hum. Genet. 36, 10-24.
12. Morris, B.J., de Zwart, R.T. and Young, J.A. (1980) Experientia 36, 1333-1334.
13. Frossard, P.M., Gonzalez, P.A., Dillan, N.A., Coleman, R.T., and Atlas, S.A. (1986) Nucl. Acids Res. 14, 6778.
14. Fritz, L.C., Ponte, P.A., Arfsten, A.E., Catanzaro, D.F., Rousseau, G.G., Eliard, P.H., Mellon, S., Cumino, F., Shine, J., Baxter, J.D., and Reudelhuber, T.L. (1986) Clin. Res. 34, 643A (abstract).