Vol. 197, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1993 Pages 197-201

RENIN GENE POLYMORPHISM ASSOCIATED WITH ALDOSTERONE RESPONSIVENESS TO THE RENIN-ANGIOTENSIN SYSTEM IN PATIENTS WITH ALDOSTERONE-PRODUCING ADENOMAS

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Received October 13, 1993

SUMMARY: Aldosterone-producing adenomas may be responsive or unresponsive to the renin-angiotensin system. In tumours from patients in the responsive subgroup, renin mRNA is expressed in greater amounts than in tumours from patients in the unresponsive subgroup, or in normal adrenals. We compared the frequency of two renin gene polymorphisms in peripheral blood DNA from the two subgroups and found a significant association (allele frequency $X^2 = 7.67$, $p<0.006$) between *BglI* polymorphism and aldosterone responsiveness. This may be a significant determinant of the biochemical behaviour of these tumours.

In 1987, we reported a subgroup of patients with aldosterone-producing adenomas (APAs) of the adrenal cortex who lacked many of the biochemical features regarded as characteristic of APA, and used to distinguish APA from bilateral adrenal hyperplasia (BAH) (1,2). In this new subgroup with angiotensin-responsive APA (AII-R-APA) aldosterone is responsive to assumption of upright posture after overnight recumbency and to infusion of angiotensin II, in contrast to aldosterone levels which fall or do not rise in response to such manoeuvres in patients with the classical angiotensin-unresponsive APA (AII-U-APA). Furthermore, urinary excretion of 18-oxo-cortisol is normal in AII-R-APA (as in BAH) in contrast to the raised levels in patients with AII-U-

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APA and in patients with glucocorticoid-suppressible hyperaldosteronism (1,2). We have also shown that this responsiveness resides within the APA since, in the week following unilateral adrenalectomy, the responsiveness to angiotensin infusion in patients with AII-R-APA is greatly diminished (3).

In tissue from AII-R-APA, we have shown that renin mRNA is expressed in greater amounts than in tissue from AII-U-APA or normal adrenals (4,5) consistent with possible involvement of the intra-adrenal renin-angiotensin system in the biochemcial behaviour and aetiology of APAs. We therefore set out to compare the frequency of renin gene polymorphisms in peripheral blood DNA from patients with AII-R-APA and AII-U-APA.

METHODS

Subjects: Genomic DNA was extracted from peripheral blood leucocytes (6) from 24 patients with AII-R-APA (19 males, 5 females, aged 38-72 years at time of sampling) and 27 patients with AII-U-APA (10 males, 17 females, aged 30-64 years).
RFLP analysis: Genomic DNA (10µg) was digested to completion with either *Hin*dIII or *Bgl*I, separated by electrophoresis in 0.7% agarose gels, and transferred to nylon membranes (Hybond, Amersham, U.K.) according to Southern (7). *Hin*dIII membranes were prehybridized for 22h at 65°C and hybridized with a 1.9kb genomic renin probe (PHRNES1.9, ATCC, 8) and incubated for 22h at 65°C. Membranes were washed for 20min at 65°C with 2XSSC/0.1%SDS, followed by washes with 0.1XSSC/0.1%SDS for 10 minutes at 65°C. Membranes were exposed to Kodak XAR film with intensifying screens and incubated at -70°C for 16h. *Bgl*I membranes were hybridized with a cDNA renin probe (a gift from Ms Yvonne Hort and Professor John Shine, Garvan Institute of Medical Research, Sydney, Australia) and treated as above.
Statistical analysis: Data were compiled according to genotype, and genotype and allele frequencies were calculated. The difference between groups was tested by X^2 analysis with one or two degrees of freedom where appropriate.

RESULTS

Bands of appropriate sizes representing homozygotes (AA, aa, BB, bb) and heterozygotes (Aa, Bb) were observed for both RFLPs (Figure 1: *Hin*dIII, 9kb and 6.2kb, left panel; *Bgl*I, 11kb and 6kb, right panel). Results of the statistical analysis are shown in Table 1. Whereas genotype and allele frequencies were similar for AII-U-APA and AII-R-APA using the *Hin*dIII RFLP, they were significantly different using the *Bgl*I RFLP. Furthermore, 63% of patients with AII-R-APA were

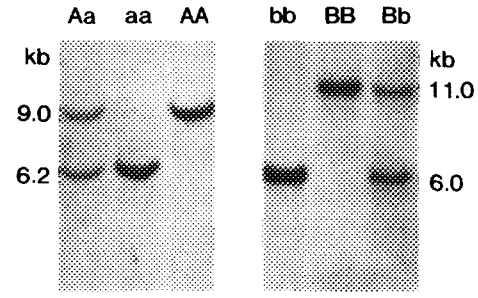

Figure 1. Representative bands showing homozygotes and heterozygotes for the *Hin*dIII RFLP (AA,Aa,aa) and *Bgl*I RFLP (BB,Bb,bb) with sizes in kilobases (kb) indicated.

homozygous for the *Bgl*I 11kb band whereas 0% were homozygous for the 6kb band. Hardy-Weinberg proportions were satisfied for the *Hin*dIII RFLP.

DISCUSSION

Since the renin-angiotensin system is a key factor in blood pressure regulation, a number of studies have attempted to find an association of RFLPs in the renin gene with essential hypertension. Perhaps because hypertension is such a heterogeneous disease, presumably with multiple genes involved, such an association has been difficult to find. In the present study, the two subgroups examined are part of a much more homogeneous population; patients with aldosterone-producing adenomas. The results support a significant association between the *Bgl*I RFLP in the renin gene and

TABLE 1: Frequencies of each renin RFLP in AII-R-APA and AII-U-APA patients

	*Hin*dIII			*Bgl*1	
RFLP	AII-R-APA	AII-U-APA		AII-R-APA	AII-U-APA
Genotypes					
AA	7 (0.29)	11 (0.41)	**BB**	15 (0.63)	9 (0.33)
Aa	16 (0.67)	14 (0.51)	**Bb**	9 (0.37)	12 (0.45)
aa	1 (0.04)	2 (0.08)	**bb**	0 (0.00)	6 (0.22)
	X^2 = 1.2 p<0.2			X^2 = 7.69 p<0.025	
Alleles					
A	30 (0.63)	36 (0.67)	**B**	39 (0.81)	30 (0.56)
a	18 (0.37)	18 (0.33)	**b**	9 (0.19)	24 (0.44)
	X^2 = 0.2 p<0.6			X^2 = 7.67 p<0.006	

aldosterone responsiveness to the renin-angiotensin system in patients with APAs. This RFLP is very interesting since it is located in Intron A of the renin gene (9). Comparing Dahl salt-sensitive and salt-resistant Sprague-Dawley rats, Wang and Rapp (10) found a 1.2kb insertion in the first intron of the salt-sensitive rat renin gene which accounted for most of the RFLPs found in the renin genes from both strains of rats, including the RFLP generated by *BglI*. They suggested that such a difference might affect renin gene expression, which could account for the lower plasma and renal renin activities, lower adrenal renin, and lack of adrenal renin responsiveness to a sodium-deficient diet that characterise salt-sensitive rats. Furthermore, in a large F_2 population derived from a salt-resistant x salt- sensitive cross raised on a high salt diet, Rapp and coworkers (11) showed that the *BglI* RFLP cosegregated with a rise in blood pressure.

In our group of patients, a similar effect may take place, such that the presence of an allele variation in the renin gene may affect adrenal renin gene expression, resulting in the observed *in vivo* responsiveness of aldosterone-producing tumours to the renin-angiotensin system, and possibly predisposing, since angiotensin is a growth factor, to development of the tumours.

ACKNOWLEDGMENTS

The present study was supported by a grant from the Australian Government Department of Veterans' Affairs Central Health and Medical Research Committee.

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