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RESEARCH ARTICLE

High dose Losartan and ACE gene polymorphism in IgA nephritis

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Abstract *Background/aims* Several studies have reported varying results of the influence of ACE gene on ACEI/ARB therapy. The efficacy of high dose ARB and its influence on ACE gene have not been explored. This is a 6 year randomised trial in IgA nephritis comparing high dose ARB (Losartan 200 mg/day) with normal dose ARB (Losartan 100 mg/day), normal dose ACEI (20 mg/day) and low dose ACEI (10 mg/day). *Results* Patients on high dose ARB had significantly lower proteinuria, 1.0 ± 0.8 gm/day compared to 1.7 ± 1.0 g/day in the other groups ($P = 0.0005$). The loss in eGFR was 0.7 ml $min^{-1}year^{-1}$ for high dose ARB compared to 3.2–3.5 ml $min^{-1}year^{-1}$ for the other three groups ($P = 0.0005$). There were more patients on high dose ARB with improvement in eGFR compared to other three groups ($P < 0.001$). Comparing patients with the three ACE genotypes DD, ID and II, all three groups responded well to therapy with decrease in proteinuria ($P < 0.002$). Only those on low dose ACEI (10 mg/day) with the I allele had increased in ESRF ($P = 0.037$). *Conclusion* High dose ARB is more efficacious in reducing proteinuria and preserving renal function when compared with normal dose ARB and ACEI, and also obviates the genomic influence of ACE gene polymorphism on renal survival.

Keywords Proteinuria · Glomerular filtration rate · Renal failure · Treatment · Angiotensin receptor blockers · ACE Gene polymorphism

The Genomics work was performed at the laboratories of the Department of Renal Medicine and the Department of Clinical Research at the Singapore General Hospital.

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Introduction

Various studies have shown that angiotensin converting enzyme (ACE) gene insertion/deletion (ID) polymorphism may play a role in the progression to end stage renal failure (ESRF) in patients with IgA nephritis (IgAN) (Hunley et al. 1996; Vleming et al. 1998; Harden et al. 1995). There have also been recent reports (Yong et al. 2006; Nonoguchi et al. 2007) that ACE ID polymorphism influences the responses of patients with IgAN to ACEI/ARB therapy. In a recent report, Dillon (2004) suggested that polymorphism of the ACE gene may have so far failed to predict either susceptibility to or progression of IgA nephropathy, but the D allele could predict a favourable response to renin-angiotensin blockade.

In the present paper, we studied the effects of high dose ARB (200 mg Losartan) compared with normal dose ARB (100 mg Losartan), normal dose ACEI (20 mg Enalapril)

and low dose ACEI (10 mg Enalapril) in patients with IgAN in the reduction of proteinuria and preservation of renal function. We also investigated the influence of the ACE gene ID polymorphism on the response of these patients to various dosages of ARB/ACEI to see if high dose ARB could override the genomic influence on therapy. The objective of this study was to establish whether the ACE I/D genotype influenced the progression of IgA nephritis over 6 years of actuarial follow up and in turn whether there was an interaction between the genotype and prescribed drug class and dose.

Methods

During the period from January 1999 to January 2001, 226 Chinese patients with biopsy proven primary IgA nephritis (IgAN) entered a randomized study with 112 patients treated with an ARB (Losartan) and 114 patients treated with an ACEI (Enalapril). Informed consent and institutional board approval (IRB) were obtained for all patients. Entry criteria included proteinuria of 1 g or more and/or serum creatinine >1.6 mg/dl and Chronic Kidney Disease (CKD) stage 3 (eGFR 30–59 ml/min). Six of the 112 patients on ARB were withdrawn from the trial (one for severe cough, one for a drug rash, one for severe giddiness and three defaulted follow up) leaving 106 patients who completed the trial. Among the 114 ACEI treated patients, 13 were withdrawn (six for severe cough, one for hypotension, one for giddiness and five defaulted follow up) leaving 101 to complete the trial. Majority of patients who entered the trial had CKD 3. There were 51 patients with hypertension in the ARB group and 50 patients in the ACEI group. There were no significant differences in the various parameters between the ARB and ACEI treated group on entry into the trial. In both groups additional BP control was achieved with atenolol, amlodipine or nifedipine. None were on steroids, cyclophosphamide, cyclosporine A or mycophenolic acid. All patients were given advice on a low salt diet. None of the patients were on treatment with aspirin, warfarin, dipyridamole, fish oil or omega 3 fatty acid.

Blood pressure control was targeted at 130 mmHg systolic and 80 mmHg diastolic. There were no significant differences in both the systolic and diastolic BP between the ARB and the ACEI groups on entry into the trial. None of the patients in the study were prescribed hydrochlorothiazide.

Patients had the following investigations performed at three monthly intervals: serum creatinine, eGFR and total urinary protein (TUP) excretion. Glomerular filtration rate was estimated using the Cockroft Gault formula for eGFR. Decrease in eGFR was expressed as millilitre of eGFR loss per year over the 6 year duration from time of entry to exit of the trial. Improvement in eGFR was taken as the positive difference between the entry eGFR and the exit eGFR over the 6 year period. End stage renal failure was equated with decline of eGFR to CKD stage five with eGFR less than 15 ml/min. Patients who did not fall into either above categories were designated as stable.

Background

An earlier pilot study of patients with IgA nephritis treated with Enalapril 20 mg or Losartan 100 mg showed that both drugs improve renal function in some patients with mild renal impairment (Woo et al. 2000). Another pilot study (Woo et al. 2005) showed that patients treated with Losartan 200 mg a day (high dose therapy) from 1996 to 1999 had dramatic decrease in proteinuria associated with improvement of creatinine clearance. These patients had not responded to therapy with Losartan 100 mg a day. The results of these studies led us to formulate a treatment regime of four drug groups comprising High dose Losartan 200 mg, normal dose Losartan 100 mg, normal dose Enalapril 20 mg with Enalapril 10 mg as a control group.

Furthermore, since our study had shown that those on Losartan 200 mg seem to do better, we decided to recruit one and a half times more patients for the high dose Losartan group. For balance, we also recruited the same number of patients for Enalapril 20 mg. Hence the ratio of 1:1.5:1:1.5 or 2:3:2:3 was used to control arm of Enalapril 10 mg, Enalapril 20 mg, Losartan 100 mg and Losartan 200 mg.

Study design

This was an open label study. About 226 patients were randomized to control arm of ACEI 10 mg, ACEI 20 mg, ARB 100 mg and ARB 200 mg in the ratio of 1:1.5:1:1.5 or 2:3:2:3. A list of random numbers generated via computer by simple randomization allocation method was used. Patients on ARB 200 mg were designated high dose ARB group, those on ARB 100 mg were designated normal dose ARB group and those on ACEI 20 mg were designated normal dose ACEI group. Those on ACEI 10 mg served as a "control group", as it is not ethical not to treat patients considering the risk of renal failure without therapy. Of the 226 randomized patients, 45, 69, 45 and 67 patients were allocated to control arm, normal dose ACEI, normal dose ARB and high dose ARB. One and a half times as many patients were randomized to normal ACEI and high dose ARB, as compared to control group and normal dose ARB, as we knew from our earlier pilot study that for high dose ARB the incidence of renal failure was lower than that of standard dose ARB and ACEI 10 mg. Two hundred and seven patients completed the trial. Six patients were

withdrawn from the two ARB arms and 13 from the two ACEI arms. Among the patients who completed the trial, 40, 61, 43 and 63 patients were in the ACEI 10 mg, ACEI 20 mg, ARB 100 mg and ARB 200 mg groups, respectively.

Determination of ACE insertion/deletion genotypes

DNA was extracted from 0.2 ml EDTA-blood using the QIAamp DNA blood extraction kit (QIAGEN, Germany). Genotyping was done using the method of (Vleming et al. 1998). The 50 µl of reaction mixture consisted of 50 ng DNA, 1× PCR buffer (Fermentas), primers concentration 0.4 µM (forward 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′; reverse 5′- GAT GTG GCC ATC ACA TTC GTC AGA T-3′), 0.2 mM dNTPs and 1 unit *Taq* polymerase (Fermentas). Amplification was carried out in an automated thermocycler (GeneAmp 9700, USA) for 35 cycles (94°C, 30 s; 60°C, 45 s and 72°C, 60 s). Products were separated in 2% agarose gel and visualised by ethidium bromide staining. Amplification of the I allele produced 1 band at 490 base pair (bp) for homozygote II. Amplification of the D allele produced 1 band at 190 bp for homozygote DD. Both bands at 490 bp and 190 bp were produced by heterozygote. Mistyping ACE heterozygotes as DD homozygotes had been reported. Therefore, all DD cases were subject to confirmation with a second PCR, performed using the insert-specific forward primer 5′-TTT GAG ACG GAG TCT CGC TC-3′ together with the same reverse primer above. A true DD genotype should give no product at the 409-bp band, whereas ID and II genotypes should. For quality control for all three genotypes, we have positive and negative controls for every PCR; the concentration of PCR template was optimized to avoid false positives. Data checking performed once after genotyping by another scientist and after this yet another level of check by a senior scientist. If there are any doubts, then a second PCR would be performed.

Sample size

The end-point chosen for the sample size calculation was the proportion of patients achieving 30% decrease in TUP. Similarly, a second sample size calculation was done to compare the rate of 30% TUP decrease between high dose ARB (200 mg) and normal dose ARB (100 mg)/normal dose ACEI. Assuming that the rate of TUP decrease to be 30% in the normal dose.

ARB/normal dose ACEI and 60% in the high dose ARB, the number of patients required in each group was 49 for a 2-sided test with $\alpha = 0.05$ and power of 80%. We expected high dose ARB to be more efficacious based on knowledge from our earlier pilot studies; hence, the choice of 60% reduction of TUP.

Statistical analysis

SPSS 10.1 for Windows was used for all analysis. Results were expressed as mean ± SD or count (%). For univariate analysis, Pearson's Chi-square test was used for comparing categorical data and ANOVA for comparing numeric data among the 4 treatment arms. ANOVA was followed by multiple comparison with Student–Newman–Keuls (SNK) range test whenever statistical significance was found among the four arms.

The three outcomes of renal failure were coded as "improved", "stable" and "ESRF". Univariate Chi-square tests for trend were done to test the effect of increasing dosage of ACEI and ARB treatment on renal failure for each genotype separately to assess the effect of treatment, controlling for genotype. Similarly Chi-square tests for trend were done to test the effect of ACE insertion/deletion (I/D) polymorphism on renal failure for each treatment type separately to assess the effect of ACE I/D polymorphism, controlling for treatment.

Finally, a multinomial logistic regression of renal function was performed to test the effect of treatment and genotype, adjusting for average systolic and diastolic blood pressure. Average blood pressures were calculated by taking the mean of all blood pressures while on medication (mean of blood pressures from 1 to 6 year).

Kaplan–Meier (K–M) survival analysis method was used to compare the time to ESRF and time to improvement of eGFR among the four treatment arms. The survival analysis was repeated for both survival end-points, adjusting for ACE genotypes. in order to investigate the effect of ACE genotype on the same two survival end-points, K–M analysis was again done, controlling for treatment arm. Log rank tests were used to compare the survival distributions between the comparison groups, i.e. firstly between treatment arms and secondly between genotypes.

Results

Univariate analysis

Table 1 compares the clinical profile of the patients in the four drug groups, those on high dose ARB (Losartan 200 mg), normal dose ARB (Losartan 100 mg), normal dose ACEI (Enalapril 20 mg) and control ACEI (Enalapril 10 mg). At the end of the study period of 6 years, using ANOVA test with post-hoc Student–Neuman–Kuels (SNK) test to compare the four treatment groups, those on high dose ARB had significantly lower proteinuria compared to the other three groups with slight decrease in eGFR in each year and there were more patients in the high dose ARB group with improvement in eGFR ($P < 0.0005$) compared

Table 1 Comparing clinical profile of patients among the four treatment groups (Mean ± SD)

	ARB (200 mg) $n = 63$	ARB (100 mg) $n = 43$	ACEI (20 mg) $n = 61$	ACEI (10 mg) $n = 40$	P value*
Sex (F:M)	34:29	18:25	26:35	19:21	NS
Age at biopsy (years)	34 ± 10	32 ± 12	32 ± 10	34 ± 11	NS
Duration of trial (months)	75 ± 3	74 ± 3	74 ± 2	75 ± 2	NS
Hypertension (yes:no)	33:30	20:23	33:29	18:22	NS
eGFR (ml/min)					
Before	63.5 ± 24.2	61.2 ± 18.4	62.2 ± 20.8	60.9 ± 19.8	NS
After	59.1 ± 31.8	40.2 ± 27.6	41.3 ± 27.9	42.3 ± 26.6	$P = 0.0005$*
	($P = 0.009$)	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	
Decrease in eGFR (ml $min^{-1}year^{-1}$)	0.7 ± 3.1	3.4 ± 3.2	3.5 ± 3.3	3.2 ± 2.6	$P = 0.0005$*
Urinary protein (g/day)					
Before	2.2 ± 0.9	2.0 ± 1.0	2.2 ± 1.6	2.3 ± 1.5	NS
After	1.2 ± 0.8	1.6 ± 0.9	1.7 ± 1.0	1.7 ± 0.9	$P = 0.0005$*
	($P < 0.001$)	($P = 0.053$)	($P = 0.004$)	($P = 0.009$)	
Blood pressure (mmHg)					
Systolic before	132 ± 12	132 ± 12	134 ± 11	132 ± 12	NS
Systolic after	128 ± 11	128 ± 11	129 ± 10	130 ± 10	NS
	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	($P < 0.002$)	
Diastolic before	84 ± 7	85 ± 7	83 ± 7	86 ± 5	NS
Diastolic after	83 ± 6	84 ± 6	83 ± 8	84 ± 7	NS
	NS	NS	NS	NS	
Outcome					
Non-ESRF	56	33	42	31	NS
ESRF	7	10	19	9	
Improvement in eGFR					
Yes	23	8	6	3	$\chi^2 = 19.8$
No	40	35	55	37	($P < 0.001$)

For all four groups, P value for systolic BP (baseline and year 6) significantly different by paired t test

* P value from ANOVA test comparing the four groups, for high dose ARB (200 mg), significantly less decrease of eGFR/year, less proteinuria after treatment and more patients with improvement in eGFR compared to other three groups

to the other three groups. However, there was no significance difference in these parameters among the three other groups using the same SNK range test.

Table 2 compares the clinical profile of the patients in the three ACE genotype groups, those with DD, ID and II genotype. There were no significant difference among the three groups in their baseline and 6 year data apart from a higher Diastolic BP at year 6 for DD genotype versus ID and II genotypes (86 ± 6 mmHg for DD genotype versus 82 ± 7 mmHg for ID genotype and 83 ± 6 mmHg for II genotype). Patients in all three genotypes responded to therapy with significant decrease in proteinuria (P <0.002 to <0.001). There were no differences in the number of patients with ESRF or those with improvement the eGFR at year 6 in all three genotype groups.

Table 3 shows the distribution of renal function for each of the four treatment arms for the subgroups of DD, ID and II patients. Patients were categorised into one of three groups in terms of renal function, those with improvement in eGFR at year 6 (designated "improved"), those with ESRF (designated "ESRF") and those with neither, designated "stable".

For ID and II patients, increasing doses of ARB had improved renal function when compared with ACEI treatment groups ($P = 0.008$ and $P = 0.012$ for ID and II, respectively, Table 3). However, for DD patients all treatment groups yielded similar renal function though, a trend towards a higher rate of renal improvement was observed in the high dose ARB group. The significance may have been missed due to the small number of DD patients ($n = 30$).

Table 4 shows that there were no significant difference in renal function among all three ACE polymorphism groups DD, ID and II for high dose ARB, normal dose

Table 2 Comparing clinical profile of patients among the three ACE genotypes (Mean ± SD)

	DD $n = 30$	ID $n = 76$	II $n = 101$	P value*
Sex (F:M)	17:13	30:46	51:54	
Age at biopsy (years)	34 ± 9	33 ± 11	32 ± 10	NS
Duration of trial (months)	75 ± 2	75 ± 2	75 ± 3	NS
Hypertension (yes:no)	17:13	38:39	49:52	NS
eGFR (ml/min)				
Before	58.6 ± 21.8	62.2 ± 22.3	61.3 ± 23.2	NS
After	47.6 ± 28.6	46.3 ± 32.9	43.7 ± 30.9	NS
	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	
Decrease in eGFR (ml $min^{-1}year^{-1}$)	1.8 ± 3.0	2.6 ± 3.5	2.6 ± 3.5	NS
Urinary protein (gm/day)				
Before	2.4 ± 1.4	2.2 ± 1.4	2.2 ± 1.2	NS
After	1.5 ± 1.0	1.4 ± 1.0	1.6 ± 1.0	NS
	($P < 0.002$)	($P < 0.001$)	($P < 0.001$)	
Blood pressure (mmHg)				
Systolic before	134 ± 10	132 ± 11	133 ± 11	NS
Systolic after	131 ± 9	128 ± 12	128 ± 10	NS
	($P < 0.002$)	($P < 0.001$)	($P < 0.001$)	
Diastolic before	84 ± 6	84 ± 7	84 ± 7	NS
Diastolic after	86 ± 6	82 ± 7	83 ± 6	$P = 0.021$*
	NS	NS	NS	(between DD and ID)
Outcome				
Non-ESRF	23	65	93	NS
ESRF	7	11	28	
Improvement in eGFR				
Yes	10	23	10	NS
No	20	53	91	

For all three genotypes, P value for Systolic BP (baseline and year 6: DD (<0.002, ID (<0.001), II (<0.001). Diastolic BP—not significant in all three genotypes (paired t test)

* P value from ANOVA test comparing the three genotypes. Significant only for Diastolic BP at year 6 between DD and ID genotype

ARB and normal dose ACEI 20 mg. But for the low dose ACEI 10 mg, there was a significant trend towards patients with increasing number of I allele, i.e. II patients as opposed to ID and DD, having increasingly worse renal function ($P = 0.037$).

Multivariate analysis

Table 5 shows the multinomial logistic regression. The effect of treatment group on renal function was significant ($P = 0.001$) but ACE polymorphism was not significant ($P = 0.490$), even with adjustment for average systolic and diastolic blood pressures. Patients on high ARB dose compared to patients on low dose ACE 10 mg were 11.3 times more likely to improve than to have ESRF ($P = 0.003$, OR = 11.34, 95% confidence interval for OR = 2.32–55.38).

Survival analysis

Time to improvement of eGFR was significantly different among the four treatment arms, being shorter in the high dose ARB ($P < 0.00005$, Fig. 1). Mean time (95% confidence interval) to improved eGFR were as follows:- high dose ARB: 67 (95% CI = 62, 73) months, normal dose ARB: 76 (95% CI = 71, 81), normal dose ACEI: 77 (95% CI = 73, 81) months, low dose ACEI: 77 (95% CI = 73, 80) months. The statistical significant difference was still present even after adjustment for ACE genotype ($P < 0.00005$). However, time for improvement of eGFR was not significant among the three ACE genotypes, DD, ID and II, controlling for treatment arm ($P = 0.1747$).

Time to ESRF was not significant among the four treatment arms, adjusting for ACE genotype ($P = 0.2418$, Fig. 2). However, when we compare specifically high dose

Table 3 The distribution of renal function for each treatment arm for the subgroups of DD, ID and II patients

Patients	Renal function	Treatment group				*P*-value *
		High dose ARB	Normal dose ARB	ACEI 20 mg	ACEI 10 mg	
DD	Improved	4 (66.67%)	0 (0%)	2 (15.38%)	2 (33.33%)	0.183
	Stable	2 (33.33%)	4 (80%)	8 (61.54%)	3 (50%)	
	ESRF	0 (0%)	1 (20%)	3 (23.08%)	1 (16.67%)	
	Total	6 (100%)	5 (100%)	13	6	
ID	Improved	8 (40%)	5 (31.25%)	1 (4.55%)	1 (5.56%)	0.008
	Stable	10 (50%)	8 (50%)	13 (59.09%)	14 (77.78%)	
	ESRF	2 (10%)	3 (18.75%)	8 (36.36%)	3 (16.67%)	
	Total	20 (100%)	16 (100%)	22 (100%)	18 (100%)	
II	Improved	11 (31.43%)	4 (16.67%)	3 (10.34%)	0 (0%)	0.012
	Stable	17 (48.57%)	14 (58.33%)	18 (62.07%)	8 (61.54%)	
	ESRF	7 (20%)	6 (25%)	8 (27.59%)	5 (38.46%)	
	Total	35 (100%)	24 (100%)	29 (100%)	13 (100%)	

The table shows the no. of patients (% of patients) with improved, stable renal function and ESRF

* *P*-value are from Chi-square test for trend

Table 4 The distribution of renal function for each genotype for the four treatment arms

Treatment	Renal failure	ACE			*P*-value*
		DD	ID	II	
High dose ARB	Improved	4 (66.67%)	8 (40%)	11 (31.42%)	0.065
	Stable	2 (33.33%)	10 (50%)	17 (48.57%)	
	ESRF	0 (0%)	2 (10%)	7 (20%)	
	Total	6 (100%)	20 (100%)	35 (100%)	
Low dose ARB	Improved	0 (0%)	5 (31.25%)	4 (16.67%)	0.848
	Stable	4 (80%)	8 (50%)	14 (58.33%)	
	ESRF	1 (20%)	3 (18.75%)	6 (25%)	
	Total	5 (100%)	16 (100%)	24 (100%)	
ACEI 20 mg	Improved	2 (15.38%)	1 (4.54%)	3 (10.34%)	0.838
	Stable	8 (61.53%)	13 (59.09%)	18 (62.07%)	
	ESRF	3 (23.08%)	8 (36.36%)	8 (27.59%)	
	Total	13 (100%)	22 (100%)	29 (100%)	
ACEI 10 mg	Improved	2 (33.33%)	1 (5.56%)	0 (0%)	0.037
	Stable	3 (50%)	14 (77.78%)	8 (61.54%)	
	ESRF	1 (16.67%)	3 (16.67%)	5 (38.46%)	
	Total	6 (100%)	18 (100%)	13 (100%)	

The table shows the no. of patients (% of patients) with improved, stable renal function and ESRF

* *P*-value are from Chi-square test for trend

ARB versus normal dose ACEI 20 mg (both groups had sufficiently large sample size), the time to ESRF was significantly longer in high dose ARB compared to normal dose ACE, with adjustment done for ACE genotype ($P = 0.0498$).

Discussion

This is a 6 year randomized study of high dose ARB (Losartan 200 mg a day) versus normal dose ARB (Losartan 100 mg a day), normal dose ACEI (Enalapril 20 mg a day) and control (Enalapril 10 mg a day). Patients on high dose ARB had significantly lower levels of proteinuria and less decrease of eGFR per year as compared to the other three groups at the end of the study.

The data also showed that patients with the various ACE genotypes, DD, ID and II responded well to therapy with ACEI/ARB. Irrespective of ACE genotypes, patients had significant decrease in proteinuria with no difference in the rate of ESRF and renal survival.

Table 5 Results of multinomial logistic regression

Renal function	Independent predictor	Coefficient	*P*-value	OR	95% CI for OR
Improved versus ESRF	Systolic bp	−0.04	0.245	0.96	0.91–1.03
	Diastolic bp	0.14	0.026	1.15	1.02–1.30
	High dose ARB	2.43	0.003	11.34	2.32–55.38
	Low dose ARB	1.19	0.153	3.28	0.64–16.76
	ACE 20 mg	0.13	0.879	1.14	0.22–5.85
	ACE 10 mg	Reference			
	ACE	1.17	0.098	3.24	0.81–13.01
	DD				
	ID	0.61	0.234	1.84	0.67–5.01
	II	Reference			
Stable versus ESRF	Systolic bp	−0.01	0.630	0.99	0.94–1.04
	Diastolic bp	0.03	0.422	1.03	0.95–1.13
	High dose ARB	0.28	0.617	1.32	0.44–3.98
	Low dose ARB	0.01	0.988	1.01	0.35–2.94
	ACE 20 mg	−0.23	0.642	0.80	0.31-2.08
	ACE 10 mg	Reference			
	ACE	0.49	0.396	1.63	0.53–5.00
	DD				
	ID	0.32	0.412	1.37	0.64–2.93
	II	Reference			

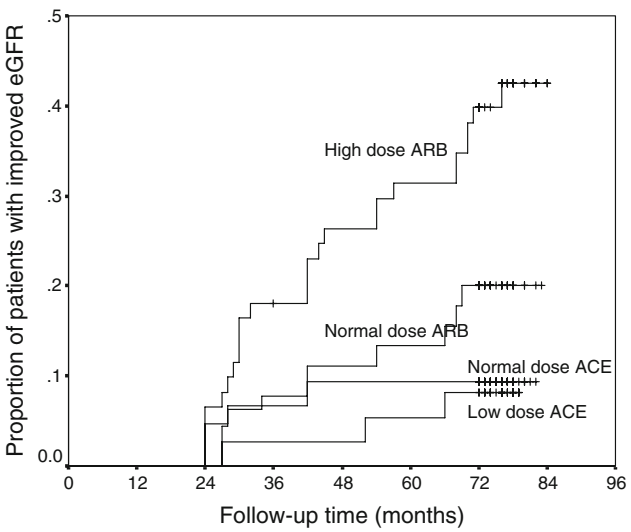

Fig. 1 Distribution of patients with improved eGFR in the four treatment groups

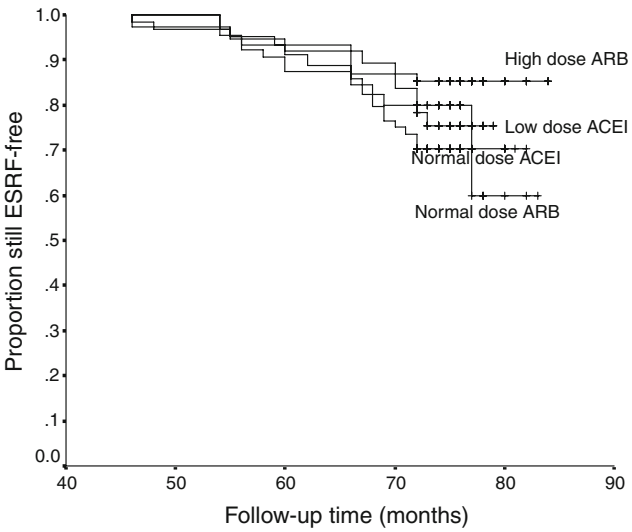

Fig. 2 Distribution of patients who have not reached ESRF in the four treatment groups

For ID and II patients, the data showed that increasing doses of ARB had improved renal function ($P = 0.008$ and $P = 0.012$) for ID and II, respectively, and not for DD genotype. However, for DD patients, the trend towards a higher rate of renal improvement observed in the high dose ARB group was still present. The significance was probably missed as the number of patients with DD genotype was small compared to those with ID and II genotype. Asian patients, compared to Caucasian patients have a much lower percentage of DD genotype (Chan et al. 2004).

There was no difference in renal function among all three ACE genotypes for high dose ARB, normal dose ARB and normal dose ACEI, but for those on low dose ACEI (10 mg), there was a significant trend towards

patients with increasing number of I allele, i.e. II patients as opposed to ID and DD, having increasing ESRF ($P = 0.037$). This study did not have a 'control' group on no treatment for ethical reasons. The closest to a 'control' group were the patients randomized to ACEI 10 mg (low dose). The high dose ARB and normal dose ARB and ACE probably over-rode the genomic influence on renal outcome, but in patients on low dose ACEI, the genomic effect was still evident, meaning that those with I allele seem to do less well than those with D allele in terms of developing renal failure. But with adequate dosage of ACEI/ARB the innate genomic effect no longer played a role in determining renal outcome.

There have been various studies (Yong et al. 2006; Nonoguchi et al. 2007) reporting on varying renal outcome in patients with IgAN in respect of their ACE gene profile. The D alelle is believed to affect the renoprotective effects of ACEI/ARB therapy. Some studies showed that patients with the DD genotype do not respond to therapy and have a higher incidence of developing ESRF. Nonoguchi et al. (2007), in a recent study (113 patients with CKD of which 75 had IgAN), reported that ARB therapy extended the time to ESRF for patients with the II and ID genotype but not the DD genotype, suggesting that DD patients have diminished response to ARB in terms of renoprotection.

Ng et al. (2005), in a meta analysis of 14,724 diabetic patients, reported a protective role of the II genotype for Asian patients with diabetic nephropathy, whereby there was a reduction in the number with ESRF when they were treated with ACEI/ATRA. In contrast, those with the D allele had a deleterious outcome in terms of ESRF. Seki et al. (2006), another group of Japanese workers, reported a similar renoprotective effect of 18 Asian patients with the II genotype with type II diabetes mellitus when treated with ACEI/ATRA, in contrast to those with the DD genotype. A study from Korea by Han et al. (2000) showed that renal preservation with ACEI was better in the DD than II genotype. Anderson et al. (2002), in a study of 54 diabetic patients reported that Losartan 100 mg was effective in reducing BP and proteinuria in both II and DD genotypes. In a previous retrospective study on a smaller group of patients ($n = 109$) with IgAN, we showed that patients with the II genotype responded better to Losartan 50–100 mg a day (Woo et al. 2008).

However, Suzuki et al. (2000) reported that I/D polymorphism in the ACE gene was not associated with renal progression in Japanese patients with IgAN. Nakao et al. (2003) in another study using ACEI/ARB on non diabetic renal disease (50% with IgA nephritis) reported that the ACE gene was not associated with decrease in proteinuria or renal progression. The percentage of patients with the DD genotype was 11%, quite similar to 14% in our study. In a recent article, Dillon (2004) concluded that polymorphism of the ACE gene may have so far failed to predict either susceptibility to or progression in IgAN, but the D allele could predict a favourable response to renin-angiotensin blockade.

In this present study on 207 IgAN patients treated with varying doses of ACEI and ARB including high dose ARB and a low dose ACEI (10 mg) as a 'control', group we have demonstrated that irrespective of ACE genotype, all three groups of patients DD, ID and II responded to therapy. The response was independent of the genotype. Patients with high dose ARB responded better and earlier in terms of renoprotection (survival and improvement). However, in the low dose ACEI (10 mg) group, the response was influenced by the presence of the I allele, those with more I allele (II) did not do as well as those with no I allele (DD), implying that the low dose ACEI did not over-ride the genomic influence of the ACE gene. The data could also support the belief that patients with DD genotype responded better as they have more circulating ACE for blockage by the ACEI/ARB therapy in contrast to the II genotype patients with less circulating ACE for blockade (Vleming et al. 1998). Conflicting results from our earlier study could be attributed to the lower doses of ARB employed (50 mg to 100 mg) as well as the smaller number of patients studied (Woo et al. 2008).

Our present data on high dose ARB suggest that it is more effective in reduction of proteinuria compared to those patients on lower doses of ARB or ACEI. This high dose ARB data are similar to those of high dose Irbesartan (Rossing et al. 2005), high dose Valsartan (Hollenberg et al. 2007) and high dose Telmisartan (Aranda et al. 2005). High dose ARBs help to further enhance reduction of proteinuria and stabilize as well as improve declining GFR in patients with CKD. Data from our present study suggest that high dose ARB induces earlier recovery of renal function with early improvement of eGFR in patients with IgAN with Stage three CKD.

With respect to the influence of ACE gene ID polymorphism on the response to ACEI/ARB therapy, our data suggest that with high dose ARB, irrespective of the ACE gene polymorphism, whether it is DD, ID or II; patients will still respond with more effective reduction of proteinuria and earlier recovery of renal impairment with regression of glomerulosclerosis.

We conclude that in these days of high dose ARB usage, the ACE gene ID polymorphism status of a patient may no longer be a matter for concern, as patients will respond to therapy as long as they are adequately treated with ACEI/ARB therapy. However, high dose ARB therapy confers the additional benefit of early improvement in renal function in some patients.

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