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Commentary

From Pharmacogenomics to Improved Patient Outcomes: Angiotensin I-Converting Enzyme as an Example

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ABSTRACT

Here we report the utility of a molecular epidemiologic approach for common, polygenic diseases. Since 1992, the angiotensin I-converting enzyme (ACE) deletion/deletion (D/D) genotype has been linked to several cardiovascular diseases, including diabetic nephropathy. Earlier, the ACE D/D genotype had been associated with excess tissue ACE activity. We have observed an association of the ACE D/D genotype with a large number of common diseases, including chronic renal failure due to non–insulin-dependent diabetes mellitus or hypertension, hypertensive peripheral vascular disease, and emphysema [chronic obstructive pulmonary disease (COPD)]. ACE inhibitors have been in clinical use since 1977 and have a well-known safety record. Armed with the knowledge that ACE overactivity was associated with their disease, we gave what was intended to be a tissue ACE-inhibitory dose of a hydrophobic ACE inhibitor to 800 Caucasian and African-American male patients with hypertension and 200 Caucasian and African-American male patients with chronic renal failure, over a period of 3 years. We here report their outcomes, which include those of two patients with end-stage hypertensive peripheral vascular disease and one patient with end-stage emphysema (COPD). As a group, the outcomes are superior to what is available in the literature. This experience suggests the power of pharmacogenomics to improve clinical outcomes for common diseases safely, quickly, and inexpensively, if effective drugs already exist.

INTRODUCTION

ALTHOUGH THERE IS WIDESPREAD AGREEMENT that knowledge of disease-predisposition genes will transform the treatment of common diseases,[1] there is little experience with how this will happen. A number of questions exist, such as (1) Will genotyping of patients always be required before any genomics-based treatment can be given? (2) Will only new drugs work, with the typically large expense in time (10 years) and money ($0.5 billion) that new drug development currently entails, or might already existing drugs prove useful for new indications? If so, patient outcomes could be improved considerably more quickly and cheaply. (3) Who will pay for Phase IV trials to test existing drugs for new indications, given the large expense in money ($100 million) and time (>5 years)[2,3] typically involved? In short, how can

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the imminent explosion in knowledge of disease-predisposition genes be translated into better clinical practice in the timeliest manner?

The experience detailed herein with the angiotensin I-converting enzyme (ACE) insertion/deletion (I/D) polymorphism suggests the following: (1) Genotyping of individual patients may not always be required for genomics-based treatments. A single drug may still be suitable for an entire disease population. The possibility of "blockbuster" drugs still exists. (2) Existing drugs may prove useful for new clinical indications. Indeed, they may be preferable to new drugs for long-term disease prophylaxis, since more will always be known about their toxicology than about that of new drugs. (3) Translation from the lab bench to the clinic can occur, literally, on the same day. However, no readily available funding source currently exists for Phase IV trials of drugs nearing the end of their patent life. For patients to receive the full benefits of pharmacogenomics, new methods of funding clinical research, and a willingness to publish less expensive clinical trials, are needed soon.

SIGNIFICANCE OF THE ACE I/D POLYMORPHISM

After the seminal obsevation[4] that the deletion/deletion (D/D) genotype of the ACE conferred a threefold higher risk of myocardial infarction than the other two genotypes combined [insertion/deletion (I/D) and insertion/insertion (I/I)], it quickly became clear that a number of other serious common diseases were also associated with the ACE D/D gentoype (e.g., Moskowitz[5]). For example, the ACE D/D genotype frequencies for end-stage renal disease (ESRD) patients are presented in Table 1, showing an excess frequency of the ACE D/D genotype, in agreement with others (e.g., Gohda et al.[6]).

Table 1. ACE I/D and Diabetic ESRD

	% D/D	Total	Odds ratio
Black men			
ESRD due to NIDDM	36.6	279	1.31
NIDDM	33.3	492	1.13
ESRD due to IDDM	37.5	32	1.36
IDDM	36.0	25	1.28
ESRD due to HTN	36.8	467	1.32
HTN	35.8	1,025	1.26
Controls	30.6	242	=1.00
Black women			
ESRD due to NIDDM	34.7	456	1.35
NIDDM	33.4	338	1.28
ESRD due to IDDM	32.0	50	1.20
IDDM		NED	
ESRD due to HTN	37.8	333	1.55
HTN	31.5	511	1.17
Controls	28.2	142	=1.00
White men			
ESRD due to NIDDM	37.5	293	1.73
NIDDM	28.1	755	1.12
ESRD due to IDDM	41.3	46	2.02
IDDM	26.9	26	1.06
ESRD due to HTN	31.6	301	1.33
HTN	29.2	1,303	1.19
Controls	25.8	125	=1.00
White women			
ESRD due to NIDDM	29.8	262	1.22
NIDDM	29.7	64	1.22
ESRD due to IDDM	33.3	33	1.44
IDDM		NED	
ESRD due to HTN	28.0	254	1.12
HTN	23.1	91	0.86
Controls		NED	

Hemodialysis patients (n = 3,959) from the southeastern United States were genotyped for the ACE I/D polymorphism. For comparison, St. Louis patients from two hospitals (n = 6,414) were also genotyped. Patients with drug abuse or viral hepatitis were taken as the control population (Moskowitz[5]). Patients were matched according to ethnicity and socioeconomic status. The ACE D/D genotype frequency is presented for each group. IDDM, insulin-dependent diabetes mellitus. NED, not enough data. Odds ratios for the ACE D/D genotype are expressed relative to the control population, except for white women, for whom the white male control group was used. Apart from white women with hypertension, all of the odds ratios were >1, suggesting that overactivity of ACE was disease-associated.

Compared with the I/I genotype, the D/D genotype is associated with twice the level of ACE activity on the plasma membrane of white blood cells.[7] The I/D genotype has an intermediate level of membrane ACE activity. This is presumed also to be the case for other tissue locations of ACE more likely to be involved in disease causation, such as the plasma membrane of vascular endothelial cells and the brush border membrane of proximal tubular epithelial cells.[8]

The amount of enzyme present in tissue, as opposed to plasma, appears to be rate-limiting for angiotensin II production. The circulating concentration of substrate, angiotensin I, is ap-

proximately 10 p*M*,[9] far below the K_m for the enzyme (16 μM).[10] In the linear region of the Michaelis–Menten curve below the K_m, the rate of product synthesis is first order with respect to the concentration of either the substrate or the enzyme.[11] So, angiotensin I concentrations being equal, ACE D/D individuals are expected to generate twice as much tissue angiotensin II as individuals with the ACE I/I genotype. This is also expected to be the case for bradykinin, ACE's preferred substrate, whose K_m is 0.18 μM.[10] The plasma concentration of bradykinin is 3 p*M*,[12] far below its K_m, so degradation of bradykinin by ACE is also first order [i.e., linearly dependent on the concentration of either substrate (bradykinin) or enzyme].

The net effect of the D/D genotype on vasoconstrictor tone is therefore multiplicative. People with the ACE D/D genotype, with twice as much endothelial cell plasma membrane ACE as those with the I/I genotype, are expected to have four times as much vasoconstrictor tone as those with the I/I genotype, the result of twice the rate of angiotensin II production and twice the rate of bradykinin degradation.

Association of increased ACE activity with a disease immediately suggests a therapeutic strategy, namely, to inhibit ACE activity. Fortunately, since the discovery of captopril in the 1970s, there has been abundant clinical experience with a variety of ACE inhibitors, both hydrophilic (such as enalapril) and hydrophobic (e.g., quinapril, ramipril). As a class, ACE inhibitors have extremely low toxicity. Indeed, the dose–toxicity curve for quinapril is essentially flat.[13]

THE IMPORTANCE OF INHIBITING TISSUE, NOT CIRCULATING, ACE

If nephrologists have been using ACE inhibitors since the mid-1980s, and if ACE is important for the pathogenesis of ESRD in most American dialysis patients (Table 1), then why are patients with chronic renal failure (CRF) still progressing to ESRD? One obvious hypothesis is that the dose of ACE inhibitor used to date has been inadequate. This has also been suggested recently[14] for the treatment of congestive heart failure. Like hypertensive (HTN) and diabetic nephropathy, congestive heart failure is also associated with the ACE D/D genotype [odds ratio of 1.34 in Caucasian men and 1.49 in African-American men (data taken from Moskowitz[5])].

What constitutes an adequate dose of an ACE inhibitor? Tissue rather than circulating ACE is the proper target for inhibition, since tissue rather than circulating angiotensin II appears to be responsible for target organ damage.[15]

ACE in the circulation consists of the N-terminal portion of the molecule, minus a membrane-spanning anchor and a relatively short C-terminal domain. Serum ACE is presumably released from the plasma membrane by an ecto-protease whose identity and regulation are still unknown. Circulating ACE can be inhibited fully by quinapril at a dose of 0.1 mg/kg of total body weight in humans (i.e., 5–10 mg/day orally in an average-sized adult). At this dose, however, tissue ACE is only 90% inhibited.[16,17] The dose of quinapril required for 100% inhibition of tissue ACE is not yet known for humans, but it is in excess of 1 mg/kg.[17] In the rat, quinapril doses of 3–10 mg/kg intravenously are required for maximal blood pressure reduction, perhaps a measure of maximal inhibition of ACE in vascular endothelial cells (see Fig. 6 in Kaplan et al.[17]).

Similar dose–efficacy studies have not yet been performed in humans, but evidence is presented below that quinapril at a dose of 2 mg/kg/day orally is significantly more effective at retarding the progression of renal failure than conventional doses of approximately 0.5 mg/kg/day (40 mg orally/day).

HYDROPHOBIC VERSUS HYDROPHILIC ACE INHIBITORS

Quinapril, which is hydrophobic,[18] inhibits tissue ACE more effectively than enalapril, which is hydrophilic.[19] There appear to be two classes of active site in the enzyme: one that is accessible to solvent and hydrophilic inhibitors such as enalapril, and a second active site that is perhaps buried in a hydrophobic environment, accessible only to hydrophobic mole-

Table 2. Florinef Regimen to Prevent Hyperkalemia

Serum [K^+] (mEq/L)	*Florinef (dose in mg)*	*Frequency of Florinef*
4.8–5.0	0.1	Once a week (e.g., M AM)[a]
5.1–5.3	0.1	Twice a week (e.g., M and F AM)
5.4–5.6	0.1	Thrice a week (e.g., M, W, and F AM)
5.7–5.9	0.1	Five times a week (e.g., M–F AM)
≥6.0	0.1	Daily[b]

[a]The frequency of Florinef should be further increased by one level each for decreased renal function [i.e., elevated serum creatinine (>2.5 mg/dL)] or advanced age.

[b]Only a daily dose of Florinef requires a diurectic, as the potassium-lowering effect of fludrocortisone acetate is seen at less than daily doses, whereas sodium and water retention occurs only with daily dosing. A loop diuretic such as furosemide (e.g., 20 mg/day for serum creatinine <2.0 mg/dL, 40 mg/day for serum creatinine >2.0 mg/dL) will of course help lower the potassium as well.

cules such as quinapril and ramipril (Wei et al.[20] and manuscript in preparation).

A hydrophobic ACE inhibitor should therefore be used for maximal inhibition of the enzyme, at both its active sites. Below we present evidence of superior patient outcomes using a hydrophobic ACE inhibitor at a dose intended to inhibit maximally tissue, rather than circulating, ACE.

PREVENTING TOXICITY

Hyperkalemia is the only dose-dependent toxicity of ACE inhibitors, but it is life-threatening since hyperkalemia, especially if it is acute, can result in sudden death due to cardiac asystole. Other serious side effects of ACE inhibitors, such as angioedema (in 0.1–1% of patients) and neutropenia (in 0.01–0.001% of patients), have an allergic basis, are usually quickly reversible upon cessation of the drug, are rare, and are idiosyncratic rather than dose-related.

For patients with reduced renal function, hyperkalemia due to use of an ACE inhibitor can be especially severe. By inhibiting angiotensin II-mediated aldosterone synthesis, ACE inhibitors exacerbate the hyperkalemia often observed in renal failure itself, which is due to so-called hyporeninemic hypoaldosteronism ("type IV renal tubular acidosis").

Replacement of aldosterone ameliorates the hyperkalemia associated with both renal failure itself, as well as the use of an ACE inhibitor (Tables 2 and 3). A fluorinated analog of aldosterone, fludrocortisone acetate (Florinef®), is effective at lowering serum potassium concentration in patients with hypoaldosteronism. At a dose of up to 0.1 mg 5 days a week, fludrocortisone acetate lowers serum potassium without the attendant sodium and water retention that is another well-known property of aldosterone. At doses of 0.1 mg daily, a loop diuretic such as furosemide (20–40 mg orally daily) is required to prevent fluid retention, which otherwise would lead to volume overload. The use of a loop diuretic also contributes to lowering serum potassium. The reason why a diuretic is not used first instead of fludrocortisone acetate to lower serum potassium concentration is because the volume depletion resulting from use of a diuretic induces a high renin, high angiotensin II state, which opposes the goal of ACE inhibition.[21]

Using the regimen in Table 2, the peak serum

Table 3. Peak Serum Potassium Concentrations

Quinapril	*Potassium (mean ± SEM)*	*Number of patients*
<80 mg/day	4.98 ± 0.07	132
≥80 mg/day + Florinef	4.87 ± 0.06	132

$p = 0.23$ (i.e., no significant difference) by two-tailed t test.

potassium concentration for patients with renal failure on higher than conventional doses of quinapril was well controlled (Table 3). There were no episodes of serious hyperkalemia (i.e., with electrocardiogram changes) or any episodes of sudden death, during a total of 3,000 patient-years of experience.

CLINICAL EXAMPLE 1: CRF

In July 1993, the author observed that fludrocortisone acetate (Florine) was effective in treating hyperkalemia in patients with CRF (Tables 2 and 3). This finding made possible the administration of higher than conventional doses of ACE inhibitors such as quinapril to patients with CRF.

In November 1993, the author observed that ESRD from hypertension or non-insulin-dependent diabetes mellitus (NIDDM) was associated with the ACE D/D genotype (Table 1). At around the same time, it became clear that angiotensin II was responsible, at least in part, for compensatory renal growth after nephrectomy.[22,23] (Norepinephrine may also be involved,[22] suggesting that adding a β-blocker to an ACE inhibitor may improve on the results shown here.) Normally, compensatory renal growth after unilateral nephrectomy, leaving 1 million nephrons, does not lead to renal insufficiency. But, insufficient nephron mass due to destruction by disease could serve as an ongoing growth stimulus[24] involving the renotropin angiotensin II, and angiotensin II could lead to apoptosis through activation of c-*myc*,[25,26] which controls entry to both the pathways of cell proliferation and cell suicide (apoptosis).

The high cost ($18 billion last year) and mortality (approximately 25% annually) of ESRD and the inability to delay the progression of CRF, especially in African-Americans,[27] who have a several-fold higher incidence of ESRD than Caucasians, are powerful incentives to develop better treatments for patients with CRF.

Despite intense efforts over >12 months, the author was unable to secure funds from research pharmaceutical companies for a Phase IV clinical trial using a hydrophobic ACE inhibitor. Doubtless one reason is that two large Phase IV trials involving ramipril had already begun, although neither used more than 10 mg ramipril daily.[2,3] An additional reason given by the pharmaceutical companies was that the patent for enalapril was due to expire in 1998. The pharmaceutical companies felt that physicians made little distinction between ACE inhibitors, so that even if quinapril or ramipril had a pronounced clinical effect, physicians would likely use the generic drug, enalapril, anyway.

Unable to secure funding for a proper large-scale, randomized, double-blinded clinical trial, the author began using a higher than conventional dose of quinapril in his own outpatient nephrology practice in March 1994. Quinapril was used as the first antihypertensive agent, except in patients with a known allergy to an ACE inhibitor. The dose of quinapril was increased over a few months to 2 mg/kg of total body weight/day, in two divided doses, before adding a second antihypertensive agent [nifedipine GITS up to a maximum dose of 120 mg p.o. twice a day (bid)], followed by a third agent (minoxidil, up to a maximum dose of 50 mg p.o. three times a day) as necessary to control hypertension.

The goals for blood pressure (110–120 mmg Hg/<80 mm Hg) and low-density lipoprotein (<100 mg/dL) were standard, as was the use of antiplatelet therapy (81 mg of enteric coated aspirin p.o. daily). Approximately 60% of the author's patients were African-American men, and the remainder Caucasian men. Beneficial effects were observed immediately. The first two patients had their serum creatinine arrested or reversed from one clinic visit to the next. As a result, in May 1994, the author began using this treatment for all of his patients with CRF ($n \sim 200$) and hypertension ($n \sim 800$). Thus, pilot experience with one or two patients was followed by a therapeutic change affecting all patients in the practice, which is common in clinical medicine.

The three groups of patients receiving conventional therapy (enalapril or quinapril at a dose of ≤40 mg/day; Table 4) were combined to form the reference group depicted in Figures 1–6.

In August 1995, losartan (50 mg/day; Table 5) was added to prevent angiotensin II production by non-ACE peptidases, especially

Table 4. Slope of 1/(Serum Creatinine) Versus Time (in Years)

	Q > 80 L[a]	*My patients <94*[b]	*Others' patients 7/97*[c]	*Others' patients 8/93*[d]
Hypertension				
White men	−0.023 ± 0.013 (15)	−0.093 ± 0.053 (7)	−0.103 ± 0.041 (10)	−0.083 ± 0.029 (8)
Black men	−0.027 ± 0.032 (26)	−0.172 ± 0.085 (7)	−0.093 ± 0.031 (14)	−0.068 ± 0.019 (14)
NIDDM				
White men	−0.101 ± 0.056 (14)	−0.139 ± 0.065 (8)	−0.101 ± 0.020 (23)	−0.204 ± 0.105 (11)
Black men	−0.043 ± 0.026 (30)	−0.128 ± 0.042 (5)	−0.125 ± 0.040 (26)	−0.110 ± 0.019 (20)
ADPKD				
White men	−0.045 ± 0.017 (7)	−0.036 (1)	−0.057 (2)	−0.043 (2)

Subjects were outpatients with serum creatinine ≥2.0 mg/dL. Data are mean ± SE values (number of patients). The larger in absolute value and more negative the number, the faster the rate of decline of renal function.

[a]Patients receiving quinapril >80 mg/day, with or without losartan (50 mg/day).

[b]Patients seen by the author before 1994 (i.e., on doses of quinapril <80 mg/day and without losartan).

[c]Patients seen by other nephrologists at the same institution as the author, using conventional doses of quinapril (≤40 mg/day), without losartan.

[d]Patients seen by other nephrologists at the same institution as the author as of August 1993, using conventional doses of quinapril without losartan.

chymase, as had been recently shown in the heart.[28] The additional hyperkalemia caused by losartan in combination with an ACE inhibitor could be managed by increasing the dose of Florinef according to the protocol outlined in Table 2.

In June 1996, losartan was stopped in all patients administratively, but this had no effect on the rate of progression of renal failure (data not shown). In June 1997, patients had their dose of quinapril administratively decreased to ≤40 mg/day (Figs. 7–11). The last serum creatinine values on all patients were obtained in December 1997.

The data presented here are observational. There was no intention of performing a randomized, prospective clinical trial since there was no funding to do so. Nevertheless, the results are highly self-consistent. The three patient groups who benefitted from "high-dose" (2 mg/kg/day) quinapril, namely, Caucasian (Fig. 3) and African-American (Fig. 4) men with HTN and African-American men with diabetic nephropathy (Fig. 6), also deteriorated once their dose of quinapril was lowered to the conventional dose of ≤0.5 mg/kg/day (≤40 mg/day; Figs. 7–9). Furthermore, the two groups who failed to benefit from "high-dose" quinapril [Caucasian men with diabetic nephropathy (Figs. 2 and 5) or autosomal dominant polycystic kidney disease (ADPKD) (Fig. 2)], also failed to show any change when switched back to "conventional-dose" quinapril (Figs. 10 and 11). The exception was Caucasian men with early diabetic nephropathy (serum creatinine <2 mg/dL), who clearly benefitted from "high-dose" quinapril (Fig. 5). In agreement with these results, others have also failed to implicate ACE as a modifying gene for ADPKD.[29]

The beneficial effect on progression of renal

FIG. 1. Average time to dialysis if creatinine = 2 mg/dL in CRF due to hypertension.

FIG. 2. Average time to dialysis if creatinine = 2 mg/dL in CRF due to NIDDM.

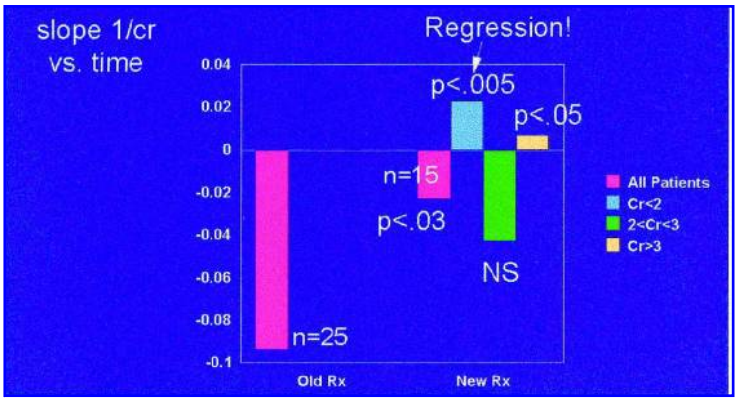

FIG. 3. Progression of CRF due to HTN in white men.

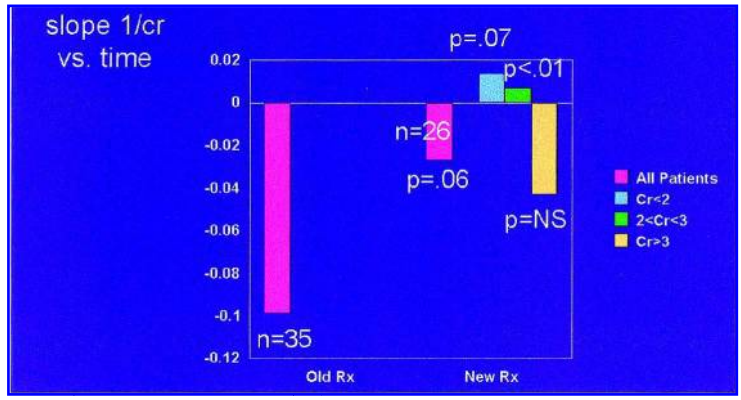

FIG. 4. Progression of CRF due to HTN in black men.

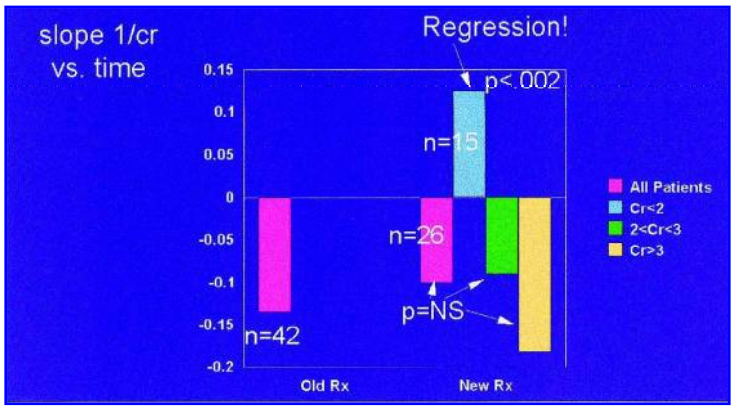

FIG. 5. Progression of CRF due to NIDDM in white men.

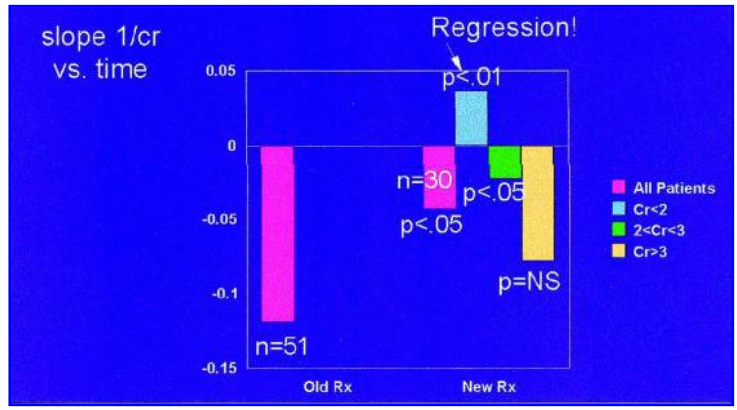

FIG. 6. Progression of CRF due to NIDDM in black men.

disease was most likely due to the elevated dose of quinapril, rather than to the combination of losartan with the ACE inhibitor. First, a markedly beneficial effect was seen in African-American men with NIDDM (Figs. 2 and 6) who took losartan for an average of only 3.7 months but high-dose quinapril for 12.3 months (Table 5). Second, patients were maintained on quinapril at high doses for 8–12 months after losartan was stopped. There was no change in their slope of (1/creatinine) versus time upon stopping losartan (data not shown). However, within 6 months of reducing the dose of quinapril from 2 mg/kg/day to $\leq$0.5 mg/kg/day, the rates of progression of renal disease had returned essentially to the same values seen in patients receiving conventional therapy (Figs. 7–9 and Table 4).

REGRESSION OF RENAL DISEASE

Regression of renal failure has not yet been described in the literature. However, if patients with hypertension or NIDDM were treated with 2 mg/kg/day quinapril before their serum creatinine had reached 2 mg/dL, regression of renal disease was seen (Figs. 3–6). In HTN, regression was also seen in more advanced disease (Figs. 4, 7, and 8). Long-term studies will need to be performed to see how long this effect lasts. These results raise the possibility that ESRD might be prevented altogether by using this treatment method early in the course of HTN or diabetic nephropathy. Approximately 11 million Americans are estimated to have a serum creatinine of $\geq$1.5 mg/dL.[30]

CLINICAL EXAMPLE 2: ATHEROSCLEROTIC PERIPHERAL VASCULAR DISEASE

Two patients (a 74-year-old African-American man and a 72-year-old Caucasian man) with symptomatic atherosclerotic peripheral vascular disease (ASPVD) due to hypertension were referred by the Radiology Service for routine prophylaxis of contrast-nephropathy, since they were found to have serum creatinine $\geq$2.5 mg/dL on the day of arteriography. Both patients were being prepared by the Surgery Service for femoral-popliteal revascularization the following day. Instead of surgery, both patients opted for medical management with quinapril 2 mg/kg/day, in two divided doses, plus aggressive lipid-lowering therapy (target low-density lipoprotein $<$100 mg/dL, target triglycerides $<$200 mg/dL). Blood pressure was maintained at $\leq$130/80 mm Hg. Pulse was reduced to 60 beats/min with a β_1-selective antagonist (metoprolol, 25–50 mg p.o. bid), since atherosclerosis is promoted by a rapid heart rate,[31] and slowing the heart rate should decrease turbulence in the arterial tree.[32]

The patients made no other change in their medications or life-style. Specifically, there was no change in the amount of cigarettes smoked or physical exercise. Nevertheless, both patients were able to defer revascularization for 4–5 years, a result not yet described in the literature.[33]

As in CRF, the magnitude of the clinical outcome contrasts with the relatively small odds ratio of the ACE D/D genotype, which for ASPVD in Caucasian men was 1.12 (data from Moskowitz[5]) and in African-American men was only 1.04 (data from Moskowitz[5]).

CLINICAL EXAMPLE 3: EMPHYSEMA (CHRONIC OBSTRUCTIVE PULMONARY DISEASE)

A 69-year-old white man with end-stage chronic obstructive pulmonary disease (COPD) due to cigarette abuse (1–2 packs per day since age 20) had been followed for 20 years in the Hypertension Clinic. In August 1994 his FEV_1 (forced expiratory volume in 1 s) was 0.87 L, and he was on 2 L/min oxygen by nasal cannulae, which had been begun in April 1994. Upon presentation in October 1995, his systolic blood pressure was only 104 mm Hg. He had 4+ pedal edema bilaterally, despite taking furosemide, 40 mg p.o. daily, consistent with severe right-sided heart failure. He was still smoking 1 pack per day. His lungs had essentially no audible air movement.

The patient's severe right-sided heart failure with systemic hypotension prevented using a

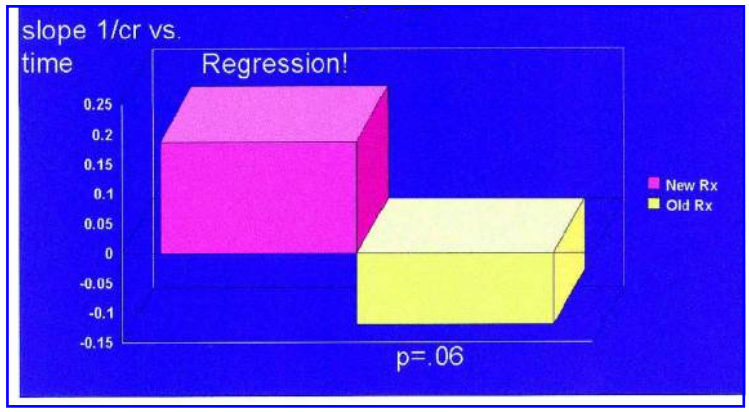

FIG. 7. Inadvertent crossover design: from new treatment to conventional treatment in white men with CRF due to HTN ($n = 22$).

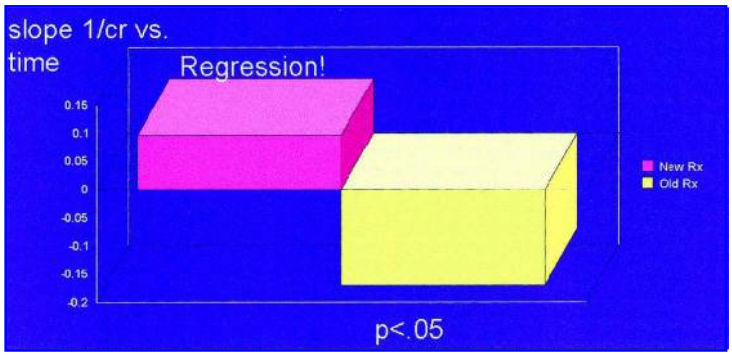

FIG. 8. Inadvertent crossover design: from new treatment to conventional treatment in black men with CRF due to HTN ($n = 13$).

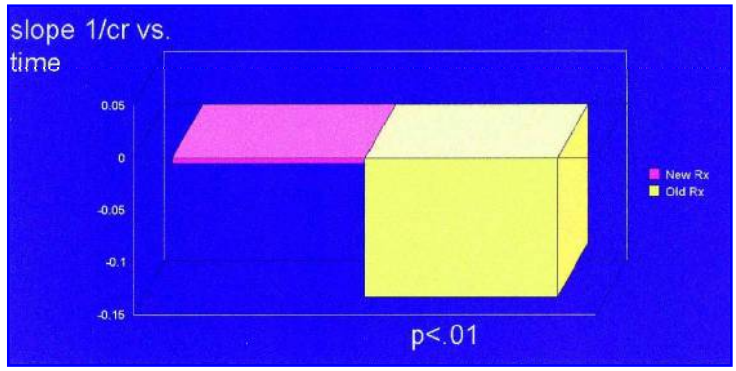

FIG. 9. Inadvertent crossover design: from new treatment to conventional treatment in black men with CRF due to NIDDM ($n = 21$).

higher dose of diuretic. Because of a recently discovered association of the ACE D/D genotype with COPD (odds ratio 1.21; data from Moskowitz[5]), the patient was begun gingerly on a hydrophobic ACE inhibitor, ramipril, at a dose of 2.5 mg orally at bedtime. The author expected the patient's systolic blood pressure to fall even lower, perhaps to 80 mm Hg, but hoped that the patient might be able to mobilize his peripheral edema. The patient's life expectancy appeared to be no more than 1 month.

When the patient was seen in the outpatient clinic a week later, his blood pressure had unexpectedly risen to 180/110 mm Hg, and his pedal edema had decreased to 2+. Ramipril appeared to have lowered his severe pulmonary hypertension, allowing adequate filling of his left ventricle. With adequate stroke volume, the patient was able to demonstrate the same systemic hypertension as in years past.

The patient's FEV_1 on August 16, 1998 was 0.78 L, compared with 0.87 L 3 years before. His most recent FEV_1 was 0.55 L (January 2001), obtained during an episode of congestive heart failure (see below). Thus he has shown relatively slow progression of COPD over the past 6.5 years. He has not been hospitalized for exacerbation of pulmonary dis-

FIG. 10. Inadvertent crossover design: from new treatment to conventional treatment in white men with CRF due to NIDDM.

FIG. 11. Inadvertent crossover design: from new treatment to conventional treatment in white men with ADPKD ($n = 4$).

ease between October 1995 and April 2002, the time of this writing.

The only other changes in his medications were an increase in his home oxygen to 3 L/min by nasal cannulae in May 1996 and the addition of a nebulizer in November 1997. Because of the patient's chronic diuretic use, losartan was begun in October 1995 to prevent hypokalemia; the combination of angiotensin receptor blocker and ACE inhibitor raises potassium more than either one alone, and has made potassium supplementation unnecessary.

The patient's weight increased by 30 lb between October 1995 and December 1997, while remaining at 2+ pedal edema, suggesting a gain of nonfluid weight, which is uncharacteristic in end-stage emphysema. He briefly cut his smoking down to ½ pack per day in 1996, but has been smoking 1 pack per day since 1997, and 1.5 pack per day since November 2000.

After the patient's dramatic hemodynamic response to 2.5 mg of ramipril in October 1995, the dose of ramipril was further titrated to keep his systemic blood pressure below 135 mm Hg systolic. This resulted in a dose of 130 mg bid by the fall of 2000. The increased requirement for ramipril appears to be due at least in part to interruption of a negative feedback loop regulating ACE gene expression.[34] In an effort to limit further increases in the dose of ramipril (generously supplied by King Pharmaceuticals), a calcium channel blocker (felodipine) was introduced in the fall of 2000, and advanced to 20 mg bid.

The patient developed marked fluid retention over a 2-week period in January 2001, leading to hospitalization for dyspnea. The patient refused intubation. His dyspnea responded to

TABLE 5. DOSE AND DURATION OF HIGH-DOSE QUINAPRIL (Q) AND/OR LOSARTAN (L) TREATMENT

	Average months		
	Q + L[a]	*Q > 80*[b]	*Max Q dose (mg/day)*[c]
White men			
Hypertension ($n = 16$)	8.9 ± 1.4	8.1 ± 2.2	134 ± 20
NIDDM ($n = 18$)	8.3 ± 1.4	7.6 ± 1.4	167 ± 15
ADPKD ($n = 7$)	8.0 ± 2.3	8.6 ± 5.3	131 ± 23
Black men			
Hypertension ($n = 32$)	5.5 ± 1.0	8.9 ± 1.5	171 ± 10
NIDDM ($n = 32$)	3.7 ± 0.9	12.3 ± 1.5	174 ± 6

Patients are those with serum creatinine ≥2 mg/dL at their first clinic visit; the number of patients is given in parentheses. Data are mean ± SE values.

[a]Duration (in months) of treatment with Q, at any dose, in combination with L, 50 mg/day.

[b]Duration (in months) of treatment with Q above a dose of 80 mg/day, given in two divided doses.

[c]Maximum daily dose of Q, in mg/day, when given without losartan.

bedrest, followed by vigorous diuresis as an outpatient of ~20 lb. Felodipine was halved and then stopped altogether, and the patient's increased blood pressure has since been controlled by increasing the dose of ramipril. The patient currently takes 400 mg bid ramipril. His estimated dry weight is now ~170 lb, considerably less than the 190 lb he weighed in 1999. In other words, he may now be losing nonfluid weight consistent with end-stage COPD.

Thus, ramipril appears to have (1) substantially reduced his secondary pulmonary hypertension; (2) delayed deterioration of his FEV_1, despite continued smoking; (3) kept him out of the hospital except for an episode of congestive heart failure precipitated by volume retention associated with use of a calcium channel blocker; (4) allowed him for several years to gain nonfluid weight; and (5) kept him alive for at least 6.5 years longer than expected.

To date, the literature would not have suggested such a positive clinical outcome in COPD using an ACE inhibitor.

SUMMARY

Markedly improved patient outcomes are reported for the first time here for four serious diseases: diabetic and hypertensive nephropathy, ASPVD, and COPD. The outcomes were inspired by pharmacogenomics. In the case of COPD especially, nothing in the literature would have suggested such a positive outcome to ACE inhibitor therapy.

The evidence presented here is clearly of an observational nature and did not involve randomization. Patients were used as their own controls, as is routine in clinical nephrology.[35] These data will need to be replicated in randomized, clinical trials, which will undoubtedly be more expensive than the experience reported here.

None of the patients described above was genotyped for the ACE I/D polymorphism. However, marked improvement in clinical outcome was observed in the majority of patients, suggesting that improved clinical outcomes can be obtained even in the absence of individual genotyping. ACE activity therefore appears to contribute to disease progression regardless of I/D genotype. Association of the ACE D/D genotype with a disease may merely indicate those with an accelerated rate of disease progression relative to some "baseline." Since two-thirds of Americans die from cardiovascular disease, and one-third from cancer, diseases that have been associated with the ACE D/D genotype,[5] the "baseline" gets disease, too.

It would of course be worthwhile to know whether the target dose of an ACE inhibitor should be adjusted for ACE I/D genotype. An even more important clinical parameter to follow serially might be the degree of inhibition of leukocyte membrane ACE activity, taken as a surrogate for tissue ACE activity.[7]

Earlier we found the ACE D/D genotype to be associated with approximately 40 common diseases,[5] including the four diseases discussed here. In particular, NIDDM and most of its complications appear to be associated with the ACE D/D genotype (Tables 1 and 6). The clinical results presented here strongly suggest that ACE activity is involved in the pathophysiology of these four diseases and raise the possibility that

Table 6. ACE I/D and Complicatons of Type 2 Diabetes Mellitus

	% D/D	*Total*	*Odds ratio*
Black men			
Retinopathy	36.0	239	1.28
Neuropathy	21.3	61	0.61
Controls	30.6	242	=1.00
Black women			
Retinopathy	34.0	47	1.31
Neuropathy	24.5	49	0.83
Controls	28.2	142	=1.00
White men			
Retinopathy	27.7	314	1.10
Neuropathy	29.4	187	1.20
Controls	25.8	125	=1.00

Outpatients and inpatients (n = 6,414) from two hospitals in St. Louis were genotyped for the ACE I/D polymorphism. Patients were matched according to ethnicity and socioeconomic status. Odds ratios for the ACE D/D genotype are expressed relative to the control populationl, as in Table 1. [Odds ratios for insulin-dependent diabetes mellitus (IDDM), NIDDM, and nephropathy due to IDDM or NIDDM were given in Table 1.] Data for complications of NIDDM in white women, and for complications of IDDM, were insufficient for analysis. These data suggest that ACE is associated with retinopathy in black and white men and black women. ACE appears to be associated with diabetic neuropathy in white men, but not in black patients with NIDDM.

ACE may be involved in the pathophysiology of all 40 common diseases (manuscript in preparation). This would lend further support to the concept that endothelial cell biology is basic to human disease (Loscalzo et al.,[32] pp. 3–38). Indeed, a reasonable explanation for the patient outcomes seen here is that in the normal course of disease progression (and perhaps aging itself), angiotensin II leads to apoptosis of downstream tissue parenchyma.[25,26,36]

This experience raises several additional points. The magnitude of a gene's contribution to a complex disease is clearly difficult to estimate. The odds ratios for the ACE D/D genotype are rather unimpressive, considering that the odds ratio for hypercholesterolemia and atherosclerotic coronary artery disease can reach as high as 3.1.[37] However, relatively weak odds ratios are to be expected for polygenic diseases involving multiple genes, and multiple polymorphisms within each gene.[38] With probably dozens of genes contributing to the development of a common, complex disease, and several polymorphisms per gene, it is perhaps not unreasonable to see odds ratios of only ~1.1–1.3 for an individual polymorphism such as the ACE I/D allelic system. Despite the rather unimpressive odds ratios for the ACE D/D genotype (<30% increased above control), the effect of adequate inhibition of tissue ACE had dramatic (~200%, Fig. 1) clinical effects.

A possible explanation for this discrepancy is that ACE functions early in the disease pathway leading to apoptosis of renal parenchyma [CRF (Bonnet et al.[25])], vascular wall [ASPVD (Hamet and deBlois[26])], and pulmonary parenchyma [COPD (Papp et al.[36] and manuscript in preparation)]. Because of the amplification inherent in biological cascades, inhibition of an early step in a disease pathway is expected to be more effective clinically than inhibition of a late step.

Second, whether a gene is causally related to a disease will ultimately require clinical proof, namely, whether pharmacological alteration of the gene product's activity in the appropriate direction alters patient outcomes for that disease. Statistical, *in vitro*, and animal data cannot be definitive. This means that clinical research, which currently receives far less funding than bench research, will be rate-limiting for understanding disease-predisposition genes.

The fastest and least expensive way to improve patient outcomes is to use an existing drug. Generally, clinicians prefer to use drugs with long clinical histories, and well-known toxicity profiles, to treat their patients. This is especially true in trying to prevent disease in completely asymptomatic patients. Such drugs are likely to be near the end of their patent life, as in the case of quinapril and ramipril. Since research pharmaceutical companies no longer have any commercial interest in such drugs, and the generic drug industry has no tradition of supporting clinical research, there is currently little funding for clinical research with such drugs. Governmental (NIH) funding has focused on basic rather than clinical research since the late 1970s. Neither Medicaid nor Medicare has a tradition yet of supporting clinical trials to improve patient outcomes. Foreign National Health Services similarly have no funds set aside for clinical trials.

Until much more funding for Phase IV trials becomes available, large randomized, placebo-controlled, double-blinded clinical studies may need to give way to much less expensive, less sophisticated studies involving simple randomization schemes (e.g., whether a person's birthdate ends in an odd or even number). "High-dose" quinapril changed the (1/creatinine) versus time slope for individual patients from one outpatient clinic visit to the next, over a period of 1–3 months. As in the experience reported here, initial patient outcomes with a new treatment based on pharmacogenomics may be so positive that randomization no longer seems ethical, and comparison instead must be made to historical or literature controls.

The example of ACE suggests that knowledge of disease-predisposition genes can transform medicine, allowing dramatic advances to be made in patient outcomes. It is certain that the new knowledge of pharmacogenomics will overwhelm the resources available for formal clinical trials, and that this will remain the case for the foreseeable future. The experience with ACE suggests that practicing physicians may need to become partners in clinical research,

adopting common-sense strategies based on pharmacogenomics, so that their patients may benefit within their own lifetimes.

NOTE

The treatment methods presented here have all been registered with the U.S. Patent and Trademark Office (Application numbers 60/310,064, 60/347,013, 60/350,563, 60/352,072, 60/352,074, and others pending). Those wishing to obtain a license to use this material are encouraged to contact the author by email at dwmoskowitz@genomedics.com, by phone at 1-877-GENOMED, or by writing to D.W. Moskowitz, M.D., Chairman and Chief Medical Officer, GenoMed, Inc., 4560 Clayton Avenue, St. Louis, MO 63110. Any licensing revenues will be used solely to fund additional research in pharmacogenomics, including clinical research.

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