Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy

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Alport syndrome inevitably leads to end-stage renal disease and there are no therapies known to improve outcome. Here we determined whether angiotensin-converting enzyme inhibitors can delay time to dialysis and improve life expectancy in three generations of Alport families. Patients were categorized by renal function at the initiation of therapy and included 33 with hematuria or microalbuminuria, 115 with proteinuria, 26 with impaired renal function, and 109 untreated relatives. Patients were followed for a period whose mean duration exceeded two decades. Untreated relatives started dialysis at a median age of 22 years. Treatment of those with impaired renal function significantly delayed dialysis to a median age of 25, while treatment of those with proteinuria delayed dialysis to a median age of 40. Significantly, no patient with hematuria or microalbuminuria advanced to renal failure so far. Sibling pairs confirmed these results, showing that earlier therapy in younger patients significantly delayed dialysis by 13 years compared to later or no therapy in older siblings. Therapy significantly improved life expectancy beyond the median age of 55 years of the no-treatment cohort. Thus, Alport syndrome is treatable with angiotensin-converting enzyme inhibition to delay renal failure and therapy improves life expectancy in a time-dependent manner. This supports the need for early diagnosis and early nephroprotective therapy in oligosymptomatic patients.

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This article is dedicated to the children and young adults included in our registry who died because of limited access to renal replacement therapy.

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Evaluation of microhematuria and microalbuminuria is common in everyday clinical practice as they are important early signs for chronic kidney disease (CKD). CKD substantially increases the risk of cardiovascular events and death.[1] Renal fibrosis is the end point of most CKDs.

Therefore, in addition to controlling hypertension,[2] therapy targeted at the prevention of renal fibrosis may be of value. Renal fibrosis due to Alport syndrome (AS) is seen in association with end-stage renal disease (ESRD) in children and young adults.[3] AS is a hereditary nephropathy characterized by progressive renal failure, sensorineural deafness, and typical ocular changes.[4] AS serves as a model of understanding progression of chronic renal fibrosis in mice[5–7] and humans.[8] The disease is caused by mutations in type IV collagen genes, leading to an abnormal composition of the glomerular basement membrane.[9] In all, 85% of Alport families have an X-chromosomal and 15% an autosomal trait of inheritance.[10] Abnormal composition of the glomerular basement membrane due to AS leads to extensive matrix deposition, inflammation, and fibrosis.[5,6] These are major components of progressive renal failure in literally all CKDs. AS inevitably leads to ESRD during adolescence or early adulthood, and ~50% of patients develop ESRD by the age of 20 years.[11]

Early diagnosis in children with AS with isolated hematuria opens a 'window of opportunity' for early intervention. Currently, there are no causal therapeutic options that are proven to delay renal failure in AS.[8] Angiotensin-converting enzyme inhibition (ACEi) has been shown to reduce proteinuria in Alport patients[12] and to delay renal failure in Alport mice,[5] suggesting that it may be of value as an effective treatment to delay renal failure in humans.[7] To test this we established the European Alport Registry to collect data over several generations of Alport families across Europe. Small children with AS first develop microscopic hematuria, proceeding to microalbuminuria, overt proteinuria, and impaired renal function, and end up with ESRD. These different steps of disease enabled us to assess if earlier introduction of ACEi at earlier degrees of disease is more effective than later therapy in delaying the time to dialysis and improving life expectancy. Our results might have the potential for generalization of the use of early nephroprotective therapy in all patients with Alport syndrome in everyday clinical practice.

RESULTS

Primary end point 'age at start of renal replacement therapy'

A total of 283 patients were followed for a mean of more than two decades (Figure 1). The mode of inheritance was within the expected range (Table 1).[10]

All 109 *noT* patients (red curve, Figure 2) were related to treated patients. Because of the genotype–phenotype correlation in AS,[3] the *noT* group with the same genotype minimized selection bias toward 'more benign' mutations in the

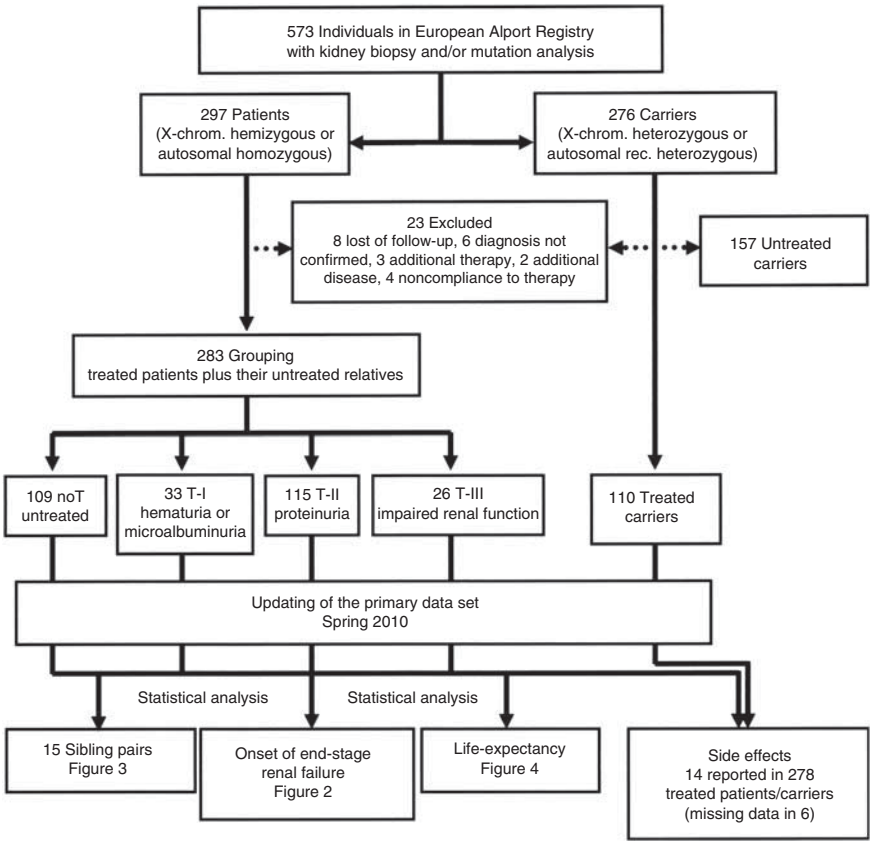

Figure 1 | Screening, assignment, follow-up, and selection for statistical analysis. Work flow of screening, assignment, updating data, and statistical analysis of Alport syndrome (AS) patients. Heterozygous carriers of Alport mutations were included in analysis of side effects of medication, but excluded from all other analyses. X-chrom., X-chromosomal.

Table 1 | Patients' characteristics

	noT	T-I	T-II	T-III	Siblings earlier T	Siblings later T	
Number of patients	109	33	115	26	15	15	All
Male	98.2% (107/109)	90.9% (30/33)	81.7% (94/115)	92.3% (24/26)	100% (15/15)	93.3% (14/15)	90.6% (269/297)
Female	1.8% (2/109)	9.1% (3/33)	18.3% (21/115)	7.7% (2/26)	0.0% (0/15)	6.7% (1/15)	9.4% (28/297)
X-chrom. inheritance	96.0% (95/99)	81.5% (22/27)	72.1% (75/104)	84.2% (16/19)	66.7% (10/15)	66.7% (10/15)	83.6% (209/250)
Autosomal-recessive inheritance	4.0% (4/99)	18.5% (5/27)	27.9% (29/104)	15.8% (3/19)	33.3% (5/15)	33.3% (5/15)	16.4% (41/250)
Proven COL4A5 mutation	88.2% (15/17)	83.3% (15/18)	70.0% (49/70)	86.7% (13/15)	66.6% (8/12)	66.6% (8/12)	83.1% (138/166)
Proven COL4A3/4 mutation	11.8% (2/17)	16.7% (3/18)	30.0% (21/70)	13.3% (2/15)	33.3% (4/12)	33.3% (4/12)	16.9% (28/166)
In-frame mutation	68.8% (11/16)	71.4% (6/8)	52.2% (12/23)	50.0% (2/4)	50.0% (1/2)	50.0% (1/2)	61.3% (38/62)
Deletion, frameshift, nonsense, rearrangement	31.2% (5/16)	28.6% (2/8)	47.8% (11/23)	50.0% (2/4)	50.0% (1/2)	50.0% (1/2)	38.7% (24/62)
Mean year of birth	1983.4 (s.d. 15.5)	1997.1 (s.d. 7.1)	1988.4 (s.d. 9.9)	1982.9 (s.d. 7.4)	1986.9 (s.d. 12.0)	1984.9 (s.d. 12.2)	
Age at start of therapy (in years)							
All patients		8.4 (95% CI 6.5–10.3; range 2–30)	15.1 (95% CI 13.5–16.8; range 1–56)	21.8 (95% CI 18.7–24.8; range 10–35)	9.5 (s.d. 8.7)	12.0 (s.d. 9.1)	
Patients with end point ESRD		None	16.6 (range 9–30)	21.2 (range 10–35)			
Median		8 (range 2–30)	13 (range 1–56)	20 (range 10–35)			
Duration of therapy (in years)							
All patients		4.0 (95% CI 2.9–5.0; range 1–14)	5.8 (95% CI 5.1–6.5; range 1–18)	7.4 (95% CI 5.3–9.5; range 1–19)	8.2 (range 1–17)	3.0 (range 1–6)	
Patients with end point ESRD		None	7.9 (range 2–15)	4.3 (1–13)	9.5 (range 2–14)	No treated patients	
Median age at start of ESRD (in years)	22 (95% CI 20–25; range 7–39)	No events	40 (95% CI 31 undetermined; range 17–40)	25 (95% CI 18–33; range 13–48)	40 (95% CI 22 undetermined)	27 (95% CI 17–29)	
Median age at start of ESRD of untreated relatives (in years)	22 (range 7–39)	22 (range 22–27)	23 (range 7–39)	18 (range 12–18)			
Initial regression of proteinuria after onset of therapy		50.0 (95% CI 28.3–71.7)	60.5 (95% CI 50.9–70.1)	52.8 (95% CI 32.3–73.3)			
Hypertension at onset of therapy	100% at ESRD (49/49)	0.0% (0/30)	13.4% (9/67)	73.3% (11/15)			
Sibling pairs							
Earlier start of therapy (*n*)	0.0% (0/15)	13.3% (2/15)	73.3% (11/15)	13.3% (2/15)			
Later start of therapy or no therapy (*n*)	80.0% (12/15)	0.0% (0/15)	6.7% (1/15)	13.3% (2/15)			

Abbreviations: chrom., chromosomal; CI, confidence interval; ESRD, end-stage renal disease.

therapy groups. The median age at onset of renal replacement therapy (RRT) in *noT* was 22 years (range and confidence intervals are shown in Table 1). *T-III* ($n=26$) had a median age at onset of ACEi therapy of 20 years and a mean duration of ACE inhibition of 7.4 years. *T-III* delayed median age at onset of RRT by 3 years to 25 years (yellow curve, Figure 2; $P<0.001$ vs. *noT*). *T-II* ($n=115$) had a median age at onset of ACEi therapy of 13 years and a mean duration of ACEi therapy of 5.8 years. *T-II* delayed median age at onset of RRT by 18 years to 40 years (green curve, Figure 2; $P<0.001$ vs. *noT*). *T-II* was more effective than *T-III* ($P<0.001$). *T-I* ($n=33$) had a median age at onset of ACEi therapy of 8 years and a mean duration of ACEi therapy of 4.0 years. None of the patients yet reached CKD stages $\geqslant 3$ (blue curve, Figure 2). *T-I* was more effective than *noT* ($P<0.001$) and *T-II* ($P<0.001$; $P=0.19$ vs. *T-III*).

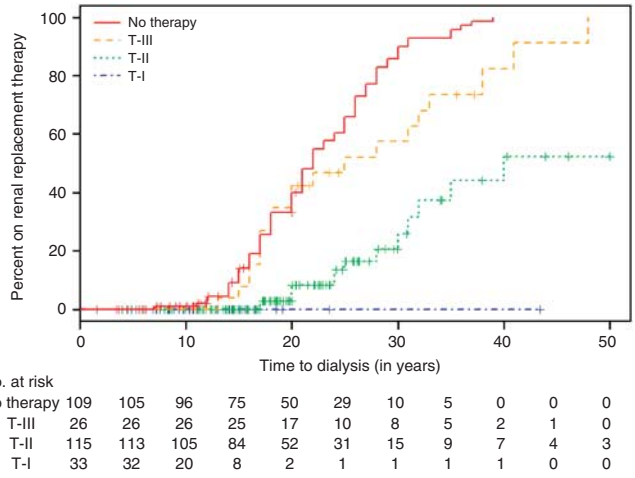

Figure 2 | Age at onset of renal replacement therapy in different treatment modalities. Untreated patients (red curve) are relatives to the treated patients (yellow, green, and blue curves) and have the same genotype. Angiotensin-converting enzyme (ACE) inhibition delays renal failure in a time-dependent manner. Tick marks indicate censoring data. Kaplan–Meier estimate.

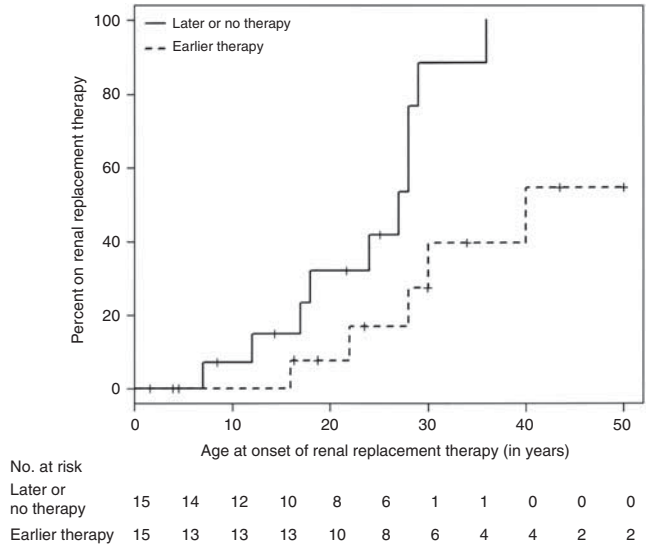

Figure 3 | Age at onset of renal replacement therapy (RRT): verification of superior outcome of earlier therapy in sibling pairs. Effect of different onset of therapy in 15 sibling pairs with identical mutations and environment. Diagnosis in the older child classically enabled earlier diagnosis in the other (younger) sibling, resulting in earlier therapy. Earlier therapy delayed onset of RRT by 13 years. Tick marks indicate censoring data. Kaplan–Meier estimate.

Verification of superior outcome of earlier therapy in sibling pairs

In order to address any selection bias, we assessed the effect of introducing ACEi at an earlier stage of disease by comparing 15 sibling pairs (Figure 3; Table 1). Each pair had the same mutation, and lived in the same environment. Typically, the older brother within these families was diagnosed quite late in the course of AS. However, diagnosis in this older sibling classically enabled earlier diagnosis in the other (younger) sibling, allowing for intervention with medication in the younger sibling at an earlier stage of disease (Table 1). The results showed that the median age at onset of RRT in the older sibling was 27 years compared with a median age at onset of RRT of 40 years in the younger ($P<0.001$).

Primary end point 'life expectancy'

Life expectancy of 101 *noT* patients was compared with 174 treated patients (T-I, -II, and -III). Patients who died because of limited access to RRT were excluded. The numbers of events in the T-I, -II, and -III groups were too low for statistical comparison between therapy groups. Median life expectancy of *noT* patients (solid curve, Figure 4) was 55 years; lifespan was found to be significantly improved by ACEi (dashed curve, Figure 4; $P=0.0369$ vs. *noT*) in the

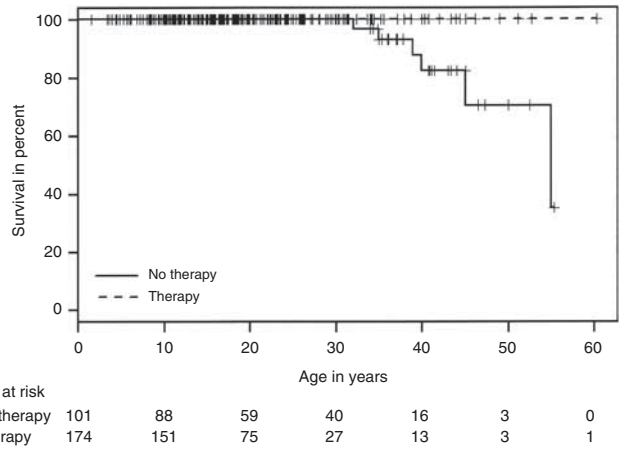

Figure 4 | Effect of treatment on life expectancy. Untreated patients (solid line) are relatives to the treated patients (dashed line) and have the same genotype. Angiotensin-converting enzyme (ACE) inhibition improves life expectancy ($P=0.0369$ vs. *noT*). Tick marks indicate censoring data. Kaplan–Meier estimate.

log-rank test. Both Figures 2 and 4 contain censored data (because of their age or slow progression of disease, patients drop out without the event RRT in Figure 2 and without the event 'death' in Figure 4).

Parameters affecting renal outcome

The natural course of AS is reflected in our levels of disease leading to therapy groups. Therefore, parameters such as *proteinuria* and *creatinine clearance* showed an effect on renal outcome. *Hypertension* usually occurs late in the course of AS. Thus, hypertension was a late result of CKD stage 4 and not an early predictor for poor renal outcome in AS (Table 1). Data on blood pressure control during treatment in these groups were not sufficient for statistical evaluation as independent risk factors in the progression of Alport CKD. As the study only included hemizygous X-chromosomal males or homozygous autosomal patients, *gender* and *mode of inheritance* were not predictors for renal outcome. The number of in-frame vs. frameshift mutations was too low in each group for statistical comparison. Year of birth was not an independent predictor for renal outcome (but affected inclusion to groups, Table 1).

Proteinuria initially decreased by a similar extent in all therapy groups (Table 1). However, in *T-II* and *T-III*, proteinuria typically climbed back to previous levels with time. In contrast to those who received therapy later, patients in *T-I* were less likely to return to pretreatment levels (31.3%, 10/32), and 12.5% (4/32) even stepped back in the course of disease and reversed to the isolated hematuria level. Thus, earlier therapy was found to be more effective than later therapy to hold—or even reverse—the natural course of proteinuria in Alport disease.

Side effects associated with medication

Side effects of the medication were reported in 14/278 (5.0%) of patients and treated carriers (missing data in 6/284; 2.1%). No severe side effects such as death from all causes, acute renal failure, or angioedema were reported, neither in the initial questionnaire nor in the data update in spring 2010. Side effects included hyperkalemia <5.5 mmol/l in 1.8% (5/284), dry cough in 0.7% (2/284), symptomatic hypotension and fatigue in 0.7% (2/284), oral ulcers, polyuria and polydipsia, aggressive behavior and agitation, sleep disorder, and withdrawal because of 'ineffectiveness' each in 0.4% (1/284 each).

DISCUSSION

The study reveals the first therapeutic option for Alport patients with progressive renal fibrosis: ESRD can be delayed by ACEi in a time-dependent manner, the earlier the better. For the first time, our data show that preserving renal function also results in a better life expectancy in patients with CKD. Thus, our observation in a rare model disease brings previous findings on the broad use of nephroprotection for everyday clinical practice in more common kidney diseases to an end; preventing microalbuminuria in diabetes in the BENEDICT trial,[13] later intervention in the REIN and RENAAL trials,[14,15] and even after onset of RRT[16] might improve life expectancy. Our data support the general need for early diagnosis and preventive therapy in CKD in yet oligosymptomatic patients with more common diseases including diabetes and hypertension.

Prospective analysis of treatment effects in AS seemed to be impossible in a disease that needs additional 20 years until ESRD develops. However, as a compromise, our European-wide longitudinal observational effort is believed to have a high quality, as (1) the study focuses on an easily verifiable primary end point in a well-defined disease always leading to ESRD; (2) *age at onset RRT* can be remembered exactly by family members, minimizing the lack of data on affected relatives; (3) this end point is most relevant for the children concerning their quality of life, social life, and life expectancy; and (4) as this end point is central to parents and nephrologists, all—irrespective their diverse languages and cultures—are willing to combine their personal destinies in order to find a cure for AS. These advantages are reflected in the high number of multinational participants, the high percentage of updated patient data, the high compliance to therapy (similar to the ESCAPE trial[17,18]), and the low rate of 'loss of follow-up.' Despite these advantages, our observational study design has the hazards of dropping relevant information on side effects and of a selection bias by 'picking' the best patients from each country with a less severe genotype into therapy groups leading to a better outcome. The high number of patients in *T-II* reduces the risk of a selection bias. The median age at onset of RRT in *noT* (22 years) is close to the median age at onset of therapy *T-III* (20 years). Therefore, we cannot exclude a selection bias. We reduce the risk by using: (1) a long average time on therapy (4.3 years in the *T-III* patients with the end point ESRD); (2) similar generations (mean year of birth does not differ significantly between groups *noT* and *T-III*, Table 1); (3) untreated relatives as 'intrafamilial controls'; (4) sibling pairs; (5) by our multinational effort including treatment data from 310 centers; and (6) by the final update of the primary data set.

Proteinuria decreased in all therapy groups; however, during the course of therapy, proteinuria returned back to or even above the level before onset of therapy in the later therapy groups, similar to the ESCAPE trial.[17] Analogous to animal models,[6–8] only early therapy was capable to prevent or reverse albuminuria in our patients. Blood pressure influences the time point of ESRD in CKD.[19] Inhibition of the renin-angiotensin system has been previously shown to delay ESRD in both diabetic and nondiabetic proteinuric patients.[20–23] However, the renoprotective effect of intensified blood pressure control is additive to the benefit conferred by ACE inhibitors in children in the ESCAPE trial.[17,18] Furthermore, an arterial pressure below the 90th percentile is a predictor for better outcome in the strict blood pressure control arm. In our trial, data on blood pressure control during treatment in these groups were not sufficient for

statistical evaluation as independent risk factors in progression of Alport CKD. Although previous trials well establish the major role of ACE inhibitors in lowering blood pressure and delaying renal failure, our study demonstrates the nephroprotective effects of ACE inhibitors even decades before overt hypertension develops. As ACE inhibitors are off-label use in normotensive children, safety issues become a major concern. No serious adverse events were reported in our study and the rate of side effects was rather low. However, future prospective trials must focus on (1) the risk–benefit balance of early therapy in oligosymptomatic children with AS and (2) prognostic factors to evaluate progression. Starting in spring 2012, the randomized, prospective, placebo-controlled EARLY PRO-TECT Alport phase III clinical trial (EudraCT number 2010-024300-10) will help to answer these questions and to find the optimal time point of an early start of therapy in the Alport population.

ACE inhibitors are not a specific therapy for AS. The nephroprotective effect of ACE inhibitors in yet oligosymptomatic, nonhypertensive patients with AS can be explained only incompletely by their antiproteinuric and antihypertensive properties.[12] Data from Alport animal models point to a major role of altered composition of the glomerular basement membrane (weakened because of the genetic defect) and of podocytes, their cytoskeleton, and their collagen receptors.[24,25] ACE inhibitors seem to have a superior potential to downregulate profibrotic factors in the Alport animal model.[4,5] Downregulation of profibrotic factors—known to be global players in all human fibrotic diseases—is independent from blood pressure and the amount of proteinuria in Alport mice.[4,5] In humans, however, this mechanism of action in AS is still speculative—further prospective trials should focus on this crucial topic in order to learn about the general mechanisms of organoprotection during fibrosis.

CKD substantially increases the risk of cardiovascular events and death.[1] Nearly one in every seven adults in the United States has CKD, with an increase of 30% over the last decade.[26] The reduction of the rate of progression and prevention of CKD is likely to have significant medical and socioeconomic impact. In 2006, >40% of incident dialysis patients in the United States had not previously seen a nephrologist[27] or been treated with either an ACE inhibitor or an angiotensin receptor blocker. Furthermore, public awareness of early kidney disease is as low as 3 to 8%.[28] In contrast, most Alport families are well aware of the 100% risk of renal failure in their affected children. Their high compliance, early diagnosis allowing preemptive therapy, and the low comorbidity make AS an ideal model to investigate the general use of early nephroprotection in CKD.[6–8] Age at onset of RRT in Figures 2 and 3 clearly spreads out in favor of earlier therapy in CKD. Similar findings from the Alport animal models make obvious that preemptive therapy before onset of renal damage (reflected by proteinuria) is most effective in delaying renal failure and improving life expectancy in everyday clinical practice for all physicians.

MATERIALS AND METHODS

Inclusion and exclusion criteria

The diagnosis of AS was proven by kidney biopsy or mutation analysis (or both). Patients were included if they were affected males with X-linked AS or patients with genetically proven homozygous autosomal AS.[9,11,29] Patients were excluded if they did not give informed consent or the diagnosis was suspected but not confirmed.

Primary data collection in the European Alport Registry and study design

Patient information, study protocol, questionnaire, consent form (in English, French, Spanish, and German; see Supplementary Material online), data collection, anonymization, and storage conform with good clinical practice guidelines were approved by the Ethics Committee (AZ 10/11/06, see Supplementary Material online; authorization of French data by the Commission Nationale de l'Informatique et des Libertés 908249). Primary data were processed by medical students, and data quality and accuracy was checked by the lead investigator (OG) using telephone interviews, email, facsimile, or by personal contact with both the physicians and patients. Questionnaires included demographic data (age, gender, country of origin, family history, and mode of inheritance) and clinical and laboratory data (how the diagnosis was made, age at onset of RRT, ACEi therapy and age at onset of therapy, renal parameters before and after onset of ACEi therapy such as proteinuria, creatinine, creatinine clearance, cholesterol, hypertension, hearing loss, eye symptoms, death from all causes, and side effects of medications such as hyperkalemia, angioedema, renal failure, cough, hypotension, rhabdomyolysis, and others).

The Registry retraces data from 310 participating centers on several decades in three generations of AS patients, hindering a prospective study design. Most pediatric nephrologists currently treat their AS patients with ACE inhibitors,[8] despite the fact that ACE inhibitors are off-label use in nonhypertensive children. As a consequence, following the concerns of the Ethics Committee and the German Society of Pediatric Nephrology regarding the legal aspects of any recommendation for this off-label use in children, this study could not be registered as a trial, but was designed as a noninterventional observational study investigating treatment effects in a purely observational manner without affecting therapy, its modality, or diagnostic decisions.

Screening, assignment, and follow-up

Screening, assignment, follow-up, and selection for statistical analysis are described in Figure 1. Data were collected from Germany, France, Spain, Belgium, Austria, Switzerland, United States (limited to only those families who recently moved from Europe to United States), Russia, Serbia, Romania, Italy, and Turkey. The primary data set was updated exclusively in spring 2010 by the lead investigator to ensure compliance to the Ethics Committee recommendations. Data were updated within 12 months of the analysis date in 86% of cases. Only 8 of 393 (2%) patients or carriers were lost to follow-up.

Outcome measures

The primary study end points were '*age at onset of RRT*' and '*life expectancy*.' Secondary end points included the decrease of proteinuria after initiation of ACEi therapy (determined as g protein per day or per g creatinine in children, depending on the center's preference), proportion of patients with a clinical diagnosis of hypertension (defined as >95th percentile for gender, age, and height in children), proportion of patients experiencing side effects

from ACE inhibitors, defined as acute renal failure (doubling of serum creatinine), angioedema, hyperkalemia >5.0 mmol/l, dry cough, symptomatic hypotension (orthostatic collapse), and others, and death from all causes. For maximum transparency in the evaluation of side effects, heterozygous carriers of Alport mutations were included in analysis of side effects, but excluded from all other analyses.

Intervention

The study investigated the treatment effects of ACE inhibitors; the control intervention was no therapy. The most commonly used ACE inhibitors were ramipril (0.025–0.1 mg/kg bodyweight) and enalapril (0.125–1.0 mg/kg bodyweight). Patients were categorized depending on renal function at initiation of therapy. Initiation of therapy was defined as:

- *T-I* starts at patients with microhematuria only (usually at birth) or microalbuminuria (30–300 mg protein per day or per g creatinine in children).
- *T-II* starts at patients with proteinuria >0.3 g/day or per g creatinine.
- *T-III* starts at patients with CKD stages III and IV.
- *noT* means no therapy until CKD stage V, on renal replacement therapy.

Statistical analysis

Distributions of continuous variables are summarized by means and 95% confidence intervals, whereas frequencies and percentages are given for categorical (including binary) variables. The primary efficacy end points '*age at onset of RRT*' and '*life expectancy*' are censored in some patients as not all patients included in the analyses have started RRT (or died). Therefore, appropriate statistical methods for censored time-to-event data were used including the Kaplan–Meier estimator and the log-rank test.[30] Life expectancy was defined as lifespan from birth to death. Only patients (irrespective of their therapy group before end-stage renal failure) with unlimited access to RRT were included in analysis of life expectancy. In the analysis of the siblings, the paired data were analyzed using a stratified version of the log-rank test.[31] Median event times are reported with 95% confidence intervals that are based on log–log-transformed confidence intervals of the event probabilities. If the confidence intervals of the event probabilities are too wide across all observed event times because of small samples, the confidence limits for the median cannot be determined. All analyses are of an exploratory nature and therefore no correction for multiple testing was applied. All reported *P*-values were two sided and those <0.05 were referred to as statistically significant. All inferential analyses were carried out using SAS, version 9.2 (SAS Institute, Cary, NC).

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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