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This is the final version of the accepted manuscript.

This is a pre-copyedited, author-produced PDF of an article accepted for publication in *Nephrology Dialysis Transplantation* following peer review. The version of record

Jan Boeckhaus, Julia Hoefele, Korbinian M Riedhammer, Mato Nagel, Bodo B Beck, Mira Choi, Maik Gollasch, Carsten Bergmann, Joseph E Sonntag, Victoria Troesch, Johanna Stock, Oliver Gross, Lifelong effect of therapy in young patients with the COL4A5 Alport missense variant p.(Gly624Asp): a prospective cohort study, Nephrology Dialysis Transplantation, Volume 37, Issue 12, December 2022, Pages 2496–2504

is available online at <https://academic.oup.com/ndt/article/37/12/2496/6505139>
or <https://doi.org/10.1093/ndt/gfac006>.

Nephrology Dialysis Transplantation
2022 DEC ; 37(12): 2496-2504
2022 JAN 12 (first published online: final publication)
doi: [10.1093/ndt/gfac006](https://doi.org/10.1093/ndt/gfac006)

Publisher: [Oxford University Press](https://www.oup.com/)

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Lifelong Effect of Therapy in Young Patients with the COL4A5 Alport Missense Variant p.(Gly624Asp): a Prospective Cohort Study

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Running Head: Lifelong effect of therapy in Alport syndrome

ABSTRACT

Background. Angiotensin-converting enzyme inhibitors (ACEis) have evolved as a first-line therapy for delaying end-stage renal failure (ESRF) in Alport syndrome. The present study tested the hypothesis of a superior nephroprotective potential of an early ACEi intervention, examining a cohort with the *COL4A5* missense variant p.(Gly624Asp).

Methods. In this observational cohort study (NCT02378805), 114 individuals with the identical gene-variant were explored for “age at ESRF” and “life-expectancy” in correlation with treatment as endpoints.

Results. All 13 untreated hemizygous patients developed ESRF (mean age 48.9+/-13.7 years) as did three very late treated hemizygotes (51.7+/-4.2 years), with a mean life-expectancy of 59.2+/-9.6 years. All 28 earlier (eGFR 60 ml/min or higher) treated hemizygous patients were still alive and still had not reached ESRF. Therapy minimized the annual loss of their glomerular filtration rate, similar to the annual loss in healthy individuals. Out of 65 heterozygotes, 4 untreated individuals developed ESRF at an age of 53.3+/-20.7 years. None of the treated heterozygous females developed ESRF.

Conclusions. For the first time, this study shows that in Alport syndrome, early therapy in individuals with missense variants might have the potential to delay renal failure for their lifetime and thus to improve life-expectancy and quality of life without the need for renal replacement therapy. Some treated patients have reached retirement age with still-functioning kidneys whereas untreated relatives have already reached ESRF at the same or younger age. Thus, in children with glomerular hematuria, early testing for Alport-related gene variants could lead to timely nephroprotective intervention.

Keywords: Alport syndrome, chronic renal failure, nephroprotective therapy, renin-angiotensin system, type IV collagen disease

KEY LEARNING POINTS

What is already known about this subject?

- ACE-inhibitors can delay renal failure in patients with the hereditary kidney disease Alport syndrome by years. The full potential of an early therapy is unclear.

What this study adds?

- To address this knowledge gap, we tested the superior kidney-protective potential of an early ACE-inhibitor therapy in a patient group with identical gene-defects. All untreated patients developed renal failure and had a reduced life-expectancy. All early treated patients were still alive and still had not reached renal failure.

What impact this may have on practice or policy?

- The study's findings provide first evidence that early therapy in adolescents with Alport syndrome has the potential to delay renal failure for their lifetime and thus to improve quality of life without the need for renal replacement therapy and life-expectancy. In children with traces of blood in the urine, testing for Alport-related gene variants could allow timely kidney-protective intervention.

INTRODUCTION

Chronic kidney disease (CKD) due to renal fibrosis affects approximately 10% of patients, impacting more than 500 million people worldwide [1,2]. In this CKD population, 2.78% of patients have hereditary type IV collagen-related nephropathy Alport syndrome (AS) as the underlying source of illness, which is the second most common monogenic cause of end-stage renal failure (ESRF) in adults [3-6]. AS is caused by disease-causing variants in the genes *COL4A3*, *COL4A4* and *COL4A5*, which encode the $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains of type IV collagen [4,7]. AS leads to ESRF early in life and is also characterized by sensorineural deafness and typical ocular abnormalities [6]. Most Alport families have an X-linked (*COL4A5*) inheritance, and up to 30% have an autosomal recessive or dominant (*COL4A3* or *COL4A4*) inheritance [4]. Female heterozygous X-linked Alport patients and heterozygous patients with autosomal dominant AS show a large variability of the clinical course [7,8].

AS serves as a model to investigate common elements of the pathogenesis of chronic progressive renal fibrosis in mice and human beings [8-11]. Abnormal composition of the glomerular basement membrane (GBM) due to AS leads to extensive matrix deposition, inflammation and fibrosis [6,12]. These are major components of progressive renal failure in all CKDs.

The angiotensin-converting enzyme inhibitor (ACEi) ramipril, if started before the onset of proteinuria, doubles the lifespan until ESRF in a mouse model of AS [10]. Registry data show a similar beneficial effect of ACEis in humans with AS [8]: Treatment starting in CKD stage 3 or 4 can delay ESRF by a median of 3 years, while treatment starting in CKD stage 2 can delay ESRF by a median of 18 years. An even earlier, i.e. pre-emptive, start has been tested in the EARLY PRO-TECT Alport trial: early initiation of ramipril therapy in oligosymptomatic children (CKD 0 or 1) with AS showed no safety concerns compared to placebo and supports the hope to slow renal failure by many years, emphasizing the value of timely therapy [13].

AS has a clearly defined course starting with microscopic hematuria, microalbuminuria, and proteinuria and progressing to renal fibrosis and ESRF [6]. AS also has a clear genotype-phenotype correlation: variants (such as large deletions) that lead to a truncated protein show the fastest progression (ESRF onset in untreated patients at approximately 20 years of age), whereas missense variants (prototypically glycine substitutions) have, on average, the slowest progression (ESRF onset at approximately 30 to 40 years of age) [14,15]. Recent data from Japan revealed that genotype also influences the response to angiotensin-targeting drugs in male patients with X-linked AS [14]. The less severe phenotype of missense variants in AS can be explained by their less severe impact on the mechanical integrity of the GBM. Missense variants account for approximately 40% of all disease-causing variants, making them the most common variant class in AS. Out of more than 460 pathogenic missense variants described in the literature for X-linked AS, the variant

NM_033380.3:c.1871G>A; p.(Gly624Asp) (chrX:g.107842023G>A, hg19) in *COL4A5* is the most common disease-causing missense variant in Europe (allele frequency of 0.0001959 in gnomAD v.2.1.1). Disease progression in p.(Gly624Asp)-associated X-linked AS is expected to be less severe [16-18].

Previous studies analyzing the beneficial effect of ACEis on Alport syndrome were not stratified by the individual genotype [8,13]. A recent study from Japan was stratified by mutation status (truncating vs. non-truncating), but not stratified by stage of kidney disease at onset of ACEi-therapy [14]. In addition, previous European registry data do not address whether missense variants responded better to ACEi therapy than truncating variants. If so, the nephroprotective potential of ACEis in patients with missense variants might be even higher than described in earlier reports, which did not distinguish between different classes of variants [8,19]. By analyzing a large number of patients with the identical p.(Gly624Asp) variant, this study eliminates the bias of genotype on the beneficial effect of ACEi therapy.

For the first time, we tested the hypothesis of whether ACEis have the nephroprotective potential to delay ESRF for one's lifetime in AS patients with missense variants, if therapy is started early, prior to significant renal fibrosis and kidney damage. This early-onnephroprotective approach could benefit most patients in the early stages of glomerular kidney diseases [2,18-21].

In 114 individuals with the common pathogenic missense variant p.(Gly624Asp) in *COL4A5*, we collected long-term observational data, including up to 29 years on nephroprotective ACEi therapy. The study explores the age at onset of ESRF and the life expectancy in the largest cohort with an identical X-linked AS variant ever studied.

MATERIALS AND METHODS

Participants and inclusion and exclusion criteria

The diagnosis of the pathogenic missense variant p.(Gly624Asp) in *COL4A5* alongside zygosity (hemi-, homo-, heterozygous) was based on a genetic consultation (including a conclusive genealogic tree for X-linked AS) and molecular genetic analysis. Individuals in which the variant could not be detected were excluded. Ten patients received an additional kidney biopsy to confirm the diagnosis of AS histologically. Patients were excluded if they did not wish to contribute or – in heterozygous females - if they donated a kidney (living donor to affected family member).

Study design and procedures

The European Alport therapy registry, which was founded in 2006, retraces medical data over several decades. Prior to 2006, the registry collected retrospective family and patient data; however, many patients were also followed in an up to 15-year prospective study design [8]. For the prospective follow-up, data were updated via telephone interviews, email, facsimile or personal contact with both physicians and patients. For ethical and data safety reasons, we recontacted only patients, who contacted us previously by email, facsimile or personally. Data were hosted centrally on a non-open access computer and were pseudonymized at the University Medical Center Göttingen. Questionnaires included demographic data and clinical and laboratory data as described previously [8]. The registry and data storage, in conformity with GCP guidelines, were approved by the Ethics Committee of the University Medical Center Göttingen (AZ 10/11/06; renewed version in 2014 and 2020; ClinicalTrials.gov Identifier NCT 02378805).

Intervention and outcome measures

An observational, non-interventional study explored the treatment effects of ACEis and angiotensin receptor blockers (ARBs), and the control intervention was no RAAS blockade. The most commonly used ACEi was ramipril (2.5 to 10 mg in adults). The study co-primary endpoints were “age at (onset of) ESRF” and “life expectancy” (lifespan until death); additional analyses included renal function (estimated glomerular filtration rate, eGFR) and proteinuria. Impaired eGFR was defined as an eGFR <60 ml/min. In children, the eGFR was calculated using the revised Schwartz equation (no formula has ever been validated in children with a GFR above 90 ml/min/1.73m²) [22]. Proteinuria was defined as >300 mg protein per gram creatinine, and micro-albuminuria was defined as 30-300 mg albumin per gram creatinine.

The start of therapy was defined as:

noT = no therapy;

T-0/I = early therapy, start before onset microalbuminuria or with microalbuminuria;

T-II = late therapy, start with proteinuria, eGFR>60 ml/min;

T-III = very late therapy, start with eGFR<60 ml/min.

Statistical analysis

For the analysis, individuals were divided into two groups. Hemizygous and heterozygous patients were separated into those who received early treatment and those who did not. Descriptive statistics are displayed as the mean and standard deviation. Statistics were calculated using SPSS Statistics version 26 (IBM, Armonk, NY). SPSS Statistics was also used to create Kaplan-Meier estimators and scatter plots. The co-primary endpoints “age at ESRF” and “life expectancy” were censored in some treated patients since not all patients included in the analyses already had reached end stage renal failure (or death).

RESULTS

The study retraces observational, retrospective clinical and therapeutic data over a time period of more than three decades in Alport families across Central Europe, and it also includes up to 15 years of prospective data. The study explores the age at onset of ESRF in the largest cohort with an identical Alport variant ever studied. Only affected family members with a definitive diagnosis of Alport syndrome were included in the data analysis (Flow Chart Fig. 1). The diagnosis was confirmed by clinical parameters and molecular genetic analysis in all included patients.

Clinical characteristics

Table 1 summarizes the clinical characteristics of the 114 patients with the identical pathogenic p.(Gly624Asp) variant in *COL4A5*, 48 hemizygous males (plus one homozygous female) and 65 heterozygous females. Twenty patients reached ESRF, 16 of which were male and 4 female. Four patients received a kidney transplant. Seven males and 4 females died (Table 1). In hemizygous patients, average age of the very-late therapy cohort was 58.5 (range 55-62) years, 44.6 (28-64) in the late therapy cohort, and 22.4 (8-59) in the early therapy cohort. Time on therapy for each cohort was 6, 11.9 and 7.4 years. Five affected children, who were yet too young and at too early a stage of CKD to have been started on RAAS blockade, were excluded from further analysis. In 29 heterozygous females, data were limited to the information that these patients did not require renal replacement therapy (RRT) (no data on present stage of disease other than “not at end-stage renal failure”). In contrast, we were able to obtain data on their present age, age at the start of therapy, current stage of

disease, and eGFR and serum creatinine levels in most hemizygous males (and data on ESRF in all hemizygous males), allowing a detailed and meaningful end-point analysis.

Most importantly, our study compared not only the therapeutic effect of RAAS blockade in individuals in Central Europe with the identical variant but also pairs or triplets of treated vs. untreated brothers or cousins originating from the same families and environment, which included earlier diagnosis and therapy in the younger sibling.

Age at onset of ESRF and life expectancy in hemizygous patients

Out of 44 hemizygous patients with detailed information about their treatment and kidney status, all 13 untreated patients had already reached ESRF, with a mean age of 48.9 \pm 13.7 years, and all 3 very late-treated patients reached ESRF, with a mean age of 51.7 \pm 4.2 years (Table 1; Fig. 2A). In contrast, none of the 12 late-treated patients and none of the early-treated patients (n=16) reached ESRF, even though 9 of the treated patients were 45 years and older. The mean times on therapy were 6.0 years in the very late therapy group, 11.9 \pm 7.9 (maximum 28) years in the late therapy group and 7.4 \pm 6.2 (maximum 24) years in the early therapy group. Remarkably, two treated patients were older than 60 years and had fair kidney function (eGFR above 40 ml/min), while one untreated brother and one untreated cousin both reached ESRF before the age of 40 years and one died after kidney transplantation before the age of 50 years. The Kaplan-Meier curves separated into all four categories are shown as suppl. Fig. S1.

The mean ages at onset of therapy were 44.0 (range 41-47) in the very late therapy group, 34.8 (14-50) in the late therapy group and 14.7 (2-39) years of age in the early therapy group. The nephroprotective effect of early RAAS blockade is also supported by the current stage of disease of individual patients in Table 1, with a majority of early-treated patients in stages 0 (isolated microscopic hematuria) and I (microalbuminuria). These data show the first evidence that the strong nephroprotective effect of RAAS blockade, if initiated pre-emptively, has the potential to withhold ESRF over the course of an individual's lifetime.

These data on ESRF are very relevantly supported by the life expectancy of our patients: age at premature death was documented in 5 out of 13 untreated patients (Fig. 2B) with a mean life-expectancy of 59.2 \pm 9.6 (range 47 to 70) years. None of the treated patients died, demonstrating that early nephroprotective therapy not only delayed ESRF but consequently also improved life expectancy.

Further analyses of long-term kidney function are visualized in Figure 3. In 11 children and adolescents between 3 and 18 years, (normal) eGFR slowly decreased over time (Fig. 3A), paralleled by a slow increase in serum creatinine (Fig. 3C). The eGFR decreased by approximately 2 ml/min per year; however, no formula has ever been validated in children with a GFR above 90 ml/min/1.73m² [22]. Similarly, the slow increase in serum creatinine in

adolescents can be a result of gaining weight and muscle mass rather than losing kidney function. Unfortunately for comparison with untreated children in the same age range, all children with a G624D variant in the EARLY PRO-TECT Alport study either were randomized to the Ramipril-arm or treated open-label with Ramipril. Therefore, we were not able to compare untreated vs. treated children in order to show a difference between slope decline of GFR in treated vs. untreated children to claim benefit.

However, the data on treated adults between 35 and 65 years of age revealed a more reliable and better interpretable result: eGFR only decreased by approximately 1 ml/min/year (Fig. 3B). Again, serum creatinine levels paralleled the eGFR results (Fig. 3D): serum creatinine only very slowly increased over time with all patients with serum creatinine levels still below 2.0 mg/dl.

Age at onset of ESRF and life expectancy in heterozygous patients

Out of 36 heterozygous patients with detailed information about their treatment and kidney status, 14.3% (4/28) of untreated patients reached ESRF at a mean age of 53.3 \pm 20.7 years (range 40 to 88 years), but none of the treated patients reached ESRF. The mean times on therapy were 9.2 years in the late therapy group and 7.5 years in the early therapy group (Table 1). In parallel, life expectancy seemed to be impaired in the untreated group, with a mean age at death of 72.3 \pm 19.6 years (range 49 to 89 years).

DISCUSSION

Monogenic kidney diseases provide opportunities to investigate treatment effects in a rather small but very well defined population [23]. The present study used the progressive hereditary kidney disease AS to investigate the effects of early intervention on ESRF and life-expectancy. The missense variant p.(Gly624Asp) in *COL4A5* is the most common disease-causing X-linked AS variant in Europe [18]. Our study tested the hypothesis that therapy with renin-angiotensin-aldosterone system (RAAS) inhibition has the potential to delay ESRF for a lifetime in AS patients with disease-causing missense variants if started prior to significant renal fibrosis and kidney damage. With 114 individuals in our investigation, we explored the largest cohort with an identical disease-causing AS variant ever studied, excluding the bias of different genotypes, which limited the informative value of previous studies [8,13,14,24,25]. The identical genotype put us in the unique situation to compare pairs or triplets of treated vs. untreated brothers or cousins originating from the same families and environments in Central Europe. The European registry data, published in 2012, were limited to retrospective information and had a risk of selection bias [8]. In contrast, the present study also used prospective data. Most importantly, the identical pathogenic missense variant p.(Gly624Asp) allowed further intra- and inter-familial comparison.

The missense variant p.(Gly624Asp) has been repeatedly described as mild or hypomorphic in some previous publications [16-18]. However, this might be a rather problematic assessment resulting from comparisons to more severe truncating variants [14,24]. Our study clearly demonstrated that the p.(Gly624Asp) variant led to ESRF in 100% of untreated or very late treated hemizygous patients. In contrast, an early or even a relatively late start of RAAS blockade prevented ESRF in all treated patients. Of note, heterozygous females with this variant, if untreated, unexpectedly showed a significant risk of progressive kidney disease, with 14.3% at ESRF. In a previous natural history study, the general risk of ESRF for heterozygous individuals with X-linked AS was approximately 30% at the age of 60 years (12% at the age of 40 years) [25]. Again, none of the treated heterozygous female patients in our study deteriorated to ESRF. Although the progression of renal disease was slower (which can be interpreted as milder), our data indicate that untreated heterozygous female patients still have a lower but still very significant risk for ESRF, which also affects their life-expectancy. RAAS blockade again seems to effectively minimize the risk for ESRF in heterozygous female patients [26]. We suggest classifying the missense variant p.(Gly624Asp) as fully causative, but well treatable, the term hypomorphic is misleading with respect to the patient's significant lifetime risk of ESRF and should be avoided.

In the ESCAPE trial, strict blood pressure control with ramipril slowed the progression of renal failure in children at late stages of CKD [27]. In the long-term follow up, a reduction in early proteinuria predicted renal survival in this population [28]. The EARLY PRO-TECT Alport trial pre-emptively treated children at early stages of CKD with ramipril [13]. This trial also resulted in a positive signal towards the long-term nephroprotective effects of early ACEi. Interestingly, 9/66 (13.6%) of children in the EARLY PRO-TECT trial had the missense variant p.(Gly624Asp) in *COL4A5* [29]. This variant was the most common variant in the trial, possibly caused by the slower course of disease in these children increasing the chance of fulfilling the inclusion criteria at very early stages of disease.

One issue our study cannot address is the presence of any intra-individual variation in the genetic background. Furthermore, the study has several additional limitations: First, possible additional risk factors such as hypertension, nephrotoxic medication or environmental factors including salt intake, animal protein intake, body weight, and physical activity are not mentioned, all of which might play an important role in aggravating of renal disease [8,12,20]. GFR-formulas in children, for example, are very likely influenced by increases in muscle mass. Age could be excluded as a potential confounder as ages between treated and untreated groups did not differ. Second, some of the data of our study are retrospective in nature. Nevertheless, our files also include up to 15 years of prospective data. The third limitation originates from the non-interventional observational design in a small population, hindering us from initiating or harmonizing therapeutic intervention. Further, the observational design only allowed us to increase evidence only for a causative association of the better

renal outcome in Alport patients under RAAS blockade [30]. Limited information hindered us to adjust or account for dosing of RAAS-blockade, however, the children in our cohort were all treated with a high dose of ramipril (about 5 mg/m², equivalent to 7.5 to 10 mg/day in adults) [13]. We tried to limit these shortcomings with a prospective follow-up design in most patients. In this context, the present data build on previous publications of retrospective data in AS [8,14,26]. The fourth limitation originates from different ages at onset of therapy in the different groups (Table 1; Suppl. Fig. S1 A), which likely also create a bias towards “evolution of standard of care over time” rather than an isolated effect on RAAS-blockade. Still, comparison of late therapy vs. untreated + very late treated revealed significant differences (p=0.006 in Log Rank Test; Suppl. Fig. S1 C and D). In the future, worldwide efforts with evidence synthesis of international retrospective and prospective data such as ASTOR (ClinicalTrials.gov NCT00481130) will hopefully further improve the knowledge base.

In elderly AS patients in our study, kidney function was still quite preserved in those who received early therapy, eGFR only decreased by approximately 1 ml/min/year (Fig. 3B), which – very relevantly - reflects about the physiological normal annual loss of eGFR in healthy individuals [31]. Thus, RAAS-blockade provides the chance for the younger generation of AS patients to have their renal function preserved for their lifetime by multi-modal therapy starting with early ACE inhibition [21,32,33].

In conclusion, in children with Alport syndrome, early therapy might have the potential to delay renal failure for their lifetime of those with missense variants, thus radically improving quality of life without the need for renal replacement therapy and life-expectancy. Alport syndrome has become a treatable disease. In addition, the best treatment effect depends on early diagnosis prior to substantial renal damage and scarring. This tremendous chance in oligosymptomatic children with AS must put pressure on national child health programs, pediatric societies, and everyday pediatric practice to obtain an early diagnostic workup in every child with microscopic haematuria [21]. Every child should be seen for the evaluation of glomerular hematuria and benefits from genetic testing for AS to allow early nephroprotective intervention.

CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract format. All authors declare no competing interests.

FUNDING

The consumables and workplace for maintenance of the European Alport therapy registry have been supplied by the University Medical Center Goettingen, Germany. The European Alport Registry has been supported by the Association pour l'Information et la Recherche sur les Maladies Rénales Génétiques (AIRG) (to O.G.) from 2006 to 2012. This work is supported by the German Research Foundation DFG (GR 1852/6-1 to OG) and has been supported by the German Federal Ministry of Education and Research BMBF (01KG1104 to OG). The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

ACKNOWLEDGEMENTS

The trial was registered at www.ClinicalTrials.gov (NCT02378805). This work is supported by the German Research Foundation DFG (GR 1852/6-1 to OG) and has been supported by the German Federal Ministry of Education and Research BMBF (01KG1104 to OG). JH is supported by the DFG (HO 2583/8-3). BB is supported by the DFG - Clinical research unit (KFO) 329 (BE6072/2-1 and BE6072/3-1). MG is supported by the DFG (Go766/18-2, SFB 1365). CB holds a part-time faculty appointment at the University of Freiburg in addition to his employment with the Limbach Group. His research lab receives support from the Deutsche Forschungsgemeinschaft (DFG) (BE 3910/8-1, BE 3910/9-1, and Collaborative Research Center SFB 1453) and the Federal Ministry of Education and Research (BMBF, 01GM1903I and 01GM1903G). The authors thank the Society for Pediatric Nephrology (Germany) and the German Society of Nephrology for their support. The authors thank the trial coordinator T. Albrecht-Nock, the national Alport patient group in Germany for their support and for sharing their individual medical history to help planning and implementing this trial. We are most thankful to our patients, our pediatric patients and their parents that they have volunteered for this very long study.

DATA AVAILABILITY STATEMENT

Please contact the corresponding author regarding data requests.

STROBE statements are provided as supplemental material.

AUTHORS' CONTRIBUTIONS

OG wrote the first draft of the article with contributions from JB, JES, JS and VT. JH, KMR, MN, BB, MC, MG, CB, JS carried out the molecular genetic analyses and contributed data about the medical family history. OG, JS and JB verified the underlying data, data of individual groups of patients were verified by JES and VT (as part of their MD-thesis). JH, KMR, MN, BB, MC, MG, CB, JS, VT and JB contributed to the development and conduct of the study. All authors edited and approved the final version of the article. OG as guarantor accepts full responsibility for the conduct of the study, had access to the data, and controlled the decision to publish.

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Characteristic	Hemizygous (n=49) ¹								Heterozygous (n=65) ³					
	untreated ²		very late therapy		late therapy		early therapy		untreated ⁴		late therapy		early therapy	
ESRF total number (percent)	13/13	(100)	3/3	(100)	0/12	(0.0)	0/16	(0.0)	4/28	(14.3)	0/6	(0.0)	0/2	(0.0)
age at ESRF mean in years and SD (range)	48.9 +/- 13.7	(27-70)	51.7 +/-4.2	(47- 55)	-	-	-	-	53.3 +/-20.7	(40-88)	-	-	-	-
received kidney transplant total number (percent)	3/13	(23.1)	1/3	(33.3)	-	-	-	-	0/4	(0.0)	-	-	-	-
death total number (percent)	7/13	(53.8)	-	-	-	-	-	-	4/28	(14.3)	-	-	-	-
age at death mean in years and SD (range)	59.2 +/-9.6	(47-70) ⁵	-	-	-	-	-	-	72.3 +/-19.6	(49-89)	-	-	-	-
age at start of therapy mean in years (range)	-	-	44.0	(41- 47)	34.8	(14- 50)	14.7	(2-39)	-	-	28.6	(9-45)	18.5	(5-32)
current age mean in years (range)	-	-	58.5	(55- 62)	44.6	(28- 64)	22.4	(8-59)	-	-	-	-	-	-
time on therapy mean in years (range)	-	-	6.0	(6-6)	11.9	(1-28)	7.4	(0-24)	-	-	9.2	(3-16)	7.5	(1-14)
current stage of disease total number (percent)														
stage 0	0	(0.0)	0	(0.0)	0	(0.0)	3	(18.8)	2	(10.5)	0	(0.0)	1	
stage I	0	(0.0)	0	(0.0)	3	(25.0)	11	(68.8)	11	(57.9)	1	(16.7)	1	
stage II	0	(0.0)	0	(0.0)	6	(50.0)	2	(12.5)	1	(5.3)	2	(33.3)	0	(0.0)
stage III	0	(0.0)	0	(0.0)	3	(25.0)	0	(0.0)	1	(5.3)	3	(50.0)	0	(0.0)
stage IV	8	(66.7)	3	(100.0)	0	(0.0)	0	(0.0)	4	(21.1)	0	(0.0)	0	(0.0)

Table 1. Patients' characteristics

Patients' characteristics includes both co-primary end-points (age at end-stage renal failure and age at death), age at start of therapy, current age, time on therapy and present stage of disease.

¹ forty-eight hemizygous males plus one homozygous female.

² five children (median age 8 years, range 3 to 15) in CKD stage 0 are not included in the table, because therapy has not yet been started.

³ no data on medical therapy in 29 heterozygous patients.

⁴ two girls in CKD stage 0 and several girls in stage I are untreated.

⁵ age at death analyzed in 5/13 patients (no data from age at death in 2 patients)

Stage 0 isolated hematuria without microalbuminuria

Stage I microalbuminuria: 30-300 mg albumin/g creatinine (gCrea)

Stage II proteinuria: >300 mg albumin/gCrea, eGFR > 60 ml/min

Stage III eGFR \leq 60ml/min

Stage IV end-stage renal failure

ESRF = end-stage renal failure

SD = standard deviation

Very late therapy = start of therapy in stage III

Late therapy = start of therapy in stage II

Early therapy = start of therapy in stage 0 or I

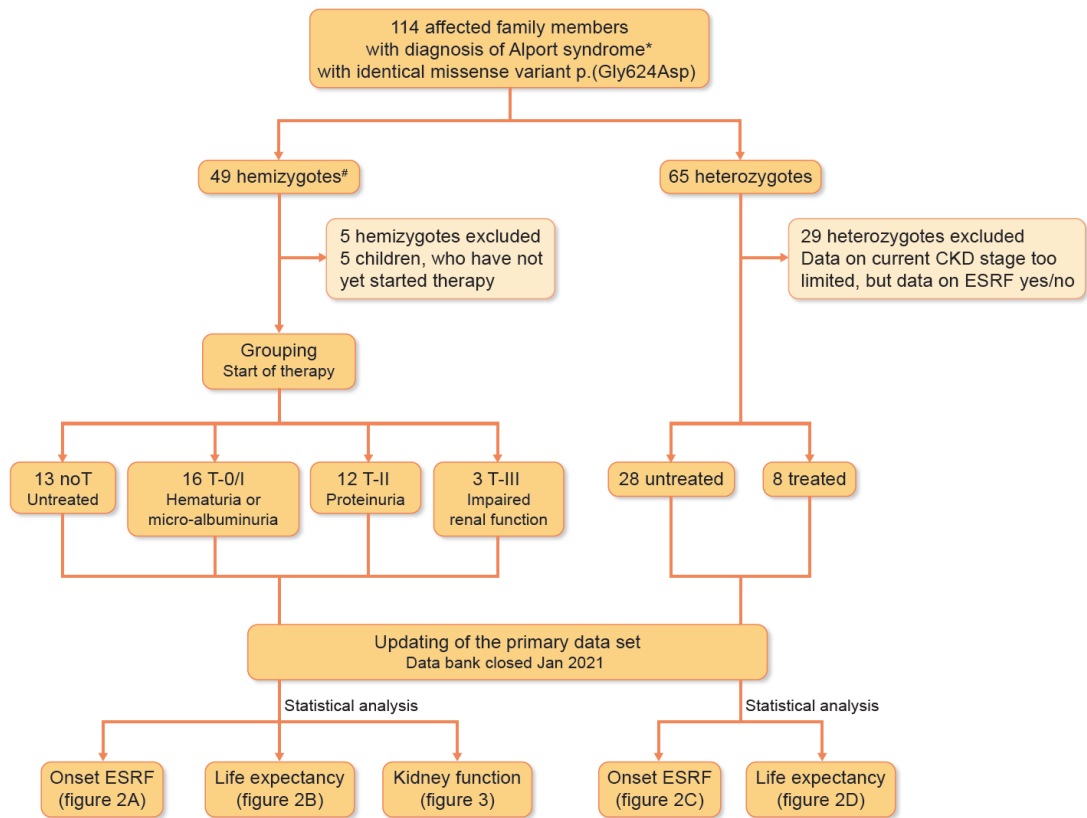


Fig. 1 Flow Chart

A total number of 114 affected family members were included in the study, all non-affected family members were excluded. Five hemizygous boys in CKD level 0 were excluded from further analysis, because therapeutic intervention had not yet been started. In addition, 29 heterozygous females were excluded from analysis of age at onset of RRT and life-expectancy, because of limited clinical information (only information about the renal status of not have reached ESRF).

* diagnosis based on clinical symptoms, kidney biopsy results and/ or mutation analysis.

48 hemizygous males plus one homozygous female

noT = no therapy;

T-0/I = early therapy, start with isolated microscopic hematuria (T-0) or with microalbuminuria (T-I);

T-II = late therapy, start with proteinuria, eGFR>60 ml/min;

T-III = very late therapy, start with eGFR<60 ml/min.

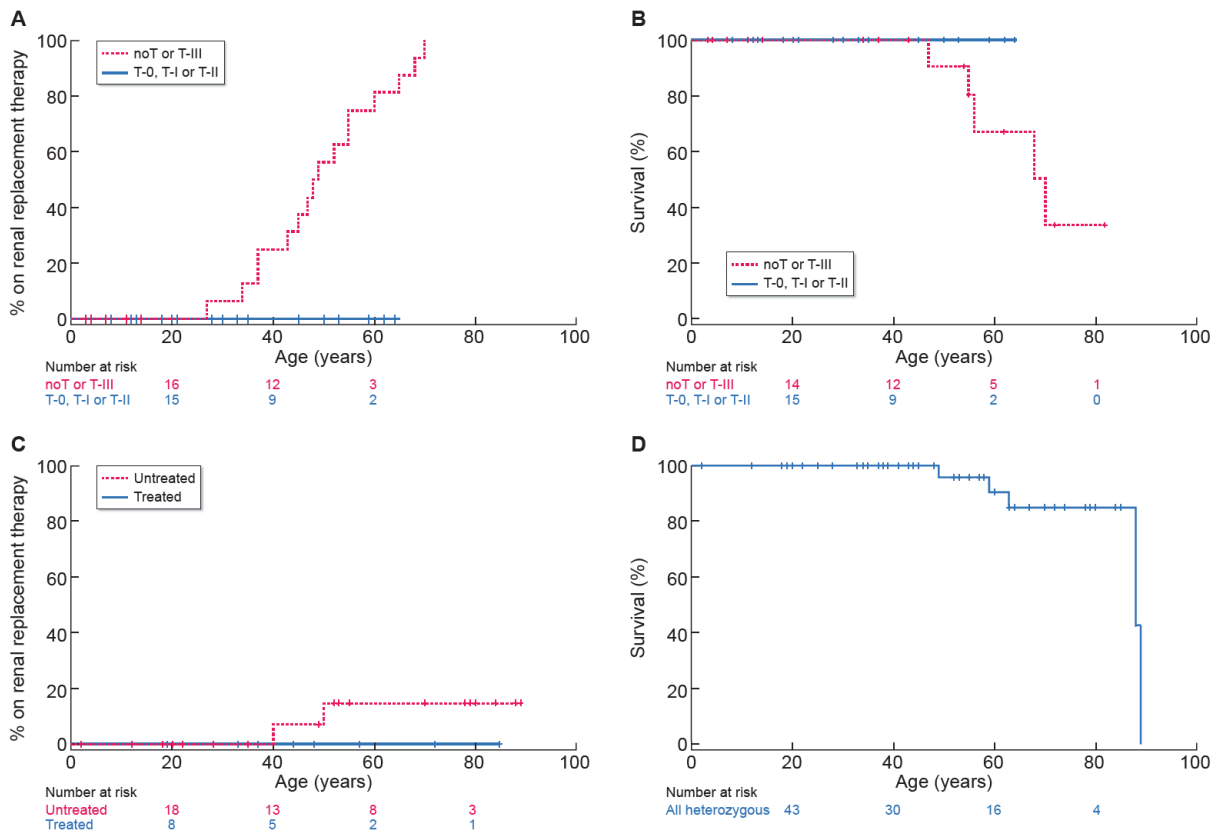


Fig. 2 Effect of therapy on renal failure and on life-expectancy

Panel A Hemizygous patients: Comparison of age at ESRF in untreated and very late treated patients (dotted line, n=21)* vs. early and late treated (solid line, n=27) demonstrates a strong nephroprotective effect of RAAS blockade, if initiated early during the course of Alport syndrome.

Panel B Hemizygous patients: Life-expectancy of untreated and very late treated patients (dotted line, n=19)* vs. early and late treated (solid line, n=27): early therapy improves life-expectancy.

*includes five children (median age 8 years, range 3 to 15) in CKD stage 0, who have not yet started therapy.

Panel C Heterozygous patients: Comparison of age at onset of renal replacement therapy in untreated patients (dotted line, n=24) vs. treated (solid line, n=10) demonstrates a nephroprotective effect of RAAS blockade.

Panel D Heterozygous patients: Life-expectancy of all patients (solid line, n=52) implicates that the heterozygous stage causing chronic kidney disease might represent a risk factor for premature death in some individuals.

noT = no therapy;

T-0/I = early therapy, start before onset microalbuminuria or with microalbuminuria;

T-II = late therapy, start with proteinuria, eGFR>60 ml/min;

T-III = very late therapy, start with eGFR<60 ml/min

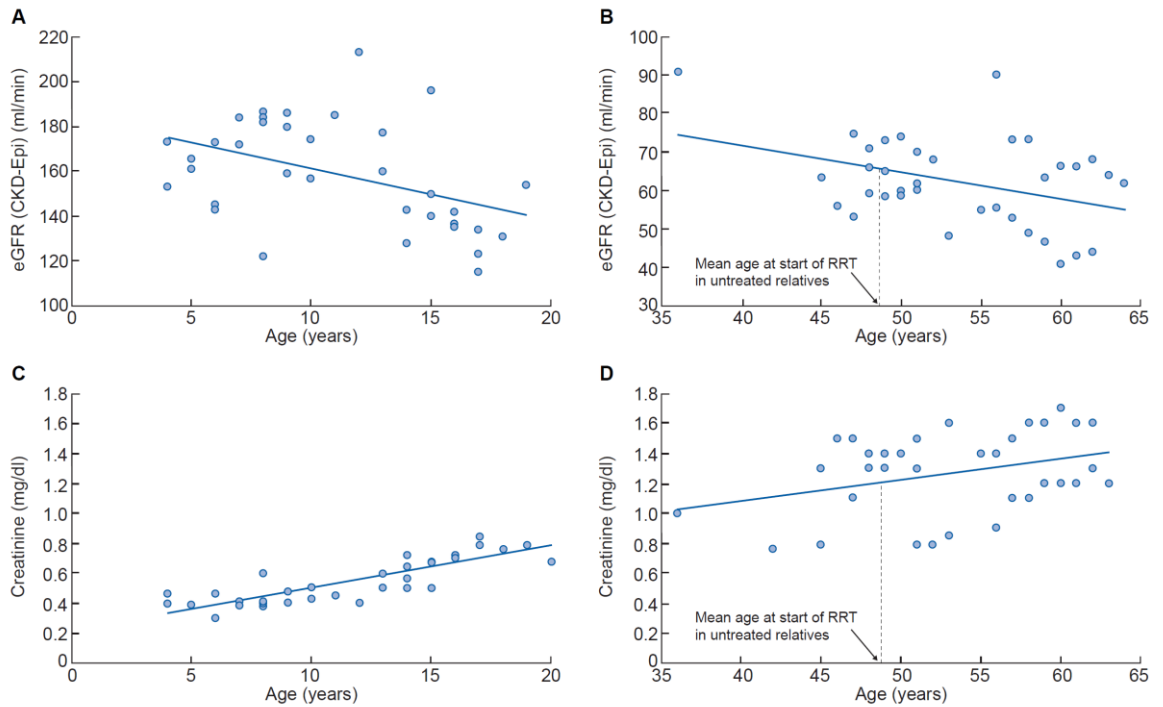


Fig. 3 Long-term decline of renal function in treated adolescents and adults

Panel A Decline of eGFR in adolescents with early therapy (T-0 and T-I) over a time span of 15 years (data values from 11 individual patients between 3 and 18 years of age, each child contributed between 2 and 6 values).

Panel B Decline of eGFR in adults with early or late therapy (T-0, T-I, T-II) over an individual follow-up time span of almost 30 years (data values from individual patients between 35 and 65 years of age). Under therapy, the annual loss of GFR is diminished to about 1 ml/min. The dotted line represents the mean age at onset of ESRF of the untreated brothers and cousins.

Panel C Slope of serum creatinine in adolescents with early therapy (T-0 and T-I) over a time span of 15 years (data values from individual patients between 3 and 18 years of age).

Panel D Slope of serum creatinine in adults with early or late therapy (T-0, T-I, T-II) over a time span of almost 30 years (data values from individual patients between 35 and 65 years of age). The dotted line represents the mean age at onset of ESRF of the untreated brothers and cousins. RRT = Renal Replacement Therapy

The solid lines represent the regression line.