Incidence of renal failure and nephroprotection by RAAS inhibition in heterozygous carriers of X-chromosomal and autosomal recessive Alport mutations

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We studied here the clinical course of heterozygous carriers of X-linked Alport syndrome and a subgroup of patients with thin basement membrane disease due to heterozygous autosomal recessive Alport mutations whose prognosis may be worse than formerly thought. We analyzed 234 Alport carriers, including 29 with autosomal recessive mutations. Using Kaplan–Meier estimates and log-rank tests, autosomal and X-linked carriers were found to have similar incidences of renal replacement therapy, proteinuria, and impaired creatinine clearance. Further, age at onset of renal replacement therapy did not differ between X-chromosomal and autosomal carriers. Both groups showed an impaired life expectancy when reaching renal replacement therapy. RAAS inhibition significantly delayed the onset of end-stage renal failure. Not only carriers of X-linked Alport mutations but also heterozygous carriers of autosomal recessive mutations were found to have an increased risk for worse renal function. The risk of end-stage renal disease in both groups affected life expectancy, and this should cause a greater alertness toward patients presenting with what has been wrongly termed ‘familial benign hematuria.’ Timely therapy can help to delay onset of end-stage renal failure. Thus, yearly follow-up by a nephrologist is advised for X-linked Alport carriers and patients with thin basement membrane nephropathy, microalbuminuria, proteinuria, or hypertension.


KEYWORDS: Alport syndrome; chronic kidney disease; collagen; familial benign hematuria; fibrosis; renal insufficiency; thin basement membrane disease

Alport syndrome is a hereditary basement membrane disorder due to mutations within type IV collagen genes. Mutations of the COL4A5 gene encoding the α5-chain of type IV collagen lead to X-linked Alport syndrome (XLAS), whereas mutations of the COL4A4/COL4A3 genes encoding the α3/α4 (IV) chains cause the autosomal recessive form (ARAS) of the disease. Men with XLAS and patients of both genders with homozygous ARAS mutations represent the completely developed pattern of the disease with end-stage renal failure during adolescence or early adulthood, including hearing loss and ocular lesions in most of them. In these patients, the course of Alport syndrome has been well studied, including their genotype-phenotype correlations and the beneficial effect of timely nephroprotective therapy.

Analysis of heterozygous XLAS carriers in the European Community Alport Syndrome Concerted Action showed a large variability of the clinical course. Hematuria was found in 96% and proteinuria in 75% of the patients; 18% reached end-stage renal failure (59% of them before the age of 40 years). These data showed that the diagnosis of heterozygous COL4A5 mutations is not equivalent to a benign course of disease. The reasons for the large inter- and intrafamilial variability in clinical manifestations in those female patients are only partially known; probably X-inactivation has a pivotal role.

Heterozygous COL4A3/COL4A4 mutations result in the phenotype of familial benign hematuria or thin basement membrane nephropathy (TBMN). Affected subjects typically present with hematuria. Histologically, TBMN is characterized by a uniformly thinned glomerular basement membrane. Having been regarded as ‘benign’ familial hematuria for a long time, these patients might have an increased risk to develop severe renal impairment—comparable to the findings in female XLAS carriers (see
above). TBMN is not a rare disease, as at least 1% of the population is affected; in a series of 76 renal transplant biopsies, even 5.2% of the kidneys presented a thinned glomerular basement membrane. For the first time, the present study compares the risk of renal impairment, end-stage renal disease, and premature death between heterozygous carriers of XLAS and ARAS mutations. In addition, the nephroprotective effect of renin–angiotensin–aldosterone system (RAAS) blockade in patients with heterozygous Alport mutations is evaluated.

RESULTS

Founded in 2006, the European Alport Registry retraces clinical and therapeutic data on several decades of Alport families across Europe. Patient information, study protocol, questionnaire (see Supplementary Material online), consent form (in English, French, Spanish, and German), data collection, anonymization, and storage conform with good clinical practice guidelines, and were approved by the Ethics Committee (AZ 10/11/06; authorization of French data by the Commission Nationale de l’Informatique et des Libertés #908249).

Heterozygous ALAS and XLAS mutations both can lead to impaired renal function and end-stage renal disease

Analysis included 234 patients, 29 of them with ARAS mutations (14 men and 15 women); the mean age of all patients was 35.1 ± 19.5 years, and the mean age at start of RAAS blockade therapy was 28.2 ± 15.7 years. In all, 3 (10.3%) of these ARAS patients are currently under renal replacement therapy, 5 (17.2%; mean age 45 years) show an impaired creatinine clearance, 10 (34.5%; mean age 35 years) present with proteinuria, and 10 (34.5%; mean age 15 years) with microalbuminuria. In all, 189 patients had a XLAS mode of inheritance (16 patients with unknown mode, but biopsy-proven thin basement membrane disease). Of them, 29 (15.4%) are under renal replacement therapy, 27 (14.3%; mean age 48 years) have an impaired creatinine clearance, 63 (33.0%; mean age 29 years) present with proteinuria, and 35 (18.5%; mean age 24 years) with microalbuminuria (Figure 1 and Table 1).

Heterozygous ARAS and XLAS mutations both can lead to renal replacement therapy late in life

A total of 41 patients (34 XLAS carriers) reached end-stage renal failure (median age at onset of renal replacement therapy (RRT) 49 years, 95% confidence interval (CI) 59–75 years; 1 male, 40 females; Figure 2). Of them, 6 had a nephroprotective RAAS blockade before start of RRT; 11 received a kidney transplant, and 3 of these kidney transplants failed (after 16, 15, and 7.5 years). There are no significant differences in age at onset of RRT between the groups.

Progressive renal failure might affect life expectancy in heterozygous carriers

Life expectancy was evaluated in 213 carriers; 21 of these patients were on RRT (transplanted or dialysis). A total of seven patients died (two transplanted patients, four under dialysis, and one under dialysis, who had undergone transplantation earlier). As this is a relatively small number of events, the median time of survival could not be determined. Therefore, survival is described by the 75% quartile, which is calculated to be 70 (95% CI 67–undetermined years). ‘Time on dialysis’ was found to affect life expectancy; therefore, end-stage renal disease in Alport carriers seems to increase the risk of premature death (Figure 3; Supplementary Materials S1 and S2 online).

RAAS blockade delays renal failure in heterozygous Alport carriers

A total of 111 patients (47%) received nephroprotective therapy (mean age at onset of therapy 28 years): 109 were treated with angiotensin converting enzyme inhibitors (ACEis), 34 with angiotensin receptor blockers, and 21 with a combination of ACEis and angiotensin receptor blockers (Figures 4 and 5). The number of ARAS heterozygotes on therapy was too low for separate analysis in Figure 4. Average time on therapy was 5.8 years (s.d. = 3.8 years, range 1–21 years). Among the patients under therapy, 53 (47.4%) currently have proteinuria, 27 (24.3%) have an impaired creatinine clearance, and 17 (15.3%) have microalbuminuria. Six patients (5.4%) patients reached end-stage renal failure.
despite RAAS blockade. In contrast, 124 patients did not receive any nephroprotective therapy, 7 of them (5.7%) showed an impaired creatinine clearance, 20 (18.0%) had proteinuria and 37 (33.3%) had microalbuminuria; 28 untreated subjects (25.2%) reached end-stage renal failure (Figure 4).

Age at onset of RRT in patients having received a nephroprotective therapy before end-stage renal failure differs from untreated patients: onset of RRT occurred significantly later in carriers who were treated with RAAS blockade (Figure 5; P < 0.0001). Whether this association is causal requires further study. Subgroup analysis revealed a significant effect of RAAS blockade in delaying onset of RRT in XLAS carriers (P < 0.0001), as well as in ARAS heterozygotes (P = 0.0231; log-rank test, Supplementary Material S1 online).

FIGURE 2 | Age at onset of renal replacement therapy in carriers with either X-linked Alport syndrome or autosomal recessive form mutations does not differ significantly. Kaplan–Meier estimate.

FIGURE 3 | Progressive renal failure leading to end-stage renal disease can affect life expectancy in heterozygous carriers of Alport mutations. Kaplan–Meier estimate for all carriers including those on renal replacement therapy (n = 21) and for the female population in Germany in 2008 (black, Human Mortality Database, University of California, Berkeley, CA, and Max Planck Institute for Demographic Research (Germany)). Available at http://www.mortality.org or http://www.humanmortality.de (data downloaded on 31 August 2011).

FIGURE 4 | Prevalence of renal replacement therapy (RRT), impaired creatinine clearance (Imp. clear.), proteinuria, and microalbuminuria (Microalb.) in patients with and without renin-angiotensin-aldosterone system blockade.

FIGURE 5 | Onset of renal replacement therapy is significantly delayed in carriers with preemptive nephroprotective therapy before end-stage renal failure (P < 0.0001). Kaplan–Meier estimate.

DISCUSSION

The aim of the study was to show potential differences in the clinical course of renal damage in heterozygous carriers with either XLAS or ARAS mutations. Further, for the first time, the study investigates the possible impact of a nephroprotective RAAS inhibition in this population.

Our study is in agreement with previous findings in 288 female XLAS carriers with a widespread range of disease severity. The risk of end-stage renal disease was about 30% at the age of 60 years; chronic renal failure appeared in 12%.9 Forty years before, Gaboardi and co-workers described similar results in 19 Alport families.20 Similar to previous investigations,9 our study might as well overestimate the risk of end-stage renal disease in XLAS carriers (as our questionnaires asked for other affected family members). Possible additional risk factors such as hypertension, nephrotoxic medication, or kidney donation21 might have an important role in aggravation of renal disease. Further studies in animal models for TBMN should focus on these risk factors in order to find new nephroprotective strategies.22
Although the increased risk for renal failure in XLAS carriers is well established, the prognosis of TBMN due to heterozygous ARAS mutations might be worse than formerly supposed. In our study, ARAS and XLAS carriers show no significant differences in the incidences of RRT, proteinuria, and impaired creatinine clearance (Figures 1 and 2). Microalbuminuria was even more common in ARAS than in XLAS carriers. Age differences between both groups were excluded as a potential confounder (as older patients show a higher incidence of renal damage caused by other reasons such as diabetes, hypertension, atherosclerosis, etc.). ARAS carriers had an even lower mean age than patients with X-linked disease (Table 1).

Our study design and our questionnaires might have overestimated the risk of end-stage renal disease in TBMN. Patients who have not been recognized as heterozygous carriers were not included in any analysis. However, every single patient with end-stage renal failure due to TBMN still presents a very significant finding, as this is in contrast to the synonym of TBMN: ‘familial benign hematuria’. Further, the high prevalence of 1:100 makes TBMN a major source of chronic renal disease in the general population.

RAAS blockade significantly delays the onset of end-stage renal failure in proteinuric Alport carriers who received therapy before onset of RRT (Figure 5). This beneficial effect is not caused by a selection bias toward ‘better’ patients in the treatment group: the number of patients with proteinuria and impaired renal function is even higher in the therapy group (as both medical conditions increase the likelihood of starting therapy; Figure 4). Because of the low numbers of ARAS heterozygotes on therapy, our study could not analyze XLAS and ARAS groups separately in Figure 4. For that reason, our results (mostly on heterozygous XLAS) should not be generalized for all ARAS heterozygotes. Further genotype-phenotype analyses in ARAS mutations would be of great use to preemptively provide patients with therapeutic options. However, one can expect a large number of patients with TBMN to remain undiscovered, because their ongoing renal disease is unrecognized (or otherwise explained by ‘unknown disease’, hypertension, atherosclerosis, or analgesica abuse). Within the next few years, the use of genetic mutation screening will become more common. Thus, TMBN patients can be easily identified, which poses the possibility to start nephroprotective therapy earlier. Accordingly to our study, widespread preemptive RAAS blockade in TBMN patients with microalbuminuria, proteinuria, or high blood pressure could prevent a large number of severe impairments of renal function and even dialysis.

Heterozygous carriers of XLAS and ARAS mutations both show an impaired life expectancy when reaching RRT. Preventive RAAS blockade might prolong life and improve quality of life in these carriers. The relatively high risk of end-stage renal disease and the impaired life expectancy of Alport carriers on RRT should lead to a greater alertness toward patients with TBMN presenting the non-benign symptoms of familial benign hematuria. The term ‘familial benign hematuria’ is misleading and has an underlying renal disease that can affect quality of life and life expectancy. As these patients reflect 1% of the total population, the sometimes non-benign course of TBMN and XLAS carriers is relevant to nephrologists. Yearly follow-up by a nephrologist and early-on nephroprotective RAAS blockade should be advised for all newly diagnosed TBMN patients with microalbuminuria, proteinuria, or high blood pressure, and heterozygous XLAS carriers.

MATERIALS AND METHODS
Inclusion and exclusion criteria
The diagnosis of the heterozygous status was proven by (1) mutation analysis or (2) kidney biopsy plus genetic consultation for decision in between XLAS or ARAS inheritance (including a conclusive genealogic tree and/or linkage analysis). Patients were excluded if they were affected males with XLAS or patients with genetically proven homozygous ARAS. Patients were excluded if they did not give informed consent or the diagnosis was suspected but not confirmed or if they donated a kidney (living donor to affected family member).

Primary data collection, assignment, and follow-up
The Registry retraces data on several decades in three generations of Alport syndrome patients, hindering a prospective study design. In all, 310 centers participated in the European Alport Registry over the years. Data were hosted centrally on a non-open access computer and were anonymized at the University of Göttingen, Germany. Questionnaires included demographic data (age, gender, country of origin, family history, mode of inheritance), 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). Data were updated via telephone interviews, email, facsimile, or by personal contact with both physicians and patients. The registry and data storage conform with good clinical practice guidelines, and were approved by the Ethics Committee (AZ 10/11/06; French data by the Commission Nationale de l’Informatique et des Libertés #908249). Data were collected from Germany, France, Spain, Belgium, Austria, Switzerland, United States of America (limited to only those families recently moved from Europe to USA), 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. Of 393 (2%) patients or carriers, 8 were lost to follow-up.

Outcome measures and intervention
The study end points were ‘RRT’, ‘age at onset of RRT’, ‘impaired renal function’ (creatinine-clearance below 60 ml/min), ‘proteinuria’ (proteinuria > 300 mg/day), and ‘age at death’. Owing to the genetic defect in Alport carriers at birth, life expectancy was defined as life span from birth to death. Impaired creatinine clearance was defined as < 60 ml/min in a 24-h urine collection. Proteinuria was defined as > 300 mg protein per day, and microalbuminuria as 30–300 mg protein per day in a 24-h urine collection. The study explored the treatment effects of ACEis and angiotensin receptor blockers; the
control intervention was with out therapy. The most commonly used ACEis were Ramipril (0.025–0.1 mg/kg body weight) and Enalapril (0.125–1.0 mg/kg body weight).

Statistical analysis

Distributions of continuous variables are summarized by means, whereas frequencies and percentages are given for categorical (including binary) variables. The efficacy end points ‘age at onset of RRT’ and ‘age at death’ 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. Median event times are reported with 95% CIs, which are based on log-log transformed CIs of the event probabilities. If the CIs of the event probabilities are too wide across all observed times because of the small sample size, 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 are two-sided, and those smaller than 0.05 are referred to as statistically significant. All inferential analyses were carried out using SAS version 9.2.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary Material S1. RAAS inhibition delays onset of end-stage renal failure in XLAS and ARAS heterozygotes.

Supplementary Material S2. Impact of ‘time on dialysis’ on ‘life-expectancy/survival’ in Alport carriers.

Supplementary Material S3. Letter from the Ethics Committee of the Medical Faculty of the Georg August University Goettingen.

Supplementary Material S4. Questionnaire: Alport syndrome. Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

REFERENCES


