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Outcome of Primary Hyperoxaluria type 1 correlates with *AGXT* mutation type: data from a large European study.

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Running title *AGXT* mutations classes and outcome

Abstract

Primary hyperoxaluria type 1 (PH1) displays a heterogeneous phenotype, likely to be affected by genetic and non genetic factors, including timeliness of diagnosis and quality of care. As previous genotype-phenotype studies were hampered by limited patient numbers the European OxalEurope Consortium was constituted. This preliminary retrospective report is based on 526 patients (410 with *AGXT* genotype). We grouped mutations by the predicted effect as null (N), missense leading to mistargeting (G170R) and other missense (M) and analyzed their phenotypic correlations. Median age of End-stage Renal Disease increased from 9.9 to 11.5, 16.9, 25.1, 31.2 and 33.9 years for the NN (n=88), MN (n=42), MM(n=116), G170RN(n=61), G170R/M(n=32) and G170R/G170R(n=71) respectively. The effect of individual missense mutations was estimated by ranking patient carrying the same mutation according to median ages at onset and at renal failure. The outcome of some recurrent missense mutations and an unprecedented number of G170R homozygotes is described in detail.

Diagnosis of PH1 is still delayed and actions aimed at increasing knowledge and screening of kidney stones is recommended. Our analysis indicates that, in addition to G170R, other causative mutations are associated with later onset of ESRD. The OxalEurope registry will provide tools necessary for characterizing those genetic and nongenetic factors through a combination of future innovative genetic, functional, and biostatistical approaches.

Keywords Primary Hyperoxaluria type 1, AGXT, missense mutation, genotype-phenotype correlations

Introduction

Primary Hyperoxaluria type 1 (PH1, OMIM 259900) is an autosomal recessive disease caused by the deficiency of the liver peroxisomal alanine:glyoxylate aminotransferase (AGT)[1]. It is the most common and severe form of the primary hyperoxalurias,[1, 2] characterized by excessive endogenous oxalate production with urolithiasis, nephrocalcinosis and progressive decline in renal function. Precipitation of insoluble calcium oxalate induces inflammation and fibrosis in kidney tissue[1, 2]. Conservative treatment with hyperhydration and urine alkalization aims to prevent precipitation of calcium oxalate. In the majority of patients the ongoing exposure to excess oxalate leads to development of end-stage renal disease (ESRD), requiring renal replacement therapy and liver-kidney transplantation[3].

More than 150 mutations in the human alanine:glyoxylate aminotransferase (*AGXT*) gene resulting in various degrees of enzyme deficiency have been documented[4]. The majority are missense mutations that would allow some therapeutic intervention on protein folding and targeting. The most common, p.Gly170Arg (G170R), accounting for 25-40% of the disease-causing alleles in Caucasian patients[5, 6], is associated with significant residual catalytic activity and immunoreactivity in liver biopsy[7]. The G170R mutation appears responsive to pyridoxine (vitamin B6 the natural cofactor of AGT) treatment[8, 9] and G170R homozygosity has been associated with a more favourable clinical course[8, 9,10]. For some other missense mutations (e.g. Ile244Thr, Phe152Ile) pyridoxine can have beneficial effects as both a cofactor and chemical chaperone *in vitro*[11], but clinical efficacy is less certain than for G170R.

Previous PH1 cohort studies addressed clinical and genetic aspects of the disorder, but validity of the findings was limited by the small numbers of patients. The present work, based on a large European cohort of PH1 patients, allows a comprehensive overview of potential genotype effects and provides more information on clinically relevant issues.

Patients and Methods

A European PH scientific consortium, established in 2008 (www.oxaleurope.org), with members obtaining IRB approval for participation. Data from 8 European countries (France, Germany, Italy, The Netherlands, Poland, Spain, Sweden and the United Kingdom) were merged to build a European database of PH1 patients. The earliest diagnosis dates from 1980 and the database was frozen on 31 December 2010 for this analysis. It comprises the available clinical, genetic and biochemical information of 526 patients (male/female: 300/226) who fulfilled the diagnostic criteria of PH1, with median time to follow-up of 11.5 years (I-III quartiles: 4.2-20.2). Countries contributed patients from different geographical origins (Table 1). Diagnosis was made by genetic and/or biochemical analyses (Table 2). Family screening identified 74 patients (14%) with a median time to follow-up of 12 years (I-III quartiles: 6.9-18.6). Complete genotype data was available for 410 patients. Urinary oxalate results were not included due to difficulties harmonizing submitted data. Time to follow-up was defined by the interval from date of symptoms to date of last clinical visit or date of death, renal survival as a function of age and gender by the Kaplan-Meier method, with patient data censored at last follow up. Pyridoxine responsiveness was recorded according to the clinician's report. Outcome was assessed in three diagnostic eras: 1 January 1980- 31December 1989, 1 January 1990- 31 December 1999 and 1 January 2000 – 31 December 2009. Outcome was compared in these subgroups, with censoring at the end of each ten year period.

In the genotype-phenotype analysis we grouped genotypes in three classes according to the expected effect on the protein product (Suppl. 1):

1. *p.Gly170Arg* (G170R): p.Gly170Arg, associated with mitochondrial mistargeting when in cis with the minor allele[12]

2. *Other missense* (M): missense and in frame ins/del that are unlikely to cause complete lack of protein production.

3. *Null* (N): nonsense, frameshift and splice-site mutations, assumed to produce no protein product.

The effect of different genotype classes on time to ESRD was estimated using Cox proportional hazards models with adjustment for gender. Proportional hazard assumption was checked using the Grambsh and Therneau test. Continuous variables were expressed as median and I-III quartiles as measure of variability; categorical variables were expressed as percentages and absolute numbers. F Test was used to evaluate clinical variables among genotype groups. Median times at ESRD and at symptoms were computed for each genotype using the method of Kaplan-Meier and differences were assessed by the log-rank test.

All statistical testing was conducted at the 0.05 level. PASW Statistics (version 20) and R (version 2.13) statistical packages were used for analysis.

Results

Clinical characteristics

The most frequent symptom at onset was urolithiasis: nephrocalcinosis and/or urolithiasis occurred in 70% of patients (Table 2). Infantile onset of symptoms (<1 year) occurred in 29 % (36.6% with ESRD at diagnosis) whereas 15% of patients experienced the first manifestation in adulthood. Median age at diagnosis was 8 years (y) (interquartile range: 2.6-22.2) and 28% of patients were diagnosed beyond 18 y. We compared the number of patients diagnosed in the last three decades and found the highest number in the most recent decade (41, 128 and 146 patients respectively) indicating either an increased awareness or improvements in diagnosis.

ESRD at diagnosis was present in 195 of 451 patients (43%, data not available for 75 patients) at a median age of 31.2y (range: 11.5–40). Of patients diagnosed in adulthood, 70% were in ESRD at the time of diagnosis, versus 29% of patients diagnosed in childhood.

Survival

The cumulative patient survival was 95, 93, 85 and 74% at 5, 10, 30 and 50 years of age respectively (Figure 1). Sixty-seven (14%) patients died. Sixty-four (96%) were in ESRD.

Among the 256 patients with preserved renal function at diagnosis (56%), 74 developed ESRD thereafter (29%). At last follow-up, 267 patients were in ESRD (58%), with data on renal function missing for 67 patients (Table 2).

Progression to ESRD was slightly, but not significantly, earlier in males than in females (log-rank test p-value: 0.155; mean age at ESRD in males: 24.3; I-III quartile: 20.3-31.3; females: 27.2; I-III quartile: 23.5-33.5) (data not shown).

Clinical features in 74 patients diagnosed by family screening

Median age at diagnosis was 6.8y (range 1.5-16). Renal manifestations at diagnosis included urolithiasis 31%, nephrocalcinosis 12%, or both 24%, other symptoms 8% (recurrence post renal transplantation 1%), unknown 5%. ESRD at diagnosis occurred in 16 % vs 43% of index cases. At the end of follow-up, ESRD had occurred in 34% vs 58% of index patients. Overall,

median age at ESRD in the relatives was 23y (range 11.5-34.4). Median ages at onset of symptoms, at diagnosis and at ESRD were not statistically different from index cases (p 0.78, 0.78 and 0.71 respectively).

Genotype-phenotype correlations

Phenotype correlation of AGXT mutation classes

Among the clinical variables considered, age at onset of symptoms, age at ESRD and AGT activity in liver biopsy were significantly different among the genotypes (Table 3).

Onset of symptoms occurred later in G170R homozygotes (median: 6y) and was intermediate in G170R/N compound heterozygotes (median: 4y) compared with the lowest values of N/N homozygotes (median: 3 y) and M/N heterozygotes (median: 2y) (Table 3).

Renal survival by censored Kaplan-Meyer analysis (Fig. 2) showed that having at least one G170R allele was also associated with a slower progression to ESRD (33.9y in G170R/G170R, 31y in G170R/M and 25y in G170R/N heterozygotes) compared with N/N homozygotes (9.9y). M/N heterozygotes (11.5y) and M/M homozygotes (16.9y) had intermediate values; such stratification of the ESRD risk was confirmed by gender-adjusted Hazard ratios (Fig. 3).

In 14/88 N/N homozygous patients, renal function was preserved to at least 16 years; seven of these developing ESRD after 20 years of age.

To explore how individual missense mutations influence age of onset and of ESRD, these were ranked as shown in Figure 4. For both outcomes, the rank position of most mutations was similar in the three series: indeed, it can be noted on simple inspection that mutations associated with early progression to ESRD (e.g. p.Gly116Arg) are recurrent in the different series and the same holds true for mutations ranked in the late progression (e.g. p.Gly47Arg). The similarity of the ranking position is statistically significant (age at onset: p=0.02, age at ESRD: p=0.008). The observed differences in age of onset and of ESRD could not be ascribed

to previously known reasons, as for the majority of mutations *in vitro* activity was reported to be very low (http://www.uclh.nhs.uk/phmd).

In addition to p.Gly170Arg, several other mutations that were found in significant numbers were considered in either homozygous or compound heterozygous states:

p.Ile244Thr. The 31 homozygotes displayed a wide age range at onset (median: 5.8y, range 0-49) and of ESRD (median: 12y, range 0-70). All four p.Ile244Thr/N compound heterozygotes had a neonatal onset, one developed ESRD in the first months of life, while three, all under 5 y, still retain renal function. Eight of the nine patients who were pyridoxine responsive had preserved renal function (mean age at last follow up: 6.7y, range: 1-17), whereas only three of the seven non-responsive patients had preserved renal function (median age at last follow up: 5y, range: 1-38).

p.Phe152Ile is also associated with a wide age range at onset of symptoms in the six homozygotes (median 20y; range: 10 - 24), in the three compound heterozygotes with G170R (median: 14,5y; range: 1.5-26) and in the ten with a Null mutation (median: 0.2y; range: 2-4.5). Three homozygotes developed ESRD after age 30y while the other three, older than 16y, still have preserved renal function (mean age: 24y). This is similar to the finding in p.Phe152Ile/G170R heterozygotes (age at ESRD: 36y in two patients and one, age 12y, with preserved renal function). Overall, there was a much later onset of renal impairment in this group.

p.Met195Arg. The ten homozygotes had early onset of symptoms (median: 2y, range: 0-3) but similar age at ESRD compared to other mutations (median: 16y, range: 9-22) and two patients have preserved renal function at 20y and 16y.

p.Asp201Glu. Five out of seven homozygous patients presented early (median age at onset: 0.5y, range: 0.5-2.7) but retained kidney function at last follow up (median age at last follow up: 13y, range: 4-15 y). The two other homozygotes, with neonatal onset of symptoms, developed early ESRD (3 months and 1 year respectively). The single heterozygous p.Arg201Glu/N patient has preserved kidney function at last follow up (10y).

p.Ser81Leu is associated with an earlier onset of ESRD when in compound heterozygosis with a Null mutation (two patients with ESRD at 10 and 14 y) than with a p.Gly170Arg mutation (2 patients older than 20 y with preserved renal function).

p.Arg36Cys. The two homozygous patients and two p.Arg36Cys/G170R heterozygotes display a wide range of onset ages (3, 6, 17, 58 years) but late ESRD (the two homozygotes at 58y; one p.Arg36Cys/G170R at 67y and the other one with preserved renal function). No compound heterozygotes with a Null mutation were present in the database.

Discussion

This study is based on the largest database of PH1 patients available currently through pan-European collaboration of centres of expertise for PH, some of which serve as diagnostic referral centres for other non-European countries. Thus the range of nationalities represented here is wider than the eight OxalEurope countries. The clinical characteristics of this series differ from those of the US registry data[13]: the median age of first symptoms was 3.9y compared with 5.5y[13]; median age at diagnosis was slightly younger (8.1y compared to 10y). While urolithiasis was the most common presenting symptom, it was less common overall than in the US study where it accounted for 90% of presenting symptoms. We analyzed PH1 patients exclusively, contrary to the US study that included all types of PH, and may explain the different age of onset of symptoms between the studies, whereas the relatively low number of patients presenting with kidney stones or nephrocalcinosis may be due to the relatively high number of patients found by family screening.

With regard to clinical findings, we found considerable diagnostic delay; it is alarming that in 3% of patients diagnosis was only made after premature failure of an isolated kidney graft. Overall, 43% of patients presented in ESRD in contrast to 20% reported in the US study although the median ages of US patients were slightly lower (23y vs 31.2y in this study). ESRD was also the presenting feature in 30% of children. Late diagnosis may depend on several factors. Firstly, detection of hyperoxaluria by metabolic analysis is less common in adult than in paediatric practice. Secondly, some patients may only experience occasional stone passage that may not be recognized as a symptom of primary hyperoxaluria. Thirdly, delayed diagnosis may be due to cumbersome biochemical procedures (collection of urine, shipment of specimen) and the multisystem character of PH1 in advanced disease.

In most PH1 patients pyridoxine alone is unable to overcome the deleterious effects of endogenous oxalate excess in the long-term where the only definitive treatment is a liver-kidney transplant, either simultaneously or sequentially[14]. As transplantation is technically challenging in the young, and not available to all PH1 patients worldwide, additional therapeutic agents are needed.

Comparison of diagnosis and follow-up during the three consecutive time decades shows more patients were diagnosed in the last one, which may indicate improved access to diagnostic services and availability of genetic diagnosis. As noted previously, better outcome was observed in patients identified by family screening[15], now advocated with the advent of more routine genetic testing. Recently published guidelines for may assist in improving outcome of PH1 patients[14]. Analysis of our database provides little evidence to support previous anecdotal reports that female patients may have a later age at onset of symptoms and progression to ESRD[16].

We found a difference, not previously described, in clinical outcome with respect to onset of ESRD associated with different genetic subclasses. Our data confirmed the majority of known geno-phenotype associations. Unsurprisingly, the most common pathological variant in our cohort was the p.G170R mutation accounting for approx 40% of alleles, with patients homozygous or compound heterozygous having a more favourable outcome particularly in terms of pyridoxine responsiveness, consistent with results of previous studies[10, 17]. In addition, patients with this mutation had later age of onset and at ESRD. However, 5/70 G170R/G170R homozygotes progressed to ESRD early (before 10 y), possibly reflecting suboptimal clinical management, presence of unidentified comorbidity, environmental factors, other possible genetic modifiers, or a deletion/gross rearrangement of the second allele that may remain undetected if only the index case is analyzed. As we found a clear association between late diagnosis, ESRD at time of diagnosis and G170R mutation, late detection with receipt of suboptimal treatment seems to be an important problem in PH1. As early conservative treatment may delay renal damage, particularly in pyridoxine responsive patients, metabolic screening for all children at first presentation of a kidney stone or nephrocalcinosis, and for all adults with recurrent stone disease, might be a most important recommendation.

At the other end of the spectrum, Null mutations were generally associated with a poor prognosis, in accordance with the complete lack of AGT protein. Nevertheless, a minority of patients with null mutations demonstrated a less severe clinical course; some were even reported to be pyridoxine-responsive although it was not clear whether the degree of response

was as currently defined[14]. However, in some of these cases pyridoxine may work on other possible glyoxylate-using enzymes.

Most, but not all of the missense mutations have now been expressed *in vitro* and the majority have little activity when expressed on the minor allele (http://www.uclh.nhs.uk/phmd and[4]) but some have significant activity on the major allele. Unfortunately the polymorphism status for many of our patients was unknown and therefore in most cases it was not possible to assess whether the polymorphic background contributed to a milder phenotype.

In the present study M/M homozygotes had a better outcome than the N/N and G170R/M heterozygosity was associated with a better outcome than G170R/N, likely due to residual enzymatic activity of the M allele. The same holds true comparing M/N with N/N (table 3, fig 2 and 3). This risk stratification indicates that at least some missense mutations do retain significant enzymatic function.

In an attempt to highlight the effects of individual missense mutations, we ordered the patients carrying the same mutation according to their median age at onset and at ESRD, and compared the rank positions in the M/M, G170R/M M/N series (Fig.4). The similarity of rank order of the missense mutations (p: 0.02) among the three series provides suggestive evidence of their graded residual activity. These findings will require confirmation as the registry expands. In several instances the severity of clinical expression of individual missense mutations as estimated by the above ranks analysis correlated well with AGT activity in liver and *in vitro* findings. Indeed, p.Ile244Thr homozygous patients displayed a wide range of age of onset and of ESRD, possibly due to the differences highlighted in functional studies, where it has been demonstrated that the mutation retains significant activity when expressed on the major haplotype, whereas with the minor haplotype it negatively influences AGT stability[18 19].

Functional studies showed that Phe152Ile AGT mutant in the monomeric state aggregates and needs to be continuously supplied with pyridoxine to remain in a catalytically competent form[17,20]. Phe152Ile associated with the minor allele might, as a result, lead to mitochondrial

mistargeting in the absence of abundant pyridoxine, like G170R[20]. In accordance with functional studies[20,21], we observed a relatively mild disease associated with this mutation.

Similarly, our clinical findings support loss of function due to impaired pyridoxine binding predicted by *in silico* analysis [22] of p.Ser81Leu.

The missense mutation p.Arg36Cys shown to retain some catalytic activity when expressed *in vitro* especially on the major allele[23], was associated with a better clinical outcome in all three patients.

The partial discordance between early functional studies and our clinical findings could be related to limitations of *in vitro* studies and suggests that new techniques are needed to inform the folding dynamics of AGT and the molecular basis of the disease[24]. The cellular assay first described by Danpure and colleagues[25] and recently updated[19] is expected to contribute to a more accurate assessment of the residual function of different mutations.

In conclusion, this study of an unprecedented number of PH1 patients showed geno-phenotype associations that have not been previously described. These findings may have important implications for the management of PH1 patients and underline the necessity of genotyping PH patients, including unambiguous assignment of minor/major allele status. We also identified considerable diagnostic delay in this disease, which may partly explain the overall adverse outcome in some patients. A limitation of this first report is the absence of some clinical data and the lack of systematic nationwide data collection, accounting for the inability to provide precise prevalence and incidence estimates. Moreover, pyridoxine sensitivity was not formally tested as is now recommended[14]. Notwithstanding these limitations, this report shows that the OxalEurope database, with establishment of regular data input, will represent an invaluable resource for delineating genotype-phenotype and other clinically relevant issues in PH. Moreover, the database will provide the backbone to design future therapies and conduct prospective clinical trials in PH ultimately providing personalized medicine to this devastating metabolic disease.

Titles and legends

Table 1. Geographical origin of PH1 patients in Europe. Number of patients given in brackets (N); percentages are rounded off the first decimal.

Table 2. Clinical characteristics of PH1 patients and relatives in the OxalEurope cohort. Number of patients/number of available data are given in brackets. For continuous variables: I quartile / median value / III quartile.

Table 3. Main clinical features for each AGXT mutation class genotypes. Number of patients given in brackets. For continuous variables: I quartile / median value / III quartile. p-value is calculated among all groups; all statistical testing was conducted at the 0.05 level. Tests used: 1Pearson test; 2Kruskal-Wallis test

Figure 1. Patient survival of the entire cohort, Kaplan-Meier survival curve.

Figure 2: censored Kaplan-Meier curves for age at onset of ESRD for each genotype class. Data was available for 355 patients, log-rank test p-value: <0.001.

Figure 3 Hazard Ratios of age at onset of ESRD adjusted for gender (estimated with Cox Model). Reference group is G170R homozygotes. The following confidence levels are all shown: 0.9, 0.95, and 0.99, using different tone of grey (from darkest to lightest).

Fig.4. Ranking of patient subgroups carrying the same missense mutation ordered according to their median a) age at onset and b) age at ESRD either in homozygous state (left panels), compound heterozygous state with G170R (middle panels) or compound heterozygous with a null mutation (right panel). The number of patients with the relevant mutation is given in parentheses. Missense mutations are coded as follow:

p.Arg36Cys	A
p.Phe152Ile	B
p.Leu384Pro	C
p.Gly156Arg	D

p.Gly82Arg	E
p.Leu101Pro	F
p.Ile244Thr	G
p.Ala368Thr	H
p.Arg233His	I
p.Trp108Arg	J
p.Gly41Arg	K
p.Gly190Arg	L
p.Glu274Asp	M
p.Gly350Asp	N
p.Met195Arg	O
p.Ser218Leu	P
p.Gly82Glu	Q
p.Arg360Gln	R
p.Cys178Tyr	S
p.Asp201Glu	T
p.Glu95dup	U
p.Met195Leu	V
p.Arg233Cys	W
p.Cys173Tyr	X
p.Ser275Arg	Y
p.Ser81Leu	Z
p.Ile220Phe	a
p.Gly116Arg	b
p.Gly362Ser	c
p.Gly109Val	d
p.Thr70Asn	e
p.Leu153Val	f
p.Gly47Arg	g
p.Leu150Pro	h
p.Gly161Ser	i
p.Ser205Leu	j
p.Ile279Thr	k

p.Gly216Arg	m
p.Gln282His	n
p.Gly41Val	o
p.Asp183Asn	p

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Table 1

		N	Range (years)
Gender:			
males	57%	(300/526)	
females	43%	(226/526)	
Diagnosis based on:			
Genetic and enzyme analysis	37.2%	(196/526)	
Genetic analysis alone	47.2%	(248/526)	
Enzyme analysis alone	8.2%	(43/526)	
Urine analysis alone (oxalate, glycolate)	0.9%	(5/526)	
Other (e.g renal biopsy, high plasma oxalate)	6.5%	(34/526)	
Symptoms:			
asymptomatic	4%	(19/526)	
urolithiasis	32%	(171/526)	
nephrocalcinosis	17%	(88/526)	
urolithiasis and nephrocalcinosis	21%	(111/526)	
other (urinary tract infection, hematuria, failure to thrive)	8%	(46/526)	
recurrence post renal transplantation	3%	(14/526)	
unknown	15%	(77/526)	
Age at first symptoms:	0.7 3.9 9.3	(calculated on 446 pts)	(0-66)
in patients presenting with urolithiasis	1.9 4.6 11.7	(calculated on 262 pts)	(0-66)
in patients presenting post renal transplantation	6.8 15.0 27.0	(calculated on 12 pts)	(6-45.4)
Symptoms < 1 year	29%	(131/446)	
Age at diagnosis	2.6 8.1 22.2	(calculated on 456 pts)	(0-72)
Diagnosis ≥ 18years	28%	(128/456)	

ESRD at diagnosis		43%	(195/451)	
ESRD at follow up		58%	(267/458)	
Death		14%	(67/477)	
	Age at death	2.35 15.5 33.9	(data missing for 3 pts)	(0.3-73.8)
Relatives (74 patients)				
	Age of first symptoms in relatives	0.3 3.9 7.1	(calculated on 58 pts)	(0.3-7.1)
	Age of diagnosis in relatives	1.6 6.8 16.0	(calculated on 72 pts)	(1.59-16)
	Age at ESRD in relatives	11.5 23.0 34.4	(calculated on 22 pts)	(11.5-34.4)

Table 2

		G170R/G170R (71)	G170R/M (32)	M/M (116)	G170R/N (61)	M/N (42)
Gender:	F	48% (34)	28% (9)	41% (47)	33% (20)	52% (2
	M	52% (37)	72% (23)	59% (69)	67% (41)	48% (2
Age at diagnosis		6.4 29.9 46.1	2.1 8.4 25.7	1.8 7.7 17.9	4.3 16.1 34.7	1.8 6.0 1
Age at onset of symptoms		0.9 6.2 22.1	0.6 3.5 10.27	0.9 3.5 7.8	1.6 4.5 12.4	0.5 2.0 5
Symptoms:	Asymptomatic	8% (5)	0% (0)	6% (6)	0% (0)	0% (0
	Urolithiasis	48% (30)	37% (10)	36% (36)	57% (31)	31% (1
	Nephrocalcinosis	11% (7)	19% (5)	16% (16)	17% (9)	19% (7
	Urolithiasis AND nephrocalcinosis	23% (14)	26% (7)	32% (32)	9% (5)	28% (1
	Other (urinary tract infection, hematuria, failure to thrive)	10% (6)	15% (4)	6% (6)	13% (7)	14% (5
	Recurrence post renal transplantation	0% (0)	4% (1)	3% (3)	4% (2)	8% (3
Age at ESRD		19.9 33.9 47.5	11.5 31.2 39.9	3.4 16.9 34.1	15.5 25.1 33.8	1.9 11. 19.9
ESRD at diagnosis		49% (30)	35% (9)	41% (40)	52% (28)	46% (1
ESRD developed during follow-up		56% (35)	54% (14)	55% (56)	62% (34)	57% (2
AGT in liver (% activity)		12.7 33.0 43.0	6.2 13.0 18.0	0.0 3.3 9.0	5.4 6.5 16.0	0.0 2.3 7
Pyridoxine	No response	40% (19)	47% (9)	43% (29)	52% (24)	67% (1
	Response	60% (28)	47% (9)	54% (36)	43% (20)	33% (9
	Not treated	0% (0)	5% (1)	3% (2)	4% (2)	0% (0

Table 3

Country	%	N
United Kingdom	17.1	(90)
Germany	13.5	(71)
Italy	9.9	(52)
France	9.9	(52)
Netherlands	9.5	(50)
Turkey	6.3	(33)
Morocco	3.2	(17)
Lebanon	3.0	(16)
Albania	1.9	(10)
Poland	1.7	(9)
Kosovo	1.5	(8)
Other countries in North Africa	3.8	(20)
Belgium	1.3	(7)
Other countries including Afghanistan, Austria, Azerbaijan, Brazil, Bulgaria, Croatia, Czech Republic, Ethiopia, Greece, India, Iraq, Jordan, Libya, Macedonia, Pakistan, Portugal, Saudi Arabia, Serbia, Slovakia, Spain, Sri Lanka, Sweden, Switzerland, Syria, USA, Yugoslavia	9.7	(51)
Missing data	0.77	(40)

Figure 1

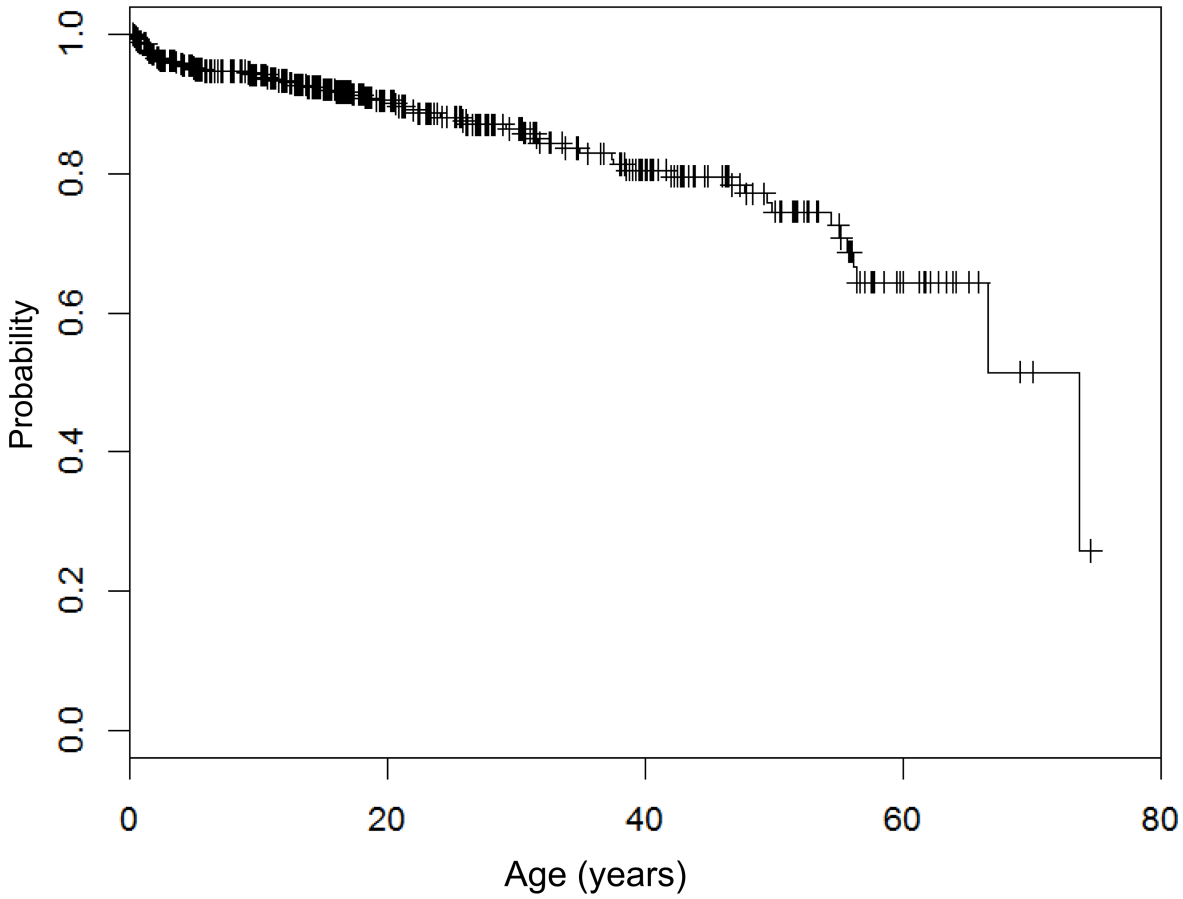


Figure 2

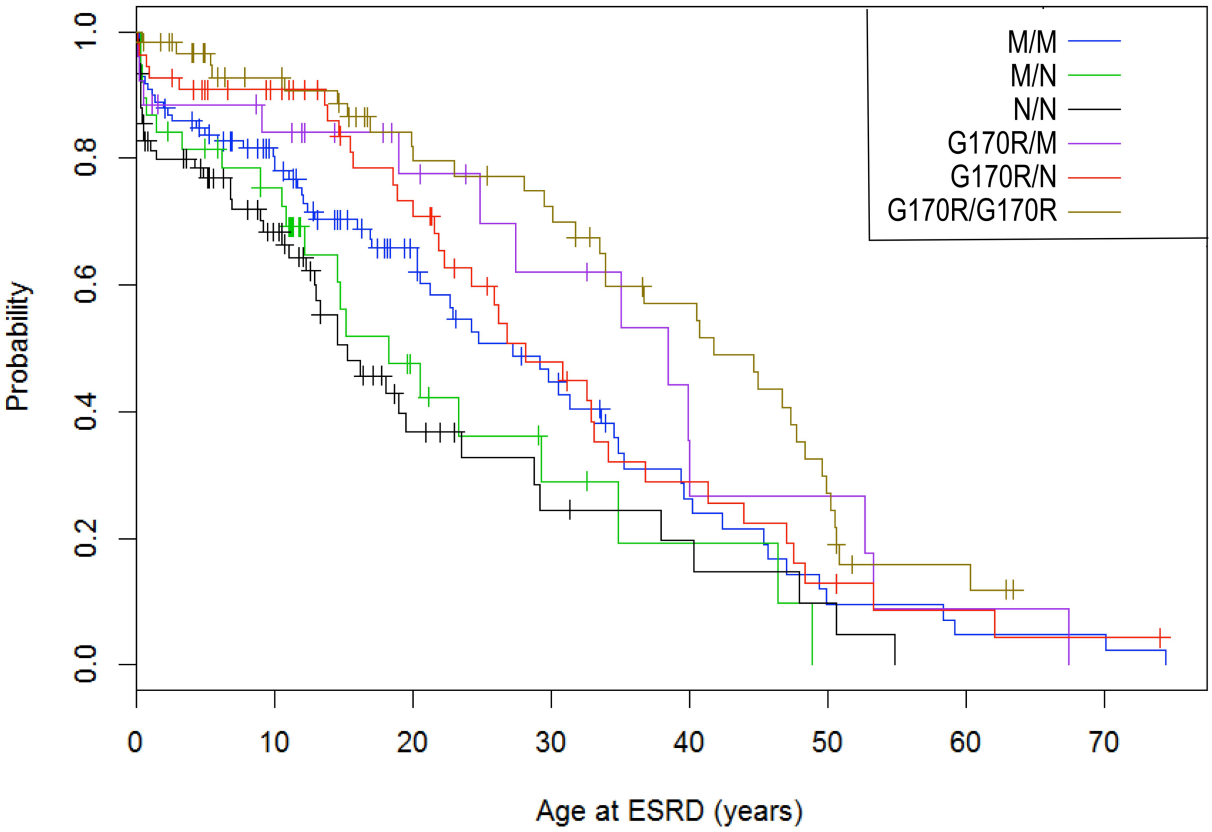
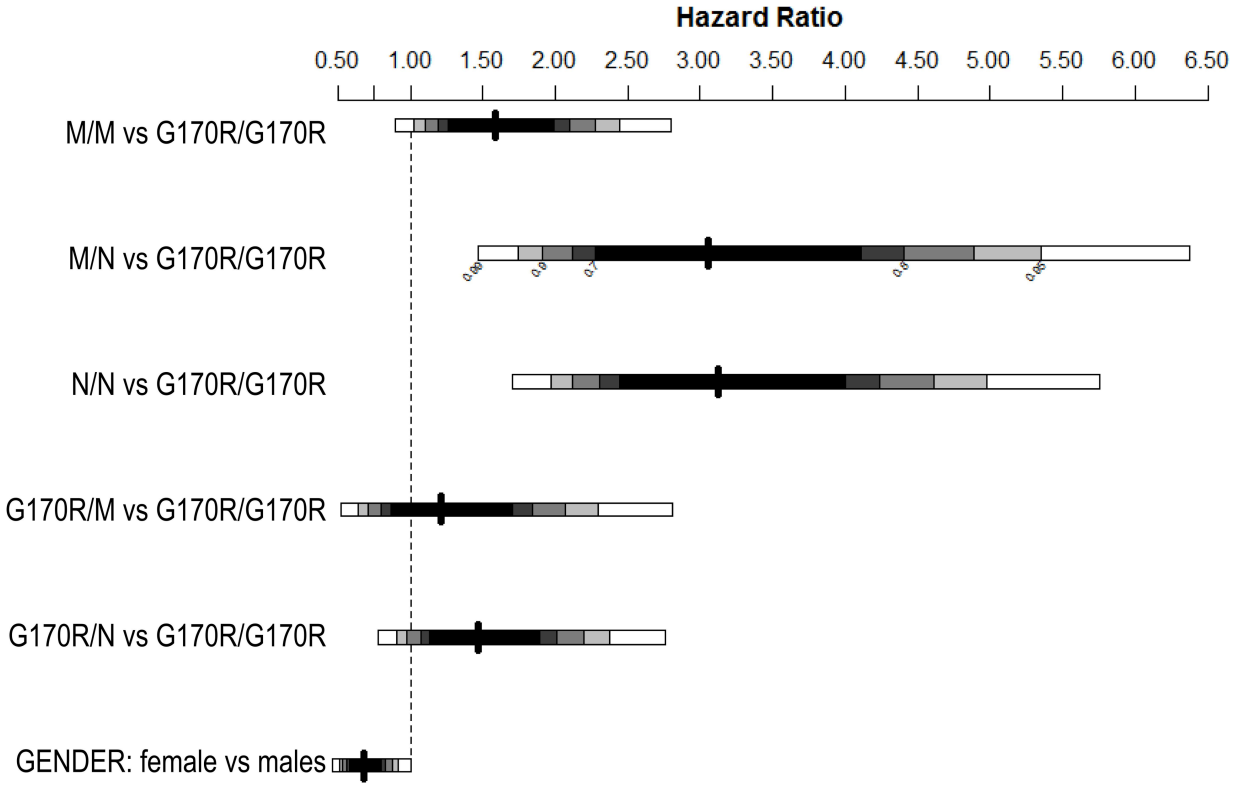

M/M
M/N
N/N
G170R/M
G170R/N
G170R/G170R
Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
70
Age at ESRD (years)

Figure 3


Hazard Ratio
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
5.50
6.00
6.50
M/M vs G170R/G170R
M/N vs G170R/G170R
N/N vs G170R/G170R
G170R/M vs G170R/G170R
G170R/N vs G170R/G170R
GENDER: female vs males