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Original Article

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Mutation Analysis of the *AGXT* Gene in Combined Liver-Kidney and Isolated Liver Transplanted Children for Primary Hyperoxaluria Type 1: A Single-Center Experience

Mehmet Türkmen[1], Demet Alaygut[2], Sinem Ağılkaya[3], Meral Torun Bayram[1], Belde Kasap Demir[1], Alper Soylu[4], Salih Kavukçu[1], Sultan Cingöz[3]

[1]Department of Pediatric Nephrology, Dokuz Eylül University, Faculty of Medicine, İzmir, Türkiye
[2]Department of Pediatric Nephrology, University of Health Sciences, Faculty of Medicine, Tepecik Training and Research Hospital, İzmir, Türkiye
[3]Department of Medical Biology and Genetic, Dokuz Eylül University, Faculty of Medicine, İzmir, Türkiye
[4]Department of Pediatrics, Dokuz Eylül University, Faculty of Medicine, İzmir, Türkiye

ABSTRACT

Objective: Primary hyperoxaluria type 1 is an autosomal recessive rare disorder, caused by mutations in the alanine:glyoxylate aminotransferase (AGXT) gene. We aimed to detect the AGXT gene mutations causing primary hyperoxaluria type I in combined liver-kidney and isolated liver transplanted children with phenotypic characteristics of primary hyperoxaluria type 1.

Methods: This study was carried out by including 6 Turkish children and their families followed by Dokuz Eylül. University Faculty of Medicine, Department of Pediatric Nephrology and diagnosed as primary hyperoxaluria with their phenotypic features. Clinical features, transplantation characteristics, and AGT catalytic activities of the cases were noted. The entire coding region including exon-intron boundaries of the AGXT gene was sequenced in patients and their family.

Results: We detected 6 mutations primary hyperoxaluria type 1 causing and 2 minor allele polymorphism in 6 patients (5 families) The entire patients had at least one primary hyperoxaluria type 1-related mutation. Patient 1 had homozygous minor allele polymorphisms Pro11Leu in exon 1 and Ile340Met in exon 10, and mutation Met195Arg in exon 5. Patient 2 had homozygous mutation c. 33_34insC in exon 1. Patient 3 was compound heterozygous for mutations Gly170Arg in exon 4 and c.846+1G>A in intron 8 and heterozygous minor allele polymorphisms Ile340Met in exon 10. Patient 4 had homozygous mutation c.823-824dupAG in exon 8. Patient 5 and 6 had homozygous mutation c.976delG in exon 10.

Conclusions: Our studies emphasize the mutation analysis of the entire coding region instead of targeted (exons 1, 4, and 7) mutation analysis of AGXT.

Keywords: AGXT, children, mutation, primary hyperoxaluria type 1

Corresponding author: Demet Alaygut ✉ alaygutdemet@gmail.com

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INTRODUCTION

Primary hyperoxaluria type 1 (PH1) is an autosomal recessive inherited disease characterized by the deficiency of a liver-specific peroxisomal enzyme namely alanine:glyoxylate aminotransferase enzyme (AGXT) which involves in glyoxylate detoxification. Deficiency of AGT results in excessive production of oxalate and glycolate.[1,2] It is a rare disease and has been reported to occur in 1-3 per million population in Europe.[3] Primary hyperoxaluria type 1 may develop as a result of low or even lack of enzymatic activity or defect during its transportation into mitochondria. Since calcium oxalate is poorly soluble in urine, PH1 is usually manifested by stones developing in the kidney and urinary system and nephrocalcinosis.

Recurrent kidney stones and nephrocalcinosis cause progressive kidney impairment. As the glomerular filtration rate decreases, oxalate excretion decreases and plasma oxalate level rises above the critical saturation point as the oxalate production rate increases. Oxalate accumulation occurs in many organs and this systemic

involvement is called oxalosis.[4] The genetic cause of PH1 disease has been determined, and so far, nearly 150 mutations have been identified in the gene that defines AGT.[5] Although genotype-enzymatic phenotype correlation is well understood, clinical phenotype-genotype correlation has not been very well explained and understood.[1]

Therefore, mutation analysis is very important in determining prognosis and treatment options. Although awareness has increased for its diagnosis, PH1 is still not known enough by clinicians and diagnosis can be only made in the long term.[6] Some of these patients can even be diagnosed after transplantation is performed due to end-stage kidney disease (ESKD).[7,8] The aim of this study is to determine the genotypes of 6 Turkish children diagnosed with PH1 based on their clinical or family histories who then underwent transplantation and compare them with their clinical findings and results.

METHODS

This study was carried out with the inclusion of 6 Turkish children followed up with the diagnosis of PH1 based on their clinical, laboratory findings, and family histories by the Dokuz Eylül University Pediatric Nephrology Clinic, and underwent isolated liver or combined liver and kidney transplantation and the families of these patients. The patients' admission complaints, their age at the time of diagnosis and at the time of transplantation, family histories, transplantation-related features, urinary oxalate concentrations, if any, stone analysis results, and AGT catalytic activity, accompanying extrarenal findings (if any) were noted from their file information. The study was conducted in accordance with the Declaration of Helsinki. Prior to the study, written permission was obtained from the center where the study was conducted; scientific ethics committee approval from the local ethics committee of the research center and written and verbal consents from the patients participating in the study via informed consent forms were obtained (2010/12-30). Financial support was received from the study center Scientific Studies Board.

MAIN POINTS

- Primary hyperoxaluria type 1 is an autosomal recessive rare disorder, caused by mutations in the alanine:glyoxylate aminotransferase (AGXT) gene.
- Primary hyperoxaluria type 1 is usually manifested by stones developing in the kidney and urinary system and nephrocalcinosis. Recurrent kidney stones and nephrocalcinosis cause progressive kidney impairment.
- Primary hyperoxaluria type I has very different clinical consequences and genotype-phenotype implications.
- It is important to perform mutation analysis of the entire coding region rather than the targeted (exon 1, 4, and 7) mutation analysis of AGXT.

Identification of Alanine:Glyoxylate Aminotransferase Gene Variants

DNA Extraction

Genomic DNA was extracted from peripheral whole blood samples of 24 individuals including 6 with and 2 without Combined liver kidney transplantation (CLKT) affected cases from 5 families by using the Genomic DNA Purification Kit (Macherey Nagel, Germany). Samples from patients, their siblings and parents were obtained after receiving their informed consent.

Polymerase Chain Reaction

We designed 10 primer pairs for 11 exons by using oligo-primer analysis software (National Biosciences, Inc.) by considering

Table 1. Primer List for *AGXT*

AGXT-exo1-2
5' CCC GCA GCA CAA GCA CAG ATA 3'
5' CAG AGG GAG GCC AGG GAG G 3'
AGXT-exo3
5' TTC TAC AGT GTG TGC GGG ACA 3'
5' AGA TGC TAG GAT GGG CTG AGG 3'
AGXT-exo4
5' GCC CCT GCT ACC TGG AGC TG 3'
5' GGG CAG AAG GAC CAG AGG GAC 3'
AGXT-exo5
5' TGC CTT CCT TGC CAG CCT GAG 3'
5' GGT GCC CAA CGC CTG ATT GAC 3'
AGXT-exo6
5' AGC AGT GCC CAG ATT TGA ACC 3'
5' CCT GTG AAC GCA GTG CCT TT 3'
AGXT-exo7
5' GCG AGA CTG CCC TGG CCT TC 3'
5' AAA GTG CCC GAG GGT GTG CTG 3'
AGXT-exo8
5' AGT TCT GAA CCC GGA CAG GAC 3'
5' TCT CTG GCA GGC TCC CTT T 3'
AGXT-exo9
5' TCT TCC TCC CGC ACC ACA 3'
5' GCT CCT GCC GAG ACT AAT CCC 3'
AGXT-exo10
5' CCG GCT CCT CTG GAA CCT GA 3'
5' GCA ATC TGG GCT TTC GGA TGA 3'
AGXT-exo11
5' CTC ATG GAC GCT GGG TGG GTG 3'
5' CGG GCT GGG TCA GTG GCT TTC 3'

the exons and exon-intron boundaries of *AGXT* gene (RefSeq NM_000030.3) (see Table 1 for primer list). After the exons of the *AGXT* gene in the DNA sample obtained from a phenotypically normal (healthy) individual were amplified by polymerase chain reaction (PCR), the products were sequenced to confirm the amplification of the right target. Polymerase chain reaction was performed using 25 ng genomic DNA in a 15 µL reaction mixture containing 2.5 mM each of deoxyribonucleotide triphosphate (dNTP), 12 pmol of each primer, 0.1 U Taq DNA polymerase (Fermentas, EU), reaction buffer (supplied by the manufacturer) in a thermocycler (MJ Research). The PCR fragments were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. The products were used as a template DNA for sequencing analysis.

DNA Sequencing

Variation analysis was performed by direct DNA sequencing of 24 individuals including 6 with and 2 without CLKT from 5 families. After the coding region and exon-intron boundaries of the *AGXT* gene were amplified, the samples were sequenced by using an automated sequencer (ABI Genetic Analyzer 3730xi). Mutations were confirmed by sequencing both DNA strands. The sequences were aligned with reference sequence of *AGXT* by using Basic Local Alignment Search Tool.

RESULTS

Six Turkish children, including five boys, were included in the study. Cases 5 and 6 were siblings. The mean age of the cases at the time of diagnosis was 123.17 ± 49.6 (42-178) months. Admission complaints were recurrent nephrolithiasis in Cases 3 and 4, abdominal pain in Cases 1 and 5, while Cases 2 and 6 presented because of family history compatible with PH1. Nephrolithiasis was detected in all patients based on their clinical, laboratory, and radiological results. Cases 1, 3, and 5 were also ESKD at the time of admission. Family history revealed the presence of recurrent nephrolithiasis in the sister of Case 1. Brother of the Case 2 died before being diagnosed because of ESKD and recurrent stones. It was learned that this ex case had oxalosis in the nephrectomy material. Family history of Case 3 could not be obtained. Brother of Case 4 had a history of recurrent stone disease. Cases 5 and 6 were siblings without any relevant family history. In addition, parents of Cases 1, 5, and 6 were relatives. Stone analysis revealed the presence of calcium oxalate in Cases 2 and 3 and whewellite stone in Case 4. Stone analysis results of other cases could not be obtained. The mean age of the patients at the time of transplantation was 146.67 ± 31 (115-180) months. In all cases transplantations were performed from live relative donors. Only isolated liver transplantation was performed in Cases 4 and 6, and combined liver-kidney transplantation was carried out in all other cases. In one of the combined transplantation cases (Case 3), acute graft loss occurred due to surgical complications (arterial thrombosis) and retransplantation was required. The first liver transplantation was performed from the mother and the second from her sister. Perioperatively, hemodialysis was performed using high flux dialyzer in patients who underwent

Table 2. Clinical Characteristics of Patients with PH1

	Case 1 (M)	Case 2 (M)	Case 3 (M)	Case 4 (M)	Case 5 (M)	Case 6 (F)
Complaint	Abdominal pain, polyuria, polydipsia	Family history	Recurrent stones	Recurrent stones	Abdominal pain	Family history
Clinical findings	Nephrolithiasis ESKD	Nephrolithiasis	Nephrolithiasis, ESKD	Nephrolithiasis	Nephrolithiasis, ESKD	Nephrolithiasis
Age of diagnosis	118 months	42 months	173 months	108 months	178 months	120 months
History of the disease in relatives	Sister with recurrent nephrolithiasis	Ex elder brother with primer hyperoxaluria	-	Brother	+	+
Age at CLKT	123 months	115 months	176 months	118 months	180 months	168 months
Donor type	Living donor (mother)	Living donor (elder sister)	Living donor (elder sister)	Living donor (Father)	Living donor (mother)	Living donor (Father)
Transplantasyon	Combined	Combined	Combined	Isolated hepatic	Combined	İsole hepatic
Urinary oxalate concentration		1.5 mmol/1.73 m^2/24 h		1.52 mmoL/1.73m^2/24 h	.	.
AGT catalytic activity	Low	.	.	Low	.	.
Extra kidney involvement	Bone	Soft tissue calcification in elder brother	Bone			

Range of urinary oxalate concentration in normal: 0.01-0.46 mmol/1.73 m^2/24 h.
CLKT, combined liver-kidney transplantations; ESKD, end-stage kidney disease; PH1, primary hyperoxaluria type I.

transplantation. Urinary oxalate concentration results of only Cases 1 and 3 were available and both were above the reference range of 0.01-0.46 mmol/1.73m^2/24 h. AGT catalytic activity could be determined in Cases 1 and 4 which was at a low level. Extrarenal involvement in bone, eye, heart, thyroid, and synovia was evaluated. There was bone involvement in Cases 1 and 3, and soft tissue involvement in Case 2. The clinical characteristics of the cases are summarized in Table 2. All coding regions of the *AGXT* gene, including exon and intron boundaries, were sequenced in patients and their families. Eleven exons were evaluated. Parents of Case 1 (Family 1) were blood relatives. Together with the index patient, the *AGXT* gene was sequenced in an affected sibling with recurrent nephrolithiasis and their parents. Clinical features of the family (Table 2) and sequence results are shown in Table 3 and Figure 1. In this family, the healthy (unaffected) mother and father were homozygous for the c.32C> T (p.Pro11Leu) variant in exon 1 and c.1020A> G (p. Ile340Met) variant in exon 10, as in the case with CLKT and the other sibling. For c.584T> G (p Met195Arg) in Exon 5, the mother was heterozygous, and both siblings were homozygous. In Case 2 (Family 2), the *AGXT* gene was sequenced in the mother and transplant recipient patient. After sequence analysis, homozygous c.33delC (p.Lys12fs) frameshift variant was detected in exon 1. The healthy mother was heterozygous for this variant. The clinical features of the family are shown in Table 2, and sequence results in Table 3 and Figure 1. In Case 3 (Family 3), *AGXT* gene was sequenced in the index case transplanted, together with 4 brothers and parents. The unaffected father carried the heterozygous splice donor variant c.846+1G>A in intron 8. The mother unaffected by the disease carried both heterozygous c.508G> A (p. Gly170Arg variant in exon 4 and also the homozygous variant c.1020A> G (p. Ile340Met) in exon 10. When we look at the pedigree segregation of heterozygous and homozygous variants in the mother and father in Figure 2, it is observed that only II: 1 and II: 4 in the family contain compound heterozygous variants. Healthy sister carried heterozygous c.846 + 1G> A variant inherited from "II: 4" father, and heterozygous variant p.Ile340Met from mother, the affected brother carried the heterozygous c.846+1G>A variant inherited from "II: 1" father, and heterozygous p.Ile340Met, and Gly170Arg variant from the mother.

In Case 4 (Family 4), the AGXT gene was sequenced in the mother, the transplant patient, and her other sibling. After the sequence analysis, it was detected that the mother carried heterozygous frameshift variant c.823_824dupAG (p.Ser275fs) in exon 4, and 2 affected PH1 children were found to be homozygous for this variant (Figure 3).

In Cases 5 and 6 (Family 5), the AGXT gene was sequenced in 2 transplant recipient siblings and their both parents. Both children of the unaffected or healthy mother and father carrying the heterozygous c.976delG (p.Val326fs) frameshift variant in Exon 10 were homozygous for this variant (Figure 3).

Table 3. Polymorphic Variants and Those of Unknown Significance, Missense Changes, Deletion/Insertion/Splice Site Mutations in the *AGXT* Gene in Our Cases

Location	Sequence Variant	Codon/ Effect	Case	Major (Pro11) or Minor (Leu11) Haplotype	In vitro AGT Activity (% of Normal) or How Proven	Frequency in Controls	SNP db	Molecular Phenotype	Reference
Exon 1	c.32C>T	p. Pro11Leu	1 (Hom.)	Minor	64% (Lumb and Danpure, 2000)	0.20	rs34116584	5% of AGT rerouted from peroxisomes to mitochondria	(Purdue et al., 1990)
Exon 10	c.1020A>G	p.Ile340Met	1 (Hom) 3 (Het)	Minor (occasionally major)	76-117% (Lumb and Danpure. 2000)	0.15	rs4426527	Little or none	(Purdue et al., 1992)
Exon 4	c. 508G>A	p.Gly170Arg	3 (Het)	Major	40% on minor, 68% on major (Lumb and Danpure, 2000)			Peroxisome to mitochondrion mistargeting	(Purdue et al., 1990)
Exon 5	c.584T>G	p.Met195Arg	1 (Hom)	Minor	No other mutation found				(Frishberg et al., 2005)
Exon 1	c.33delC	p.Lys12fs	2 (Hom)	Major				frameshift	(Pirulli et al., 1999)
Exon 8	c.823_824dupAG	p.Ser275fs	4 (Hom)	Unknown				frameshift	(Yuen, et al., 2004)
Exon 10	c.976delG	p.Val326fs	5,6 (Hom)	Major				frameshift	(Pirulli et al., 1999)

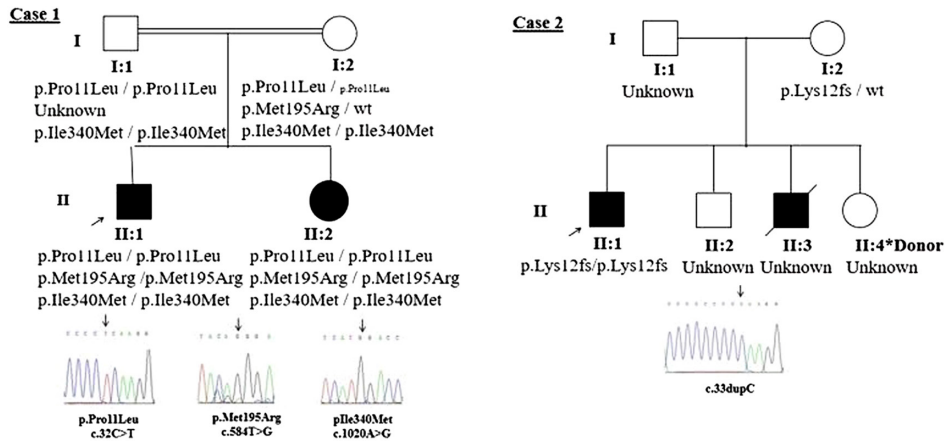

Figure 1. Sequence results of Case 1 and Case 2.

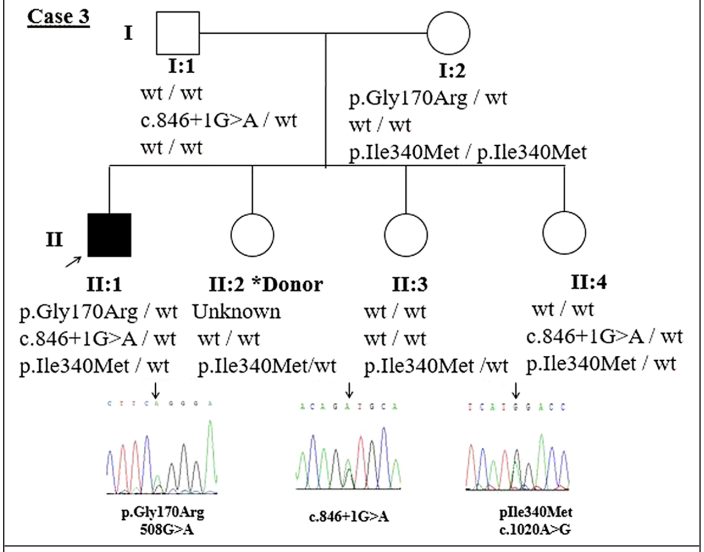

Figure 2. Sequence results of Case 3.

DISCUSSION

Primary hyperoxaluria type 1 has very different clinical results. Patients who died in early infancy and also cases diagnosed with nephrolithiasis in adulthood have been reported.[9] In this study, 6 children who presented with different complaints and clinical findings and underwent combined liver-kidney or isolated kidney transplantation were evaluated for the retrospective analysis of *AGXT* gene. As previously reported, PH1 can manifest 5 different clinical features. The first is nephrocalcinosis and kidney failure (26%) in the early infantile period. Secondly, progressive kidney failure may develop in adolescence or early adulthood following a recurrent stone disease (30%). The third group of patients are diagnosed by examination of the stone passed in adulthood (21%). The fourth and perhaps the most tragic group is diagnosed after posttransplantation recurrence of stone disease (10%) and the fifth group of patients are diagnosed by family screening (13%).[4] Our study group was already made up of children, and they were diagnosed in the preadolescent period. Our study group did not contain any infants.

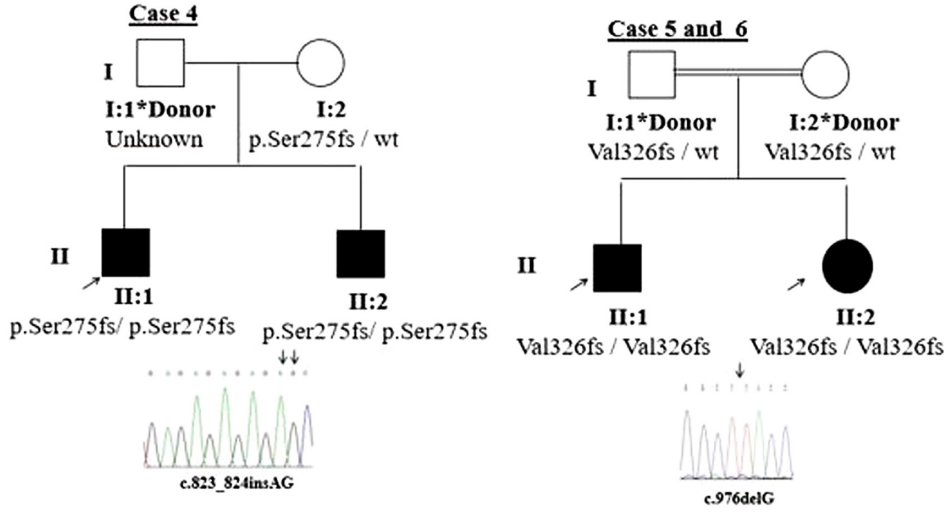

Figure 3. Sequence results of Cases 4, 5, 6.

All patients had a history of stones, but the main admission complaints of 2 cases were not related to a stone disease, and cases that were followed up and even lost due to PH1 were detected in their families. Nephrolithiasis and nephrocalcinosis are the first findings in many cases. All cases with a history of recurrent stones and nephrocalcinosis should be evaluated in terms of PH1. All cases in this study group also had a history of stone disease. The examination in these patients is done by recurrently measuring concentrations of oxalate or glycolate in 24-hour urine samples or evaluating plasma oxalate or glycolate concentrations in patients who develop ESKD.[10,11] Again, it is useful to analyze the crystalluria or evaluate the contents of the stone samples obtained.[12,13] Approximately 40% of cases have ESKD and extrarenal symptoms at the time of diagnosis during childhood and adolescence. This rate reaches 60% in adulthood. Even a considerable proportion of adults are diagnosed after isolated kidney transplantation. Therefore, if there is a family history of recurrent nephrolithiasis and the cause of ESKD is unknown, these patients should be evaluated in terms of PH1. It should be remembered that measuring urinary oxalate clearance will not make sense after development of ESKD. Genetic or enzymatic diagnosis should be used in all cases with systemic oxalosis and high plasma oxalate levels (usually > 80 µmol/L in ESKD).[1] Primary hyperoxaluria type 1 is a life-limiting condition. The mortality rate was reported to be 28% in a Swiss cohort and 19% in a Dutch cohort.[14,15] There was no mortality in our patients. This may be due to the absence of our infant PH1 cases.

As far as we concern, the most important reason was that we were working in a center where combined (liver-kidney) transplantation can be performed. The possibility of performing combined transplantation improves the prognosis perfectly.[16] We performed combined liver transplantation in our 4, and isolated liver transplantation in 2 cases. Transplantation is the most curative form of treatment of choice for these cases.

Since oxalate storage cannot be prevented by dialysis, in our cases, peritoneal dialysis and hemodialysis were temporarily performed and maintained up to the time of transplantation. Primary hyperoxaluria type 1 is a heterogeneous disease at the molecular level. *AGXT* gene is the only gene identified. With target mutation analysis, 50%-70% of mutations in *AGXT* gene, and with sequence analysis, all (100%) mutations in this gene can be identified.[5] Many mutations and polymorphisms have been described. There is significant molecular heterogeneity among patients. Mutations can occur anywhere along the gene and 75% of them are point mutations.[4] In a study by van Woerden et al.[17] 33 patients with PH1 were compared in terms of genotype and biochemical phenotype and heterogeneity was detected clinically. Even in the presence of the same homozygous mutations in the same family, we can encounter different clinical pictures. Hoppe et al[10] reported that combined liver and kidney transplantation was performed in a 14-year-old male patient with p.Gly170Arg (c.508G> A) mutation, although urinary oxalate excretion was normalized with pyridoxine treatment, and hematuria or nephrocalcinosis could not be demonstrated. Amoroso et al[18] performed sequence analysis in 23 Italian patients with the diagnosis of PH1 and found 13 different mutations in exons 1, 2, 4, and 10. These mutations are the same as those previously reported in the literature.[19] The most common *AGXT* mutation is pGly170Arg. The pGly170Arg disrupts traffic between peroxisome and mitochondria resulting in pathological disorders.[4] The p.Pro11Leu is another defined mutation. This mutation alone is responsible for the delocalization of 5% of the protein to the mitochondria in the homozygous state.[20] However, when it is accompanied with p.Gly170Arg, it is responsible for 90% of AGT not being able to be transported to mitochondria.[21] Missense mutations are commonly followed by small insertion/deletions (indels), most notably c.33dupC.[22] It is known that this mutation is mostly related to ESKD that occurs in infantile period. This mutation creates a stop codon, leading to nonfunctional truncated protein. This duplication has no ethnic and geographic relationship.[23] Although no relationship between mutations and ethnicity has been demonstrated, the p.Ile244Thr mutation is seen in people with North African and Spanish descent.[5] van Woerden et al[17] performed sequence analysis in 57 Dutch patients with PH1 and detected the 3 most frequently observed mutations (p.Gly170Arg, p.Phe152Ile, and c.33insC) in their patients. They reported that Val336Asp was accompanied by the p.Gly170Arg mutation as a minor allele in 2 patients, while Gly82Arg mutation was present as a new mutation in 3 patients. Harambat et al[1] evaluated a cohort of 155 patients, 72 patients had undergone a total of 97 transplantations, while they detected p.Gly170Arg mutations in 136 patients. Patients with this mutation had demonstrated slower progress to ESKD and had a better clinic. However, there are publications stating that *AGXT* gene mutations are not guiding in terms of clinical differences of the disease. Early onset, severe disease findings can be detected in some patients, while some individuals can remain asymptomatic until 40-50 years of age.[24] Although siblings diagnosed with PH1 usually have similar mutations, different signs of disease have been described in these patients. While 6 siblings in a family were homozygous for the c.33dupC mutation, 1 of them was diagnosed prenatally, 4 were diagnosed between the ages of 7 and 2 years, and 1 remained asymptomatic until the age of 20.[25] Possible causes for these variations appear to be related to differences in the activity levels of other enzymes involved in oxalate synthesis, modified genes, dietary oxalate precursors, kidney oxalate involvement, dietary oxalate absorption, hydration status, infections, and urinary crystallization factors.[24] Although it is more important and meaningful to discuss the mutations of *AGXT*, *AGXT* has an allele variant called the common and minor allele. It is known that this allele is functional and has a pathological significance.[5]

There are different polymorphic variants defined in different exons. Some polymorphic variants form the minor allele haplotype (AGT-Mi). The primary unchanged variant in the allele is

the c.32C> T variant, which causes the replacement of leucine in codon 11 with proline and takes the name of the major allele (AGT-Ma).[5] The catalytic activity of the minor allele is lower than that of major allele and is between 50-60%.[26,27] The Leu11 variant affects the mitochondrial targeting sequence at the N-terminal of the presumably weak, indivisible protein. The frequency of AGT-Mi is population-dependent and is detected in 10%-20% in Caucasians, but only 2% in Japanese people. There are no studies showing the incidence of these mutations in Türkiye. In a study conducted in our country, 82 patients with suspected clinical PH1 were evaluated and 15 mutations in 13 families were detected. Three of the mutations detected in this patient group are new mutations. However, polymorphism was not evaluated in this study.[28] It was known that 3 different variations detected in Family 1 in our cases were defined as minor alleles and affected the catalytic effect of the enzyme less than the major allele.[25] As seen in the pedigree of case 1 (Family 1) in Figure 1, unlike the healthy mother and father, the siblings additionally carried the homozygous (p.Met195Arg) variant. It has been thought that the effects of 3 minor variants might affect the sick cases in this family. In addition, transplant recipient case II: 1 showed severe phenotypic properties, while case II: 2 was characterized by recurrent nephrolithiasis. Although the 2 siblings contained the same changes in the AGXT gene, it was thought that being phenotypically different could be due to the potential of female individuals to tolerate some changes. The first variant in Case 2 (Family 2) was defined by Pirulli et al in 1999. It is one of the pathogenic variations defined in the *AGXT* gene.[29] The presence of only p. Ile340Met in the allele inherited from the mother in the Family 3 was not sufficient to induce loss of *AGXT* function. Pathogenic effects of c.846+1G> A and p. Gly170Arg variants are prominent. Variation screening in 3 healthy siblings in addition to the family gives information about the pathogenic effects of variants. These data are also important in terms of identifying the domestic donor in the family.[30] It is known that this variant, which causes changes in the protein structure encoded by the AGXT gene in the Family 4, is pathogenic and rarely seen.[31] Also in Family 5, the variant that causes changes in the protein structure encoded by the AGXT gene is known as a rarely seen pathogenic variant.[32] The clinical significance of genetic tests has been discussed in many articles, and as a result, it has been determined that it will be important not only to diagnose but also to evaluate the response to clinical course and pyridoxine treatment. Maybe it will lead to the evaluation of other drugs that can be used for its treatment in the future.[33] All of our patients had received pyridoxine treatment during the pre-transplant period.

In conclusion, our findings are important for the confirmation of the pathogenic and likely pathogenic effects of the AGXT variations detected previously. Especially segregation findings in Family 3 have shown that p.Gly170Arg variant can be associated with the disease phenotype. Briefly, we confirmed the presence of potentially pathogenic and benign variations in the families by performing sequence and segregation analysis in healthy and unhealthy children in the families we included in our study These data will be important for the correct selection of domestic donors. Mutational analysis of the *AGXT* gene in PH1 patients can be a useful tool for establishing the diagnosis and choosing an appropriate therapeutic strategy. Our studies emphasize the mutation analysis of the entire coding region instead of mutation analysis of *AGXT* in targeted exons (i.e., exons 1, 4, and 7).

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Dokuz Eylül University (Date: November 12, 2010, Decision no: 12-30).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

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