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Screening for Fabry disease in patients undergoing dialysis for chronic renal failure in Turkey: Identification of new case with novel mutation

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ABSTRACT

Background: Chronic renal failure (CRF) is a serious complication of Fabry disease (FD). The aims of the present study were to determine the prevalence of unrecognized FD in Turkish hemodialysis population and to investigate the molecular background.
Method: Primarily, α-galactosidase A (α-Gal A) activity was investigated on DBS in 1136 patients of both sexes who underwent dialysis for CRF in Turkey. The disease was confirmed by analyzing enzyme activity in leukocyte and *GLA* gene sequencing in all patients in whom α-Gal A level was 40% of normal or less.
Results: Mean age of the patients (44.5% female, 52.5% male) was 56.46 ± 15.85 years. Enzyme activity was found low with DBS method in 12 patients (four males, eight females). Two men, but no women, were diagnosed with FD by enzymatic and molecular analysis. In consequence of genetic analysis of a case, a new mutation [hemizygote c.638C>T (p.P214S) missense mutation in exon 5] was identified, which was not described in literature. Family screening of cases identified six additional cases.
Conclusion: As a result of this initial screening study performed on hemodialysis patients for the first time with DBS method in Turkey, the prevalence of FD was detected as 0.17%. Although the prevalence seems to be low, screening studies are of great importance for detecting hidden cases as well as for identifying other effected family members.

1. Introduction

Fabry disease (FD) (OMIM, ID 301500) is a rare X-linked lysosomal storage disorder caused by deficient activity of the enzyme α-Gal A resulting from mutations affecting the *GLA* gene (OMIM, ID 300644). It is characterized by severe multisystemic involvement that leads to major organ failure and premature death in affected men and in some women. The α-Gal A deficiency results in progressive accumulation of undegraded glycosphingolipids, predominantly globotriaosylceramide (Gb3), within cell lysosomes throughout the body (Desnick et al., 2001; Mehta et al., 2009). The condition has conventionally been considered to be rare, affecting only 1 in 40,000 to 1 in 238,000 male individuals (Meikle et al., 1999; Poorthuis et al., 1999; Poupetová et al., 2010). However, a newborn screening study suggested that the incidence of FD might be 1 in 3100 to 1 in 4100 births for late-onset disease and 1 in 37,000 births for classic phenotype (Mechtler et al., 2012; Spada et al., 2006). The frequency of FD is obviously higher in high-risk populations, such as patients with CRF requiring dialysis, left ventricular hypertrophy (LVH), and stroke (Linthorst et al., 2010).

In patients with classic phenotype, characteristic findings of the disease occur as a result of accumulation of substrate in endothelium. Significant findings of the disease, such as angiokeratoma, hypohydrosis, corneal and lenticular opacities, and acroparesthesia begin in childhood. In patients at the second or third decade, progressive proteinuria, decline in glomerular filtration rate (GFR), and tubular damage occur usually, and renal failure develops in the fourth decade. Life-threatening renal, cardiac, and cerebrovascular diseases are added in later decades. Primary cause of death commonly seen in the fourth and fifth decades is renal failure, particularly in affected male patients (Eng et al., 2006; Sessa et al., 2001). When compared to normal population, death occurs approximately 20 years earlier in male patients, and 15 years earlier in

Abbreviations: α-Gal A, alpha-galactosidase A; CKD, chronic kidney disease; CRF, chronic renal failure; DBS, dried blood samples; DNA, deoxyribonucleic acid; DP, dialysis patients; EDTA, ethylenediaminetetraacetic acid; FD, Fabry disease; Gb_3, globotriaosylceramide; GFR, glomerular filtration rate; HD, hemodialysis; HGVS, Human Genome Variation Society; LVH, left ventricular hypertrophy; MI, myocardial infarction; PCR, polymerase chain reaction; PD, peritoneal dialysis; RF, renal failure.

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Table 1
Distribution of gender and age, mean enzyme activities, and results of screening test of patient and control groups.

	Age (Mean ± SD)	Enzyme activity in DBS (μmol/L/st)	Positive screening test[a] (n)	Patients with FD[b] (n)
Dialysis patients				
Female (n = 521)	56.13 ± 17.01	2.98 ± 2.31	8	0
Male (n = 615)	56.73 ± 14.83	3.01 ± 2.58	4	2
Total (n = 1136)	56.46 ± 15.85	2.98 ± 2.43	12	2
Control (n = 30)	32.40 ± 8.86	3.13 ± 1.28	–	–

[a] Number of cases with enzyme activity in DBS 40% lower than control group.
[b] Number of cases (n) confirmed with intra-leukocyte enzyme analysis and genetic analysis.

female patients (Mehta et al., 2009). Therefore, early diagnosis is of vital importance in cases with FD.

Although the disease has an X-linked recessive inheritance pattern, female carriers may present with some symptoms, and rarely, with complete clinical manifestations of the disease related to random X-chromosomal inactivation (Wilcox et al., 2008). As in other lysosomal storage diseases, clinical findings in FD also differ from case to case. In milder cardiac and renal variants, residual α-Gal A is found, symptoms begin in adulthood, and clinical findings are limited to heart or kidneys (Meroni et al., 1997; Nakao et al., 2003). Diagnosis of FD is made by measuring α-Gal A activity in plasma or leukocytes. Enzyme replacement therapy, that became available especially in recent years, has changed and improved natural course of the disease by preventing deposition in the organs (Schaefer et al., 2009). Recognition of FD before clinical findings emerged has become crucial, for the success in its therapy and development of new therapy methods. Therefore, patients with stroke of unknown cause and cardiovascular diseases and those who undergo dialysis for CRF at an early age should be considered high-risk group and screened for Fabry disease, thus, early diagnosis of the patients in the family of the patients detected should be ensured by family screening.

The present study aimed to screen Fabry disease enzymatically and molecularly in a patient group that underwent dialysis for CRF in Ankara city, Turkey. Although there are reports about the screening of this target population in various countries, there is lack of data from Turkey in the literature.

2. Method

Totally, 1136 patients (521 females, 615 males) who underwent dialysis for CRF in 16 dialysis centers in the province of Ankara were recruited in the study. The study was initially approved by the Local Ethical Committee of Gazi University Medical School, and written consent was obtained from the patients before the samples were collected. Distribution of gender and age, mean enzyme activities, and results of screening test of dialysis patients and control groups are given in Table 1.

2.1. Blood collection

Primarily, venous blood samples of patients on dialysis treatment were collected for evaluation with a syringe before dialysis. Four drops of the blood were transferred to a filter paper, allowed to dry at room temperature, and stored at 2–4 °C before being centrally processed. Enzyme activity in leukocytes was analyzed by collecting 10 cc blood samples into test tubes containing ethylenediaminetetraacetic acid (EDTA) from the patients whose α-Gal A activity in DBS samples was 40% lower than control sample. DNA sequencing of the *GLA* gene was carried out after collecting 3 cc blood samples into test tubes containing EDTA from patients with low the α-Gal A activity. All laboratory tests were performed in Pediatric Metabolic Disorders and Genetics Laboratory, Gazi University, School of Medicine, Ankara, Turkey.

2.2. Measurement of α-Gal A activity

Measurement of enzyme activity in dried blood spot samples was performed using the method by Chamoles et al. (2001). The enzyme activities were calculated in μmol/L/h.

The determination of α-Gal A activity in leukocyte was done using the technique previously described by Desnick et al. (1973). Values between 9–31 nmol/h/mg proteins were considered normal.

2.3. DNA sequencing of the GLA gene

Sequence analysis was carried out for the *GLA* gene, using genomic DNA samples. Genomic DNA was extracted from buffy coat cells using the QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The genomic primers covered the intron–exon boundaries as well as the exons. Primers are available upon request. The PCR amplification of the *GLA* exons 1 to 7 was performed in a 50 μL reaction mixture which contained 100 ng of genomic DNA, 25 mM $MgCl_2$, 10 mM of each deoxynucleotide triphosphate, 2.5 U of DNA polymerase (Gold Taq, Applied Biosystems), and 20 pmol of each primer. PCR products were resolved by electrophoresis on a 1% agarose gel. After cleaning-up, the products were sequenced in both directions

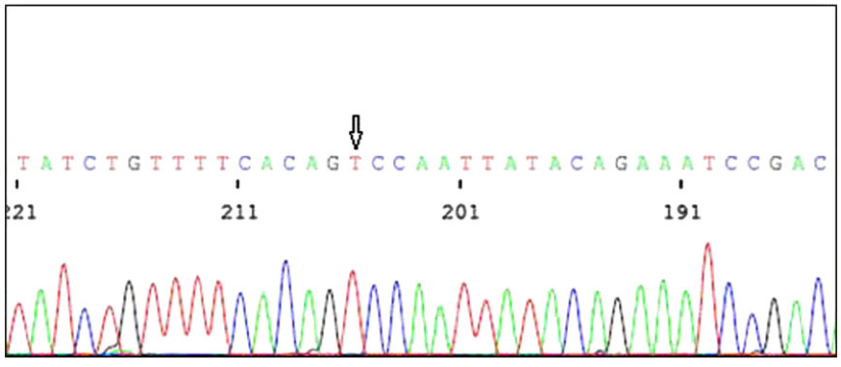

Fig. 1. The hemizygote c.638 C>T change in *GLA* gene of the case 2, which it has not been reported previously in FD.