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(Citation)
Therapeutic Apheresis and Dialysis,26(6):1187-1192

(Issue Date)
2022-12

(Resource Type)
journal article

(Version)
Accepted Manuscript

Word count; Abstract; 149, Text; 1848
Tables; 1, Figures; 3

Screening for Fabry disease among male patients on hemodialysis in Awaji Island

Running title: Screening for Fabry disease in a remote island

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Acknowledgements

The authors would like to thank for their collaborators: Dr. Masafumi Saito, Saito Internal Medicine Clinic; Dr. Satoshi Saika, Saika Clinic; Dr. Yoshiharu Nakayama, Takayama Clinic; Dr. Masahiro Okada, Sumoto Itsuki Hospital; Dr. Yoshiaki Nakabayashi, Nakabayashi Hospital; and Dr. Katsuyuki Tome, Tome Clinic.

Funding statement

The authors received no specific funding for this work.

Conflict of interest disclosure

H.F. received lecture fees from Sumitomo Dainippon Pharma Co., Ltd.

Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine received a scholarship donation from JCR pharmaceuticals Co., Ltd.

Ethical approval statement

The trial was approved by the Institutional Review Committee for the Protection of Human Subjects in Research at Kobe University (No. 180302) and was conducted in accordance with the principles of the Helsinki Declaration.

Patient content statement

Written informed consent was obtained from all enrolled patients.

Clinical trial registration

This study is not registered in a public trials registry because this is a cross sectional study.

Abstract

Background

Fabry disease (FD) manifests decreased α-galactosidase A (α-Gal A) activity and multiorgan damage. There are some undiagnosed cases of the condition among patients on dialysis. The prevalence of FD may also vary with the region.

Methods

Among 227 male patients undergoing maintenance hemodialysis in Awaji Island, a remote island in Japan, 201 (88.5%) were included in this study. Patients with α-Gal A activity <5.0 pmol/hr/disk proceeded to secondary screening. Patients with positive secondary screening underwent further genetic analysis.

Results

The number of patients with a family history of cardiac, cerebrovascular, and kidney diseases was 31 (15.4%), 23 (11.4%), and 31 (15.4%) patients, respectively. Although three patients (1.5%) had low α-Gal A activity, none of them was positive in the secondary screening.

Conclusion

We could not identify any male hemodialysis patient with FD in Awaji Island, even though some patients

had a family history of kidney and cardiovascular diseases.

Keywords: Fabry disease, hemodialysis, α-galactosidase A activity, remote island

Introduction

Fabry disease (FD) is a lysosomal storage disorder resulting from a deficiency in the activity of α-galactosidase A (α-Gal A) [1]. This enzyme deficiency causes the intracellular accumulation of glycosphingolipids, mainly ceramide trihexoside (CTH), in various tissues. Subsequently, FD manifests as acroparesthesia and hypohidrosis in childhood, and major organ involvement such as renal, cerebral, and myocardial damage is advanced in adulthood. In general, these organ disorders are usually more severe in male patients than in female ones because their α-Gal A activities are extremely reduced.

It is well-known that the progression of organ involvement leads to a short lifespan. However, there are currently some available therapies for FD. Enzyme replacement therapy and oral pharmacological chaperon therapy could reduce the accumulation of CTH and prevent further disease progression in FD patients. Therefore, early diagnosis and start of treatment are important to improve the mortality of those with FD. However, it is difficult to diagnose atypical cases of FD because of the absence of typical signs and symptoms of the condition. Particularly, in patients with FD on dialysis, the cause of end-stage kidney disease (ESKD) is sometimes either unknown or poorly identified. Thus, screening tests for FD are important for patients on dialysis.

Various studies have reported that the prevalence of FD ranges from 0% to 1.7% in patients on dialysis [2, 3]. Generally, the prevalence of the inherited disease could be affected by genetic and geographical factors. We previously performed the screening test for patients on dialysis in the south part of Hyogo

Prefecture, Japan [4]. However, we did not identify any FD patients.

Therefore, we investigated the prevalence of FD among male patients on dialysis in Awaji Island, a remote island located in Hyogo Prefecture, Japan.

Materials and Methods

Study design and population

Awaji Island is a remote island in Hyogo Prefecture, Japan (Figure 1). This island has a population of 130,000 people and a surface area of approximately 592 km^2. In March 2019, 227 male patients underwent maintenance hemodialysis at all seven dialysis facilities in Awaji Island. Among them, 201 (88.5 %) who gave their informed consent were included in the present study. In women, α-Gal A activity may be normal and is often false negative in screening, making it difficult to evaluate, therefore we included only male hemodialysis patients in our study. The screening test was performed from March to April of 2019.

The trial was approved by the Institutional Review Committee for the Protection of Human Subjects in Research (No. 180302) and was conducted in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from all enrolled patients.

Data collection

Patients' information was reviewed from the medical records in each dialysis facility. Information about

age, dialysis duration, primary cause of ESKD, medical history, and family history was extracted. The medical history included the presence of cardiac disease, cerebrovascular disease, diabetes, and hypertension. Furthermore, the family history included the presence of cardiac disease, cerebrovascular disease, kidney disease, diabetes, and hypertension. No information was collected on whether or not a kidney biopsy had been performed in the past.

Measurements of α-Galactosidase A activity and Genetic analysis

Measurement of α-Gal A activity was performed using dried blood spot samples. After collecting blood samples at the beginning of dialysis, four drops of blood were transferred to a filter paper in each patient (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Subsequently, the filter paper was dried at room temperature and stored at 2 °C–4 °C until the measurement of the enzyme activity. The α-Gal A activity was measured by a fluorescence assay using 4-methylumbellifenyl [5]. In the primary screening, patients with α-Gal A activity <5.0 pmol/hr/disk were considered positive. The secondary screening re-checked α-Gal A activity for the positive patients for the primary screening. Consequently, α-Gal A activity <12.0 Agal U was considered positive for the secondary screening (Figure 2). Among the positive patients for the secondary screening, we would proceed to a genetic analysis of the α-Gal A gene in the case of obtention of the patient's informed consent. The presence of a genetic abnormality for α-Gal A was considered as a definitive diagnosis of FD.

Statistical analysis

Statistical analyses were performed using SPSS version 27 (IBM Corporation, Armonk, NY, USA). We made tables for categorical variables. Continuous data were presented as the mean ± standard deviation while categorical data were presented as frequencies and percentages. A *P*-value of <0.05 was considered statistically significant.

Results

Patient characteristics

The clinical characteristics of the patients in this study are shown in Table 1. The mean age of the study participants was 67.7 ± 13.0 years. The most common cause of ESKD was diabetic nephropathy (N = 84: 41.8%), followed by chronic glomerulonephritis (N = 45: 22.4%), nephrosclerosis (N = 27: 13.4%), and unknown causes (N = 30: 14.9%). The mean duration of dialysis was 7.7 ± 7.1 years. As for the medical history, the number of patients with cardiac and cerebrovascular diseases was 113 (56.2 %) and 38 patients (18.9 %), respectively. In terms of the family history, the number of patients with a family history of cardiac, cerebrovascular, and renal diseases was 31 (15.4%), 23 (11.4%), and 31 patients (15.4%), respectively. Furthermore, 8 (4.0%) patients had a family history of kidney disease and cardiac or cerebrovascular disease, and 1 (0.5%) patient had a family history of renal, cardiac, and cerebrovascular disease.

α-Gal A activity screening

Primary screening revealed that the mean α-Gal A activity was 20.5±10.9 (range 4.61–90.77) pmol/hr/disk in all the study patients, among whom 3 patients (1.5%) had low α-Gal A activity (below the cutoff value of 5.0 pmol/hr/disk) (Figure 3). These three patients were considered positive during the primary screening. The mean of their α-Gal A activity was 4.73 pmol/hr/disk, and one of them had a family history of kidney disease. Subsequently, we conducted secondary screening for the three patients who were positive during primary screening. Their α-Gal A activity was 13.1, 18.7, and 19.2 Agal U, respectively. All three patients had normal α-Gal A activity (above the cutoff value of 12.0 Agal U). Therefore, genetic analysis was not performed for these patients.

Discussion

We screened male patients on dialysis for FD in a remote Japanese island, Awaji Island, and our results revealed several findings as follows: (1) three patients had low α-Gal A activity in the primary screening, (2) the secondary screening showed that all of the three patients had normal α-Gal A activity, and (3) we could not identify patients on hemodialysis with FD although 8 patients (4.0%) had a family history of renal disease and cardiac or cerebrovascular disease, and 1 patient (0.5%) had a family history of renal, cardiac, and cerebrovascular disease.

We focused on the regional differences in the prevalence of FD and performed a screening test for

patients on dialysis in Awaji Island. To date, the prevalence of FD in patients on hemodialysis by a screening test has been reported from various countries. Among European countries, it has been reported that the prevalence of FD was 0.53% (19/3551) by Russian study [6], 0.26% (4/1516) by Austrian study [7], 0.22% (1/508) by Dutch study [8], 1.7% (1/59) by French study [3], and 0% (0/155) by United Kingdom study [2]. On the other hand, there are only a few reports in Asian countries. The studies from Thailand and China have reported that the prevalence of FD was 0% (0/81) [9] and 0.23% (2/876) [10], respectively. In Japan, there are 11 screening tests for patients on dialysis. These previous studies showed that the prevalence of FD in Japan varies from 0% to 1.2% [4, 11, 12]. The variation in the prevalence of FD in each country seems to be due to several factors. First, the sample size and the race of the study patients differed between the studies. Second, there is a possibility that the prevalence of the disease might differ not only with the country but also with the region, even in the same country. In fact, the previous studies examining gene variants of GLA have reported that mutations frequently differ between the east and the south regions in Japan [13, 14]. Furthermore, in addition to the genetic factors, geographical factors such as accessibility and mobility in transportation may affect the prevalence of hereditary diseases. Therefore, we conducted a screening test for FD in male patients on hemodialysis on a remote island, Awaji Island, one of the remote islands in Japan. Our results demonstrated that three patients had decreased α-Gal A activity in the primary screening, and the secondary screening revealed that there were no patients with decreased enzyme activity. Although we could not completely conclude that there were no patients on hemodialysis with FD in Awaji

Island since no further genetic analysis was performed for our study patients, we supposed that there is a low possibility of the presence of male hemodialysis patients with FD in Awaji Island.

In general, it is considered that taking the patient's family history is an important first step toward the diagnosis of genetic diseases. Particularly, FD takes a long time to be diagnosed because the symptoms are often slowly progressive and various [15]. Accordingly, it has been reported that family history could be one of the crucial clues to the diagnosis of FD [16]. However, in most of the previous studies, screening for FD was mainly performed by the enzyme activity measurement, while there are few descriptions of family history. Therefore, we evaluated a family history of cardiovascular, cerebrovascular, and kidney diseases. In our study, 31 patients (15.4%) had a family history of kidney disease. In addition, eight patients (4.0%) had a family history of renal disease combined with cardiac or cerebrovascular disease, and 1 patient (0.5%) had a family history of renal combined with cardiac and cerebrovascular diseases. When screening for FD, it is recommended that FD should be diagnosed with reference to not only α-Gal A activity but also other tests, such as plasma lyso-Gb3 and genetic analysis, especially in the strongly suspected cases with a contributive family history. Thus, we are going to proceed with the further genetic analysis of FD among these patients in the future if they request such analyses. The limitation of this study is the small sample size. In recent years, high-risk screening of patients with renal, cardiac, or neurological manifestations has been conducted in Japan [17, 18, 19], and their sample size are big compared with this study. However, we speculate that both the genetic factors and geographical factors influence the prevalence of Fabry's disease.

Therefore, even though the sample size was small, we believe the strength of this study lies in the fact that it was conducted on a remote island and that almost all male dialysis patients on the island participated in the study.

In conclusion, we could not find out any male patients on hemodialysis with FD in Awaji Island, one of the remote islands in Japan. However, we consider that further genetic analysis is needed, especially in the strongly suspected cases, for the accurate assessment of the prevalence of FD because of the limited α-Gal A activity testing.

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Table 1: Patient characteristics of patients on dialysis

	N = 201
Age (years)	67.7 ± 13.0
Dialysis duration (years)	7.7 ± 7.1
Clinical diagnosis of ESKD	
Chronic glomerulonephritis	45 (22.4%)
Diabetic nephropathy	84 (41.8%)
Nephrosclerosis	27 (13.4%)
ADPKD	4 (2.0%)
RPGN	1 (0.5%)
Chronic pyelonephritis	0 (0%)
Others	10 (5.0%)
Unknown	30 (14.9%)
Past medical history	
Cardiac disease	113 (56.2%)
Cerebrovascular disease	38 (18.9%)
DM	99 (49.3%)
HT	183 (91.0%)

ESKD: end-stage kidney disease, ADPKD: autosomal dominant polycystic kidney disease, RPGN: rapid progressive glomerulonephritis, DM: diabetes mellitus, HT: hypertension

Figure legends

Figure 1 Geographical location of Awaji island in relation to mainland Japan.

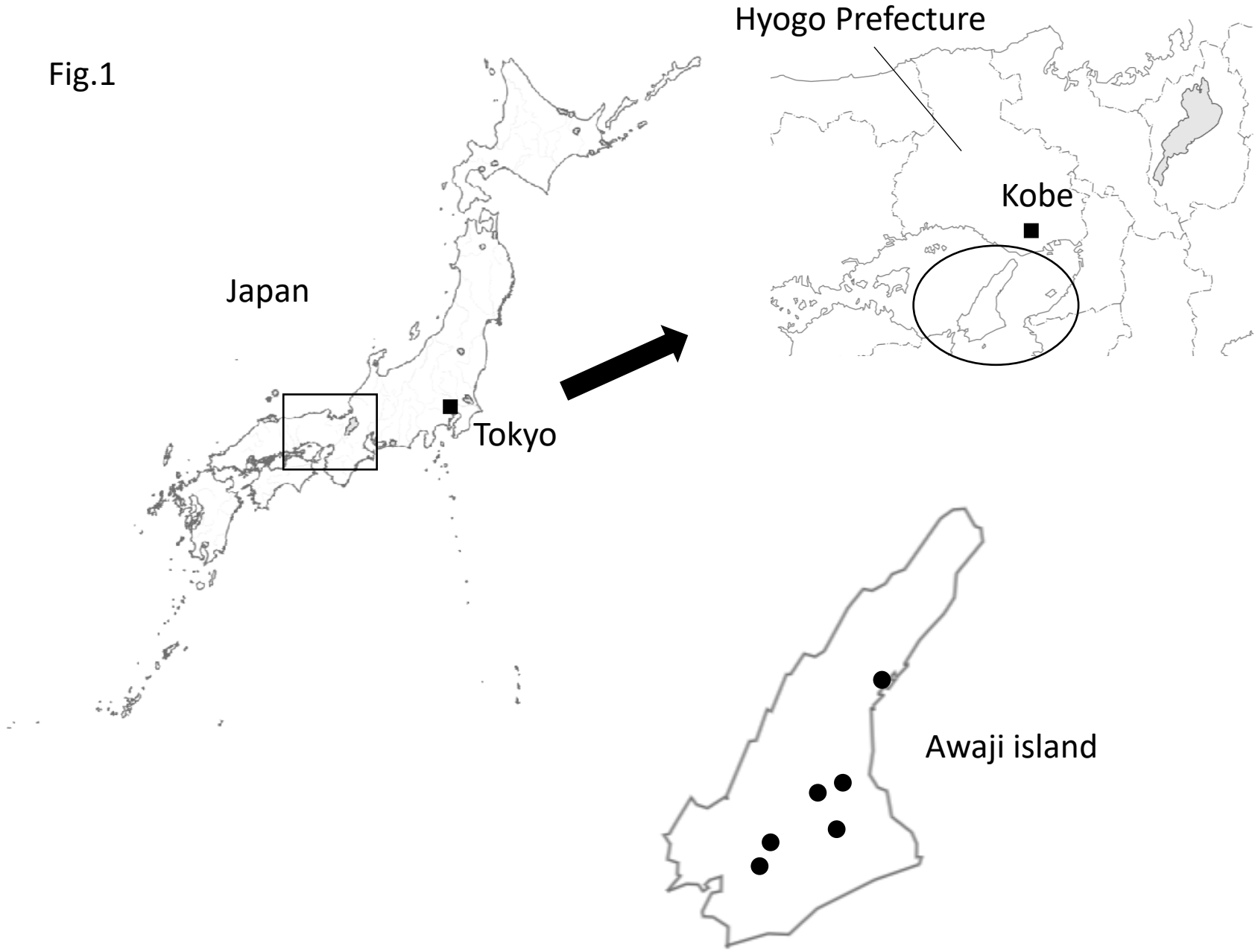
The black dots indicate the locations of the facilities that participated in this study. This figure was created by processing a map from the Geospatial Information Authority of Japan.

Figure 2 Flowchart of the screening procedure for Fabry disease

Figure 3 Distribution of α-Gal activity in the primary screening for Fabry disease

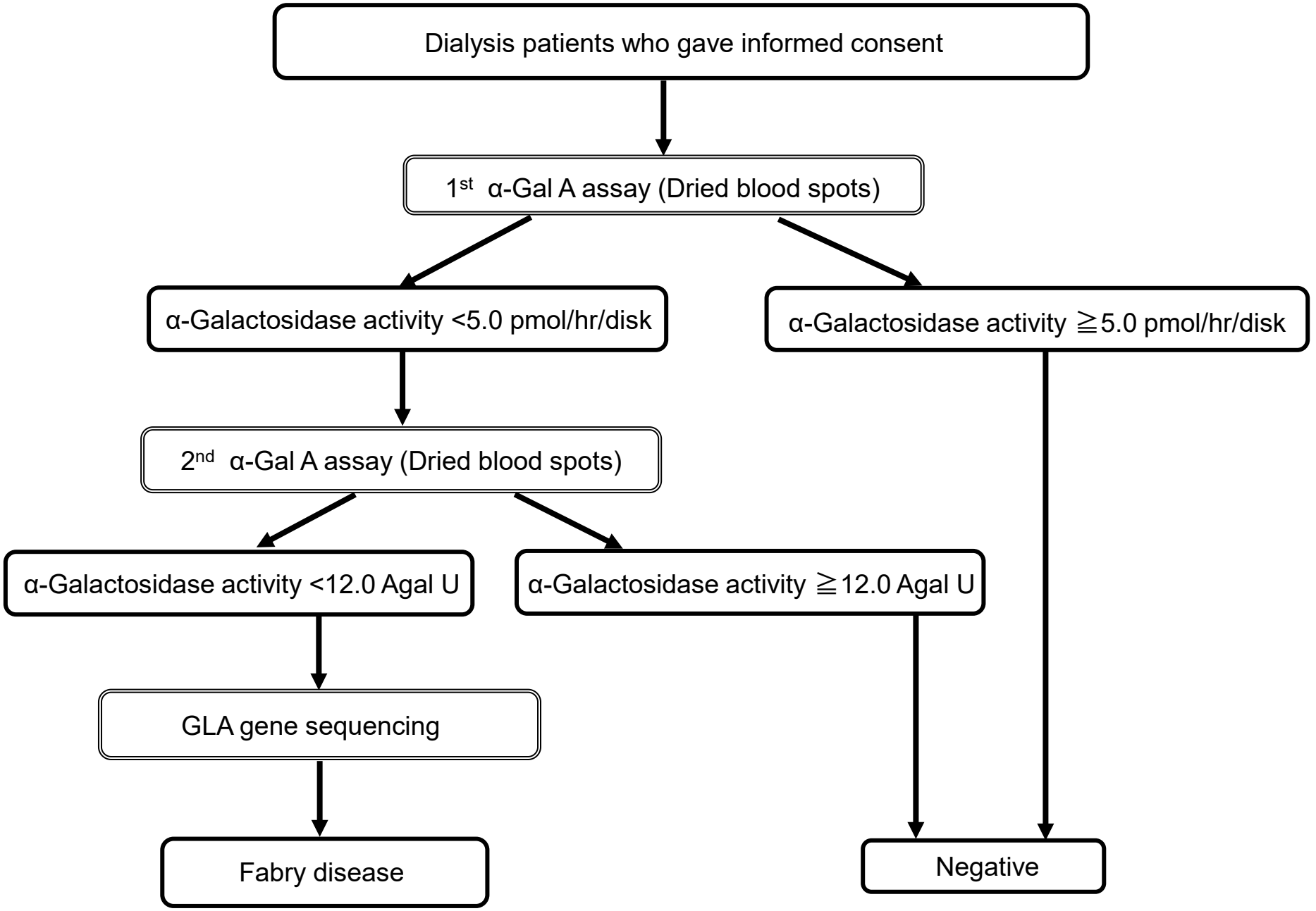
Dashed line: cutoff level (<5.0 pmol/hr/disk)

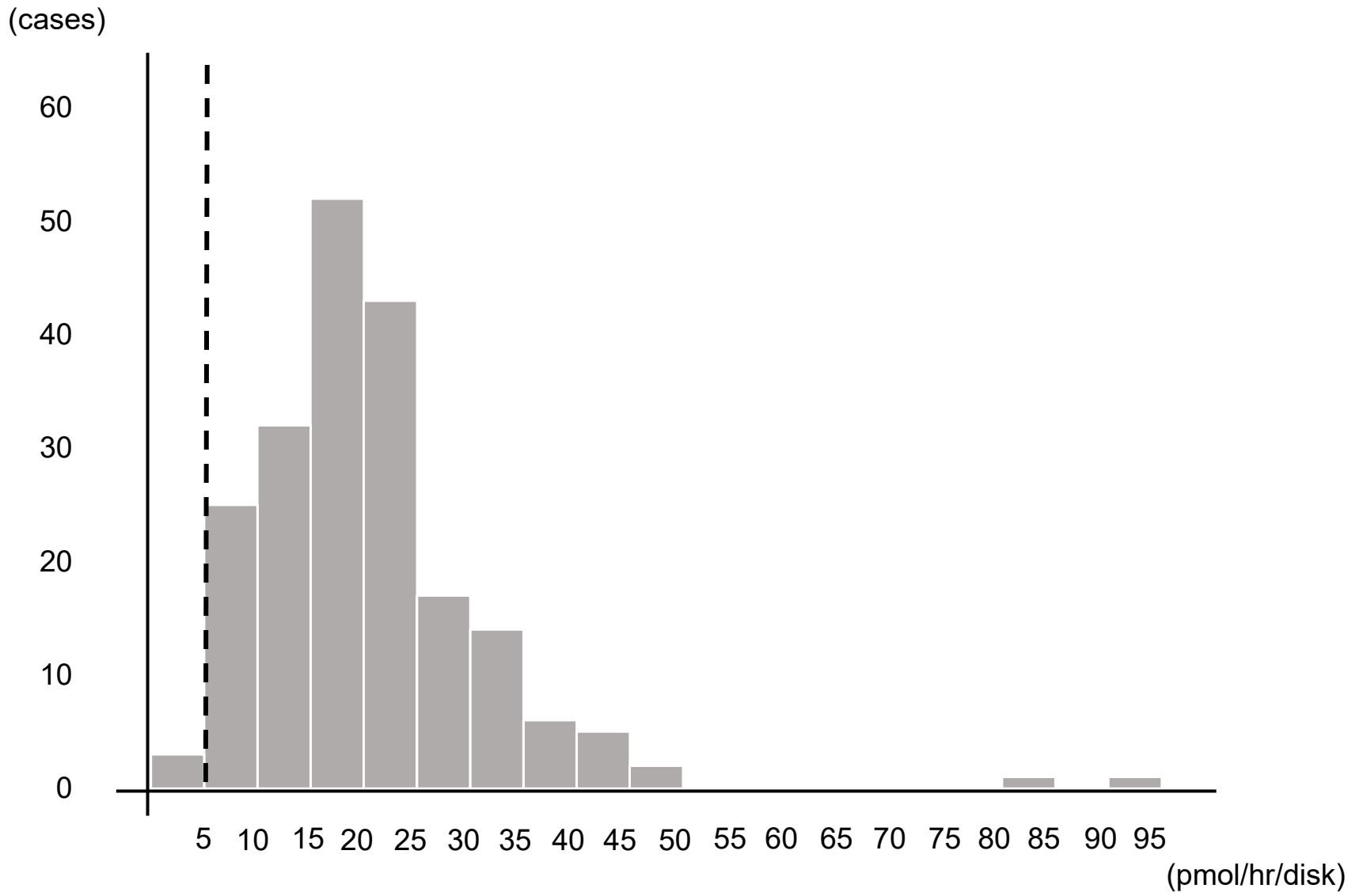
Fig.1



図の説明　(地図の出典・加工したことの記載が必要)

Fig.1: Geographical location of Awaji island in relation to mainland Japan. The black dots indicate the locations of the facilities that participated in this study. This figure was created by processing a map from the Geospatial Information Authority of Japan.


Dialysis patients who gave informed consent
1st α-Gal A assay (Dried blood spots)
α-Galactosidase activity <5.0 pmol/hr/disk
α-Galactosidase activity ≧5.0 pmol/hr/disk
2nd α-Gal A assay (Dried blood spots)
α-Galactosidase activity <12.0 Agal U
α-Galactosidase activity ≧12.0 Agal U
GLA gene sequencing
Fabry disease
Negative


(cases)
60
50
40
30
20
10
0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
(pmol/hr/disk)