Systematic cascade screening in the Danish Fabry Disease Centre: 20 years of a national single-centre experience

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Abstract

The lysosomal storage disorder Fabry disease is caused by deficient or absent activity of the GLA gene enzyme α-galactosidase A. In the present study we present the molecular and biochemical data of the Danish Fabry cohort and report 20 years’ (2001–2020) experience in cascade genetic screening at the Danish National Fabry Disease Center. The Danish Fabry cohort consisted of 26 families, 18 index patients (9 males and 9 females, no available data for 8 index-patients) and 97 family members with a pathogenic GLA variant identified by cascade genetic testing (30 males and 67 females). Fourteen patients (5 males and 9 females; mean age of death 47.0 and 64.8 years respectively) died during follow-up. The completeness of the Fabry patient identification in the country has resulted in a cohort of balanced genotypes according to gender (twice number of females compared to males), indicating that the cohort was not biased by referral, and further resulted in earlier diagnosis of the disease by a lower age at diagnosis in family members compared to index-patients (mean age at diagnosis: index-patients 42.2 vs. family members 26.0 years). Six previously
unreported disease-causing variants in the GLA gene were discovered. The nationwide screening and registration of Fabry disease families provide a unique possibility to establish a complete cohort of Fabry patients and to advance current knowledge of this inherited rare lysosomal storage disorder.

Introduction

Fabry or Anderson-Fabry disease (OMIM #301500) is a rare underdiagnosed X-linked lysosomal disorder resulting from either deficient or absent activity of the lysosomal enzyme α-galactosidase A (α-galA) [1], with universal accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb₃), in lysosomes, resulting in a multisystemic disorder [2]. α-galA is encoded by the GLA gene (HUGO Gene Nomenclature Committee ID: 4296; Gene Entrez: 2717; NCBI reference sequence: NM_000169.2).

Fabry disease was first independently described in 1898 by the German dermatologist Johannes Fabry and the English surgeon William Anderson [3, 4]. The “familial” clustering and inherited nature of the disease were recognized in 1947 [5]. Subsequently, accumulation of Gb₃, and galabiosylceramide inside lysosomes as well as involvement of α-galA in Gb₃ metabolism were identified [1, 6, 7]. Introduction of an assay determining α-galA activity and identification of the genomic sequence encoding the enzyme (GLA), rendered biochemical and molecular confirmation of Fabry disease feasible [8–10]. Variants of the GLA gene were initially reported in 1989 [11].

The disease phenotype can be classified into a severe, classical phenotype, and a generally milder nonclassical, late-onset phenotype. Hemizygous males with pathogenic GLA variants associated with the classic phenotype have little or absent α-galA activity, marked accumulation of Gb₃ and globotriaosylsphingosine (lyso-Gb₃) in tissues and organs and clinical manifestations typically appearing in childhood or adolescence. In contrast, hemizygous males with the late-onset phenotypes have residual α-galA activity, and do not show the early manifestations of classical Fabry disease. Heterozygous females with pathogenic GLA variants may be asymptomatic or develop clinical manifestations as severe as those in males early or at a late point in life.

The first report of Fabry patients in Denmark published in 1972 [12], included a family with five male members with pain and/or peripheral paresthesia provoked by fever or overheating, angiookeratomas, proteinuria, involvement of the central nervous system, cardiac involvement and the characteristic corneal opacities. Three of the five patients died at young age (54, 33 and 38 years), and the remaining two (17 and 33 years old), were seriously affected [13]. Subsequently, when α-galA activity measurements became available in Denmark in the early 1970s, the Danish Fabry patients could be diagnosed biochemically. Nevertheless, the diagnosis of female heterozygotes remained challenging. If the X chromosome with the abnormal allele is predominantly inactivated obligate heterozygotes with no or mild symptoms could have α-galA in the normal range. The diagnostic issue was resolved in the mid-1990s, when sequencing of the GLA gene was introduced allowing specific identification of the disease-causing variants in the Danish patients [14]. Nevertheless, the classification of GLA variants of yet unknown significance as (likely) pathogenic or (likely) benign variants is still clinically challenging and requires further clinical, biochemical and, in some cases, histological investigations.

The production of α-galA by genetic engineering for direct enzyme-substitution therapy in 2001 launched a new era in the treatment of Fabry disease. The Institute of Medical Genetics
at Copenhagen University, transferred the known Fabry families to a clinical facility, Department of Endocrinology and Metabolism, Rigshospitalet, which has since served as the National Danish Fabry referral center, being specialized and licensed to treat patients with multi-organ involvement as well as inherited endocrine disorders, such as diabetes mellitus, adrenal and pituitary diseases, and multiple endocrine neoplasias [15]. Children with Fabry disease are treated in Centre for Inherited Metabolic Diseases, Departments of Clinical Genetics and Pediatrics, Rigshospitalet in collaboration with above department.

Cascade screening is a systematic process offering genetic counseling and testing of relatives of an index-patient for identification of individuals at risk for a hereditary condition based on the a priori likelihood of a positive test [16, 17]. Fabry disease as an X-linked disorder has a high penetrance, though not 100% in female heterozygotes [18]. The X-linked nature of Fabry disease is suitable for an efficient, high diagnostic yield cascade screening over at least three generations surrounding an index-patient. In the present study, we summarize and present the molecular and biochemical data of the Fabry patients from the Danish National Fabry Disease Center spanning 20 years of screening experience.

**Materials and methods**

**Study design**

All Fabry patients diagnosed and followed at the Danish Fabry Disease Centre, Department of Endocrinology and Metabolism, and Centre for Inherited Metabolic Diseases, Departments of Pediatrics and Clinical Genetics, Copenhagen University Hospital, Rigshospitalet between June 2001 and June 2020 were included in the study. We also obtained data, when possible, from studies performed in the Fabry patients before 2001 at Institute of Medical Genetics, Copenhagen University. The diagnosis of hemizygous males was confirmed by identification of the enzyme deficiency and the pathogenic GLA gene variant. Heterozygous females were diagnosed by demonstrating the pathogenic GLA gene variant. A GLA gene variant was classified as pathogenic based on its presence in the GLA-specific databases [19, 20]. In principle, only male family members served to determine or confirm the phenotype and thus the clinical impact of the family variant (classical or late onset).

The Kingdom of Denmark consists of Denmark, Greenland and the Faroe Islands (with a population of 5.8 million, 56,648 and 50,844 inhabitants, respectively) [21–23]; thus, patients living in Greenland and the Faroe Islands are entitled to be referred to Danish hospital expertise at a tertiary referral center, the Danish Fabry Disease Centre.

**Cascade screening strategy**

The genetically verified families transferred to Department of Medical Endocrinology and Metabolism had all received prior genetic counseling and were offered assessment of the phenotypic clinical diagnosis of Fabry disease. In all other index-cases with suspicion of the disease, genetic counseling was offered before testing for the variant. In recent years, the genetic diagnosis was often obtained via the use of gene panels for specific organ manifestations such as inherited causes of cardiomyopathy. In such cases genetic counseling was done after referral to the National Fabry Centre. At the first visit a three-generation pedigree of the family was drawn with identification of relatives to be offered cascade screening. The index-cases/family members with pathogenic GLA variant were subsequently encouraged to contact their relatives and transfer the information regarding the genetic Fabry disease diagnosis. Afterwards the relatives were contacted and offered genetic counseling. Family members eligible to cascade screening were initially informed by the index patient and the Fabry center contacted the family members if consent was obtained. However, in 2012 the Danish Council on Ethics has
introduced five criteria for unsolicited contact with relatives [24], which are met in the case of Fabry disease and therefore our center is obliged to disclose the information to the family members in case the patient with the pathogenic GLA variant is not willing to do so. Only one confirmed index-case, diagnosed before 2012, refused to inform his/her family members, as well as any further contact with him/her.

The following procedure was utilized for the identification of relatives at risk of Fabry disease (Fig 1): (i) In male patients with the pathogenic variant, his mother, his daughter and all his siblings were offered testing; (ii) In females with the pathogenic variant, both her parents, and all her children were offered testing and depending on the parents’ result, either only her female siblings in case of a father with pathogenic GLA variant or all her siblings in case of a mother with pathogenic GLA variant were tested (♂ = male, ♀ = female).

Evaluation of phenotype

All family members with a pathogenic GLA variant, independent of gender underwent comprehensive assessment of clinical symptoms and organ manifestations immediately after the genetic diagnosis was obtained. This included the following: A complete medical history and physical examination to assess features such as “Fabry facial features”, dermatologic evaluation for associated skin lesions (angiokeratomas), acroparesthesias/neuropathic pain; cardiological work-up with electrocardiography, echocardiography and later Holter monitoring; nephrology

Fig 1. Flow chart of the principle of the procedure of the cascade screening. Cascade screening begins once an index-case has been identified. Three generations surrounding the index-case are genetically screened. Each time a family member with a pathogenic GLA variant is identified, the procedure is repeated. When the index-case/family member with a pathogenic GLA variant was male, his mother, his daughter and all his siblings were offered testing. When the index-case/family member with a pathogenic GLA variant was female, both her parents, and all her children were offered testing and depending on the parents’ result, either only her female siblings in case of a father with pathogenic GLA variant or all her siblings in case of a mother with pathogenic GLA variant were tested (♂ = male, ♀ = female).

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evaluation with related biochemical investigations including urinary albumin excretion, measured and estimated glomerular filtration rate; brain magnetic resonance imaging evaluation for presence of lacunar infarcts; ophthalmological evaluation for orbital traits associated with Fabry disease (cornea verticillata, retinal vessel tortuosity); sweat test for the evaluation of anhidrosis or hypohidrosis; lung function test; audiogram for the evaluation of hearing thresholds. Therefore, due to the wide spectrum of clinical manifestations, Fabry patients are followed by the Copenhagen Fabry Multidisciplinary Team, consisting of doctors and nurses from a number of different specialities (cardiologist, nephrologists, ophthalmologists, neurologists, paediatricians, radiologists, dermatologists, gastroenterologists, pulmonologists, audiology specialist, psychiatrists, genetic counselors, primary care providers, clinical nurse specialists).

**GLA gene analysis**

Genetic testing was initially centralized in a research setting at the Department of Medical Biochemistry and Genetics, University of Copenhagen. Through 2005–8 the analysis was gradually transferred to a clinical setting at the Department for Clinical Genetics at Copenhagen University Hospital. Over time a variety of different techniques have been used for variant detection [11, 25–29]. The discovered variants were classified according to severity as classical or late-onset by the gene-specific databases when they were known [19, 20]. In case the identified GLA variant was absent from the gene-specific databases, the hemizygous male family members served to classify the GLA variant by phenotype assessment. NM_000169.3 was used as reference sequence.

**Measurement of α-galactosidase A activity in leukocytes**

α-GalA activity was determined in mixed leukocytes using a fluorimetric assay with 4-methylumbelliferyl-α-D-galactopyranoside as substrate [26, 30, 31]. The fluorescence intensity was measured in a Fluostar Optima plate reader (BMG Labtech) at excitation and emission wavelengths of 360 nm and 450 nm, respectively.

**Measurement of plasma Gb₃ and Lyso-Gb₃ and urine Gb₃**

Gb₃ measurements were performed at Sahlgrenska University Hospital, Sweden up to 2006 by densitometric evaluation after high performance thin layer chromatography for plasma Gb₃ and orcinol detection in the case of urine Gb₃ [32] and afterwards in Genzyme’s laboratories, using a rapid liquid chromatography with tandem mass spectrometry LC/MS/MS method for quantification of total plasma- lyso and urine Gb₃ [33]. Gb₃ units from Sahlgrenska were given in mg/L (reference: 1.6–3.3) while more recent measurements from Genzyme were in μmol/L (reference: <7.0). The calibration of methods has changed over time, but no attempts were made to correct values for unification. Therefore, each individual measurement was compared to the respective method- and laboratory derived normal reference range. Lyso-Gb₃ was not measured in all patients before treatment start, because these measurements were not available routinely in 2001. Early participants had the measurements later in the course of the disease either during treatment or untreated. Lyso-Gb₃ was only measured in Genzyme’s laboratory.

**Statistical analysis**

Due to the nature of the data as well as low numbers in all subgroups, descriptive statistics were computed as mean (range and/or standard deviation) and/or median (range and/or interquartile range) for continuous variables and as counts and percentages for categorical variables.
Ethics

The study was approved by the Danish Health and Medicine Authority (3-3013-667/1), the Regional Health Research Ethics Committee (H-3-2014-FSP8) and the Danish Data Protection Agency (2014-641-0055).

Results

Patients—Cascade screening

A total of 115 patients were included in the Danish Fabry Disease Centre up to June 2020, born between 1936 and 2019, of whom 39 were males and 76 females. The 115 patients belong to 26 families. During the follow up period all patients resided in Denmark, Greenland or the Faroe Islands. Fourteen patients (5 males and 9 females) died during follow-up. Mean age of death for all causes was 58.5 (range 36.6–77.2) years in all patients, 47.0 (range 36.6–51.9) years in males and 64.8 (range 47.5–77.2) years in females. One hundred and one patients (34 males and 67 females) were alive at the end of the follow up period. The prevalence rate of Fabry disease in the Danish population is estimated to be 1:58000 (males 1:85000, females 1:44000). In general, family members complied to the genetic cascade screening, and only six abstained from further follow-up.

Pathogenic GLA variants

In total 24 different pathogenic GLA variants were identified (Table 1); 15 missense variants (Fig 2), 4 variants were predicted to introduce a premature stop codon, one indel variant (c.369+3_c.547+954del4096insT), one splice site deletion (c.999+1del), one deletion (c.885del) and one duplication (c.864dup) both leading to a frameshift. Two families shared the p.N34S and two the p.R301X variants, without any recognized relationship between the families. Five of the Danish pathogenic gene variants have not been registered in the fabry-database.org [19] nor in the International Fabry Disease Genotype-Phenotype Database [20] and another one does not appear in the International Fabry Disease Genotype-Phenotype Database (Table 1).

Index-patients

Three patients were not of Danish origin: one male was from Middle East, one male from the United States of America, and one female from South America. None of the index-patients in these three families were diagnosed in Denmark and therefore not registered in the Danish Fabry Disease Centre (Table 1). The remaining 23 index-patients were of native Danish origin. We could not with certainty obtain data regarding the index-patients of four families as they died before 2001. One index-patient did not want baseline assessment, further contact and follow-up and refused to disclose the information to his/her family members. Another index-patient did not want further contact and follow-up after being evaluated at baseline but he/she informed his/her family members. The mean follow-up period of the living index-cases was 8.4 years (range 0.3–19.5 years).

From the 18 known index-cases, 9 were males and 9 were females, with a mean age (±standard deviation) at referral 32.9±14.6 and 51.5±19.0 years, respectively (range: 10.0–54.0 and 15.9–72.7 years, respectively). One female index-patient died during the follow-up period at the age of 71.6 years. The mean age (±standard deviation) at the end of the follow-up period for the alive male and female index-patients was 42.0±13.3 and 57.2±13.6 years, respectively (range: 19.3–61.0 and 30.4–73.8 years, respectively) (Table 2). The mean age of the family members was substantially lower than that of the index-patients especially at diagnosis but also at follow-up (Table 2).
Table 1. The 26 families, their 115 Fabry disease patients and their corresponding GLA-gene variants of the Danish Fabry disease register. Type of the GLA gene pathogenic variants, protein nomenclature, colloquial nomenclature, coding sequence (according to http://varnomen.hgvs.org), site of mutation and genotype classification (according to International Fabry Disease Genotype-Phenotype Database (dbFGP) http://dbfgp.org/dbFgp/fabry/ and the http://fabry-database.org) are presented. Sex, age (in years) and the primary clinical manifestation at Fabry disease diagnosis of the index-cases are presented.

| Family ID | Amino acid change | Nucleotide change | Exon | Likely pheno type | N (Deceased) | Index-cases |
|-----------|-------------------|-------------------|------|-------------------|--------------|-------------|
| A         | p.Asn34Ser        | N34S              | 1    | Classic           | 4            | F (27) neuropathic pain |
| B         | p.Asn34Ser        | N34S              | 1    | Classic           | 2 (1) 2       | M (?) N/A deceased |
| C         | p.Gly85Asp        | G85D              | 2    | Classic           | 26 (7) 8 (2) 18 (5) | F (?) N/A deceased |
| D         | p.Arg112Cys       | R112C             | 2    | Classic           | 15 3 12      | M (12) neuropathic pain |
| E         | p.Ala156Thr       | A156T             | 3    | Classic           | 13 (3) 10 (2) M (33) | N/A deceased |
| F         | p.Gly171Ser       | G171S             | 3    | Late-onset        | 2 1 1 M (28) nephropathy |
| G         | p.Ile232Thr       | I232T             | 5    | Likely classic    | 10 (1) 4 6 (1) M (36) proteinuria |
| H         | p.Trp236Cys       | W236C             | 5    | Classic           | 3 2 1 F (51) cardiomyopathy |
| I         | p.Gly261Asp       | G261D             | 5    | Classic           | 6 2 4 F (68) cardiomyopathy |
| J         | p.Gly271Ser       | G271S             | 6    | Classic           | 4(1) 3 (1) F (66) cardiomyopathy |
| K         | p.Ser276Asn       | S276N             | 6    | Classic           | 1 1 - F (?) cardiomyopathy |
| L         | p.Gln279Arg       | Q279R             | 6    | Classic           | 1 1 - M (54) cardiomyopathy |
| M         | p.Ser297Phe       | S297F             | 6    | Classic           | 2 1 1 F (73) cardiomyopathy |
| N         | p.Arg301Gln       | R301Q             | 6    | Late-onset **     | 2 - 2 N/A |
| O         | p.Ile317Thr       | I317T             | 6    | Classic           | 2 1 1 M (40) cardiomyopathy, nephropathy, neuropathic pain |
| P         | p.Asn355Lys       | N355K             | 7    | Classic           | 2 1 1 M (36) nephropathic pain |
| Q         | p.Lys168Ter       | K168*             | 3    | Novel*            | 1 - 1 F (63)*** cardiomyopathy |
| R         | p.Arg227Ter       | R227*             | 5    | Classic           | 2 - 2 M (?) N/A deceased |
| S         | p.Arg301Ter       | R301*             | 6    | Classic           | 3 - 3 F (53) cardiomyopathy |
| T         | p.Arg342Ter       | R342*             | 7    | Classic           | 4 1 3 M (33) neuropathic pain |
| U         | (large deletion)  | c.195-?_801 +? del | 2–5 | Novel*            | 1 1 - M (47) cardiomyopathy |
| V         | (deletion-insertion) | c.369+3_c.547+954del| 3–4 | Classic           | 1 - 1 F (16) migraine |
| W         | p.Phe295Leufs*22 | c.885del          | 6    | Novel*            | 3(1) 2 (1) 1 M (10) neuropathic pain |
| X         | (splice site deletion exon 6 skipping) | c.999+1del* | Novel* | 1 1 - M ** non-Danish |
| Y         | p.Ile289Tyrfs*10 | c.864dup          | 6    | Novel*            | 1 1 - M ** non-Danish |

Total 115 39 76

* Novel variants that have not previously been reported in the literature nor in the Fabry Genotype-Phenotype Databases (International Fabry Disease Genotype-Phenotype Database (dbFGP) (http://dbfgp.org/dbFgp/fabry/) and http://fabry-database.org)

** Reference 25

† never referred to the Department

‡ does not live in Denmark

*** no contact with the family

†† unknown

N/A not available

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Fig 2. Structure of human GLA and positions of the amino acid substitutions resulting from the missense pathogenic variants identified in the Danish Fabry patients. The backbone is displayed as a ribbon model, and the ligand and sugars as a stick model. The amino acids involved in the substitutions and the catalytic residues (D170 and D231) are indicated as a space-filling calotte (Corey-Pauling-Koltun CPK) model. Front view (top) and back view (bottom).

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Index-cases were referred to the Department from cardiologists, nephrologists and neurologists. Eight were investigated for Fabry disease due to cardiomyopathy, five due to neuropathic pain, two due to kidney disease, one for migraine and one had cardiomyopathy, kidney disease and neuropathic pain. The reason for referral was unknown for one index-patient.

α-galactosidase A activity

Enzyme activity in leukocytes was available in 35 (90%) male and 61 (80%) female patients. All males had low to absent leukocyte α-galA activity (Fig 3). The median enzyme activity in males was 1.6 nmol/h/mg protein (25th–75th percentile: 1.1–2.0 nmol/h/mg protein; range: 0.3–11.0 nmol/h/mg protein; normal α-galactosidase A activity range: 20–65 nmol/h/mg

|                    | Index-cases (n = 18) | Family members (n = 97) |
|--------------------|----------------------|-------------------------|
| Available data N   | 18                   | 61                      |
| Age at diagnosis (years) |                     |                         |
| Mean (SD)          | 42.2 (19.0)          | 26.0 (19.4)             |
| Range              | 10.0–72.1            | 0.0–72.5                |
| Sex (M/F)          | 9/9                  | 30/67                   |
| Deceased           |                      |                         |
| Sex (M/F)          | -/1                  | 5/8                     |
| Age at diagnosis (years) |                  |                         |
| Mean (SD)          | 66.4                 |                         |
| Age of death Mean (years) (SD) |                |                         |
| Age of death Range | -                    | 36.5–77.2               |
| Alive              |                      |                         |
| N                  | 17                   | 84                      |
| Sex (M/F)          | 8/9                  | 59/25                   |
| Age at diagnosis (years) |                  |                         |
| Mean (SD)          | 40.8 (18.6)          | 26.0 (19.4)             |
| Age at diagnosis (years) Range |            |                         |
| Age of death Mean (years) (SD) |         |                         |
| Age of death Range | 19.3–73.8            | 0.7–83.9                |
| First manifestation |                      |                         |
| Cardiomyopathy     | 8                    |                         |
| Neuropathic pain   | 5                    |                         |
| Kidney disease/proteinuria | 2                |                         |
| Migraine           | 1                    |                         |
| Cardiomyopathy, neuropathic pain, kidney | 1   |                         |
| Unknown            | 1                    |                         |

Index-patients: 17 + 3 unknown from abroad + 1 with no contact + 4 died before 2001 + 1 died after 2001 = 26 families
Cohort: 84 relatives alive + 18 index-patients (17 alive + 1 dead) + 13 dead (1 dead index-patient) contact = 115 patients
M: male; F: female; SD: Standard deviation

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protein), whereas in female patients, the enzyme activity showed a wide range (1.6–39.0 nmol/h/mg protein; normal α-galactosidase A activity range: 20–65 nmol/h/mg protein). Of the 61 female patients tested, 17 (28%) presented with normal enzyme activity at diagnosis. The median enzyme activity in females was 15 nmol/h/mg protein (25th–75th percentile: 10–21 nmol/h/mg protein). No statistically significant difference in enzyme activity was found between females with missense and nonsense mutations (n = 52 vs. 7; median: 15.0 vs. 17.0 nmol/h/mg protein, respectively), but numbers were too small for reliable conclusions to be drawn. Comparison of enzyme activity between males with missense vs. males with nonsense mutations was not feasible because only one male had a nonsense mutation (29 males with missense mutation vs. 1 male with nonsense mutations).

**Gb₃ in plasma and urine and Lyso-Gb₃ in plasma**

Gb₃ in plasma and in urine was available in 102 (36 males and 66 females) and 88 (34 males and 54 females) patients, respectively. Lyso-Gb₃ in plasma was available in 84 patients (29
males and 55 females). Due to the use of different laboratories and assays during the reported period, the results were used only in relation to the overall assessment of each individual index-patient’s disease severity. Nevertheless, males had plasma Gb$_3$ and lyso-Gb$_3$ concentrations, respectively, higher than the normal upper limit (high vs normal plasma Gb$_3$: 24 (67%) vs 12 (33%); high vs normal plasma lyso-Gb$_3$: 25 (86%) vs 4 (14%)), while the opposite was true for the female Fabry patients (high vs normal plasma Gb$_3$: 17 (26%) vs 49 (74%); high vs normal plasma lyso-Gb$_3$: 22 (40%) vs 33 (60%)).

**Discussion**

In this retrospective study we have presented data on genetic screening from the Danish Fabry Disease Centre at Department of Endocrinology and Metabolism at Copenhagen University Hospital, Rigshospitalet, which has served as national reference center for Fabry disease patients in the Kingdom of Denmark since 2001. The department is responsible for diagnosis and follow-up of adult Fabry disease patients as well as for genetic counseling and screening of their families while children with Fabry are followed in collaboration with the Centre for Inherited Metabolic Diseases at the same hospital. The Danish Fabry Disease Centre provides a comprehensive overview of Fabry disease patients’ medical history and health condition and constitutes a significant research tool for this rare disease with the help of the population-based National Patient Register, which contains detailed medical information on admissions to somatic hospital departments since 1977 [34]. More than 25 published papers have so far been based, totally or partially, on data from the Danish Fabry Disease Centre [26, 30, 35–50] including randomized clinical trials [51–55]. In this paper we aimed to report the 20 years of experience in diagnosing patients with Fabry disease in Denmark and describe the genetic counseling and cascade screening procedure conducted in their family members. Follow-up was done concerning age (calculated as mean) at baseline and end of the study period as well as status of dead or alive.

The vast majority of patients in the Danish cohort were identified after family cascade screening: 18 index-patients and 97 genetically identified family members with Fabry, or in mean 5.4 affected family members per index-case. This number is slightly higher than the one reported in a recent literature review on family genetic testing in Fabry disease, which revealed the identification of an average of 4.8 additional affected family members per index-patient (365 index-patients and 1744 affected family members) [56]. In addition, it indicates high efficacy of the cascade family screening for the identification of Fabry patients. Other valuable tools for the identification of undiagnosed Fabry patients are newborn screening or screening of at-risk populations (e.g., hypertrophic cardiomyopathy, hemodialysis patients, stroke). It has been suggested that the combination of the cascade family screening with the newborn/at-risk populations screening could identify several additional undiagnosed Fabry patients [56, 57]. However, screenings conducted in newborns or in at-risk populations carry a high risk of identifying numerous likely benign and benign GLA variants [56].

Most of the families in this study were of the classical Fabry genotype, but those referred from genetic variant screening programs for e.g. hypertrophic cardiomyopathy are mostly of later onset types and of milder phenotypes [56, 58]. The great majority of the variants identified in our cohort were unique (‘private’). Most of the alleles were missense variants (15/24) and we found in addition 4 nonsense variants, 1 large deletion, 1 indel (blend of insertion and deletion), and three single nucleotide deletions/duplications affecting splice sites or reading frame. The distribution reflects the overall reported pathogenic variants (HGMD Professional 2021.3 database): 67.7% missense/nonsense [59], fabry-database.org: 71% missense, 17% nonsense variants [19]. None of the missense variants were novel whereas one nonsense and four
of the remaining variants were novel. Four of the novel variants are loss-of-function variants (large deletion, deletion-frameshift, splice site deletion exon 6 skipping, duplication-frameshift) and the index-cases harbouring them were males with confirmed phenotypic Fabry disease metabolic defects. Therefore, these four variants have been interpreted as pathogenic according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines [60]. The fifth novel variant is a nonsense variant, and the index-patient was a female with normal enzyme activity, high concentrations of plasma lyso-Gb₃, hypertrophic cardiomyopathy (endomyocardial biopsy revealed Fabry disease specific abnormalities) but without manifestations from the kidney. No male family member harbouring the variant has been identified. This variant can be interpreted as at least likely pathogenic (class 4) as a loss-of-function variant. It should be noted that in general, nonsense variants are associated with the classic phenotype [61].

In X-linked inheritance disorders, the number of heterozygotes is expected to be the double of homozygotes. In our cohort, we identified twice as many heterozygotes as homozygotes. This underlines the completeness of the family screening. Nevertheless, because Fabry disease is an X-linked recessive disorder, the heterozygotes present with a heterogenous phenotypic spectrum, from asymptomatic to as severely affected as their male relatives. A possible explanation for this wide clinical spectrum may be a skewed X chromosome inactivation, which results in preferential inactivation of the X chromosome carrying the normal allele [62].

Index-patients were referred to the Department from other clinics, mainly from cardiology where they had been diagnosed during genetic investigation for cardiomyopathy [41]; to a lesser extent, from neurologists due to atypical neuropathic pain or migraine, or from nephrologists due to incidental findings in a kidney biopsy. In some countries many of the Fabry patients with first manifestations from a stroke or neuropathic pain would be identified by screening [63, 64]. A few countries perform newborn screening [65], which has resulted in disclosing many variants of unknown significance in the GLA gene and only few patients with classical Fabry disease. None of these screenings have been performed in Denmark.

The heterogeneity and the shared clinical manifestations of Fabry disease with other diseases often result in delayed diagnosis. Cascade genetic screening in known families is a simple and effective method for early diagnosis of the disease.

Early diagnosis of treatable rare genetic diseases is important when effective therapies exist, that can delay the progression of the disease and improve outcomes. Previous studies have shown that Fabry disease patients with classical GLA variants that have initiated treatment in early stages had better outcomes compared with those having started treatment later, especially when irreversible organ damage was already present [66–70]. Moreover, early diagnosis provides the opportunity to offer follow-up in a multidisciplinary and genetic counseling team [56]. Systematic and proper cascade screening requires thorough genetic counseling of all family members. In young Fabry patients it is important to counsel early to be able to provide them with a good basis for reproductive choices, including information about the inheritance pattern, recurrence risks, the individually different phenotype/genotype relationships and the availability of preimplantation and prenatal genetic diagnostics.

Performing proper genetic and management counseling also requires a thorough knowledge of the phenotype/genotype relationship as well as treatment options and their possible outcomes in relation to the individual phenotype of the patient. In the beginning of the era of the first enzyme replacement therapy, it was still not recognized that females could be as affected of Fabry disease as males. It was also not recognized that a severely affected individual could have an asymptomatic child and vice versa—this was only gradually published [71, 72].

In our Fabry cohort, two confirmed index-cases refused further investigation (and follow-up) and in addition one of them refused to provide information on close family members.
general, index-cases, as well as the family members identified via family screening were overwhelmingly compliant to inform their relatives. There is an ongoing discussion about patients' “right not to know” and “right to refuse treatment”. Arguments defending and criticizing the “right not to know” are based on the values of autonomy, liberty and duty [73]. One can argue that patients should take responsibility for decisions regarding the management of their health and lives enacting their “right not to know” and “right to refuse treatment”, however, it should not be neglected that this could negatively affect their family members that might carry the same pathogenic variant, and thus deprive their family members of their “right to know”.

The mean age of death in our cohort was 47.0 years for males and 64.8 years for females, compared with a life expectancy at birth (2000–2020) of 74.3–79.6 and 79.0–83.6 years, respectively, in the general population in Denmark [74]. The mean age of death revealed in our cohort was lower in males but higher in females than the one reported in the Fabry Fabrazyme Registry (53.7 years in males and 60.9 years in females) [75]. This could be due to a number of factors, small cohorts with low statistical power being the most likely. Data from the same registry revealed that the most common cause of death in Fabry disease patients was cardiovascular disease, followed by cerebrovascular events (i.e. stroke). Unfortunately, in our cohort the cause of death was not known in all deceased patients.

Strengths and limitations

To the extent of our knowledge, we present one of the most complete and unbiased genetic cascade screenings in a nationwide Fabry cohort. All adult Danish patients with Fabry were followed in a single center, which ensures internal consistency across all examinations. More importantly all relevant family members were offered genetic testing once a Fabry positive diagnosis had been made and complete organ assessment was performed in all patients irrespective of symptoms. A strength of the study is that we have verified our unbiased genetic inclusion by having twice the number of females in the national cohort compared to males.

Due to suboptimal compliance issues some index-cases and family members lacked the initial assessments which is one of the limitations. Furthermore, given that Gb3 measurements were not available in 2001, these were initiated later and subsequently obtained from two laboratories, both of which changed methodology over time. Finally, a substantial data amount was collected prior to the lyso-Gb3 era why most patients did not have these measurements initially.

Conclusions and perspectives

In this paper, we present descriptive outcomes of 20 years of experience in diagnosis, mortality and cascade screening of Fabry disease patients in the Danish Reference Centre for Fabry disease, resulting in as complete family screening as possible, taking into consideration consent from patients, by including twice the number of females as males due to X-linked inheritance. The completeness of the Fabry patient identification in the country has as such resulted in a cohort of balanced genotypes according to gender, indicating that the cohort was not biased by referral, and further resulted in earlier diagnosis of the disease by a lower age at diagnosis in family members compared to index-patients; such is rarely seen in other Fabry cohorts [66, 76]. Six previously unreported disease-causing variants in the GLA gene were discovered.

This nationwide screening and registration of Fabry disease families provides a unique possibility to establish a complete cohort of Fabry patients and to advance current knowledge of this rare lysosomal storage inherited disorders.
Supporting information

S1 Data.
(SAV)

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