Identification of patients with Fabry disease using routine pathology results: PATHFINDER (eGFR) study

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Abstract

Aims: Lysosomal α-galactosidase A deficiency (Fabry disease (FD)) was considered an X-linked recessive disorder but is now viewed as a variable penetrance dominant trait. The prevalence of FD is 1 in 40000-117,000 but the exact frequency is disputed depending on ascertainment of late-onset cases and degree of female penetrance. Its prevalence in the general population, especially in patients with abnormal renal function is unclear. This study attempted to identify the prevalence of FD in patients with abnormal results identified from laboratory databases. Methods: Electronic laboratory databases were interrogated to identify from clinical biochemistry records patients with a phenotype of reduced estimated glomerular filtration rate categorised by age on one occasion or more over a 3-year time interval. Patients were recalled and a dried blood spot sample was collected for determination of α-galactosidase A activity by fluorimetric enzyme assay in men and mass spectrometry assays of α-galactosidase A and lyso-globotriaosylceramide (lyso-GL-3) concentrations in women. Results: Samples were obtained from 1084 patients identified with reduced renal function. No cases of FD were identified in 505 men. From 579 women one subject with reduced α-galactosidase activity (1.5 μmol/l/hr) and increased Lyso-GL-3 (5.5 ng/ml) was identified and shown to be heterozygous for a FD mutation (c.898C>T; p.L300F; Leu300Phe). It was later confirmed she was a relative of a known affected patient. Conclusions: Pathology databases hold routine information that can be used to identify patients with inherited errors of metabolism. Biochemical screening using reduced eGFR has a low yield for unidentified cases of Fabry Disease.

What is known:

- Fabry's Disease is a treatable rare multisystem disorder affecting renal function with complex inheritance.
- Its prevalence in the general population is unclear.

What this article adds:

Screening laboratory databases using age-adjusted renal function measures can identify patients with Fabry's disease but at a low yield

Introduction

Lysosomal α-galactosidase A deficiency causes Fabry disease (FD)[1-3], which was considered to be X-linked recessive and therefore females were simply carriers but more recently, it has been shown to be a variable penetrance dominant trait as up to 70% heterozygous females are symptomatic[4,5]. The prevalence of Fabry's disease is 1 in 40000-117,000 but the exact frequency is disputed depending on ascertainment of late-onset cases, female penetrance and variants of unknown significance[6,7]. Deficiency of lysosomal α-galactosidase A activity causes accumulation of globotriaosylceramide (Gb3), globotriaosylsphingosine (Lyso Gb3) and related glycosphingolipids throughout the body[8]. Clinical symptoms are variable depending on age of onset

and include abdominal pain in 60%, neuropathic pain and sensorineural deafness, multiple angiokeratomas, renal failure, cardiomyopathy and stroke[1-3].

Identification of patients with rare diseases is difficult. Case-finding depends on the prior possibility of disease. Populations with end stage renal disease (ESRD) on dialysis show a 10-fold Fabry Disease enrichment compared to the general population[7,9] and cases are found in cardiology and stroke clinics[7] but unidentified cases may be difficult to locate in the wider population. As enzyme replacement is now available, it would preferable to identify patients prior to onset of renal failure[10]. The PATHFINDER project is based on the premise of using routine laboratory results to identify patients who should be tested for inherited errors of metabolism. The project design has been validated in patients with other disorders[11]. This paper describes the use of age and creatinine-based estimated glomerular filtration rate (eGFR) as screening biomarkers for FD.

Methods

Subjects

Subjects were identified at 9 hospitals serving approx. 4,575,000 people by interrogating laboratory databases to identify individuals in three specific age bands with reduced eGFR calculated using the Modified Diet in Renal Disease (MDRD; 4 variable) or chronic kidney disease epidemiology (CKDEPI) equations. Thresholds were chosen empirically by estimating a feasible population size that could be sampled based on the distribution of eGFR results in a sample of data from Queen's Hospital, Burton.

- Age 20-49, eGFR 15-54 mL/min/1.83m^2
- Age 50-59, eGFR 15-49 mL/min/1.83m^2
- Age 60-69, eGFR 15-39 ml/min/1.83m^2

Laboratory database searches were performed using creatinine measurements over a maximum a 6-year time period interval limited by site-specific data access issues. Once provisionally identified, subjects were invited to take part in the study as they had been identified as having abnormal results in routine pathology testing. They were asked if they would meet with a research nurse to allow collection of a blood sample for lysosomal α-galactosidase activity. At the research visit, patients provided informed consent, and basic demographic details were collected. An EDTA blood sample was collected and single drops were spotted on a dried blood spot (DBS) collection card. Subjects indicated their preference for anonymity or if they wanted to receive a copy of their results.

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Male samples

Analysis of DBS cards was performed at a regional inherited errors of metabolism laboratory. A 3 mm diameter disc was punched from the DBS and extracted in sodium taurocholate by shaking at 4°C for 60 min. Sample extract was transferred to black 96-well microplates and incubated with substrate for 20 hours at 37°C. Samples were analysed for α-galactosidase, and acarbose-inhibited acid α-1,4-glucosidase, total acid α-1,4-glucosidase (measured as a reference enzyme) activities measured simultaneously in different wells on the same plate[12]. The enzymatic reactions were stopped by the addition of 100 µL/well sodium carbonate buffer (pH 9.5). Assays were performed in duplicate, and one blank was assayed for each sample. To prepare blanks, extract from each sample was added to incubated substrate solution after the enzymatic reactions were stopped. The relative fluorescence (excitation 360 nm, emission 460 nm) of each well was measured using a Biotek Synergy HT fluorescence plate reader. Fluorescence readings were corrected for the blank and compared with a 4- calibrator (supplied from Sigma)

The absolute amount of whole blood per spot is not known accurately but is comparable between samples, so enzymatic activities are expressed as pmol of substrate hydrolysed per punch per hour (pmol/punch/hour). α-galactosidase A activity below the reference range for males (6.3 – 47.0 pmol /punch/ hr) was considered significantly reduced. If activity in the first sample was low, patients were invited to return for repeat

sampling and a fresh whole blood EDTA sample was sent for analysis. Repeat samples were analysed in duplicate for alpha galactosidase activity in leucocytes and plasma with beta-galactosidase and beta-hexosaminidase (A and B) analysed as reference enzymes respectively. Repeat assessment of blood spot activities of α-galactosidase and α-1,4-glucosidase was also performed. Reference ranges had been established by the local laboratory and had been validated across UK specialist laboratories. Results were collated by Queen's Hospital, Burton and communicated to participating sites.

Female Samples

Fabry disease is X-linked but females can still be affected. There is overlap at the lower end of the α-galactosidase A activity reference range between affected and unaffected cases so a biomarker is also used. Samples from females only were sent to ARCHIMEDlife laboratories (Vienna, Austria) for assay of α-galactosidase A activity and Lyso-globotriaosylceramide (lyso-GL-3), a biomarker that is increased in Fabry disease, by Tandem mass spectrometry[8]. Results from the female samples showing either a low enzyme activity, increased lyso-GL-3 or both resulted in genetic sequence testing. α-galactosidase activity below the reference interval for females (1.2 – 50.0 μmol/l /hr) or Lyso-GL-3 above the reference limit (0-3.5ng/ml) were considered significant. (comment: ref interval for a gal enzyme activity analysed for the female samples at Archimed changed about the start of June 2019 to 2.8 - 50.0 umol/l/hr, as per letter issued, so if there are any samples that were sent for analysis after this timeframe the new range will have been used).

Results

Database searches identified 1109 potential patients, with results available for 1105. Protocol deviation was found in 21 cases with 3 subjects aged below 20yrs, and 18 over 70yrs leaving 1084 subjects in total. Demographic information is shown in table 1.

Table 1 here

In males α-galactosidase A activity was 15.0±5.23 (range 3.2-36.6) pmol/punch/hr. Reduced α-galactosidase activity (<6.3pmol/punch/hr) was found in 7 male subjects from 505 tested. Low activity in DBS samples can be due to many factors including a low white blood count, or inadequate sample drying. These subjects were recalled for a second analysis: 2 subjects declined and 5 repeat tests were normal. In females α-galactosidase activity was 5.2±2.4 (range 0.7-28.8) μmol/l/hr and 1 subject from 579 tested had reduced α-galactosidase activity (<1.2 μmol/l/hr). No pathogenic mutations were found in patients with low α-galactosidase activity. A further 16 samples from females with normal range α-galactosidase activity had elevated Lyso-GL-3. One female subject (age 55, eGFR 40ml/min/1.73m^2) had α-galactosidase activity 1.5 μmol/l/hr, but Lyso-GL-3 5.5 ng/ml and was identified as heterozygous for a FD mutation (c.898C>T; p.L300F; Leu300Phe). She knew that her mother had Fabry disease but had not been previously tested.

Discussion

The prevalence of FD has been reported to be 1 in 40,000 -117,000[6] but some sources suggest that as many as 1 in 50,000 males are affected and when late-onset disease is included the incidence may be as high as 1 in 4600[13,14]. The prevalence of FD in patients with ESRD is 0.12-0.70%. Earlier detection would allow for treatment prior to the development of renal disease. Neonatal blood spot screening for Fabry's is available in some US states and Japan[15]. This suggest a prevalence of 1 in 12,000 (8,000 if variants of unknown significance considered pathogenic). Screening for FD does not form part of UK neonatal screening protocols.

Most case detection studies for FD have been conducted in patients with significant renal disease or cardiomyopathy. Screening for Fabry's disease in patients with CKD3b-4 from 0.2-0.6%[16-18]though some studies fail to find any cases[19]. A study of the prevalence of FD in a secondary care population under the care of renal specialists is underway in Australia[6]. However, data on the prevalence in earlier stages of renal disease (not under the care of specialist renal physicians) is lacking. This study found one new female case of FD from 1084 patients (505 male; 579 female) from primary care and non-specialist secondary care based on age-related mild-severe renal disease diagnosed on reduced eGFR. However α-galactosidase activity for females

with FD overlaps with the bottom of the normal range resulting in up to 30% false negatives so addition of lysoGL-3 is recommended in females[20,21]. Enhanced screening with LysoGL-3 biomarker confirmed one female was heterozygous for FD (c.898C>T; p.L300F ; Leu300Phe)[22]. Further work identified that she was related to a known affected case but had not been contacted for family screening.

The PATHFINDER study pathology-based electronic health record data to identify patients from primary care and non-specialist secondary care mostly CKD stage 2-3(a). This limited this study to use only creatinine (eGFR) data rather than complete renal disease profiles[23]. No data on albuminuria or (less relevant) haematuria. Albuminuria is considered a useful marker for FD but many early cases of FD lack significant albuminuria[24]. The cohort sampled was lower risk than previous studies and included both primary and non-specialist secondary care settings. Though underpowered to detect significant numbers of cases, it suggests that enrichment for prior renal disease using a CKD3 cut-off and possibly additional biomarker stratification[25] is required before screening for FD is viable.

PATHFINDER Project Collaborator group:

Collaborating Centre	Research team	Creatinine assay and eGFR calculation method	Approx population served	No. of Recruits
Ipswich Hospital	Dr Taruna Likhari (PI), Genessa Peters (RN)	Enzymatic creatinine CKD-EPI	385000	104
Princess Royal Hospital, Telford	Dr Nigel Capps (PI), Louise Tonks (RN)	Enzymatic creatinine MDRD until 30/10/18, then CKD-EPI	490000	104
Queen's Hospital, Burton	Professor Tim Reynolds (PI), Louise Wilcox (RN), Clare Mewies (SA)	Jaffe creatinine CKD-EPI	360000	68
Russell's Hall Hospital, Dudley	Ms Jackie Smith (PI & RN)	Enzymatic creatinine CKD-EPI	450000	430
Oxford University Hospital	Mrs Nicky McRobert (PI), Jamie Burbage (RN)	Enzymatic creatinine CKD-EPI	655000	85
University Hospital North Midlands	Dr Anthony Fryer (PI), Loretta Barnett, Susan Hendy (RN)	Enzymatic creatinine CKD-EPI	900000	50
Royal Berkshire Hospital	Julie Sutton (PI & RN)	Enzymatic creatinine CKD-EPI	600000	162
Brighton & Sussex University Hospitals	Prof Gordon Ferns (PI) , Mel Smith, Valentina Toska (RN)	Jaffe creatinine MDRD	460000	40
West Suffolk Hospital	Prof Patrick Twomey (PI) Joanne Kellett (RN)	Enzymatic creatinine CKD-EPI	275000	62

Collaborating Centre	Research team	Creatinine assay and eGFR calculation method	Approx population served	No. of Recruits
Total number of recruits	Total number of recruits	Total number of recruits	4,575,000	1105

Population served extracted from individual hospital CQC reports Abbreviations: Principal Investigator (PI); RN = Research Nurse (RN); Study administrator (SA)

Contributors : The study was designed by TMR, who also ran the study data gathering and management of the sample and results. Specialist biochemical and genetic analyses were performed by KT and KB. Data were analysed by TMR and ASW, and they wrote the study manuscript. The final manuscript as submitted was approved by all the authors.

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Competing interests : TMR is currently in receipt of project grants from Genzyme Therapeutics, Oxford, UK (now Sanofi Genzyme, Oxford, UK); Shire Pharmaceuticals, Basingstoke, UK, now Takeda Pharmaceutical Ltd; and Synageva BioPharma, Watford, UK (now Alexion Pharma UK, Uxbridge, UK).

Patient consent for publication : Not required.

Ethics approval : The project received ethics approval from the National Research Ethics Service Committee East Midlands - Northampton (UK Integrated Research Application System project number 158121; 14/EM/1153; 14/10/2014). It was included in the National Institute for Health Research portfolio (UKCRN ID: 17588) and transferred to Health Research Authority on 15 June 2016.

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