Screening for patients with Gaucher's disease using routine pathology results: PATHFINDER (ferritin, alkaline phosphatase, platelets) study

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Abstract

Aims: Lysosomal β-glucocerebrosidase (GBA) deficiency causes Gaucher disease (GD), a recessive disorder caused by bi-allelic mutations in *GBA*. The prevalence of GD is associated with ethnicity, but largely unknown and potentially underestimated in many countries. GD may manifest with organomegaly, bone involvement and neurological symptoms as well as abnormal laboratory biomarkers. This study attempted to screen for GD in patients using abnormal platelet, alkaline phosphatase (ALP) and ferritin results from laboratory databases.

Methods: Electronic laboratory databases were interrogated using a 2-4 year time interval to identify from clinical biochemistry records patients with a phenotype of reduced platelets ($<150x10^9$/L) and either elevated ALP (>130iu/L) or ferritin (>150 (female) or >250µg/L(male)). The mean value over the screening window was used to reduce variability in results. A dried blood spot sample was collected for the determination of GBA activity in patients meeting these criteria. If low GBA activity was found then the concentration of the GD-specific biomarker glucosyl-sphingosine (lyso-GB1) was assayed, and the *GBA* gene sequenced.

Results: Samples were obtained from 1058 patients; 232 patients had low GBA activity triggering further analysis. No new cases of GD with homozygosity for pathogenic variants were identified but 12 patients (1%) were identified to be carriers of a pathogenic variant in *GBA*.

Conclusions: Pathology databases hold routine information that can be used to screen for patients with inherited errors of metabolism. However, biochemical screening using mean platelets, ALP and ferritin has a low yield for unidentified cases of Gaucher Disease.

What's already known about this topic?

- Gaucher Disease may manifest with organomegaly, bone involvement, neurological symptoms as well as abnormal laboratory biomarkers.
- The prevalence of Gaucher disease in the general population is unclear.

What does this article add?

- Screening for Gaucher disease using simple laboratory data identified a number of patients with low enzyme activity but genetic testing found only carriers of disease but no overt cases.

Introduction

Lysosomal storage disorders are a group of biochemically detectable often treatable diseases of which Gaucher's disease (GD) is one of the commonest [1]. Lysosomal β-glucocerebrosidase (GBA) deficiency causes GD resulting in the accumulation of glucocerebroside [2]. This is a recessive condition caused by mutations of the GBA gene on chromosome 1. It is particularly common in Ashkenazi Jews in whom the carrier rate is 8.9% whereas in the general US population the carrier rate is approximately 1%[3]. The prevalence is generally thought to be approximately 1 in 40,000 in the general UK population, but may be as high as 1 in 1000 in Ashkenazi Jews[2,3]. The manifestations of glucocerebroside accumulation include hepatomegaly, splenomegaly, skeletal disorders in type 1 and added neuronal damage in types 2 and 3. Splenomegaly results in platelet sequestration, hepatic involvement can cause increased serum ferritin[4], and bone involvement in increased serum alkaline phosphatase (ALP) activity[5].

Identification of patients with rare diseases is problematic as the possibility of the rare disease has to be considered before a highly specialist test is ordered. 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[6-8]. This paper describes the use of ferritin concentration, alkaline phosphatase (ALP) levels, and platelet count as a screening biomarker panel for GD in the PATHFINDER project.

Methods

Subjects

Subjects were identified at 9 hospitals serving approximately 4.4 million people by interrogating laboratory databases to identify individuals who had results for platelets, ALP and ferritin. Depending on the laboratory computer systems in use a time window of 2-4

years was used, and the averages of platelet count, ALP and ferritin results were calculated. Individuals who had average platelet count below the lower limit of the reference range (LLN) and either average ALP activity or average ferritin concentration above the upper limit of the reference range (ULN) were identified as potential participants. Reference range cut-offs applied were: platelets (Male(M) & Female (F)) <150 $x10^9$/L; ALP (M &F) >130 iu/L; ferritin (M) >250 µg/L, (F) >150 µg/L.

Once provisionally identified, subjects were sent an invitation letter indicating that they had been identified as having abnormal results in routine pathology testing and asked if they would be prepared to meet with a research nurse to collect a blood sample for analysis of lysosomal β-glucocerebrosidase activity, and if deemed appropriate Lyso-Gb-1, and genetic sequencing. 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 whether they would prefer to remain anonymous or if they would like to receive a copy of their results.

Analysis

DBS cards were analyzed for β-glucocerebrosidase activity (GBA) with a fluorometric assay using 4-methylumbelliferyl-β-D-glucopyranoside as a substrate [9] at Centogene GmbH (Rostock, Germany). Normal enzyme activity was defined as ≥4.1µmol/L/h, which represents activity 0-15% of 'normal'. Those samples which showed reduced GBA activity were then analysed for the biomarker Lyso-Gb1 by liquid chromatography mass spectrometry[10] A lyso-GB1 concentration ≤6.8 ng/ml was defined as normal. Any individuals with low lyso-GB1 activity had DNA extracted for sequencing using an amplicon-based next-generation sequencing (NGS) approach. Missing regions or regions of poor quality were re-analysed with classical Sanger sequencing to achieve 100% coverage. Potential pathogenic variants in *GBA* were identified and *GBA* variants were reported according to the guidelines

of the American College of Medical Genetics [11,12] using NM_0001573.3 as the reference transcript.

Results

Database searches identified a large number of potential candidates. At Burton, 449 candidates were identified of whom 150 agreed to participate. Response rates are not available for other centres. In total, recruiting centres saw 1060 patients, for which GBA results were available for 1058 individuals. Demographic information is shown in table 1. Twenty-one women identified that the blood tests leading to identifying them to take part in the study appeared to derive from tests carried out whilst they were pregnant.

Table 1 here

Average GBA activity was 5.9±3.0μmol/L/hr; median (Inter-Quartile Range (IQR)) 5.4(4.2-7.0) μmol/L/hr (reference range >4.1μmol/L/hr). In 232 cases, GBA activity was below the LLN, so Lyso-GB1 was assayed and the *GBA* gene was sequenced. In those cases, Lyso-GB1 concentration was 3.7±1.1ng/mL; median (IQR) 3.6 (2.9–4.4) ng/mL (reference range <6.8ng/mL). Only one patient (identified as a mutation carrier) had a Lyso-GB1 concentration >6.8 ng/ml. A further 11 patients with Lyso-GB1 within the reference range were also identified as carriers of pathogenic variants (Table 2). All carriers identified met the low platelet criterion; 1 had increased ferritin; 4 increased ALP and 7 both increased ferritin and increased ALP.

The twelve individuals identified with *GBA* variants carried a total of 7 distinct variants. The common observed pathogenic variants were c.1226A>G; p.N409S (Asp 409 Ser; n=4) and c.1504C>T; p.R502C (Arg 502 Cys; n=3) and three others were also classified

as pathogenic or likely pathogenic. Two others were classified as variants of uncertain significance (VUS) (Table 2).

Table 2 here

Discussion

Inherited errors of metabolism occur in 2% of the population but comprise more than 2000 disorders which carry orphan disease status. Newborn screening is conducted for 6–20 disorders using neonatal DBS in different countries including for lysosomal storage disorders[13]. However, no systematic approach exists for identifying adults with inherited metabolic disorders and mass screening is not likely to be cost- or time-effective. Detection of patients with inherited metabolic errors is challenging as the phenotypic spectrum of these disorders is considerably greater than classical descriptions would suggest. The rationale of the PATHFINDER study is to use routinely measured laboratory data to enrich the group being screened so that specialist testing including NGS[14] becomes more practical.

Studies of screening for GD carried out in Israeli Ashkenazi Jews, a high-risk group, found 83 carrier couples in a study of 28893 individuals in an organised carrier screening programme giving a carrier frequency of 5.7%[15]. The carrier frequency in low-risk groups is significantly lower. A study from Japan in 102 patients with neurological symptoms identified 2 neuronopathic patients with low GBA activity who were confirmed to have GD[16].

In a European, mostly French cohort, pathogenic *GBA* gene variants were found in 3 of 391 control patients (0.77%)[17]. Given a similar prevalence neonatal screening for GD is not recommended in the UK[18]. In our study 12 of 1058 subjects had single pathogenic

variants (1.13%). There was no significant difference between our carrier rate and that found in a European cohort (P=0.54)[17], and was also similar to that found in the US [3].

This study used routinely measured biological markers of bone, liver and bone marrow involvement. which have all been identified as key markers in consensus group statements on GD [19,20]. Platelets are decreased as the accumulation of Gaucher cells in the bone marrow results in thrombocytopaenia[21] and as a result enhanced detection rates for GD are found in tertiary centre haematology clinics[4]. Ferritin and ALP are elevated in GD due to reticuloendothelial and hepatocyte liver involvement[22,23]. However, some studies show that GD can be associated with decreased ALP secondary to decreased osteoblastic activity in later stage disease when bone remodelling disorders such as the Ehrlenmeyer flask deformity are evident[5,24]. This study was designed to detect minimally symptomatic individuals and the presence of bone pain and deformity would already have led to prior investigation of the patient so only elevated liver markers were used.

The 12 cases with single pathogenic variants (carriers) were identified using low platelets and elevated ferritin in 1 case, low platelets and elevated ALP in 4 cases, and both in 7 cases. With the exception of 1 patient with ferritin >1000μg/L, the mean biomarker levels in most cases were only minimally abnormal. Therefore, we can be quite certain that there was no significant enrichment of our sampling pool for patients carrying mutations of the *GBA* gene. Given the low incidence of GD, this study was underpowered to detect significant numbers of cases but the lack of enrichment of asymptomatic carriers does suggest that this biomarker combination would be ineffective as a mass screening tool. A study in 160 secondary care haematology centres in Japan recruited 995 patients using criterion of platelets <120 $x10^9$/L and found 76 cases with initial low GBA activity of which 11 (1.2%) had a second low activity level[25]. One patient was diagnosed with GD clinically and confirmed on genetic testing and one extra heterozygote was identified. Similar findings were

found using the more comprehensive Gaucher earlier diagnosis consensus point-scoring system which includes clinical as well as laboratory data as 42 individuals scoring >7.5 points and with reduced lysoGB1 (<1.2ng/mL) were found but no new cases of GD were identified from 170,000 individuals in a Finnish biobank[26].

Conclusion

This study identified no new cases of GD and 12 carriers from 1057 pre-stratified screenees (1%). Mean biomarker levels were usually minimally abnormal. No significant enrichment was found for pathogenic variants in the *GBA* gene using this pre-screening algorithm. Given the low incidence of GD, this study was underpowered to detect significant numbers of cases but the lack of enrichment of asymptomatic carriers does suggest that this biomarker combination would be ineffective as a mass screening tool.

PATHFINDER Project Collaborator group:

Collaborating Centre	Research team	Approx population serving	No. of Recruits
Ipswich Hospital	Dr Taruna Likhari (PI), Genessa Peters (RN)	385000	274
Princess Royal Hospital, Telford	Dr Nigel Capps (PI), Louise Tonks (RN)	490000	171
Queen's Hospital, Burton	Professor Tim Reynolds (PI), Louise Wilcox (RN), Clare Mewies (SA)	360000	150
Russell's Hall Hospital, Dudley	Ms Jackie Smith (PI & RN)	450000	121
Oxford University Hospital	Mrs Nicky McRobert (PI), Jamie Burbage (RN)	655000	1
University Hospital North Midlands	Dr Anthony Fryer (PI), Loretta Barnett, Susan Hendy (RN)	900000	77
Doncaster and Bassetlaw Hospital	Dr Pankaj Chaturvedi (PI) Veronica Maxwell, Amy Neal (RN)	420000	1
Brighton & Sussex University Hospitals	Prof Gordon Ferns (PI) , Mel Smith, Valentina Toska (RN)	460000	37
West Suffolk Hospital	Prof Patrick Twomey (PI) Joanne Kellett (RN)	275000	219
		4395000	1060

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. VS and CB performed the specialist assays and genetic analysis. 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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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

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