Targeted urine microscopy in Anderson-Fabry Disease: a cheap, sensitive and specific diagnostic technique

Mathu Selvarajah1, Kathy Nicholls1,2, Tim D. Hewitson1,2 and Gavin J. Becker1,2

1Department of Nephrology, The Royal Melbourne Hospital, Parkville, Melbourne and 2Department of Medicine, University of Melbourne, Parkville, Melbourne, Australia

Correspondence and offprint requests to: Kathy Nicholls; E-mail: Kathy.Nicholls@mh.org.au

Abstract

Background. Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder resulting from the deficiency of trihexosylceramide α-galactosidase (α-Gal A). The diagnosis is often missed or delayed, and specific diagnostic tests (serum α-Gal A activity, genotyping or biopsy) are expensive and not widely available. We evaluated the diagnostic potential of urine microscopy in AFD.

Methods. We studied 35 male and female AFD patients across a wide phenotypic spectrum and 21 controls with other renal diseases. Fresh urine sediment was examined under phase-contrast microscopy using polarized light for Maltese cross (MC) particles, anti-CD77 antibody to detect globotriaosylceramide (GL3, the substrate of α-Gal A), and anti-podocalyxin antibody to assess podocyte excretion.

Results. Characteristic MC 2 particles and anti-CD77 binding within vacuolated urinary epithelial cells were both detected in AFD with high sensitivity and specificity (MC 2 detection sensitivity 100%, 95% confidence interval (CI) 85.4–100%, specificity 100%, CI 80.8–100%; anti-CD77-binding sensitivity 97.1%, CI 83.3–99.9, specificity 100%, CI 80.8–100%). Albuminuria (urinary albumin-to-creatinine ratio, ACR) correlated with quantitative particle excretion—in low, intermediate and high MC excretors, and median ACR was 1.6, 6.9 and 20.0 mg/l mol, respectively (analysis of variance P = 0.017). Podocyte staining was positive in ~50% of all AFD patients and was similar in those with and without clinical Fabry nephropathy (FN), whether or not treated with enzyme replacement.

Conclusions. Targeted urinary microscopy is a non-invasive, inexpensive, accessible and rapid diagnostic technique.
technique, especially applicable where serum x-Gal A activity and genotyping are not affordable or available. As the number of urinary MC 2 particles increases with rising albuminuria, the technique may also be useful in assessing FN burden.

**Keywords:** fabry disease; GL3; urinary microscopy; CD77; podocyte excretion

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**Introduction**

First described by Anderson and Fabry, the clinical features of this X-linked lysosomal storage disorder result from mutations in the gene encoding the lysosomal enzyme trihexosylceramide x-galactosidase (EC 3.2.1.47), previously known as x-galactosidase A (x-Gal A) [1, 2]. The predominant substrate (globotriaosylceramide; GL3) accumulates in various cell types and tissues, resulting in variable organ dysfunction and heterogeneous phenotype. Chronic kidney disease, cardiac dysfunction and ischaemic stroke comprise the major life-threatening burden of disease.

‘Renal variants’ have also been reported [3]. Estimates of incidence of Anderson-Fabry disease (AFD) in the general population range from ~1:40 000 to 1:60 000 males [4]. Prevalence is population dependent and is higher in dialysis and renal transplant patients. A recent review of 18 screening studies in a total of 21 256 patients on renal replacement therapy found a combined prevalence of AFD of 0.1%, while in individual studies, the incidence of AFD among male patients ranged from 0 to 1.6% [5]. Renal lesions are found in both (hemizygous) males and (heterozygous) females. Renal symptoms in women typically occur later than in males, with considerable variation in phenotype being probably due to variable X chromosome inactivation [6]. Histological renal lesions are characterized by glycolipid deposits in tubular epithelial, mesangial, interstitial, vascular endothelial and smooth muscle cells and podocytes [7, 8]. Early diagnosis enables optimal genetic counselling and organ protection strategies, as well as earlier access to specific enzyme replacement therapy (ERT) with recombinant human x-Gal A [9]. Definitive diagnosis in males can be made by genotyping, by assaying leucocyte or plasma x-Gal A activity or via electron microscopy of renal biopsy tissue. In female AFD patients, enzyme levels vary widely, overlap normal ranges and are not reliable for diagnosis. Mutation testing and enzyme assays are expensive and not widely available. At a clinical level, the diagnosis is often missed: diagnosis in UK males with AFD lags a mean of 8.2 years from the onset of neuropathic pain and 10.7 years from the noting of angiokeratoma [10]. In 366 European patients with AFD participating in the Fabry Outcome Survey, the mean delay to diagnosis after symptom onset was 13.7 years for males and 16.3 years for females [11]. A simple screening test for evidence of clinical AFD disease would be diagnostically useful. While eye examination for corneal verticillata [12] identifies some undiagnosed patients, corneal changes are not universal, and slit lamp examination is not universally available or applied.

Reports suggest that urine microscopy may be a useful tool for the non-invasive diagnosis of AFD, but it remains underutilized and has not been systematically evaluated. Clinicians caring for AFD patients represent diverse specialties, and research into renal AFD involvement at a cellular level is relatively underdeveloped. Beyond diagnosis, examination of urinary cells may also be useful in assessing disease progression. Desnick et al. [13] and Nagao et al. [14] described the common variety of birefringent Maltese cross (MC) pattern (oval fat bodies) in the urine sediment of AFD patients when viewed under a polarized microscope. This finding is most commonly seen in patients with proteinuria from any glomerular disease [14, 15], although starch and talc particles also produce a distinguishableMC appearance [16–18]. Birch et al. [15] and later Branton et al. [18] further described characteristic internal lamellation and irregular surface protrusions of MC particles in AFD. Most (76%) of the cells present in the urine of AFD patients are renal tubular epithelial cells [19]. Characteristic lipid vacuoles within urinary epithelial cells of patients with AFD can be visualized using the Papanicolaou stain, and immunohistochemistry has also been used to localize glycosphingolipid [20]. Utsumi et al. [20] monitored the effect of therapy in AFD using qualitative changes in urinary oval fat bodies and anti-GL3 immunofluorescence.

Loss of podocytes into urine is another potential window to Fabry nephropathy (FN). Podocyte shedding into the urine, as detected by podocalyxin (PDX) staining, occurs in progressive glomerular disease, may be involved in the development of progressive glomerulosclerosis, is increased in active glomerular injury and may be a useful non-invasive marker of activity in glomerular disease [21–26]. We examined the urine of AFD patients, across a broad spectrum of disease activity, using three separate techniques: polarized light microscopy to detect and quantify the characteristic MC particles, tissue stains to detect sphingolipid and immunohistochemistry to localize and quantify GL3 in urinary epithelial cells and to assess podocyte excretion. We aimed to evaluate these methods as diagnostic and monitoring tools in patients with AFD and to compare changes in urinary cells with treatment.

**Materials and methods**

The study was approved by Melbourne Health Human Research and Ethics Committee, and participants gave informed consent. Consecutive patients attending the Fabry clinic at the Royal Melbourne Hospital over 12 months were studied. Control urine samples were collected from patients attending the nephrology outpatient clinics and from patients admitted to the nephrology ward at the same hospital. Samples were not collected if patients had active urinary tract infection. Urine was delivered to the laboratory then assigned a serial number before processing. Concomitant urine biochemistry results and serum creatinine (SCR) were recorded. Macrolubimunuria was defined as urine albumin to creatinine ratio (ACR) $>$ 26.4 mg/mmol, microalbuminuria as ACR 2.64–26.4 mg/mmol and normalalbuminuria as ACR $<$ 2.64 mg/mmol ($<$ 30 mg/g). Estimated glomerular filtration rate (eGFR) was calculated using the modified MDRD formula [27] and renal dysfunction was defined as an eGFR of $<$ 60 mL/min.

Patients with renal transplants were excluded. Urine samples were studied within 4 h of voiding. Mid-stream urine was used in all patients, after pilot studies indicated that complete void or 24-h urine collections were extensively contaminated with vaginal epithelial cells in female
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patients. Pilot studies also examined differential retention of sphingolipid with the use of various fixatives, the role of carbowax to increase cell yield, the impact of impurities and contamination [17, 28] and the use of specific antibodies for the identification of target cells in urine.

**Slide preparation for microscopy**

A measured volume (20–100 mL) of fresh mid stream urine was centrifuged (Beckman™, model TJ-6 centrifuge) at 1000 g for 10 min. A 10 mL aliquot of the supernatant was sent to the biochemistry laboratory for routine measurement of ACR using automated methods. The cell pellet was re-suspended in 500 µL of the supernatant, and 25 µL of the suspension was used to prepare each of the four slides for examination under polarized light.

A phase-contrast microscope (Nikon Eclipse 80i) with polarizing filter was used to identify birefringent MC particles. The numbers and morphological subtypes of MC particles in whole slides were counted and arbitrarily scored using four groups (Table 1). Control slides were also prepared in the same manner using distilled water in place of urine, to evaluate the impact of impurities and contamination (talc and powdered gloves) on MC particle assessment [17, 28].

Thereafter, 2 mL of phosphate-buffered saline (PBS) was added to the suspension and was re-spun (1000 g for 10 min). After supernatant removal, 2 mL of 2% polyethylene glycol (carbowax) was added. Six thousand one hundred microliter of this suspension was spun on to a glass slide by cytocentrifuge (Shandon™ Cytospin 2; Thermo Fisher Scientific, Wallingford, MA) at 1000 r.p.m. for 10 min, then air-dried (10 min). Slides were then fixed in ice-cold ethanol (15 min) and air dried for podocyte staining and in Neutral Buffer Formalin (30 min) for lipid and GL3 staining.

**Urine cytology**

For the staining of urinary sediment cytorepreparations were examined with standard Papanicolaou and Periodic acid–Schiff (PAS) staining techniques.

**Immunochemistry**

Rat monoclonal anti-CD77 antibody (Abcam, Cambridge, UK) was used to locate GL3 accumulation [29, 30]. CD 77 is expressed in vascular endothelium and in some peripheral blood mononuclear cells in healthy individuals [31]. Anti-human PDX monoclonal antibody, PHM5 (Clone 18.29; Chemicon catalogue, Millipore, Billerica, MA) antibody, was used to identify podocytes [21–23].

Immunocytochemical localization of GL3 and PHM5 was performed on the slides using a standard immunoperoxidase technique. After quenching endogenous peroxidase activity with 0.3% H2O2 in methanol for 20 min, slides were incubated for 10 min with horse normal serum (Vectastain™; Vector, Burlingame, CA). Excess serum was blotted and the slide was then incubated with primary antibody; rat monoclonal antibody to CD77 (GL3) or mouse monoclonal anti-PHM5 for 2 h. Slides were then incubated with biotinylated anti-rat IgG (Vector) or biotinylated anti-mouse IgG (Vector), as appropriate, for 10 min, followed by ABC Elite kit reagents (Vectastain™; Vector) for 15 min. After each step, slides were washed twice with PBS for 5 min. Finally, using 3,3′-diaminobenzidine tetrahydrochloride (Dako, Glostrup, Denmark) as the chromogenic substrate, the slides were incubated for 2–7 min and washed in tap water.

Nuclei were counterstained with Harris haematoxilin for 90 s and washed in tap water for 5 min. Slides were mounted with DePex™ (BDH, Poole, UK) and cover-slipped, after dehydrating in graded alcohol and HistoClear™ (National Diagnostics, Atlanta, GA).

For each specimen, all the mononuclear non-squamous cells in a cytopsins slide were counted at X400 magnification. Positive cells were expressed per 100 total non-squamous mononuclear cells. Each procedure was performed by a single author (M.S.).

**Statistical analysis**

Data are reported as median and range or mean ± SD as appropriate. Specificity and sensitivity are expressed with 95% confidence intervals (CI). Statistical analysis was performed using SigmaPlot™ (SPSS, Chicago, IL), with P < 0.05 considered statistically significant.

**Results**

The baseline characteristics of the 35 AFD patients are detailed in Tables 2 and 3. Seventeen patients were on ERT, either agalsidase alpha (Replagal™; Shire HGT) or agalsidase beta (Fabrazyme™; Genzyme). Ten of these 17 patients had received ERT for at least 5 years. Six patients, including four female heterozygotes, had no clinical or biochemical evidence of FN (normal albuminuria, SCr and eGFR). Urinary ACR exceeded 100 mg/mmol in five patients, and renal function was impaired in four. One male patient was on maintenance haemodialysis, with residual renal function. No patient had a history of treatment with chloroquine or gentamicin.

The control group comprised 21 non-Fabry subjects: 20 patients (11 males) across a spectrum of biopsy-proven renal disease: lupus nephritis (Classes 3–5, n = 5), diabetic nephropathy (n = 2), nephrotic minimal-change disease (n = 1), focal glomerular sclerosis (n = 2), membranous nephropathy (n = 1), crescentic nephritis (n = 2), Anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis (n = 2), thrombotic thrombocytopenic purpura (TTP, n = 1), acute tubular necrosis (n = 3), IgA nephropathy (n = 1) and one normal subject who had not undergone renal biopsy and in whom albuminuria and SCr were normal. Within the control group, urinary ACR exceeded 100 mg/mmol in 12 patients.

**Microscopy**

Examination of urinary specimens under phase-contrast microscopy using a polarized light filter was performed in 29 AFD patients—technical microscopy problems precluded examination in the initial six patients. Examination

| Table 1. Scoring system for urinary MC 2 particle excretion |
|-----------------|-----------------|
| Score           | Number of MC particles per slide |
| 0—None          | None            |
| 1—Low           | <100            |
| 2—Moderate      | >100 and no clumps |
| 3—High          | >100 and clumps present |

**Table 2. Baseline characteristics of AFD study group**

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<tr>
<td>Age, mean in years (range)</td>
<td>44.7 ± 13.8 (18–67)</td>
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<tr>
<td>Gender, males (%)</td>
<td>16 (46%)</td>
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<tr>
<td>Time since diagnosis, mean</td>
<td>10.9 (0.1–27)</td>
</tr>
<tr>
<td>On ERT (%)</td>
<td>18 (51%)</td>
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<tr>
<td>On angiotensin blockade (%)</td>
<td>16 (46%)</td>
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**Table 3. Severity of renal involvement in AFD study group**

<table>
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<tr>
<th>Severity of renal disease</th>
<th>N</th>
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<tr>
<td>Females with normal albuminuria and normal eGFR/SCr</td>
<td>4</td>
</tr>
<tr>
<td>Males with normal albuminuria and normal eGFR/SCr</td>
<td>2</td>
</tr>
<tr>
<td>Presence of microalbuminuria</td>
<td>15</td>
</tr>
<tr>
<td>Presence of (macro)albuminuria</td>
<td>9</td>
</tr>
<tr>
<td>Presence of renal dysfunction</td>
<td>4</td>
</tr>
<tr>
<td>ERT</td>
<td>4</td>
</tr>
<tr>
<td>On haemodialysis</td>
<td>1</td>
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revealed three morphologically distinct populations of auto-fluorescent, birefringent MC particles:

i) MC 1 particles (Figure 1A) were rounded, non-lamellated, without protrusions or internal structure, and with symmetrical MC limbs. The numbers of MC 1 particles varied from a few to thousands per slide. Clumps were seen when high numbers of cells were present. These particles tended to congregate towards the edge of the cover-slip. Size also varied: the largest could be seen under \( \times 100 \) magnification. MC 1 particles were seen in the urine of both AFD and proteinuric control patients, including patients with diabetic nephropathy, IgA nephropathy, lupus nephritis, crescentic nephritis, ANCA-positive vasculitis, TTP, membranous nephropathy and minimal change nephrotic syndrome. They were not seen in the patients with acute tubular necrosis or membranous nephropathy in remission (urine ACR \( = 26 \text{ mg/mmol} \)).

ii) MC 2 particles (Figure 1B and 1C) had a lamellated appearance with protrusions, best appreciated with minor adjustments in focal length, and resembled ‘mosquito coils’. Larger MC 2 particles showed larger protrusions. Protrusion shape varied from hook-like to spherical. Under the polarized light filter, separate MCs were visible in the larger protrusions as well as in the main MC 2 body (Figure 1C). At high density, MC particles tended to form clumps, within which it was difficult to differentiate MC 2 from MC 1 particles. MC 2 particles were found in urinary specimens from 28 of the 29 AFD patients, but not in any of the 21 controls (\( \chi^2 P < 0.001 \)). MC 2 detection sensitivity was 100% (CI 85.4–100%) and specificity 100% (CI 80.8–100%). Their presence was independent of gender, albuminuria, renal dysfunction and ERT status.

MC 2 particle excretion correlated with urinary albumin level in AFD patients: low MC 2 particle excretors had a median ACR of 1.6 mg/mmol (range 0.49–5.08), intermediate excretors 6.9 mg/mmol (range 3.48–48.4) and high excretors 20.0 mg/mmol (range 1.05–110). There was a

Fig. 1. (A) Clump of MC 1 particles under polarized light filter, (B) MC 2 particle (arrow) under phase-contrast microscopy, (C) combined polarized light filter and phase-contrast microscopy. (D) Powder contamination, phase-contrast microscopy with polarized light filter. Scale bar in all photomicrographs = 50 \( \mu \text{m} \).

Fig. 2. MC 2 particle excretion in relation to ACR.
statistically significant increase in the urinary albumin excretion between low and high excretors (Figure 2, analysis of variance $P = 0.017$).

MC 2 particle excretion did not differ between the AFD patient group on ERT ($n = 15$, excretion score = 2.5 ± 0.5) and those not on ERT ($n = 14$, excretion score = 2.2 ± 0.8, $t$-test $P = 0.36$).

iii) MC 3 (Figure 1D) particles appeared round or polygonal and larger, flatter and duller than MC 1 and MC 2 particles, with nucleus-like centres and asymmetrical MC limbs [20]. MC 3 were easily distinguished from MC 1 and MC 2 and did not form clumps. Initially noted incidentally, MC 3 were confirmed to be due to contamination with talc, as their number reduced dramatically when powdered gloves and patient talc use were avoided.

**Staining of urinary sediment**

Although light microscopy of Papanicolaou-stained smears from patients with AFD showed characteristic enlarged tubular epithelial cells with vacuolated cytoplasm and vesicular or condensed nuclei (Figure 3A), Papanicolaou and PAS staining were non-contributory due to low sensitivity and specificity. Glycogen storage in squamous vaginal epithelial cells confounded assessment in females, and many tubular cells did not stain.

**Immunocytology**

i) Anti-CD77

With anti-CD 77 antibody, positively stained vacuolated mononuclear cells were easily identified in almost all AFD patients, but in none of the controls ($\chi^2 < 0.001$). Positive cells appeared enlarged with numerous vacuoles were clearly visible (Figure 3B and C).

The size and shape of positive cells varied, with some as large as squamous cells (Figure 3B). Vacuoles appeared either waxy or as empty space, reflecting the degree of lipid extraction during processing. Some cells were seen with detached cell fragments. Vacuolated cells were clearly distinguishable from any non-vacuolated cells at higher magnification (Figure 3C). Dense cytoplasmic staining of some neutrophils and mononuclear cells was seen in some control patients (Figure 3D), especially those with proliferative nephritis of any aetiology, but these were consistently smaller and once again easily distinguished from the vacuolated cells in AFD specimens.

In patients with AFD, CD 77-positive cells were seen even in those with normal eGFR and normoalbuminuria and in our youngest patients. CD 77-positive cells were detected in a single urine sample in 34 of the 35 patients ($\chi^2 P < 0.001$), with the single exception being a patient with normal renal function and ACR, in whom the total urinary cell count was very low. Examination of a repeat specimen did reveal clearly positive cells in her urine but is not included in statistical analysis. Anti-CD77 binding was 97.1% sensitive (CI 83.3–99.9%) and 100% specific (CI 80.8–100%).

GL3 cell excretion was demonstrated across all ages (18–67 years) and across a broad spectrum of AFD.

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**Fig. 3.** (A) Papanicolaou-stained vacuolated urinary epithelial cell. Scale bar = 50 µm. Immunoperoxidase staining for CD 77 shows (B) positive (arrow) staining of a urinary epithelial cell. Squamous epithelium (example shown with double arrows) is unstained. Intracellular vacuoles are clearly seen in the CD77–positive cell at higher magnification (insert) (scale bar = 50 µm in main micrograph, 125 µm in insert). (C) A negatively stained urinary epithelial cell, and adjacent vacuolated epithelial cell are seen at low and high magnification (scale bar = 50 µm in main micrograph and 125 µm in insert). (D) CD 77–positive leukocytes from a control patient with glomerular crescents under low and high (insert) magnification. Leucocyte staining is morphologically distinct from that seen in vacuolated epithelial cells (scale bar = 50 µm in main micrograph and 125 µm in insert).
individuals, from heterozygote females with normal renal function and normal albuminuria to end-stage kidney disease on haemodialysis. Excretion of GL3 cells per 100 mononuclear non-squamous cells ranged from 0.5 to 29.4 cells, with median 6.0. The GL3 cell excretion level of patients with AFD but without clinical nephropathy (n = 6) at 4.2 ± 4.4 was not statistically significantly different from that in AFD patients with FN (n = 29, GL3 cell excretion level = 10.4 ± 8, P = 0.078). Likewise, the GL3 cell excretion rate for the patient group not on ERT (median 4.2, range 0.5–23.3) did not differ statistically from those on ERT (median 7.6, range 2.2–29.4, P = 0.08).

Discussion

Of the three morphologically distinct MC particles seen in urine under phase-contrast microscopy with polarized light filter, MC 2 were highly specific (100%) and sensitive (97%) and probably represents fragmentation of shed nephronal epithelial cells containing lysosomal GL3. The quantitation of urinary MC 2 particles could be a useful adjunct to renal biopsy in assessing renal disease burden, especially if relevant software was developed. A prospective study with serial assessment of MC 2 particles would be required to investigate its use in monitoring treatment. Confirmation of test specificity and sensitivity in a separate AFD population, and estimation of inter- and intra-observer variability, would also be important to further validate the technique.

Renal cellular GL3 accumulation in AFD begins early in life. We were able to detect CD77/GL3-positive cells (GL3 cells) and GL3 as MC 2, even before the development of FN in both male hemizygotes and asymptomatic female heterozygotes. This would suggest that specialized urinary sediment examination is more reliable than α-Gal A assay in heterozygotes.

Not surprisingly, routine laboratory urine assessment did not report oval fat bodies or MC particles in any of the samples from AFD patients, as routine urine microscopy does not include polarized light examination. MC particles can be easily missed when microscopy is limited to phase contrast without polarizing filter and will be universally missed without the use of either technique. Routine laboratory urinary cell counts were normal in 32/35 AFD patients. Thus—except for variable albuminuria—routine urine examination would be passed without comment in most AFD patients.

Characteristic cell detection in urine of patients with AFD with anti-CD 77 antibody in patients with AFD is also highly sensitive (97%) and specific (100%). Like MC 2 particles, GL3 cells were seen even in patients without clinical nephropathy and in the patient on haemodialysis, indicating applicability of this methodology across a broad range of disease presentation.

The number of GL3 cells or MC 2 particles excreted are expected to depend on renal disease burden, the degree of renal scarring and probably on ERT, including the duration of ERT in relation to cellular turnover time. In advanced renal failure, a reduction in functional nephron number will lead to reduction in number of desquamated cells, explaining the lower excretion of GL3 cells seen in this subgroup. In earlier or less severe disease, we found that the number of GL3 cells increased with the onset of albuminuria. This is consistent with the finding of proximal tubular cell deposits in females with FN only in those patients with moderate to severe nephropathy [32].

Males tend to have a higher disease burden than females within a similar age group. However, we could not demonstrate any clear quantitative differences in GL3 cells or particles. Our patient group may be subject to selection bias in that sicker females are more likely to visit the clinics than are asymptomatic heterozygotes, many of whom decline invitations to attend clinical care even after diagnosis and counselling.

Monitoring of therapy in AFD is vexed by the lack of a good biomarker: currently, clinical monitoring of the disease and its therapy is based on target organ damage. Urinary GL3, expressed in relation to urinary creatinine excretion, is of limited clinical use, especially in females [33]. Renal biopsy studies before and after ERT initiation show reasonably rapid clearance of GL3 from vascular endothelial cells, glomerular mesangial cells and cortical interstitial cells; moderate clearance from vascular smooth muscles cells but limited clearance from tubular cells and podocytes after nearly 1 year of ERT [34]. This also suggests that nephronal epithelial cell GL3 clearance could be a good non-invasive marker of long-term ERT response. We demonstrated the presence of MC 2 particles and GL3 cells even after 8 years of regular ERT. Assessment of urinary MC 2 particles or GL3 cells could be more useful as a long-, rather than short-term monitoring tool, and may have some inherent advantages over biopsy in assessing clearance from tubular cells, as it reflects total renal GL3 burden. Even though biopsy studies have shown significant GL3 clearance from renal distal tubular cells after 4.5 years of ERT [35], biopsies may not detect patchy lesions. Patients with FN progress despite ERT, sometimes to end-stage kidney disease, and the optimal treatment regimen is unknown. A long-term prospective
study with serial urinary GL3 cell monitoring is needed for validation.

Clinical studies have recorded the prevalence of PDX-positive cells in urine as 0–44% in healthy, 53–80% in diabetic nephropathy and ~88% in other glomerular disease [21, 23, 24]. Our finding of relatively low-grade podocyte excretion (54%) in single spot urine samples from patients with AFD is consistent with a glomerular injury which is slowly progressive but does not endorse the use of podocytopathy as a diagnostic or monitoring tool in AFD.

Vacuolated epithelial cells can be detected with Papainalou stain in the urine of patients with AFD but are not pathognomonic, being seen also in crescentic nephritis. As this technique is also technically difficult, particularly for quantitation, we would not recommend it for routine use.

It is likely that AFD is missed even more commonly in less developed countries than in Australia. Even where available, the current costs of ~$120 AUD for enzyme assay and $110 AUD for each specific mutation tested (with >300 known mutations) are prohibitive in the developing world. The cost involved in using urinary microscopy for diagnosis is offset by multiple potential uses for the microscope and by the potential benefit of specific diagnosis to the patient. Even if specific therapy is not available, cheaper non-specific therapy such as renin–angiotensin system blockade is widely accessible and likely to be of benefit. The value of urine microscopy in this setting is not widely appreciated, even among nephrologists, and warrants emphasis. Polarized light microscopy requires patience and careful observation, but we estimate that the training time required for a nephrologist is ~5 h. Specimen preparation and examination is complete within 20 min. Diagnosis using CD77 staining utilizes standard laboratory techniques and could potentially be automated.

In this study, we have revisited and updated an old technique. Diagnosing AFD using phase-contrast microscopy and immunocytochemistry is not difficult and is highly sensitive and specific, permitting easy, cheap and rapid diagnosis of AFD across many countries.

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