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Diagnosing Fabry Disease – Delays and Difficulties within Discordant Siblings

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Introduction

Fabry disease, or Anderson-Fabry disease, was first described in 1898 by two separate physicians, William Anderson (London) and Johannes Fabry (Germany)¹ ². They independently described patients with ‘angiookeratoma corporis diffusum’, the now well recognized characteristic dermatological manifestations of this condition. It is perhaps more commonly known as Fabry disease since Johannes Fabry suggested a systemic disorder was responsible. We now know that Fabry disease is a multi-system progressive X-linked lysosomal storage disorder due to alpha-galactosidase deficiency, which can present in numerous ways to different clinicians³ ⁴. As a result the diagnosis is often delayed for many years or even decades⁵.

Fabry disease is often only clinically recognised in the classical form of the disorder, associated with undetectable alpha-galactosidase activity, resulting in impaired degradation of neutral glycosphingolipids, particularly globotriaosylceramide (GL-3)⁵. Progressive accumulation of these substances in various tissues and body fluids produces end organ damage, which subsequently leads to presentations with neurological, dermatological, renal, cardiac or cerebrovascular signs or symptoms, in combination or isolation, from the first decade of life onwards.

Average age of symptom onset in males is 9 years and 13 years for females yet the age of diagnosis is 23 and 32 years respectively. Life expectancy is reduced by 17 years in affected males and by 6 years in affected females. It is now recognised that the ‘classical’ form of the disease represents only one end of a spectrum associated with impaired glycosphingolipids metabolism. At the other end of the spectrum are asymptomatic females, whilst in between exist variant forms of the disease in affected males and females. Variant forms of the disease are associated with detectable but low enzyme levels, with isolated renal and cardiac forms described. The variation in disease manifestations, over time and between different organs, helps to explain the delay in achieving a diagnosis.

Making the correct diagnosis avoids harm from unnecessary procedures and investigations undertaken to evaluate symptoms but most importantly allows consideration of enzyme replacement therapy (ERT). ERT has been shown to stabilise progression of the disease, but having only been in existence since 2001, no mortality benefit has been demonstrated to date. As yet there is no international agreement on ERT but there is a consensus view (Mehta et al). Given the difficulties in reaching the correct diagnosis in a timely fashion, the wide presentation of disease and the questions remaining regarding ERT, it is clear that a greater understanding of this complex multi-system single gene disorder is desirable.
We have identified a family where three affected brothers (X, Y & Z) have markedly different variants of Fabry disease yet unusually share an identical gene mutation. Such wide phenotypic variation within siblings, or varied phenotype-genotype correlation, has not previously been reported and offers the opportunity to enhance our understanding of the disease.

This report also demonstrates the heterogeneity of Fabry disease presentations and phenotypes, whilst serving as a reminder to all physicians, whether general or specialised, of the value of exploring family histories, even after several years. It also highlights that Fabry disease is not such a rare disorder when one considers both classical and variant forms, since the true number of variant forms is unknown but more common than previously reported6.

**Identifying Familial Disease**

A routine clinic review of patient X, known to our department for over 30 years, with a cadaveric renal transplant and presumed hypertensive renal failure, identified an unusual family history, where both surviving brothers were under investigations by cardiologists for non-ischaemic heart disease. Patient X, the eldest of 3 brothers, had presented to our department in 1975, aged 25 with severe hypertension, requiring dialysis in 1978 and then received a cadaveric kidney transplant in 1980.

Separately, younger brothers Y & Z had been investigated for a variety of symptoms over several years. Cardiological assessment of sibling Y had revealed unexplained severe left ventricular hypertrophy, refractory to anti-hypertensive therapy, prompting consideration of other diagnoses. His cardiologist referred the patient for a tertiary centre cardiac assessment with a view to possible cardiac biopsy. However no additional investigations were performed at that time.

**Confirming Familial Disease**

As both renal and cardiac clinicians became aware of the emerging family history and entered into correspondence, Fabry disease was considered. Blood spot or serum alpha-galactosidase levels were assessed using standard techniques (Figure 1A) and showed alpha-galactosidase deficiency (but importantly not absence of enzyme activity). Classical Fabry disease is associated with undetectable enzyme levels, whereas atypical or variant disease is associated with low enzyme levels3, suggesting our siblings had a variant form of the disease. Pedigree analysis revealed an inherited disorder compatible with X-linked disease (Figure 1B). The elder male sibling had died from cancer in his fifth decade and we are therefore unaware of his status.

Genetic mutation analysis of the entire alpha-galactosidase gene revealed that all 3 siblings shared an identical missense mutation (Figure 1C). The base pair mutation identified, R301Q in exon 6, results in the substitution of arginine with glutamine (Figure 1C). This mutation was first described in 19907 and, although it results in targeted early degradation of an aberrantly folded alpha-galactosidase enzyme, is associated with a degree of residual enzymatic activity8 – in keeping with the serum enzyme assay results obtained in this family (Figure 1A) and previous observations in variant forms of the disease8.
Phenotypic Characterisation

With the molecular genetic diagnosis confirmed, we sought to characterise disease manifestations in each sibling, summarised in Table 1. Initial assessment clinically suggested that sibling X had a renal variant of Fabry disease whereas siblings Y and Z had a cardiac variant. Both cardiac and renal variants of Fabry disease have been described in patients carrying the R301Q mutation but no reports exist of both variants within the same family.

Renal disease involvement

Fabry disease affects the kidney via the accumulation and deposition of uncleaved neutral glycosphingolipids in various essential specialised cells (podocytes, endothelial, mesangial and interstitial cells). This results in several abnormalities of renal function but typically in microalbuminuria and proteinuria, which worsens with age, ultimately progressing to end stage disease.

Sibling X originally had a native renal biopsy performed in 1975 but in our centre it is routine practice for samples to be discarded after 30 years, hence original biopsy samples were not available for retrospective analysis. However samples were available from bilateral native nephrectomies performed in 1984 for severe hypertension, four years post-cadaveric renal transplantation, when patient X was age 34.

Analysis was limited, as the specimens were not processed for diagnostic assessment at that time. Examination of haematoxylin and eosin (H&E) stained sections revealed features of end stage renal disease (Figure 2A) only but electron microscopy images revealed a myelin figure or zebra body in keeping with a diagnosis of renal Fabry disease (Figure 2B). These appearances represent deposits of glycosphingolipids within enlarged secondary lysosomes as lamellated membrane structures, composed of concentric layers with a periodicity of 3.5 to 5 nm and with an onion skin appearance.

Thus, sibling X had renal disease that progressed to end-stage requiring renal replacement therapy, initially in the form of dialysis and subsequently renal transplantation. His renal transplant is still functioning well 32 years post-transplantation, in keeping with renal Fabry disease, as a donor kidney should have normal alpha-galactosidase levels. Ultra structural changes in keeping with Fabry disease have been reported in donor kidneys, possibly via migration of recipient endothelial cells, but not in sufficient amounts for recurrent disease to be evident or change graft function.

Despite the early severe renal disease in sibling X, both siblings Y and Z appear to have normal renal function when most recently assessed by serum creatinine (Table 1). In addition, given that serum creatinine is a poor marker of early renal dysfunction, neither sibling Y or Z have significant proteinuria. The lack of renal involvement for these siblings provides a stark phenotypic contrast to their older sibling sharing the same genotype.

Cardiac disease involvement
Cardiac involvement in Fabry disease includes left ventricular hypertrophy (LVH), arrhythmia, angina and dyspnoea in approximately 40-60% of patients\textsuperscript{12}. Together renal and cardiac disease account for the reduction in life expectancy observed in Fabry disease; which is approximately 17 years for males and 5 years for females\textsuperscript{13}. Over time, cardiac disease has increasingly accounted for the mortality observed, as patients with renal failure survive longer on renal replacement therapy in the form of dialysis and transplantation\textsuperscript{13}.

Typically LVH is concentric and progressive myocardial fibrosis is seen, with replacement fibrosis typically seen in the posterior-lateral wall and mid-myocardium\textsuperscript{3,12}. Cardiac MRI performed when siblings X, Y & Z were 61, 59, & 51 years old respectively, showed these characteristic findings, with more severe cardiac disease in siblings Y & Z, despite younger age and much shorter duration of hypertension (Figure 3A & B). As described earlier, sibling Y had been successfully treated for hypertension for 1 year, yet without any appreciable change in LVH and guidance suggests that patients with unexplained or atypical LVH should be tested for Fabry disease\textsuperscript{14}.

Malignant arrhythmias account for a significant proportion of cardiac deaths in Fabry disease\textsuperscript{15}. Sibling Z is under consideration for ICD implantation for resistant ventricular dysrhythmias, as he has not responded to treatments offered thus far, despite ERT. Neither sibling X or Y appear to have had symptomatic arrhythmias. The differences in hypertrophy observed do not explain the resistant arrhythmias seen in sibling Z. It should be remembered that the images portray a cross-section of the sibling's myocardium only (Figure 3A & B). Coronary angiography in siblings Y and Z demonstrated normal coronaries, whilst full analyses of the MRI images show more extensive myocardial infiltration in sibling Z (data not shown) despite similar degrees of LVH. This tends to support the hypothesis that ventricular arrhythmias are related to fibrotic scars rather than LV hypertrophy or systolic dysfunction alone\textsuperscript{15}.

These images also show that patient X does not have a renal limited variant. A Japanese study examining screening dialysis patients for Fabry disease showed that six of 514 patients had previously undiagnosed variant forms of Fabry disease\textsuperscript{16}. Of these 6 patients, 5 had LVH and all had missense mutations other than the R301Q mutation with low alpha-galactosidase levels\textsuperscript{15}. Thus sibling X appears to have a recognised variant form with predominant renal involvement and cardiac disease also.

**Other systems involvement and Enzyme replacement therapy**

Clinical assessment of these siblings to date suggests that in addition to renal and cardiac involvement, there appear to be some neurological manifestations but no other features of Fabry disease (Table 1). Acroparathesia has been the predominant form of neurological involvement, with MRI brain analysis not suggestive of Fabry disease (data not shown). These siblings seem to have variant disease involving 3 systems to differing degrees of severity.

ERT appears to be a safe and effective therapy if initiated early enough, with the ability to stabilise disease in other organ systems, even for those requiring dialysis for
example. Patient X has declined ERT at present, whilst patient Z is receiving therapy and patient Y has declined further assessment or management.

Discussion

Delays and difficulties in reaching a diagnosis of Fabry disease, recently described as the ‘new great imposter’, are common. Patient X required 35 years before diagnosis and his discordant siblings 7 years. This study highlights the value of revisiting historical diagnoses and the importance of family history in assessment, including organ disease other than that of the specialist area concerned.

Our study also describes discordant phenotypic variants of Fabry disease within 3 siblings sharing an identical mutation, which has not previously been reported. This adds further complexity to this disorder and raises the obvious possibility of genetic or environmental factors that can modify the course of variant disease. The R301Q mutation localises to a mutation hotspot and having been previously identified in both cardiac and renal variants of Fabry disease, it seems congruous that this mutation could produce the phenotypes observed in this family, with perhaps modifier genes or environmental factors influencing disease manifestations. We are presently attempting to identify genetic factors involved in these discordant siblings.

Pursuing a diagnosis of Fabry disease, and in particular characterising the underlying gene mutation in each family, should now be considered routine clinical practice, given the advent of ERT and other treatment options under development. In vitro and pre-clinical studies of non-enzyme replacement based treatments try to chemically enhance the stability of the mutant aberrantly folded protein, by reducing early-targeted degradation of the abnormal protein (“chaperone therapy”). As this form of the protein retains enzymatic activity, this is proposed to result in less accumulation of glycosphingolipids, lowering disease burden and hopefully slowing progression. Clinical studies are awaited.

Raising awareness of the variant forms of Fabry disease and the possibility of discordant familial disease is important, as despite the availability of effective treatments, it remains common for long delays between presentation and diagnosis. Early initiation of ERT is essential for maximum benefit and delayed diagnosis can result in irreversible disease unresponsive to ERT. Delays in diagnosis can also result in trials of inappropriate and potentially harmful alternative treatments, such as beta-interferon or systemic corticosteroids. Guidance exists for how to investigate or when to consider a diagnosis of Fabry disease but ultimately all general physicians should be aware of ‘The Great Imposter’.

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**Conflicts of Interest** None
References


3. Germain PD. Fabry Disease. *Orphanet Journal of Rare Diseases* 2010; 5:30


Figure Legends

Figure 1. Serum alpha-galactosidase levels, Family structure and mutational analysis

A. Confirmation of significant α-galactosidase deficiency, but not absence of enzyme activity, was confirmed using standard tests.

B. The family pedigree is shown with affected brothers in shaded squares. Unaffected family members are shown in unshaded symbols. Female carriers are shown with a circle with a dot in the centre.

C. Sequencing of the GLA gene in all 3 affected males confirmed a hemizygous change in the GLA gene, leading to a missense variant Arginine to Glutamine at amino acid position 301. Female carriers are heterozygous for this variant (data not shown).

Table 1. Siblings sharing R301Q mutation have discordant phenotypic disease
Clinical assessment and characteristics of siblings X, Y and Z compared to systems affected in classical Fabry disease (RRT renal replacement therapy).

Figure 2. Retrospective analysis of native nephrectomy specimens for Sibling X

A. The native renal biopsy specimen (performed in 1984 for severe resistant hypertension) from patient X was reviewed and revealed no obvious features on H&E stained sections.

B. Electron microscopy revealed a myelin figure suggestive of Fabry disease.

Figure 3. Variant cardiac disease demonstrated by MRI imaging

A. Typical delayed hyper-enhancement in posterior basal myocardium from post gadolinium enhancement images

B. CINE derived end diastolic images demonstrating generalised left ventricular hypertrophy with more severe cardiac disease in siblings Y & Z, despite younger age and shorter duration of hypertension.
Figure 1.

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GLA gene variation
Transition from G to A in exon 6
c.902G>A, p>R301Q