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Twin pairs showing discordance of phenotype in adult Gaucher's disease

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Summary

Background: Non-neuronopathic (type 1) Gaucher's disease, a recessive disorder caused by glucocerebrosidase deficiency, shows marked variability in the severity and extent of clinical expression: many individuals who harbour two mutant alleles remain mildly affected or asymptomatic. Despite much effort, it is not possible accurately to predict disease severity from the genotype, or to identify those patients destined to develop severe disease and meriting early treatment.

Aim: To determine the degree to which variance in Gaucher disease is determined by non-heritable factors.

Design: Case reports of monozygotic and dizygotic twin pairs.

Results: For the monozygotic twin pair, homozygous for the frequent N370S glucocerebrosidase allele, there was no evidence that significant lipid storage was ever initiated in the unaffected twin. In contrast, pathological storage of glucocerebroside has been present in the macrophages of both members of the dizygotic twin pair (compound heterozygotes for the N370S and L444P alleles) from an early age but, by the age of 57 years, only one has developed symptoms.

Discussion: Non-heritable factors influence Gaucher disease expression in genetically predisposed individuals. Understanding the interactions between heritable and non-heritable factors will be critical for an analysis of pathogenesis, and the treatment of individuals predisposed to Gaucher disease.

Introduction

Gaucher's disease (OMIM nos. 230800, 231000 and 230900), a monogenic recessive disorder, is caused by mutations in the gene encoding the lysosomal hydrolase, glucocerebrosidase (acid β-glucosidase EC 3.2.1.45). The residual glucocerebrosidase activity appears to determine the clinical phenotype: hydrops fetalis and congenital ichthyosis result from the most disabling mutations;[1] neuronopathic (type 2 and 3) disease occurs where there is some residual enzyme activity;[2] and mutations that only partially inactivate glucocerebrosidase give rise to non-neuronopathic (type 1) Gaucher's disease.[3,4]

The clinical features of type 1 Gaucher's disease

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are protean and variable.[3] Gaucher cells, which are pathological macrophages, primarily infiltrate the spleen, liver and bone marrow. Patients may be identified first in childhood with hypersplenism and growth retardation, or come to light in old age with trivial thrombocytopenia or isolated splenomegaly. On the other hand, patients of all ages may develop severe skeletal, or life-threatening visceral, disease. The assumption that the nature of the mutation in the glucocerebrosidase gene would determine disease severity has prompted the search for genotype/phenotype correlations to explain this wide variation in the clinical course of the disease.[5–8] It has been suggested that such correlations might assist in treatment decisions for patients with type 1 Gaucher's disease. Attempts to correlate specific mutations with Gaucher's disease phenotype have, however, been generally disappointing[9,10] and the penetrance of many genotypes is variable, as demonstrated by studies of the two most frequent mutant human glucocerebrosidase alleles.

The N370S allele, present in over 70% of Ashkenazi Jewish and about 43% of non-Jewish Gaucher's patients,[11] generally gives rise to a mild disease phenotype.[5,12,13] When the frequency of the N370S allele in the Jewish population as a whole is calculated, it is predicted that about two-thirds of homozygous individuals remain asymptomatic and are never diagnosed with Gaucher's disease.[10,11] Nonetheless, some patients with this genotype develop disabling Gaucher's disease—including widespread skeletal injury. The L444P allele[14] is present in about 12% of non-neuronopathic patients, and more than half of patients with neuronopathic disease are L444P homozygotes.[10,15] Early studies suggested that homozygosity for this allele would inevitably lead to neurological manifestations.[5] It has subsequently been shown, however, that in the Japanese, as well as other populations, homozygosity for L444P can also be associated with non-neuronopathic disease.[16]

Here we report two sets of twins, one monozygotic and the other dizygotic, who are highly discordant for the manifestations of Gaucher's disease.

Case histories

Monozygotic twins

M1 presented at the age of 69 with rapid onset of fatigue, abdominal swelling and weight loss. She was a single woman, and had lived with her identical twin sister (M2) all her life. Physical examination revealed pallor, hepatosplenomegaly and evidence of easy bruising. There was generalized pigmentation and pingueculae—a characteristic ocular sign of Gaucher's disease. Results of investigations are shown in Tables 1 and 2; a bone marrow aspirate confirmed the diagnosis of Gaucher's disease. Genotyping at the glucocerebrosidase locus showed that she was homozygous for the N370S allele. Within three years symptomatic thrombocytopenia developed and thereafter she underwent total splenectomy with rapid correction of the pancytopenia (Table 2). The patient remained well until the age of 80, when she suffered multiple crush fractures of her thoraco-lumbar spine. MRI imaging demonstrated bone marrow infiltration by Gaucher cells (Figure 1, a and b). The patient has also suffered a pathological fracture of the neck of humerus, which healed well, following which she was commenced on bisphosphonate therapy with supplementation of calcium and vitamin D3.

Her twin sister, M2, had no evidence of Gaucher disease. She had a normal physical examination and no evidence of hepatosplenomegaly. Her blood counts were normal until the age of 83, when she developed a mild normochromic normocytic anaemia (Table 2).

Both twins developed senile dementia and, aged 83, entered residential care. Twin M1 died at the age of 84, and her sister died 6 months later.

We have confirmed that the two sisters were monozygotic twins by DNA minisatellite fingerprinting[17] (Cellmark Diagnostics). Lymphocyte gene rearrangement studies (Dept. of Haematology, University of Cambridge, Addenbrooke's Hospital) showed no evidence of expansion of clonal B-cell or T-cell populations in either twin (data not shown). X-chromosome inactivation studies[18] showed a 90% skewed inactivation of one parental chromosome in the affected twin M1 (which would be expected in about 10% of the female population), and equal Lyonization of both X-chromosomes in M2.

Table 1 Diagnostic investigations for Gaucher's disease

	Leucocyte glucocerebrosidase†	Glucocerebrosidase genotype*
Monozygotic twins		
M1	0.70	N370S/N370S
M2	0.62	N370S/N370S
Dizygotic twins		
D1	1.3	N370S/L444P
D2	0.2	N370S/L444P

†Normal ranges for assays: 8.4–32.8 and 9–19.6 nmol/h/mg, for assays conducted by different reference laboratories for the monozygotic and dizygotic twins, respectively. *Determined by sequencing.

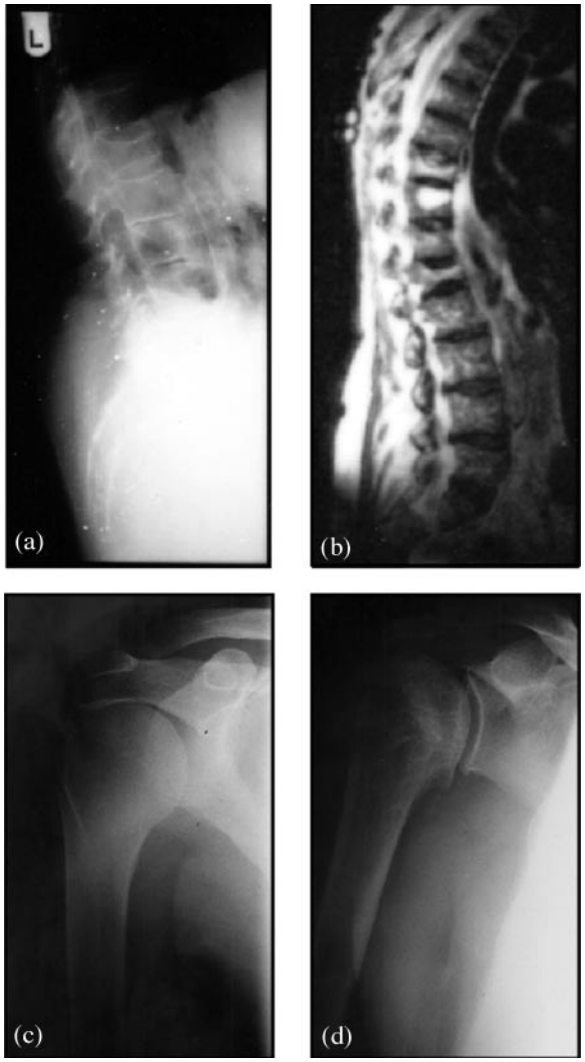

Figure 1. Radiological investigations. **a** M1: spine X-ray showing vertebral crush fractures. **b** M1: spine MRI showing vertebral crush fractures and bone marrow infiltration by Gaucher cells. **c** D1 (unaffected twin): X-ray of right shoulder. **d** D2: X-ray of right shoulder showing avascular necrosis of the humeral head.

The two sisters cohabited all their lives, and the only discordant medical history is that M1 underwent tonsillectomy and adenoidectomy at the age of seven years. Serological studies showed that both sisters had IgG antibodies to Ebstein-Barr virus (EBV) and varicella zoster virus (VZV), but no antibodies to cytomegalovirus (CMV) or herpes simplex virus (HSV).

Dizygotic twins

D1 was found to have splenomegaly at the age of 15 years, when he contracted cat-scratch fever; no investigations were undertaken. The presence of Gaucher's disease was identified when, as a medical student, the patient had a diagnostic marrow aspirate after palpating his own spleen. At the age of 56, the patient complained of increasing fatigue. He had not suffered any other complications of Gaucher's disease. On examination, pingueculae were present, the spleen tip was palpable, and he had 2 cm hepatomegaly. Sample investigations are detailed in Tables 1 and 3 and Figure 1c. Genotyping by DNA sequencing showed that subject D1, like his twin brother D2, was a compound heterozygote for the L444P and N370S mutations. Serological testing confirmed that IgG antibodies against HSV, VZV and EBV were present but no antibodies to CMV were detectable.

D1 was commenced on ERT with imiglucerase, and his symptoms responded well to treatment. After 6 months of treatment, his liver volume decreased from 2.35 l to 1.87 l, and his spleen volume from 0.71 l to 0.47 l. The biochemical response is shown in Table 3.

His dizygotic twin D2 was first evaluated at the age of 49 years. Splenomegaly had been noted when he had scarlet fever at the age of six, but Gaucher's disease was only identified as the cause when his twin brother was diagnosed. When 21 years old, the patient had an episode of prolonged bleeding following extraction of a tooth, and was found to be thrombocytopenic. Ten years later, he suffered a presumed episode of splenic infarction with sudden onset of left upper quadrant abdominal pain. This settled spontaneously, but he briefly became jaundiced. Bone marrow examination at this time showed Gaucher's cells. At the age of 47 years, he suffered avascular necrosis of his right humeral head (Figure 1d), leaving him with a limited range of movement and some residual aching.

On examination, he had numerous bruises and pingueculae. There was marked hepatosplenomegaly. Results of specimen investigations are shown in Tables 1 and 3. Serological testing confirmed the presence of IgG antibodies to HSV, VZV, EBV and CMV.

D2 was started on ERT with alglucerase (later converted to imiglucerase), and has since made a good response with marked reduction in organomegaly (liver and spleen volumes of 2.41 l and 1.71 l prior to the commencement of ERT, and 1.48 l and 0.48 l, respectively, after 6 years of therapy) and restoration of blood counts and markers of disease activity (Table 3). There have been no further episodes of bone infarction.

Discussion

While twin M1 is affected by her Gaucher disease, twin M2 has no splenomegaly and there is no

Table 2 Laboratory features in monozygotic twins

	M1				M2				Normal range
Age (years)	69*	75**	76**	82**	71	75	76	82	
Haemoglobin	9.1	12.0	11.2	12.1	12.7	12.5	12.8	10.1	11.5–16.5–g/dl
Leucocyte count	2.1	6.3	5.6	4.9	5.3	5.8	6.0	4.14	$4–11 \times 10^9$
Platelets	26	258	244	181	246	235	271	250	$150–450 \times 10^9$
Sedimentation rate						18			5–15 mm/h
C-reactive protein		3				3			< 6 mg/l
IgG		14.9	14.8			8.2	8.2		6.0–13.0 g/l
IgA		2.3	2.6			1.5	1.3		0.8–3.7 g/l
IgM		1.6	1.3			1.6	1.4		0.4–2.2 g/l
Electrophoretic pattern		PC	OC			N	N		
Angiotensin converting enzyme		137	108			81	68		15–70 U/l
Total acid phosphatase		11.4	6.7			8.9	5.8		0.5–6.0 U/l
Chitotriosidase		U				U			0–140 µmol/l/h
Ferritin		1697				129			15–235 µg/l

*Pre-splenectomy; **Post-splenectomy. PC, polyclonal; OC, oligoclonal; N, normal; U, undetectable (null genotype).

Table 3 Laboratory features in dizygotic twins

	D1			D2				Normal range
Age (years)	50*	54*	57**	49*	50**	54**	56**	
Haemoglobin	16.1	16.5	15.4	14.1	15.1	15.8	15.4	13–18 g/dl
Leucocyte count	6.4	6.4	6.7	5.8	6.4	6.4	6.6	$4–11 \times 10^9$
Platelets	125	140	170	71	94	164	210	$150–450 \times 10^9$
IgG	14.1	13.0	12.8	22.3	18.2	18.9	18.2	6.0–13.0 g/l
IgA	2.7	2.3	1.9	3.1	2.7	2.5	2.4	0.8–3.7 g/l
IgM	> 3.9	2.7	2.7	> 3.9	3.7	3.1	2.8	0.4–2.2 g/l
Electrophoretic pattern	PC	PC	PC	PC	PC	PC	PC	
Angiotensin converting enzyme	99	97	74	304	148	101	61	15–70 U/l
Total acid phosphatase	14.6	13.9	10.1	22.8	11.1	4.3	6.3	0.5–6.0 U/l
Chitotriosidase		2607	2016		8181	2222	1590	0–140 µmol/l/h

*Pre-ERT; **Post-ERT. PC, polyclonal; OC, oligoclonal.

clinical evidence that significant storage of glycolipid in the macrophage system has ever been established. In contrast, in both twins D1 and D2, the disease process appears to have been established in childhood, with the accumulation of Gaucher cells in their viscera since an early age. Both have had documented splenomegaly since childhood and bone marrow examinations which confirmed the presence of Gaucher cells. Twin D2 exhibited the first symptoms attributable to hypersplenism some 15 years after splenomegaly had been detected, and it was another 15 years before his skeletal disease became symptomatic with the development of avascular necrosis of the humeral head. Remarkably, his brother D1 has had splenomegaly for at least 35 years, but has remained entirely asymptomatic for Gaucher's disease. The phenotypic difference between these dizygotic twins appears to be due to factors influencing the progression of the disease, rather than the initiation of glycolipid storage.

Previous investigators have suggested that genetic factors other than those that determine expression of the glucocerebrosidase gene, such as the activator saposin C,[19,20] are responsible for the variations in disease phenotype seen between individuals carrying identical mutant glucocerebrosidase alleles. The only genetic difference, however, between twin M1, with symptomatic Gaucher's disease including skeletal involvement, and her unaffected sister is a partially skewed inactivation of the X chromosome. Although it is theoretically possible that differential expression of a gene on the X-chromosome may be important, it seems unlikely that any germline allele is responsible for this discordance, particularly as marked variation in the manifestations of Gaucher's

disease is observed between male and female siblings as well as unselected male and female patients.

It is possible that acquired somatic mutation might account for the differential penetrance of mutant glucocerebrosidase alleles. Raised immunoglobulin levels with oligoclonal or monoclonal bands, as seen in M1, are a common feature of Gaucher's disease and indicate that these patients may generate significant clonal populations of B lymphocytes. Theoretically, such clones might be able to influence disease expression, perhaps by means of secreted factors such as interleukin 6 which could act on the macrophage-derived Gaucher cells.[21] In these discordant monozygotic twins, however, gene rearrangement studies have revealed no evidence of significant clonal expansion of blood lymphocyte populations in either twin.

The other possibility is that environmental factors determine the penetrance of disease. It has been reported that EBV infection can trigger the development of disproportionately massive splenomegaly in patients with previously undiagnosed Gaucher's disease,[22] and it is possible that chronic infection with EBV, or other viruses, might be a trigger for the accumulation of Gaucher cells.[23] Twins M1 and M2, however, are both seropositive for EBV IgG antibody.

M1 and M2 lived together all their lives, and appear to have had the same exposures to infectious and environmental agents. It is noteworthy that the symptomatic twin, M1, unlike her sister, underwent tonsillectomy and adenoidectomy in childhood. This suggests that she suffered from recurrent infections and a degree of chronic inflammation at that time. It was another 60 years before she developed symptomatic Gaucher disease, so it seems unlikely that these childhood infections were directly responsible. This history does, however, suggest that the immune system of M1 may have responded to infections differently from that of her sister. It remains unclear what factors resulted in the initiation of glycolipid storage in one twin and not the other.

For twins D1 and D2, comparison of chitotriosidase activities before treatment for D1 and after treatment for D2 clearly reflect the clinical evaluation of disease severity and confirm the great disparity in disease progression between them. Interestingly, they differ in their serology to CMV, with the affected twin showing evidence of CMV infection. CMV infection can be transmitted vertically, but is normally acquired in adolescence or early adulthood through close personal contact with other infected individuals. Latent virus persists in cells of the monocyte/macrophage lineage, and thus persistent infection with CMV could affect the expression of Gaucher's disease. In N370S homozygote patients who are seropositive for CMV and/or EBV, there is reportedly no correlation between the levels of antibody to the viruses and disease severity.[24] To our knowledge, no cross-sectional study of CMV seropositivity in symptomatic and asymptomatic patients with genotypes that predispose to Gaucher's disease has been reported.

Our findings show that in Gaucher's disease, as with several 'monogenic' disorders,[25–27] it is not possible reliably to correlate genotype and phenotype. The diagnosis of Gaucher's disease needs to be made on clinical and not solely genetic grounds. Screening of high-risk populations for mutations at the glucocerebrosidase locus has been advocated as a means of identifying Gaucher's patients at a presymptomatic stage so that ERT can be instituted early in the course of the disease. However, on account of variation in disease expression, such as described here, there are many individuals who possess mutations in both parental glucocerebrosidase alleles who will never develop symptomatic Gaucher's disease. In addition, of those patients who do eventually develop the disease, many will remain asymptomatic for many decades. Furthermore, once the predisposing genotype has been identified, there is evidence that, in the Ashkenazim at least, the clinical condition may remain stable for many years.[28,29] Even where it has been demonstrated that clinical decline occurs during observation,[30,31] there is no evidence that individuals gain any health benefit from ERT during the asymptomatic period. Thus, at present, we consider that ERT should only be given to patients who have clear clinical indications for treatment.

The twins here reported with Gaucher's disease confirm that interacting factors are at least as important as the nature of the mutant glucocerebrosidase alleles in influencing the initiation and progression of the disorder. The nature of these factors is far from apparent, and may involve genetic as well as environmental contributions that are not inherited in the germline. However, defining the relevant environmental influences will be critical for determining to what extent and at what age any given individual with glucocerebrosidase deficiency will be affected—and may ultimately assist in the allocation and planning of treatment programmes.

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