Phenotype/genotype relationships in sickle cell disease: a pilot twin study

M. W. WEATHERALL*, D. R. HIGGS†, H. WEISS‡, D. J. WEATHERALL†, G. R. SERJEANT*

* MRC Laboratories (Jamaica), University of the West Indies, Kingston, Jamaica
† Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK
‡ Infectious Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, London, UK

Summary
The roles of genetic and non-genetic factors in the haematology, growth and clinical features of sickle cell disease have been studied in nine identical twin pairs (six homozygous sickle cell disease, three sickle cell-haemoglobin C disease). A comparison group of 350 age–gender matched sibling pairs, selected to have an age difference of <5 years, was used for assessing the concordance of numerical data. Attained height, weight at attained height, fetal haemoglobin, total haemoglobin, mean cell volume, mean cell haemoglobin and total bilirubin levels showed significantly greater correlation in identical twins than in siblings. Twins showed similarities in the prevalence and degree of splenomegaly, susceptibility to priapism, and in onset of menarche, but other clinical complications were discordant in prevalence and severity. These findings suggest that physical growth and many haematological characteristics are subject to genetic influences, but that non-genetic factors contribute to the variance in disease manifestations.

Keywords
Sickle cell disease, twin studies, concordance

Introduction
The great variability in clinical and haematological features of sickle cell disease provide a challenge for understanding the pathophysiological mechanisms involved but may also hold the key for therapeutic interventions in this condition. The genetic basis of sickle cell disease is undisputed but variability in the disease is influenced by non-genetic factors such as the role of folate deficiency in exacerbating the anaemia, skin cooling in precipitating painful crises, and skin trauma in initiating chronic leg ulceration. Furthermore, much research has focused on the striking changes in red cell morphology and less attention has been paid to the pathological role of the high counts and abnormal function of white cells and platelets. The recent interest in pulmonary hypertension and in venous incompetence in the persistence of chronic leg ulcers also illustrates the potential importance of other mechanisms not directly related to the red cell changes. It is now clear that sickle cell disease, far from being a condition dominated by the abnormal red cell morphology, is affected by a host of genetic and environmental factors and their interaction. In this situation, studies of identical twins have proved a powerful tool in distinguishing the relative roles of genetic and non-genetic influences and it is surprising that twin studies in sickle cell disease are confined to two reports of single twin pairs, one with homozygous sickle cell (SS) disease (Amin et al., 1991) and other with a severe form of sickle cell-β+ thalassaemia of Italian/American origin (Joishy, Griner & Rowley, 1976). Even within the database of over 5500 patients with sickle cell disease attending the Jamaican Sickle Cell Unit, it has only been possible to identify nine pairs of identical twins, but because of the potential importance of this model, we now present data from this group.

Subjects and methods

Subjects
The patients attended the Sickle Cell Clinic of the University of the West Indies, Kingston. At the time of
the study in May 1997 (with later review), there were 12 twin pairs in whom DNA analysis suggested that nine twin pairs were identical, six with homozygous SS disease and three with sickle cell-haemoglobin C (SC) disease. Two of these twin pairs (one SS and one SC) participated in the Cohort Study, with prospective review of clinical information every 3 months and haematological analysis every 6 months. The remaining seven identical twin pairs were followed less rigorously in the Sickle Cell Clinic, with appointments scheduled every 6 months and haematological analysis every 2–3 years unless clinically indicated. Because of the paucity of non-identical twins, 350 gender–genotype matched sibling pairs with age difference of <5 years, were used as a comparison group for examining the degree of concordance between numerical data in the two groups.

Clinical methods and definitions

None of the subjects had received bone marrow transplants or were on interventions such as chronic transfusion or hydroxyurea, likely to affect haematology or clinical course. Height was measured on a wall-mounted stadiometer and analysed observations were restricted to maximum attained height in subjects where both members of the pair (twins or sibling pairs) had been followed to at least the age of 15 years or were the same age at measurement. Weight was measured on a lever balance and the weight at the time of maximum height was used with the above restrictions.

Aplastic crisis was defined as a markedly lowered haemoglobin level with absence of reticulocytes, or if present, a daily increase consistent with the recovery phase, and confirmed by serological evidence of human parvovirus infection. Acute splenic sequestration (ASS) was defined as the combination of a sudden increase in spleen size (usually >3 cm below the costal margin), a fall in haemoglobin levels of >2 g/dl to levels usually below 4.5 g/dl, and an increase in the reticulocyte count to 2–3 times the steady state value, with decrease in splenomegaly following transfusion or spontaneous resolution of the attack. Acute chest syndrome was defined by radiological confirmation of a new pulmonary infiltrate or signs of consolidation, usually associated with a history of cough and dyspnoea. Priapism was an involuntary painful erection unassociated with sexual desire which could be stuttering (usually <6 h) or major (usually >24 h). Painful crisis referred to bone pain of sufficient severity to limit function, which usually resulted in admission to hospital or daycare centre. Dactylitis (hand–foot syndrome) was a painful swelling of the digits, or the dorsum of the hand or foot. Avascular necrosis of the femoral head referred to radiological evidence of articular surface disruption of the femoral head. Leg ulcers were defined as ulcers around the ankles persisting for a minimum duration of 3 months.

Haematological analysis

Haematological data were obtained electronically (Coulter counter models ZB16; S plus 4 and MaxM Coulter Electronics, Miami, FL, USA). Reticulocytes were counted manually after staining with brilliant cresyl blue, serum bilirubin by the method of Lathe and Ruthven (1958), fetal haemoglobin (HbF) by the alkali denaturation method of Betke, Marti and Schlicht (1959) and HbA₂ as described by Millard et al. (1977). Only steady-state values (those taken while the patient was well and without acute symptoms) were included in the analysis.

DNA analysis

α-Globin genotypes were determined as reported by Higgs et al. (1982). Nine multiallelic loci were determined by either Southern blot analysis or polymerase chain reaction including α-globin 3′-HVR (Higgs et al., 1981), APO B, IgH (Decorte et al., 1990), pG3 (Smith et al., 1998), PMS 600i, PMS 600ii, MS 620, MS 617 (Flint et al., 1995) and 608 JF (J. Flint, unpublished data). Assuming that, on average, the variable number of tandem repeat sequences have four alleles each with a frequency of 0.25, the probability of homozygosity at all nine loci in non-identical twins can be conservatively estimated at 5000 : 1. On this basis, the nine pairs of twins homozygous at all nine loci were considered identical.

Statistical analysis

The small number of identical twins did not allow variance components analysis to estimate the proportion of variation because of genetic and environmental factors. Instead, correlation coefficients were calculated for identical twins and for sibling pairs (including three non-identical twins) and a P-value was calculated for the difference in correlations (Gardner & Altman, 1988). Where repeated measurements were available, analysis was confined to the maximum height and weight attained and the minimum steady state haemoglobin values. No attempt was made to assess correlations of clinical features in the two groups but the agreement between clinical courses in the identical twins was qualitatively reported.
Results

Analyses of numerical data (Table 1) indicate that correlations between identical twin pairs were significantly greater than for sibling pairs, this effect being strong for attained height, weight, HbF, mean cell haemoglobin (MCH), and total bilirubin and weaker for haemoglobin and mean cell volume (MCV). Only reticulocyte counts and conjugated (direct reacting) bilirubin were not more closely correlated in identical twins.

Some clinical data, α-globin gene number, haematology and major clinical episodes in the identical twin pairs designated A-H, J are summarized in Table 2.

Bone disease

Dactylitis is usually limited to subjects below 5 years and was analysed in the six twin pairs (A-D, G, H) observed over this interval. A history of dactylitis occurred in at least one twin of four SS twin pairs, two were discrepant (A: twin I had a single episode, twin II none; C: twin I had four episodes, twin II none), and in two, both twins were affected but at different times (B: twin I had a single event at 8 m, twin II had three events between 2 and 8 m; D: twin I had a single event at 10 m, twin II a single event at 34 m). In only a single attack there was concurrence in time between twins (B: both twins affected 2 days apart). Dactylitis did not occur in either of the two SC twin pairs. Painful crises did not occur in two SS twin pairs (A, B) or two SC twin pairs (G, H), but the relative frequencies in the five other twin pairs (four SS, one SC) were often discrepant (Table 2). Avascular necrosis of the femoral head occurred in one twin of a single twin pair (I twin II). Painful crisis and probably dactylitis are known to be precipitated by environmental factors especially skin cooling which is consistent with discordance in twin pairs.

Splenic pathology

Of six SS twin pairs, E followed from ages 10 to 22 years and F from ages 22 to 28 years were never observed to have splenomegaly or a history of ASS. In B, splenomegaly developed at age 8–9 months and disappeared after 2 years of age in both twins, without evidence of ASS. Of three SC twin pairs, two (G, H) were never observed to have splenomegaly, and in J, both twins had persistent splenomegaly, 7 cm (twin I) and 9 cm (twin II) when last observed at 25 years of age. Neither had evidence of ASS, but twin II had two attacks of splenic pain consistent with splenic infarction.

In D, twin I developed ASS at 13 m and 16 m with splenectomy at 18 m, whereas twin II showed transient and intermittent splenomegaly between 12 and 49 m but no evidence of ASS. In two pairs (A, C), both twins developed ASS. In A, both twins developed ASS at 9 m within 13 days of each other, twin I had a second attack at 15 m and splenomegaly then resolved in both. In C, both twins had ASS simultaneously at 12 and 16 m; twin I had a third episode at 15 m with splenectomy at 17 m and twin II had further episodes at 14 and 18 m with splenomegaly at 18 m. With the exception of this latter family, most events did not occur simultaneously and even the latter coincidence is probably more readily explained by an environmental factor based on close contact between twins possibly superimposed on a genetically determined predisposition.

Acute chest syndrome

There were 10 events in five subjects of four twin pairs. In only one twin pair (J) were both members affected, twin I with a single attack at 25.1 years and twin II with three attacks between 13.3 and 16.4 years. In the others, single attacks occurred in B twin II and D twin II and four

Table 1. Correlations between twin pairs and sibling pairs for growth and selected haematology

<table>
<thead>
<tr>
<th>Variable</th>
<th>Identical twin pairs</th>
<th>Sibling-pairs</th>
<th>P-value for difference between correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attained height</td>
<td>9 0.997 (0.98–1.00)</td>
<td>207 0.59 (0.50–0.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight</td>
<td>9 0.94 (0.72–0.99)</td>
<td>203 0.48 (0.37–0.58)</td>
<td>0.005</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>9 0.96 (0.82–0.99)</td>
<td>289 0.79 (0.74–0.83)</td>
<td>0.03</td>
</tr>
<tr>
<td>Fetal haemoglobin</td>
<td>8 0.98 (0.89–1.00)</td>
<td>238 0.63 (0.55–0.70)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean cell volume</td>
<td>9 0.92 (0.66–0.98)</td>
<td>288 0.60 (0.52–0.67)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean cell haemoglobin</td>
<td>9 0.98 (0.90–1.00)</td>
<td>288 0.60 (0.52–0.67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>8 0.81 (0.32–0.96)</td>
<td>221 0.50 (0.39–0.59)</td>
<td>0.20</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>8 0.81 (0.25–0.96)</td>
<td>221 0.51 (0.41–0.60)</td>
<td>0.005</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>8 0.95 (0.74–0.99)</td>
<td>221 0.51 (0.41–0.60)</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Table 2. Basic haematological, genetic and clinical data in identical twin pairs

<table>
<thead>
<tr>
<th>Twin pair</th>
<th>Follow-up (year)</th>
<th>Age (year)</th>
<th>β-Globin genotype</th>
<th>α-Globin genotype</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>Reticulocytes (%)</th>
<th>HbA2 (%)</th>
<th>HbF (%)</th>
<th>Total bilirubin (µmol/l)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Complications</th>
<th>Admission pain crisis</th>
<th>Last spleen size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1–3</td>
<td>3</td>
<td>SS</td>
<td>αα/αα</td>
<td>7.5</td>
<td>84</td>
<td>29</td>
<td>17</td>
<td>3.1</td>
<td>12.4</td>
<td>16</td>
<td>97.9</td>
<td>16.8</td>
<td>2 ASS, dactyl.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>6.8</td>
<td>83</td>
<td>29</td>
<td>9</td>
<td>3.3</td>
<td>9.8</td>
<td>14</td>
<td>98.3</td>
<td>17.2</td>
<td>ASS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.7–11</td>
<td>11</td>
<td>SS</td>
<td>αα/αα</td>
<td>7.4</td>
<td>75</td>
<td>25</td>
<td>12</td>
<td>3.3</td>
<td>3.2</td>
<td>24</td>
<td>118.6</td>
<td>20.5</td>
<td>dactyl., aplasia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>6.6</td>
<td>72</td>
<td>25</td>
<td>13</td>
<td>3.6</td>
<td>2.9</td>
<td>27</td>
<td>120.6</td>
<td>21.3</td>
<td>3 dactyl., aplasia, ACS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C, coh</td>
<td>0–20</td>
<td>20</td>
<td>SS</td>
<td>αα/αα</td>
<td>7.3</td>
<td>92</td>
<td>32</td>
<td>17</td>
<td>3.1</td>
<td>5.2</td>
<td>82</td>
<td>162.5</td>
<td>44.4</td>
<td>3 ASS, 4 dactyl., ulcer, aplasia, priapism, TIA</td>
<td>5</td>
<td>Splenectomy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>7.3</td>
<td>92</td>
<td>32</td>
<td>17</td>
<td>2.9</td>
<td>5.0</td>
<td>72</td>
<td>162.4</td>
<td>42.4</td>
<td>4 ASS, ACS, aplasia, priapism</td>
<td>4</td>
<td>Splenectomy</td>
</tr>
<tr>
<td>D</td>
<td>1–16</td>
<td>16</td>
<td>SS</td>
<td>αα/αα</td>
<td>7.8</td>
<td>87</td>
<td>30</td>
<td>12</td>
<td>3.8</td>
<td>5.1</td>
<td>43</td>
<td>157.7</td>
<td>39.2</td>
<td>2 ASS, dactyl., meningitis</td>
<td>0</td>
<td>Splenectomy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>7.4</td>
<td>86</td>
<td>29</td>
<td>11</td>
<td>3.7</td>
<td>4.4</td>
<td>24</td>
<td>151.6</td>
<td>37.4</td>
<td>ACS, dactyl., ulcer</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>10–22</td>
<td>22</td>
<td>SS</td>
<td>αα/αα</td>
<td>9.1</td>
<td>92</td>
<td>33</td>
<td>13</td>
<td>3.0</td>
<td>7.8</td>
<td>32</td>
<td>165.2</td>
<td>44.0</td>
<td>AGN</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>22–28</td>
<td>28</td>
<td>SS</td>
<td>αα/αα</td>
<td>9.5</td>
<td>93</td>
<td>33</td>
<td>13</td>
<td>2.8</td>
<td>8.7</td>
<td>21</td>
<td>167.5</td>
<td>48.5</td>
<td>Nil</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>9.0</td>
<td>91</td>
<td>29</td>
<td>16</td>
<td>2.7</td>
<td>9.7</td>
<td>21</td>
<td>158.5</td>
<td>44.6</td>
<td>Nil</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0.8–17</td>
<td>17</td>
<td>SC</td>
<td>αα/αα</td>
<td>9.2</td>
<td>87</td>
<td>28</td>
<td>7</td>
<td>–</td>
<td>0.7</td>
<td>17</td>
<td>159.6</td>
<td>55.2</td>
<td>Nil</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>10.2</td>
<td>90</td>
<td>28</td>
<td>8</td>
<td>–</td>
<td>1.0</td>
<td>19</td>
<td>159.6</td>
<td>52.6</td>
<td>Nil</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>H, coh</td>
<td>0–28</td>
<td>28</td>
<td>SC</td>
<td>αα/αα</td>
<td>12.0</td>
<td>77</td>
<td>26</td>
<td>3</td>
<td>–</td>
<td>0.6</td>
<td>15</td>
<td>177.3</td>
<td>66.6</td>
<td>Nil</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>11.8</td>
<td>77</td>
<td>26</td>
<td>3</td>
<td>–</td>
<td>0.7</td>
<td>13</td>
<td>176.4</td>
<td>66.5</td>
<td>Cholestatic episode</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>12–25</td>
<td>25</td>
<td>SC</td>
<td>αα/αα</td>
<td>12.0</td>
<td>82</td>
<td>28</td>
<td>6</td>
<td>–</td>
<td>1.3</td>
<td>24</td>
<td>169.8</td>
<td>64.9</td>
<td>ACS</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>αα/αα</td>
<td>12.5</td>
<td>83</td>
<td>28</td>
<td>5</td>
<td>–</td>
<td>1.5</td>
<td>17</td>
<td>170.8</td>
<td>69.0</td>
<td>3 ACS, priapism, ANFH</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

In all twin pairs, the first line represents twin I.

Coh, cohort study subjects; ASS, acute splenic sequestration; ACS, acute chest syndrome; dactyl., dactylitis; ANFH, avascular necrosis of the femoral head; TIA, transient ischaemic attack; AGN, acute glomerulonephritis; painful crises, number of admissions to hospital/daycare centre.
attacks in C twin II but none in the corresponding twins. These data do not support a strong genetic role.

**Priapism**

Stuttering priapism occurred in three subjects of two twin pairs. In C, both twins developed stuttering priapism at 13.3 years which persists intermittently in twin II but twin I suffered a major attack lasting 6 days at 16.0 years although he now has normal erectile function without further priapism. In J, twin II had typical nocturnal stuttering priapism once or twice monthly from the age of 20.1 years and a single episode lasting 10 h at 21.1 years; twin I was unaffected. The data in twin pair C are consistent with a genetic predisposition but the subsequent natural history in the two twins differed.

**Aplastic crisis**

The B19-human parvovirus confirmed aplastic crises occurred in two SS twin pairs (B,C), affecting both twins on the same day in B and 10 days apart in C. The lowest recorded haemoglobin levels were 3.1 and 5.4 g/dl in B and 4.0 and 2.8 g/dl in C. The temporal concurrence in this established environmental factor illustrates the difficulties of using concurrence to distinguish between genetic and environmental factors in causation.

**Cerebrovascular events**

There were no typical strokes, but a transient ischemic event involving altered consciousness and disorientation which resolved in 24 h occurred in C twin I at age 19.3 years. No event has occurred in twin II during the follow-up to 23.5 years.

**Gallstones**

Annual gallbladder ultrasounds from the age of 5 years in the cohort study provided prospective data in two twin pairs (C,H). In C, gallstones were present at the first examination at 5.3 years in twin I but did not develop in twin II until 17 years. In H, neither twin showed gallstones during follow-up to 25 years of age.

**Leg ulcers**

Ulceration has persisted intermittently in C twin I from 15.3 years but no ulcers have occurred in twin II over observation up to 23.5 years. In D, twin I developed a small ulcer at 16.2 years which healed in 4 m later and twin II had a traumatic ulcer at 16.0 years which healed in 6 m. These data are too limited to form conclusions on genetic and environmental contributions although trauma is a clear environmental factor.

**Menarche**

Of the four female twin pairs (three SS and one SC) the interval between onset of menarche in the three SS pairs was 1 month (D), 2 months (F) and 11 months (E). In the latter pair, twin II commenced menarche at aged 16.5 years when weighing 48.5 kg. At that age, twin I weighed 44.0 kg and did not reach the menarche until 17.4 years when she weighed 47.8 kg. In the SC twin pair (G), the interval was 2 months.

**Other major clinical events**

Abdominal painful crisis with distension and absent bowel sounds occurred in one subject (D twin I at age 6 years). An episode of deep jaundice (serum total bilirubin >500 μmol/l), suggestive of acute cholestasis, affected H twin II but not twin I. In twin pair E, impetigo developed in both twins at age 13.7 years and in twin I was associated with acute glomerulonephritis. In B, both twins had congenital deafness, a white forelock and blue eyes (Waardenburg’s syndrome).

**Discussion**

It may seem surprising that the potentially powerful tool of twin studies has not been previously used to explore the relative roles of genetic and non-genetic factors in the variability of sickle cell disease but part of the reason lies with the relative infrequency of suitable twin pairs. The estimated twin rate for African-Americans is 45–50 per 1000 live births but the rate for monozygotic twins is similar in all ethnic groups at three to four per 1000 live births (Hall, 2002). The Jamaican Clinic with a population of 5500 patients with sickle cell disease would therefore be expected to yield 16–22 identical twin pairs. The estimated twin rate for African-Americans is 45–50 per 1000 live births but the rate for monozygotic twins is similar in all ethnic groups at three to four per 1000 live births (Hall, 2002). The Jamaican Clinic with a population of 5500 patients with sickle cell disease would therefore be expected to yield 16–22 identical twin pairs. However, to be eligible for study, both twins would have to survive until presentation to the clinic and sufficiently long to provide data for analysis. As twins have a lower birth weight and higher early mortality, the current nine identical twin pairs may not be grossly discrepant from those available. The size of the population served by the Jamaican Clinic substantially exceeds the subjects available to the Cooperative Study in the USA indicating that greater numbers of identical twins are unlikely to become available in the foreseeable future. Although the current

data set falls short of that usually used in twin studies, these approximate calculations indicate that the Jamaican data are likely to be the best available.

The paucity of longitudinal data especially in the non-Cohort SC patients was a further concern as was the young ages of several twin pairs. However, although this implies that they may yet develop other complications of the disease, recall of the frequent complications of early life such as dactylitis will be more accurately recorded.

Despite these limitations, identical twin pairs showed closer concordance than sibling pairs indicating a strong genetic component in attained height and weight, and in HbF, MCH and total bilirubin levels and weaker, but still significant, relationships occurred with haemoglobin level and MCV. The haemoglobin level in patients with SS disease is influenced by at least two genetic factors, α-thalassaemia and the level of HbF (Serjeant et al., 1996). α-Thalassaemia is associated with a low MCV, MCH and MCHC, and ameliorates some clinical features of SS disease (Higgs et al., 1982). However, its association with higher haemoglobin levels may be detrimental unless there is a coincident increase in HbF, and high haemoglobin levels are a risk factor for painful crisis and some other features. As genetic factors influence both the degree of haemolysis and of bilirubin conjugation (Haverfield et al., 2005), the greater concordance of total bilirubin values in identical twin pairs compared with sibling pairs was expected. The rate of bilirubin generation is therefore under at least partial genetic control and is also believed to be a risk factor for gallstone formation, the disparity in age incidence in twin pair C (one developed gallstones before age 5 years and the other at age 17 years) indicates that non-genetic factors significantly affect gallstone formation.

If concordance applies to growth and many of the haematological indices, it is equally clear that much discordance occurs in clinical features. A history of dactylitis or the hand–foot syndrome occurred in both twins of two pairs but in only one twin of another two pairs. Painful crisis frequency was concordant in two pairs and clearly discordant in another pair. ASS occurred in both twins of two pairs but in only one twin of another pair. Even concordance cannot be assumed to favour genetic factors, as it occurs in an undisputed environmental factor such as human parvovirus B19 infection and the associated aplastic crisis.

As might have been expected, many manifestations of the disease have both genetic and non-genetic components. For example, genetic factors influence haemoglobin level which is a risk factor for painful crisis (Baum et al., 1987; Platt et al., 1991) but the environment determines skin cooling which is a major precipitating factor (Redwood et al., 1976; Serjeant et al., 1994). This work directed towards a better understanding of the genetic factors involved in phenotypic variability in sickle cell disease must therefore, be accompanied by a major effort towards a better understanding of the role of the environment.

Overall, these results are consistent with those of the two previously reported single identical twin pairs (Joishy et al., 1976; Amin et al., 1991). While limited, these data suggest that genetic factors may be important in growth and in some haematological and biochemical indices, but that environmental factors assume greater importance in the clinical expression and complications of the disease. The small sample size is a clear limitation of this study but considering that these were all the identical twin pairs available to one of the largest sickle cell clinics in the world, it is unlikely that a larger data set will become readily available.

Acknowledgements

We thank Dr Thomas Walker of the Department of Radiology, Royal Berkshire Hospital, Reading for the gallbladder ultrasound data. David Weatherall thanks the Leverhulme Trust and Graham Serjeant thanks the Sickle Cell Trust (Jamaica) for support.

References


