THE OCCURRENCE IN A FAMILY OF SICILIAN ANCESTRY OF THE TRAITS FOR BOTH SICKLING AND THALASSEMIA

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ALTHOUGH in recent years the blood disorders sickle cell disease and thalassemia have both been studied extensively from the genetic standpoint, thus far the simultaneous occurrence within a single family group of the genes responsible for these two diseases has not been reported in the medical literature of the United States. The significance of the discovery and study of such families in elucidating the relationship between these genetically similar blood dyscrasias is obvious. Since 1944 we have had under observation three generations of a family of Sicilian ancestry displaying both the sickle cell trait and thalassemia minor.* The finding in Mike D., the first member of the family studied, of a severe hemolytic...
anemia with sickle cells in both ordinary stained blood smears and in sealed wet preparations led to the examination of the remaining available members of his family and the discovery of the situation to be described below.

CASE HISTORY

Mike D., a 38 year old male of Sicilian ancestry, was first seen in April, 1944, with complaints of recurrent pain and aching in his bones and joints dating back to early childhood. At the age of 8 months he was supposed to have had infantile paralysis and had always been in poor health since that time. Attacks of pain in his bones and joints had occurred every few months for many years, often accompanied by fever and a yellowish discoloration of his skin. Each attack lasted from four days to several weeks. In addition to the ordinary contagious diseases of childhood the patient recalled one attack of pneumonia and pleurisy in early life.

**Physical examination:** Weight, 117 lbs.; temperature, 98.6 F; pulse, 60; respirations, 20; blood pressure, 120/80. The patient was a small white male, not acutely ill. The sclerae were slightly yellow. No swelling of any of the joints could be detected. The left upper extremity showed muscular atrophy and diminished deep reflexes. There was some muscular weakness of all four extremities. The spleen and liver were barely palpable.

**Laboratory findings:** Urine, normal. Kline test, negative. Sedimentation rate (Westergren), 10 mm./1 hr. Serum calcium, 10 mg./100 ml. Serum phosphorus, 5 mg./100 ml. Serum alkaline phosphatase, 9 K.A. units. RBC, 4.0 million/mm.² Hgb, 8.3 Gm./100 cc. blood. Hematocrit, 30 per cent. Reticulocytes, 10.0 per cent. MCV, 75 cu. microns. MCH, 21 micromicrograms. MCHC, 28 per cent. Differential, N:51, L:43, M:5, B:1. Stained smear (fig. 1B), numerous target cells and normoblasts. Occasional oval and stippled cells present. Occasional sickle cells. Serum bilirubin: total 0.3 mg.; direct 0.3 mg. Hypotonic fragility test of patient, 0.36-0.10 per cent; control, 0.46-0.32 per cent. Sickling test, 0 per cent sickling per forty-eight hours in sealed wet preparations. Sternal marrow, marked normoblastic hyperplasia.

**X-ray findings:** Gall bladder, calculi present. Chest, lung fields negative, heart size within normal limits. Abdominal film, calcification in region of the spleen. All long bones, both feet, and the entire spine showed thickening of the bony trabeculae throughout. The majority of the vertebrae were flattened and there was mushrooming of the head of the left femur. The skull showed finely granular osteoporosis throughout.

The patient has been seen periodically since 1944. The administration of ferrous sulphate in adequate dosage had no apparent effect on his blood picture. A cholecystectomy performed in August, 1946, resulted in some improvement in his general health, but the patient still complained of the recurrent pains in his back and extremities.

FAMILY STUDIES

The paternal and maternal grandparents were born near Palermo, Sicily. The paternal grandparents came to the United States where F. D., Mike’s father, was born. Mike’s mother, Mrs. F. D., was born in Sicily but was brought to this country by her parents in childhood. So far as the patient knew, no other member of his family had suffered from any chronic disease.

One brother, N. D., had been killed in an automobile accident. A younger brother, J. D., had died in 1928 at the age of 17 years with a severe anemia. About one month before entrance to the hospital, J. D. began to feel generally weak and noticed that he tired easily. Three weeks later he experienced pain beneath his lower rib margins bilaterally, extending almost entirely around his body. This pain persisted for several days and then disappeared to be followed by a gradually increasing weakness of his lower extremities. This progressed to a complete flaccid paralysis of the lower extremities with loss of bladder control. On physical
examination he was found to have a fever of 101 F., some stiffness of his neck, complete flaccid paralysis of the lower extremities, and absence of abdominal, cremasteric, and patellar reflexes. Urinalysis was negative. The blood count showed a hemoglobin of 48 per cent (Dare), red cell count of 2.76 million, leukocyte count of 3,150 with 79 per cent lymphocytes, 18 per cent neutrophils, and 3 per cent monocytes. A few nucleated red cells were seen on the blood smears and lymphoblasts were thought to be present. The spinal fluid showed a cell count of 27 neutrophils with a negative Kolmer and normal Lange test. Smears and cultures of the spinal fluid were negative. He went rapidly downhill with spiking fever up to 106 F. and died one week after admission. Permission for necropsy was not obtained.

Although one is tempted to assume that the anemia in J. D. was in some way related to the blood disorders shown by Mike D. and certain other members of the family, the information on J. D.'s hospital record does not support such an opinion. All one can say with certainty is that this individual had a transverse myelitis and an anemia of unknown etiology, possibly on the basis of acute leukemia, with, probably, a terminal septicemia.

Ten other living members of the family have been studied to date with the findings recorded in table 1. Mike D.'s father, F. D., was found to show only the sickling trait while the mother exhibited thalassemia minor (fig. 2A). Mike's wife, of German ancestry, was entirely normal. Mike's sons, F. J. D. and M. W. D., both were found to have thalassemia minor (fig. 2C). Mike's brother, B. D., showed the sickling trait; B. D.'s wife, of Italian ancestry, was entirely normal; B. D.'s daughter, M. F. D., and one son, F. W. D., showed the sickling trait while the other son, J. M. D., was regarded as normal. The spleen was not palpable in any member of the family except Mike. Complete roentgenographic stud-
TRAILS FOR SICKLING AND THALASSEMA IN SICILIAN FAMILY

ies of the bones were made on all members of the family, and no alterations from
normal were noted other than in Mike.

No history of any admixture of Negro blood could be elicited and the appear-
ance of all members of the family was that of Caucasians.

Table 1.—Summary of Hematologic Findings in D Family

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>RBC</th>
<th>Ht.</th>
<th>Hct.</th>
<th>Hemat.</th>
<th>MCV or MCH</th>
<th>MCHC% or MCV micros.</th>
<th>Stained Smear</th>
<th>Hypotonic Fragility</th>
<th>Van den Bergh</th>
<th>Sickling Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. D.</td>
<td>67 M</td>
<td>5.3</td>
<td>15</td>
<td>45</td>
<td>85</td>
<td>30</td>
<td>33</td>
<td>0.5 7.5</td>
<td>Normal</td>
<td>0.12%-0.12%</td>
<td>D—0.0 mgm.</td>
<td>Positive</td>
</tr>
<tr>
<td>Mrs. F. D.</td>
<td>60 F</td>
<td>6.0</td>
<td>12</td>
<td>44</td>
<td>73</td>
<td>20</td>
<td>27</td>
<td>2.8 8.1</td>
<td>Target and oval cells</td>
<td>0.44%-0.28%</td>
<td>D—0.0 mgm.</td>
<td>Negative</td>
</tr>
<tr>
<td>Mike D.</td>
<td>41 M</td>
<td>4.0</td>
<td>8.5</td>
<td>70</td>
<td>75</td>
<td>20</td>
<td>28</td>
<td>10.0 6.0</td>
<td>Target, oval and stippled cells. Rare sickle cells.</td>
<td>Normal</td>
<td>0.40%-0.10%</td>
<td>D—0.3 mgm.</td>
</tr>
<tr>
<td>Mrs. Mike D.</td>
<td>34 F</td>
<td>4.5</td>
<td>13</td>
<td>95</td>
<td>29</td>
<td>30</td>
<td>0.9 7.4</td>
<td>Normal</td>
<td>0.44%-0.32%</td>
<td>D—0.0 mgm.</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>F. J. D.</td>
<td>15 M</td>
<td>5.3</td>
<td>15</td>
<td>78</td>
<td>23</td>
<td>30</td>
<td>2.0 5.7</td>
<td>Target and oval cells</td>
<td>Normal</td>
<td>0.49%-0.16%</td>
<td>D—0.0 mgm.</td>
<td>Negative</td>
</tr>
<tr>
<td>M. W. D.</td>
<td>14 M</td>
<td>6.2</td>
<td>13</td>
<td>46</td>
<td>21</td>
<td>31</td>
<td>2.3 6.1</td>
<td>Target and oval cells</td>
<td>Normal</td>
<td>0.46%-0.22%</td>
<td>D—0.2 mgm.</td>
<td>Negative</td>
</tr>
<tr>
<td>B. D.</td>
<td>39 M</td>
<td>4.8</td>
<td>15.5</td>
<td>45</td>
<td>93</td>
<td>32</td>
<td>34</td>
<td>0.9 6.2</td>
<td>Normal</td>
<td>0.42%-0.30%</td>
<td>D—0.0 mgm.</td>
<td>Positive</td>
</tr>
<tr>
<td>Mrs. B. D.</td>
<td>36 F</td>
<td>4.5</td>
<td>13.5</td>
<td>43</td>
<td>95</td>
<td>30</td>
<td>31</td>
<td>0.8 5.3</td>
<td>Normal</td>
<td>0.44%-0.32%</td>
<td>D—0.1 mgm.</td>
<td>Negative</td>
</tr>
<tr>
<td>M. F. D.</td>
<td>15 F</td>
<td>4.7</td>
<td>14</td>
<td>46</td>
<td>97</td>
<td>30</td>
<td>30</td>
<td>0.6 8.1</td>
<td>Normal</td>
<td>0.44%-0.32%</td>
<td>D—0.0 mgm.</td>
<td>Positive</td>
</tr>
<tr>
<td>F. W. D.</td>
<td>12 M</td>
<td>4.6</td>
<td>14.5</td>
<td>43</td>
<td>93</td>
<td>31</td>
<td>33</td>
<td>0.9 8.5</td>
<td>Normal</td>
<td>0.46%-0.32%</td>
<td>D—0.0 mgm.</td>
<td>Positive</td>
</tr>
<tr>
<td>J. M. D.</td>
<td>10 M</td>
<td>4.4</td>
<td>12</td>
<td>41</td>
<td>93</td>
<td>27</td>
<td>29</td>
<td>0.8 7.4</td>
<td>Slight hypo-</td>
<td>0.46%-0.32%</td>
<td>D—0.0 mgm.</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Control fragility 0.46%-0.33%
then fixing with formalin; (2) the traditional technic of sealing a drop of blood under a cover slip with vaseline and observing at intervals up to forty-eight hours; (3) the Hansen-Pruss method whereby a small amount of a dye such as methylene blue or janus green is added to a slide, allowed to dry, then a drop of blood added, with observations at intervals up to forty-eight hours; and (4) the vitamin C and sodium bisulphite methods recently proposed by Daland and Castle. Those recorded in table 1 as negative for sickling failed to sickle with any of the above methods, while those recorded as positive showed sickling to a greater or lesser degree with all the methods tried.

Serologic tests were done for the A-B-O blood groups, M and N factors, Rh factors, the S factor, and the Kell-Cellano factor. The results of these tests are recorded in the genealogic chart, figure 1. The reactions of the cell suspensions of several of the individuals studied with the anti-S serum were somewhat equivocal; the present interpretation must be regarded as tentative. The tests provide no evidence that in any case the legal parent is not the biological parent. The use of these data in linkage studies will be discussed below.

Table 2 records the results of studies aimed at determining whether there existed between the 'sickling' members of the family significant differences in the ease with which the sickling phenomenon could be induced. It is a well known fact that most individuals who sickle at all will exhibit under suitable conditions 90–100 per cent sickling. In the experiment recorded in table 2 an attempt was made to establish sub-optimal conditions for the production of sickling, on the hypothesis that possible differences between the various sickling members of the family would in this way be accentuated. For each individual a dilute cell suspension was prepared from a coagulated specimen of blood, obtained forty-eight...
hours previously. In the studies by the Beck and Hertz method a single drop of this suspension was added to 1 cc. of saline suspension, which was then incubated

Table 1.—Representative Series of Tests for Sickling. Column 2 gives averages for three preparations, one a plain sealed preparation, one prepared by the Hansen-Pruss technic using methylene blue, and one prepared by the Hansen-Pruss technic using Janus green.

<table>
<thead>
<tr>
<th>Name</th>
<th>1. % Sickled cells in Beck and Hertz Preparations</th>
<th>2. % Sickled cells in Sealed Cover Slip Preparations</th>
<th>3. % Sickled cells in Vitamin C Preparations (Daland and Castle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. D</td>
<td>23</td>
<td>30 41 43</td>
<td>27 36</td>
</tr>
<tr>
<td>Mrs. F. D.</td>
<td>0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Mike D</td>
<td>87</td>
<td>3 34 34</td>
<td>40 100</td>
</tr>
<tr>
<td>Mrs. Mike D</td>
<td>0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>F. J. D.</td>
<td>0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>M. W. D.</td>
<td>0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>B. D.</td>
<td>1</td>
<td>1 17</td>
<td>0 0</td>
</tr>
<tr>
<td>Mrs. B. D.</td>
<td>0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>M. F. D.</td>
<td>26</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>F. W. D.</td>
<td>28</td>
<td>1 1 28</td>
<td>2 99</td>
</tr>
<tr>
<td>J. M. D.</td>
<td>0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Fig. 3.—A comparison of the type of sickling produced by the Beck and Hertz method in F. D. (a) and his son, the patient, Mike D. (b). Note the predominantly holly-leaf type of sickle cell in F. D. and the long, filamentous form of cell in Mike D.

at 32° C. only eighteen hours instead of the more usual twenty-four. In the “sealed cover slip preparations” the same dilute suspensions were used; the element of stasis so successfully introduced by Scriver and Waugh for accelerating the sick-
ling phenomenon was of course absent. The vitamin C tests were conducted as described by Daland and Castle. All the tests recorded in the table were run simultaneously except those on F. D. It is apparent that Mike D. tended to sickle more readily than any other member of the family. F. D., Mike D.'s father, appeared to exceed his son on one of the three tests. The fact that the determinations on F. D. were unfortunately of necessity not run simultaneously with those on all the other members of the family detracts from the weight to be attached to this finding.

Not only did Mike D. sickle more readily than the other members of the family; there also appeared to be qualitative differences in the type of sickling observed. This is illustrated by figure 3. The sickling in Mike D. tended to be of the long, filamentous type, whereas other members of the family, of whom F. D. was typical, showed a marked preponderance of the "holly-leaf" type.

**Discussion**

An appreciation of the significance of this family requires a brief review of currently accepted theory regarding the inheritance of the two hematological abnormalities here represented. The consensus regarding thalassemia is that there exists, predominantly among persons living in or derived from the northern Mediterranean regions, a gene which in the heterozygous condition produces a mild hematologic disorder variously termed target-oval cell syndrome, microcytemia or thalassemia minor, and in the homozygous condition a much more severe disease known as Cooley's anemia, Mediterranean anemia, erythroblastic anemia, or thalassemia major. More recently, evidence has been presented that a comparable genetic situation exists with respect to sickle cell disease, i.e., it is postulated that there exists, predominantly among Negro populations, a gene which in the heterozygous condition produces only the sickle cell trait, and in the homozygous condition, sickle cell disease. Except for the absence of leukocytosis, the clinical and hematologic findings in Mike D., the patient, are typical of sickle cell disease. Since, however, both of his sons have thalassemia minor, we must conclude that Mike himself also has a thalassemia gene, inherited from his mother, plus a gene for the sickling phenomenon, inherited from his father. Inasmuch as either of these genes by itself has very minor effects, the occurrence in Mike D. of a severe hemolytic-type anemia clinically indistinguishable from sickle cell disease is a noteworthy finding.

Although this appears to be the first family to be studied in this country in which the simultaneous segregation of the sickle cell and thalassemia genes has been recognized, Silverstromi and Bianco have reported from Italy a series of five families which exhibit the same phenomenon. These families contain a total of eight individuals whose history and findings closely resemble those of Mike D. In four of the families one parent is known to have thalassemia minor and the other the sickle cell trait; in the fifth family one parent has the sickle cell trait and the other parent, deceased at the time of the study, may be assumed to have had thalassemia minor because of siblings who appear to have this condition.
However, none of Silvestroni and Bianco’s eight cases had children, so that the crucial genetic evidence that they had received a thalassemia gene is lacking.

What, now, can be the explanation of the hematologic condition encountered in Mike D.? At least three alternatives must be considered.

1. Let us designate the gene responsible for the sickling phenomenon as Sk, and the gene responsible for thalassemia as Sa. The first possibility is that these two genes are inherited quite independently of one another, being located on different chromosomes, and that we have here a type of non-allelic factor interaction, such as is seen not uncommonly in experimental material (cf. Neel). If we permit sk to represent the normal allelomorph of Sk and sa the normal allelomorph of Sa, then we can diagram the marriage which resulted in Mike as:

\[
\begin{array}{c}
\text{F. D. Mrs. F. D.} \\
\text{Sk/sk sa/sa} \\
\times \\
\text{Sk/sk Sa/sa} \\
\text{B. D. Mike D.} \\
\text{Sk/sk sa/sa} \\
\text{Sk/sk Sa/sa}
\end{array}
\]

2. The literature contains reports (review in Chini and Valeri) of individuals who although heterozygous for the thalassemia gene actually exhibited a rather severe anemia. The possibility exists that the genetic situation is as diagrammed for (1), but that the anemia in Mike D. (and in Silvestroni and Bianco’s cases) is not due to factor interaction, but represents an extreme variant of thalassemia “minor,” with the occurrence of sickling an incidental finding. Or, conversely, it is possible that in a few persons a single Sk gene may rarely produce “sickle cell disease.” Present evidence indicates that persons with sickle cell disease always are homozygous for the sickling gene, but exceptions may yet be found. Were this so, then this would be sickle cell disease, with the coexistence of the thalassemia gene of no etiological significance.

3. The third possibility is that the Sk and Sa genes are members of a series of multiple allelomorphs, in which the normal allelomorph may be designated So. The marriage which produced Mike then may be diagrammed as:

\[
\begin{array}{c}
\text{F. D. Mrs. F. D.} \\
\text{Sk/so} \\
\times \\
\text{Sa/so} \\
\text{B. D. Mike D.} \\
\text{Sk/so} \\
\text{Sk/So}
\end{array}
\]

Under these circumstances, the extreme picture in Mike D. finds an explanation in the well known genetic fact that in a series of three allelomorphs where A is normal and A’ and A” result in departures from the norm, the phenotype of A’A” usually is a more marked departure from normal than AA’ or AA”. The situation here is somewhat complicated by the fact that if the two genes in question were genetically linked, being situated on the same chromosome, they might in limited data appear to behave as allelomorphic factors.
It is impossible to reach a decision as to which of the above described possibilities is correct on the basis of the information now at hand. However, from the study of a series of children who are the offspring of individuals such as Mike D. and a normal spouse, we might be in a position to formulate an opinion, basing such an opinion on the following lines of reasoning.

1. If the first explanation is correct, then the children resulting from marriages between an individual like our patient and a normal individual should be of four types occurring in equal numbers, namely, normal, those with the sickling trait, those with thalassemia minor, and those with a hemolytic anemia like that seen in our patient.

2. If the second explanation is correct, then by no means all the individuals who receive both a sickle cell gene and a thalassemia gene should show an anemia, while the children of such persons might include some showing severe thalassemia "minor" in the absence of sickling. The fact that the eight cases of Silvestroni and Bianco plus our own have all shown a uniformly severe anemia tends to lessen the weight to be attached to this second explanation, although, on the other hand, it must be borne in mind that there would be a disproportionate tendency for the more severe cases to come to medical attention.

3. Finally, if the third explanation is correct, then the children resulting from marriages between an individual like our patient and a normal person should be of only two types occurring in equal numbers, namely, those with thalassemia minor, and those with the sickle cell trait. But as stated above, genetic linkage of the $5k$ and $5a$ genes would in the absence of observed crossing-over between the two genes be confused with allelism.

A critical decision between these three possibilities must await the accumulation of a number of pedigrees comparable to the one reported here. There is, however, a supplementary line of attack on the question which should not be overlooked. Snyder, Russell, and Graham have reported a genetic linkage between the genes responsible for the $MN$ blood group factors and the gene responsible for the sickling phenomenon. If linkage studies on thalassemia were to reveal that the responsible gene is also linked with the genes producing the $MN$ blood group factors, then here would be evidence that both the $5k$ and $5a$ genes were located on the same chromosome—a necessary step in establishing allelism, although, of course, not in itself proof positive. Actually, as the Rh controversy demonstrates so well, the decision as to whether in man one is dealing with linked or allelomorphic genes may be very difficult. In this particular instance, a decision might be possible if the two genes, although both linked with the $MN$ genes, were linked to different degree. This would require an extensive body of data.

The literature contains at least 26 case reports of sickle cell disease in presumably entirely Caucasian individuals. Of the 12 families involved, 16 have been of Greek or Italian extraction. It seems quite possible that many of these cases are comparable to Mike D., since in Greece and Italy the probability that a person who receives the $5k$ gene from one parent will receive an $5a$ gene from the other is greater than the probability that he receives another $5k$ gene from the second parent.
1. A 38 year old male of Sicilian ancestry with a chronic, hemolytic anemia clinically indistinguishable from sickle cell disease is described. Family studies extending over three generations and including all persons indicate that this individual has received from his father a gene for the sickling phenomenon, and from his mother a gene for thalassemia.

2. Three alternative hypotheses are advanced to account for the severity of the anemia present in the patient. The bases whereby a decision can be reached as to which of the three hypotheses is correct are discussed. It is indicated that no decision is possible until the results of studying a number of families comparable to the present are available.

3. It is suggested that many of the reported cases of sickle cell disease in Caucasians actually involve a genetic situation comparable to the one reported in the present paper.

REFERENCES

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