The Genetics of Sickling

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It is known that Bahrain is one of the countries where haemoglobinopathies (especially sickling) form real health problems. This drains the health resources and drastically affects family and personal life. It is essential that there is far more understanding of sickle cell disease.

The sickle cell disease patient differs from the normal human only in the structure of the haemoglobin. The normal human haemoglobin is a tetramere with a molecular weight of 64,500. It consists of two alpha and two non-alpha globin polypeptide chains, each of which has a single covalently bound haem group. Each of the four haem groups is made of an iron atom within a protoporphyrin ring.

In sickle cell disease, when oxygenated, the cells are biconcave discs like red blood cells of a normal individual. However, when they are deoxygenated, they become elongated, filamentous and sickle shaped.

Heterozygous individuals who receive the abnormal gene from one parent but its normal allele from the other, would be expected to have sickle cell trait. Homozygous individuals who receive the abnormal gene from each parent, would develop sickle cell anaemia. When mating occurs between two individuals with sickle cell trait, the ratio between normal children, children with sickle cell trait and children with sickle cell anaemia is 1:2:1: [1].

Hb S differs in its electrophorectic properties from Hb A. The difference lies in the protein part. The nature of the difference was found by Ingram (1957-1959). That a particular position in the amino acid sequence of the B-polypeptide chain, which is occupied by a glutamic acid residue in Hb A, is occupied by a valine residue in Hb S. If normal Hb A is written as Alpha 2 Beta 2, the structure of Hb S may be written as Alpha 2 Beta 2 6 glu→ val. and it was found to be due to a single mutational step which is the smallest unit difference in the primary structure of a specific protein.

At the chromosomes level, there are 6 globin genes coding for the 6 globin chains, Alpha, Beta, Gamma, Delta, Epsilon, Zeta [2]. Epsilon, Gamma, Delta chains are similar to a Beta chain. While Zeta is similar to an Alpha chain. There are 9 different genetic loci which code for the 6 globin genes. In addition, there are at least 3 pseudo genes which have sequences similar to other globin genes, but which differ in that they are not expressed into globin protein.

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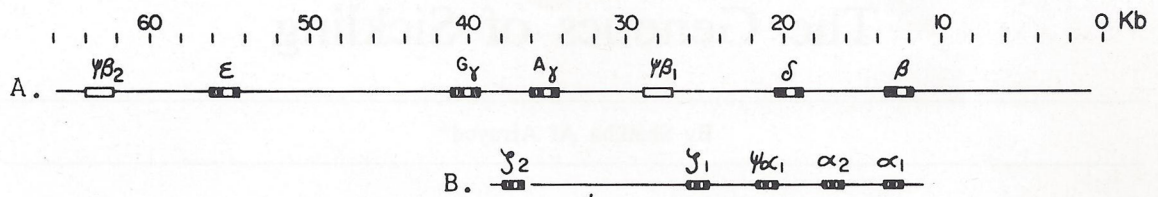Salmaniya Medical Centre
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Fig. I — Globin genes complexes. (A) β--gene complex on chromosome 11. (B) ∝-gene complex on chromosome 16. Distances along the chromosome are measured in kilobases (kb) at top.

The region of chromosome 11 coding for Beta and Beta like genes (Epsilon, Gamma A, Gamma G, Delta) has been thoroughly mapped by restriction endonuclease analysis, and these genes have been sequenced.

At the DNA level, the full base sequence messenger RNA for the polypeptide chain has now been determined [1]. Starting from the $\bar{5}$ end it consists of a sequence of 50 bases which are not translated into aminoacid sequence, the initiator codon (AUG) which codes for methionine and is later removed from the polypeptide chain, the sequence of 438 bases which code in triplets for the sequence of the 146 aminoacids of the normal Beta polypeptide, the chain termination codon (UAA), a sequence of 132 bases not translated into a polypeptide sequence, and finally a poly A sequence which is also not translated.

The DNA base sequence is even longer because it includes two intervening sequences (IVS). These IVS in the DNA are transcribed into RNA but are removed during processing. So, they are not represented in the final messenger RNA molecule which binds to the ribosomes and serves as a template for polypeptide synthesis.
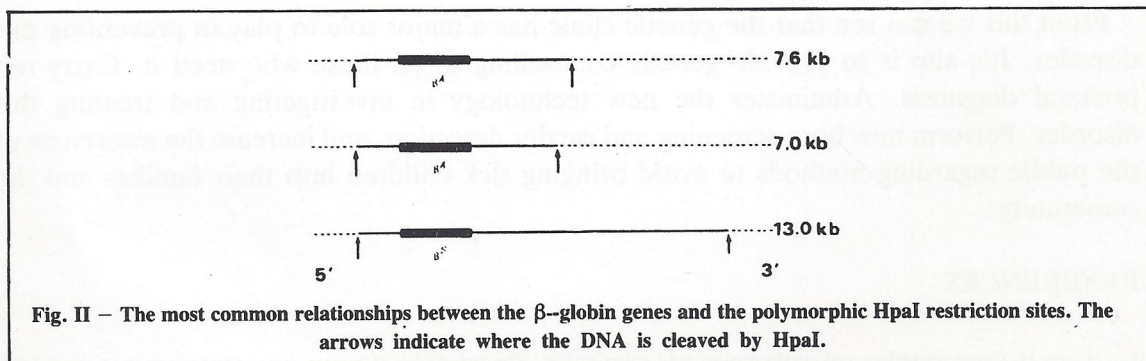
Sickle cell mutation which results in substitution of glutamic acid by valine at position No. 6 in the polypeptide chain can be attributed to a change from RNA codon GAG to GUG. This change, resulting from the substitution of A (Adenine) by U (Uridine) at position No. 20 in the messenger RNA sequence, can in turn be attributed to a mutational change from T (Thymine) to A (Adenine) in the corresponding position in the DNA sequence of the gene itself. So, all the sickling phenomena is a result of a single base change at the level of DNA.
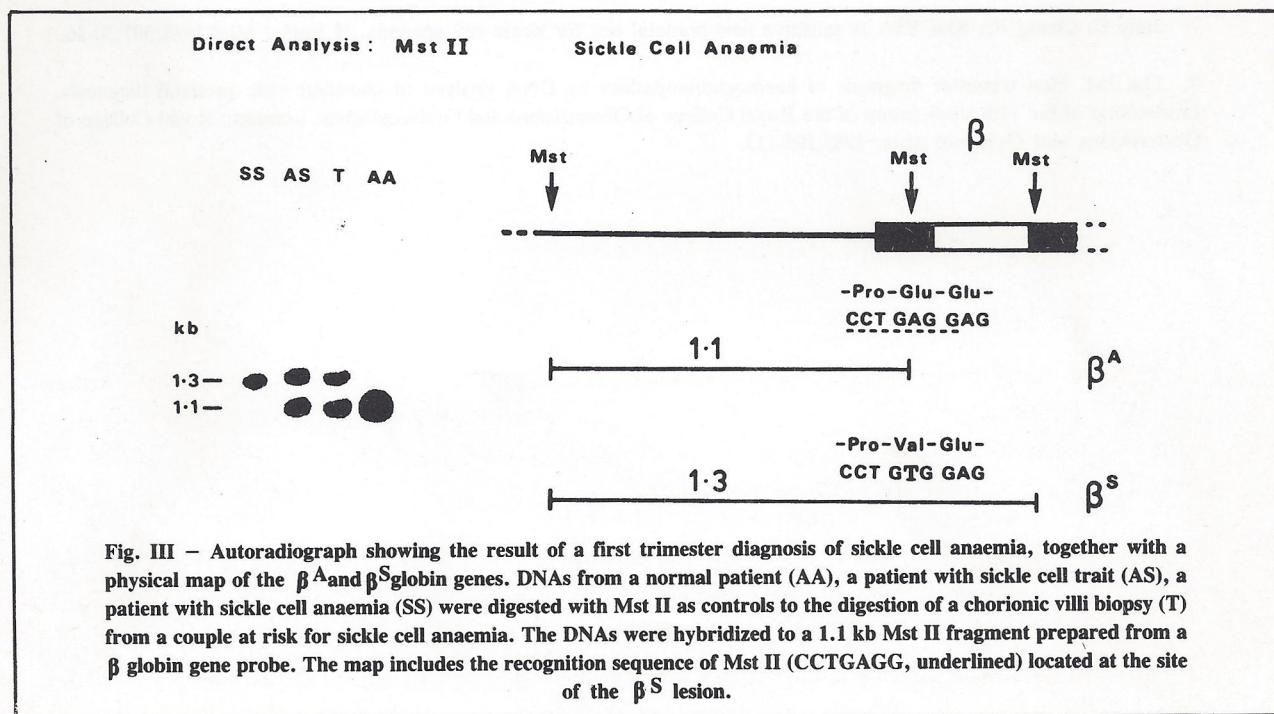
The antenatal diagnosis of sickle cell disease

The antenatal diagnosis of sickle cell disease can be used to detect the status of the fetus early in pregnancy [3,4,5]. The parents then know the exact condition of the fetus, whether it is normal, a carrier or diseased. Provided with this information, they have the right to decide whether to continue the pregnancy or not.

Fetal blood sampling : The antenatal diagnosis of sickling has been carried out by fetoscopy, fetal blood sampling and analysing the globin chain synthesis since 1975. It requires a very specialised technique carried out in very few medical centres. It can only be done after 18 weeks of gestation and it has a 5-10% risk of fetal mortality.

DNA analysis of amniotic fluid cell : a) By using HpaI endonuclease. In 1978 Kan and Dozy [6] described a DNA polymorphism located extremely close to the Beta globin gene on chromosome 11. The DNA was digested with restricted endonuclease HpaI, the Beta globin gene was contained in a fragment of DNA 7.6 Kilobase in length. We expect the person with Hb A to have only the 7 or 7.6 Kb fragments, while persons with sickle cell trait will have the 7 Kb and 13 Kb fragments. Those with sickle cell disease will have only the 13 Kb fragment.

Fig. II — The most common relationships between the β--globin genes and the polymorphic HpaI restriction sites. The arrows indicate where the DNA is cleaved by HpaI.

b) By using Mst II endonuclease. It was mentioned that Hb S results from a single base mutation from an A to a T. This base change happens to occur in the middle of a recognition site for the restriction endonuclease Mst II. This enzyme recognises the sequence CCTNAGG (N is any base) in the normal Beta globin chain. Mst II normally cuts $\bar{5}$ to the B globin gene and at the GAG codon to generate a 1.1 Kb fragment. The base change from A to T destroys the recognition site resulting in a larger fragment of 1.3 Kb. Therefore, Mst II can be used to diagnose sickle cell anaemia directly, and we can expect to see the 1.15 Kb fragment in the AA genotype. The 1.35 Kb fragment in the SS and both the 1.15 Kb and the 1.35 Kb fragment in the HbAS.

Fig. III — Autoradiograph showing the result of a first trimester diagnosis of sickle cell anaemia, together with a physical map of the β A and β S globin genes. DNAs from a normal patient (AA), a patient with sickle cell trait (AS), a patient with sickle cell anaemia (SS) were digested with Mst II as controls to the digestion of a chorionic villi biopsy (T) from a couple at risk for sickle cell anaemia. The DNAs were hybridized to a 1.1 kb Mst II fragment prepared from a β globin gene probe. The map includes the recognition sequence of Mst II (CCTGAGG, underlined) located at the site of the β S lesion.

Chorionic villi biopsies and the first trimester diagnosis of sickling [8]. Dr. J.M. Old developed a method of extraction of a small amount of fetal DNA from chorionic villi biopsies which can provide a first trimester (8-10 weeks) antenatal diagnosis by DNA analysis. The DNA was best prepared from a fresh chorionic villi biopsy or one that had been quickly frozen and transported in dry ice. This DNA is then subjected to endonuclear Mst II for analysis.

From this we can see that the genetic clinic has a major role to play in preventing this disorder. It's aim is to provide genetic counselling to all those who need it. Carry out prenatal diagnosis. Administer the new technology in investigating and treating this disorder. Perform new born screening and carrier detection, and increase the awareness of the public regarding methods to avoid bringing sick children into their families and the community.

REFERENCES

1. Harris H. Gene mutation and single amino acid substitution. The principles of human biochemical genetics. 1980;5:35.

2. Phillips JA, Kazazian HH. Haemoglobinopathies and thalassaemias. In : Emery AEH, Rimoin DL, eds. Principles and practice of medical genetics. Vol 2. Edinburgh: Churchill Livingstone, 1983;2:1019-1093.

3. Alter BP, Modell CB, Fairweather D, et al. Prenatal diagnosis of haemoglobinopathies: a review of 15 cases. N Engl J Med 1977;295:1437-1443.

4. Alter BP. Prenatal diagnosis of haemoglobinopathies: a status report. Lancet 1981;2:1152-1155.

5. Corninne D, Boch M. Prenatal diagnosis using DNA polymorphism. N Engl J Med 1983;308:1054-1058.

6. Kan YW, Dozy AM. Antenatal diagnosis of sickle cell anaemia by DNA analysis of amniotic fluid cell. Lancet 1978;2:910-911.

7. Judy C, Chang JC, Kan YW. A sensitive new prenatal test for sickle cell anaemia. N Engl J Med 1982;307:30-36.

8. Old JM. First trimester diagnosis of haemoglobinopathies by DNA analysis of chorionic villi: prenatal diagnosis. Proceedings of the 11th study group of the Royal College of Obstetricians and Gynaecologists. London : Royal College of Obstetricians and Gynaecologists, 1983;105-113.