

## Beta Thalassaemia

### **PART A – BACKGROUND (Read this first):**

The haemoglobin in the erythrocytes (red blood cells) of humans is made of four haem protein chains (or residues) – containing an iron atom each – and four globin residues. Oxygen is carried by the haem residues.

The haemoglobin of human embryos is made up of 2 alpha globin residues and two epsilon residues. As the foetus develops epsilon residues are no longer produced. Instead gamma residues are synthesised. In time the foetal erythrocytes contain haemoglobin that is made up of 2 alpha chains and two gamma chains.

Young children have haemoglobin molecules made up of 2 alpha chains and 2 delta chains.

Adult haemoglobin molecules are made up of 2 alpha chains and 2 beta chains.

The switch from delta to beta chains takes place at about age five years over several months.

This sequence involves a series of gene switches. The gene for alpha haemoglobin remains switched on for life. The gene for epsilon haemoglobin is switched off in foetuses and that for gamma haemoglobin is switched on. In time the gene for gamma haemoglobin is switched off and that for delta haemoglobin is switched on. Then the gene for delta haemoglobin is switched off and that for beta haemoglobin is switched on.

A series of mutations can occur along the gene coding for beta haemoglobin. One occurs at the site of the gene that codes for the amino acid glutamine. This particular mutation causes the codon for glutamine to be replaced by a STOP codon. This STOP codon causes the production of the polypeptide to stop early, after amino acid 38, so the polypeptide is shorter than that for normal beta globin. This inevitably means that the polypeptide will not form the normal protein. The function that the protein normally carries out will not take place. The normal function for the haemoglobin molecule is to carry oxygen to cells. Without oxygen the cells cannot do their work and the individual will become ill!

This disorder, beta thalassaemia, is common in Greek and Italian families. It is an autosomal (chromosome 11) disorder.

If you are homozygous for thalassaemia, (i.e. you inherit two copies of the beta chain mutation, one from mum and one from dad) then you will have Thalassaemia major and be severely anaemic and have growth and bone deformities.

If you are heterozygous for thalassaemia (i.e. you inherit only one copy of the beta chain mutation from one parent and a healthy beta chain from the other parent) then you will have Thalassaemia minor. You will be a carrier of the disease but not show any symptoms.

## PART B – VIEWING THE STRUCTURE OF HAEMOGLOBIN

Go to: <http://www.ncbi.nlm.nih.gov/Structure/index.shtml>

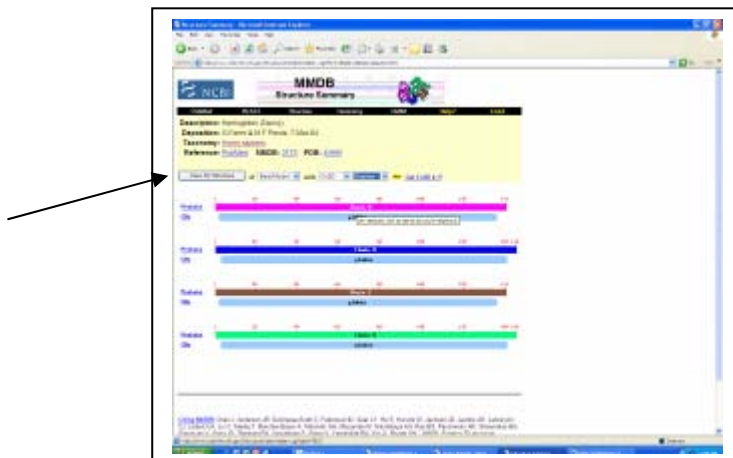
**Step 1.** Search Entrez for <Structure> for<4hhb> Hit the Go button



**Step 2.** Tick: 1. 4HHB  
Hemoglobin (Deoxy)  
[mmdbid:3173]

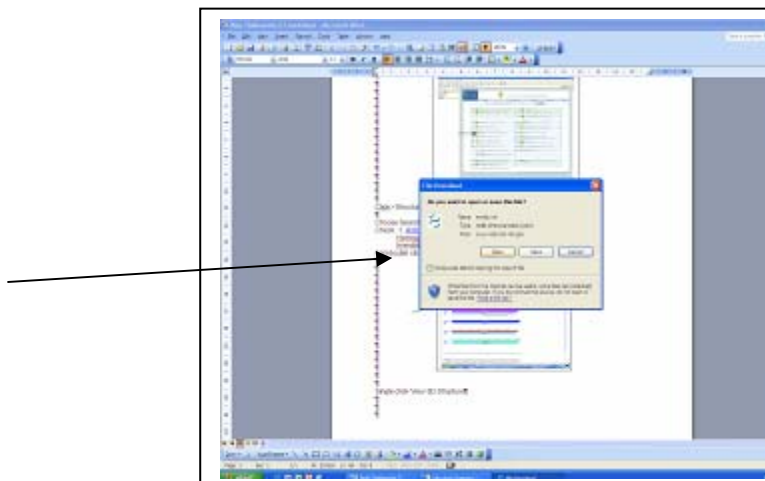
Double click: **4HHB**

This will take you to MMDb Structure Summary Page:



**Step 3.** Single click: View 3D Structure.

**Important Note:** If you do not have Cn3D 4.1 downloaded onto your computer, do this by clicking on Get Cn3D 4.1! and follow instructions. It's free. Then return to view 3D Structure.



**Step 3 Extra step if required.** Click: File Download Open (or Save)

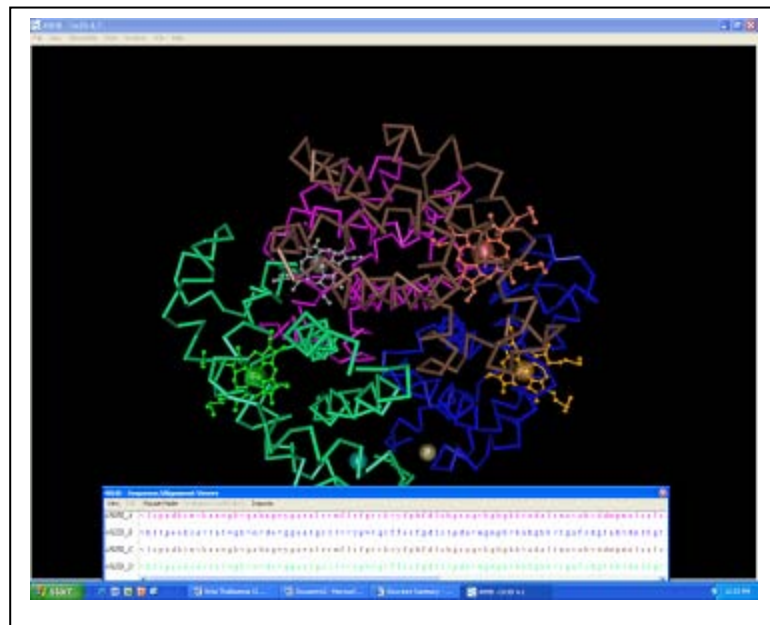
If it does not load correctly and open in the window, chose the Save option, park the file somewhere and load it that way.

**Step 4.** Go to: Show/Hide  
Click on: Show Everything

**Step 5.** Go to: Style  
Click on: <Rendering Shortcuts> then <Tubes>

**Step 6.** Go to: Style  
Click on: <Colouring Shortcuts> then <Molecule>

**Step 7.** Go to: View  
Click on: <Animation> then <Spin>



Look at the haemoglobin molecule. Look for the four iron atoms – one in the centre of each of the four heme groups.

**Step 8.** Go to: View  
Click on: <Animation> then <Stop>

The DNA for adult haemoglobin A and B is found on chromosome 11.

**Step 9.** Compare the colours of the four polypeptides (4HHB\_A, 4HHB\_B, 4HHB\_C and 4HHD\_D) shown in the Sequence Alignment/Viewer with the colours of the four globin residues (the alpha and beta hemoglobins, HBB\_A to HBB\_D) of the haemoglobin molecule. Identify the two beta globin residues. You can do this by clicking on 4HHB\_1 through to 4HHD\_D in order. Now, by clicking and dragging on the molecule you can rotate it manually.

**Step 10.** To make the beta globin chains easier to see, highlight the two beta chains in a colour. This can be done in two ways:

1. drag along the sequence 4HHB\_B, hold Control, and drag along the 4HHB\_D sequence  
or

2. look at Sequence Alignment/Viewer, Mouse Mode, Select Rows (the default is Select Rectangle) then click on the two beta sequences (that is, 4HHB\_B and 4HHB\_D)

**Qn:** In the SAV window what do you notice about the letter **sequence** of the two polypeptides 4HHB\_B and 4HHB\_D?

**Qn:** What do you notice about the **shape** of the two polypeptides 4HHB\_B and 4HHB\_D?

## **PART C – VIEWING A MUTATION IN THE BETA GLOBIN MOLECULE THAT CAUSES BETA THALASSEMIA**

One variation of **beta thalassemia major** is caused by being homozygous for a mutation whereby the DNA sequence that encodes amino acid #39 is altered. Instead of coding for a glutamine (Q) it codes for a STOP codon. This means that only the sequence 1 to 38 is made in both beta chains.

**Qn:** What sort of mutation is this?

**Step 1.** Drag the molecule around a bit so that you re-familiarise yourself with its shape.

**Step 2.** Go to Sequence Alignment/Viewer and select amino acid number 39 (~~pwt**q**rff~~) in both 4HHB\_B and 4HHB\_D. Use Control to select both at one time. Look at the site of the mutation in the molecule (two small yellow highlights).

**Step 3.** Now highlight amino acids 1-38 (vh1~~vvpwt). This selects the truncated (shortened) beta sequences in chains 4HHB\_B and 4HHB\_D. Go to Show/Hide then click Show Selected Residues. You are now looking at the only section of the Hb beta residue that is synthesised in people with this mutation.

**Step 4.** Now highlight the entire beta sequence before the q residue (#39) (do this by selecting 'select rectangle' under mouse mode and highlighting the area of beta globin sequence you want and using the control key to select the second sequence). Next select the two alpha sequences (do this by selecting 'select rows' under mouse mode and then click on the alpha sequences using the control button and your mouse). Go to Show/Hide and click on Show Everything. The yellow areas are the thalassemia haemoglobin molecule. The other colours show the part of the beta chain not produced in this mutation.

**Step 5.** Click and drag on the molecule to spin it around and view the iron (Fe) groups. These are the groups that bind oxygen.

**Qn:** What happens to the binding site of the heme group (that contains the iron atom) in the truncated (shortened) beta chain sequence?

**Qn:** What do you think will happen to the oxygen carrying capacity of the mutated Hb molecule?

**Qn:** What effect will this have on the life expectancy of the affected individuals?

### **Something to think about....**

If the gene for delta haemoglobin can be fiddled with so that it is never switched off (it generally happens at about age five years old) there is a possibility that the child affected with beta thalassemia can continue to produce foetal haemoglobin (2 alpha and 2 delta polypeptides) and may live a somewhat normal life. Sounds good, so what is the problem with this idea?