A 4 base pair TGAT insertion at codon 116 of the beta globin gene caus¬es ,0 - Thal¬assaemia

A new β^0 thalassaemia allele caused by a TGAT insert in codon 116 of exon III was detected in a patient compound heterozygous for β^0 thalassaemia/Hb D Los Angeles and his father. The mutation unexpectedly causes a classical thalassaemic phenotype. The compound heterozygousity leads to mild microcytic anemia and no further clinical signs.

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Sir,

We report a new, thalassaemia mutation detected in a patient compound heterozygous for Hb D $_{\text{Los Angeles}}$ and β^0 thalassaemia. A 3 - year old boy was referred to our hematology unit for evaluation of a microcytic anemia of unclear origin. Initial routine laboratory studies revealed a Hb of 96 g/L with a normal reticulocyte count and absence of red cell inclusions (i.e. Heinz bodies). On clinical examination no significant abnormalities were found except for a slight pallor despite the dark skin colorit. The hematological parameters of the propositus, his parents and one sister are reported in Table 1.

Table 1. Hematological parameters of propositus and his family (ND: not determined).

	Hb(g/L)	Ec(x1012/L)	MCV(fL)	MCH(pg)	Retics($\times10^9$/L)
Propositus	96	5.31	63	18	122
Father	121	5.76	68	21	ND
Mother	120	4.29	77	28	ND
Sister	125	4.31	81	29	ND

Poly CAT A cation exchange HPLC[1] showed only a peak in position of Hb D $_{\text{Los Angeles}}$ (96.5%), 3.0% Hb A_2 and 0.5% Hb F. No normal Hb A could be detected. Additionally, in isoelectric focussing[2] no normal Hb A was detectable (*data not shown*). The father of the propositus was a carrier of β thalassaemia (Hb A_2 5.3% Hb F 0.4%) and the mother was heterozygous for Hb D $_{\text{Los Angeles}}$ (Hb A_2 2.5 %, Hb D 46.0%).

To determine the nature of the corresponding thalassaemia mutation and to confirm the nature of the structural variant we analyzed the β globin genomic DNA. DNA extraction and PCR were done using standard methods. A 527 bp long PCR product containing exon III of the , globin gene generated with primers 5'-GGGTTAAGGCAATAGCAAT -3' (located at +1139 to 1159 relative to the Cap site) and 5'-CACTGAGACCTCCCACATTCCC-3' (position 1666 to 1647) was first analyzed by non-radioactive SSCP analysis.[3] Strong heteroduplex and single strand signals indicated the presence of a deletion / insertion. This was confirmed by direct sequencing using an ABI310 Prism sequencer and Big Dye chemistry (Applied Biosystems, Foster City, CA, USA). The overlapping signal started at the second base of codon 116. The PCR product was electrophoresed on agarose and the corresponding band eluted using the QIAquick Gel Extraction Kit (Qiagen, Basle, Switzerland). The fragment was denatured for 5 min at 95°C and collected on ice. Ligation into pCR - TOPO vector and transformation were performed using the TOPO TA Cloning Kit (*In vitro* gen, Groningen, The Netherlands) according to the protocol of the manufacturer. Direct forward and reversed

Figure 1. Forward sequence of cloned PCR product using forward PCR primer as sequencing primer. Wildtyp sequence is shown as shaded area. The TGAT insert between nt 1379 and 1380 is shown on white background; The wild type positions are given relative to the cap site.

sequencing of cloned products show¬ed a TGAT insert between nt 1379 and 1380. The result of forward sequencing is shown in Figure 2. The Hb D $_{\text{Los Angeles}}$ mutation at codon 121, GAA→CAA, was confirmed by sequencing of the corresponding PCR product of the mother and by reversed sequencing of the double stranded PCR product of the propositus.

The 4 bp TGAT insert in Codon 116 leads to a premature stop at wild type codon 138/139. The father of the propositus shows the classical picture of a β^0 thalassaemia carrier with no signs of hemolysis or reticulocytosis, there was no sign of a mutant hemoglobin in peripheral blood. The frameshift does not produce, like many other frameshift or nonsense mutations in exon III, a more severe form of thalassaemia[4] or even a dominant type.[5] Whether this mild phenotype is caused by a transcript sensitive to nonsense mediated mRNA decay[6] and/or yet unclear mechanisms as described for other exon III nonsense[7] or frameshift[8] mutations remains unclear.

On re-examination of the compound heterozygous patient at the age of 11 years he again showed a borderline anaemia with a Hb of 115–120 g/L and a pronounced microcytosis but no abnormal clinical findings. This confirms several previous reports about the relatively benign nature of the compound heterozygous state of Hb D $_{\text{Los Angeles}}$ and classical β^0 thalassaemia.[9,10] Unfortunately, since the family members are Tamil refugees residing in Switzerland, we were unable to study the patient's grandparents in order to determine whether this mutation is a *de novo* insertion or inherited.

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