A de novo Deletion Causing $\epsilon\gamma\delta\beta$ -Thalassemia in a Dutch Patient

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The $\epsilon\gamma\delta\beta$ -thalassemias are rare forms of deletional mutants characterized by large deletions of or within the β -globin gene cluster. Heterozygotes have hematological parameters resembling those of a heterozygote for β^0 -thalassemia (β -thal) with severe microcytosis and hypochromia but with normal levels of HbA₂ and HbF [1]. In some patients the deletion is larger than 150 kb and encompasses the entire β -globin gene cluster including the LCR. Here we describe a de novo $\epsilon\gamma\delta\beta$ -thal in a Dutch female with a deletion of at least 200 kb or larger, extending from ~ 60 kb 5' to ϵ to more than 90 kb 3' to β .

The patient was followed for several years at the University Hospital in Rotterdam, The Netherlands. Hematological examination showed a moderate anemia with microcytosis and hypochromia but with normal HbA₂ and HbF values (Hb 9.3 g/dl; RBC $4.18 \times 10^{12}/l$; MCV 67.0 fl; MCH 22.2 pg; HbA₂ 3.0%; HbF < 0.5%). The identity of the thalassemia was determined at the laboratories in Leiden, The Netherlands and in Augusta, Ga., USA. For that purpose blood samples from the patient and her parents were repeatedly collected in vacutainers with EDTA as anticoagulant; informed consent was obtained. The denaturing gradient gel electrophoresis (DGGE) analysis [2] and the α/β in vitro chain synthesis ratio (α/β ratio: 1.75; 1.89 in duplicate analyses) were

determined at the University of Leiden, The Netherlands. The number of α -globin genes were analyzed by both PCR and gene mapping [3]. Six enzymes were used to analyze the β -globin gene cluster, namely BamHI, BglII, HindIII, EcoRI, XmnI, and TaqI. Seven different probes, spanning the β -globin gene cluster, were used to delineate the size of the deletion; the β -IVS-II probe (a 0.95-kb EcoRI/BamHI fragment), the 5' ϵ probe (a 1.45-kb BamHI/HindIII fragment 16

Gene mapping analyses of the β -globin gene cluster revealed that in all instances the intensities of the bands for the DNA of the patient were about half of those seen for her parents without observing an additional band of different size. No intensity differences were seen when the α -probe was used in the hybridization experiments. These data indicate the presence of a large deletion of at least 200 kb with a 5' endpoint upstream from the γ B1 probe (58 kb from ϵ) and the 3' breakpoint more than 90 kb 5' to the β -gene. This deletion was only observed in the proband but not in her parents; nonpaternity was unlikely and thus, the deletion occurred as a de novo event.

Figure 1 compares this deletion with those observed in several other patients of different nationalities [from 4]. It resembles most closely that described in a large Canadian family, although there are quite some uncertainties about the locations of both the 5' and the 3' ends of all these large deletions. However, such naturally occurring deletional mutants may contribute to the understanding of the mechanisms leading to such abnormalities, and therefore, additional sequence data should be collected. The data published for the Canadian family are of importance because they showed severe anemia in the newborn at an age of 30-40 days when the HbF level was still about 70%.

Fortunately, recovery was already evident at 3 months of age (40% HbF) and adult heterozygotes showed the same hematological data as observed for our proband. Indeed, the presence of the proposed deletion of more than 200 kb with its ill-defined 5' and 3' breakpoints fully explains the clinical and hematological observations that were made for this young female.

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$\epsilon\gamma\delta\beta$ -Thal in a Dutch Girl

Acta Haematol 1996;96:108-109