A rare case of compound heterozygosity for δ+27 and Hb Neapolis (Dhonburi) associated with an atypical β-thalassemia phenotype

We report an unusual β-thalassemia phenotype associated with a novel interaction of rare globin gene defects (δ+27 and Hb Neapolis). The phenotype/genotype relationship in this case illustrates the potential pitfalls in genetic counseling in areas such as southern Italy, where thalassemia is characterized by a wide range of molecular defects and is an important medical-social problem.

β-thalassemia with normal HbA2 and thalassemia-like red cell indices is a rare form of β-thalassemia trait, related to mild β-thalassemia defects or to co-inheritance of mutations, associated or not on the same chromosome, that decrease both β- and δ-globin gene function.[1]

We report the molecular basis of a mild hypochromic microcytic anemia in a 5-year-old boy from Naples, Italy. Cation exchange high performance liquid chromatography (HPLC) revealed normal HbA2 (2.49%) and no HbF. The patient had normal osmotic resistance and normal serum iron and transferrin concentrations, but persistently high serum ferritin level (510 ng/mL). Bone marrow biopsy was normal except for large iron storages in macrophages. The patient was negative for the most common hemochromatosis point mutations. His father had normal red blood indices and iron balance, low HbA2 (1.5%) and normal HbF (0.3%). His mother had mildly hypochromic microcytic red blood indices, normal HbA2 (2.7%) and HbF (0.6%) (Table 1). These findings suggest that the propositus was heterozygous for both δ- and β-thalassemia or a carrier for α-thalassemia.

DNA was extracted from peripheral blood leukocytes of the patient and his parents. Southern blot analysis of the α-globin gene cluster revealed no rearrangements in any subject. The propositus did not carry any of the most common Mediterranean β-thalassemic mutations, i.e. β°39, β+IVSI-110, β°IVSI-1, β+IVSI-6, β+IVSII-745, β°IVSII-1, β+-87, Cod 6-A, analyzed by reverse dot-blotting (kit BeTha Gene 1, Bio-Rad Laboratories, Hercules, CA, USA).[2] Globin chain synthesis analysis was performed[3] to discriminate between δβ- and α-thalassemia carrier status. The α/β ratio was 2.27 for the patient, and 1.66 and 0.96 for his mother and father, respectively. The patient and his mother had an anomalous reverse phase HPLC peak that recalled Hb Neapolis (β 126 (H4) Val→Gly). Hb Neapolis is an unstable hemoglobin variant undetectable by conventional hematologic HPLC screening and showing mild thalassemic features. Subjects heterozygous for this variant have mild microcytosis and slightly increased HbA2 levels.[4] We examined the propositus and his family for the mutation causing the synthesis of Hb Neapolis (codon 126: T→G) and of δ+27 (G→T), the most common Mediterranean δ+-thalassemia mutation,[5] by allele-specific amplification (ARMS) reactions[6] and verified the results with sequence analysis (3100 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). To generate the ARMS amplicon for each mutation analysis, normal or mutant allele-specific primer was coupled to a common primer (Table 2). The reaction mix included two other primers (control B and control E) to generate an internal positive control fragment.

The genomic DNA (0.5 μg) was amplified in a 50 μL reaction volume containing 50 pmol of each allele-specific and common primers, 10 pmol of each control primer, 200 μM dNTPs (Amersham Pharmacia Biotech, England, UK), 2.5 U of Taq polymerase (AmpliTaq, Applied Biosystems) and 1× PCR buffer supplied by the manufacturer. The PCR conditions were: 93°C (30 seconds), 65°C (15 seconds), 72°C (45 seconds) for a total of 25 cycles (final extension at 72°C for 7 min) on a 9600 Applied Biosystems thermal cycler apparatus.

Table 1. Hematologic data.

Subject	*Hb (g/dL)*	*MCV (fL)*	*MCH (pg)*	*A_2 (%)*	*F (%)*	*α/β*
Mother	12.2	73.12	24.02	2.7	0.6	1.66
Father	14.8	85.8	30.4	1.5	0.3	0.96
Propositus	10.4	71.5	21.2	2.49	0.6	2.27

These investigations revealed the δ+27 (G→T) mutation in a heterozygote state, in the patient and his father, and the beta-globin codon 126 (T→G) that produces the Hb Neapolis variant in the patient and his mother, thus confirming the origin of the anomalous HPLC peak. These results indicate that the atypical β-thalassemia phenotype with a normal HbF level was caused in our patient by coinheritance in trans of δ+27 (G→T) and β–globin codon 126 (T→G) mutations. The δ-thalassemic trait normalized HbA2 level, thus almost completely silencing the mild β-thalassemic phenotype produced by Hb Neapolis (Table1). This rare Hb variant is relatively frequent in Naples, where in one case it was associated with Hb Lepore-Boston.[7] To our knowledge, this is the first report of interaction of Hb Neapolis with a δ-thalassemic defect.

The mother's HbA2 level is lower than expected for Hb Neapolis carriers,[4] probably because of iron deficiency (serum iron: 42 μg/dL), which is known to reduce Hb A2 levels.[8] This condition might have masked the underlying mild β-thalassemia carrier status. Consequently, a detailed hematologic analysis should be performed in such cases.

This case is a further example of the remarkable molecular

Table 2. Primers for detection of the δ+27 (G→T) and Hb Neapolis (codon 126: T→G) mutations by ARMS analysis (underlined bases are mismatches with respect to the wild type sequence).

Primer	*Sequence (5'→3')*	*Position*
δ+27 (G→T)		δ-globin gene
Normal	TTATAACCTTGATACCAACCTGCCCAGCGC	+161 to +130
Mutant	TTATAACCTTGATACCAACCTGCCCAGCGA	+161 to +130
Common	CAACTGCTGAAAGAGATGCGGT	-232 to -211
β+ cod 126 (T→G)		β-globin gene
Normal	CAGCCACCACTTTCTGATAGGCAGCCTCCA	+1439 to +1410
Mutant	CAGCCACCACTTTCTGATAGGCAGCCTCCC	+1439 to +1410
Common	TATCATGCCTCTTTGCACCATTC	+1093 to +1115
Control primers		β-globin gene
Control B	GAGTCAAGGCTGAGAGATGCAGGA	+1948 to +1925
Control E	GAAGGTGAGGCTGCAAACAG	+1740 to +1759

heterogeneity and consequent wide clinical variability of thalassemia syndromes in our region. It also highlights the potential pitfalls in genetic counseling in areas where globin gene disorders are most common.[9,10]

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