null allele of the SPTA1 gene in trans. Br J Haematol 2004;127:118-22.
11. Wichterle H, Hanspal M, Palek J, Jarolim P. Combination of two mutant α spectrin alleles underlies a severe spherocytic hemolytic anemia. J Clin Invest 1996;98:2300-7.
12. Wilmotte R, Maréchal J, Morlé L, Baklouti F, Philippe N, Kastally R, et al. Low expression allele αLELY of red cell spectrin is associated with mutations in exon 40 (α V/41 polymorphism) and intron 45 and with partial skipping of exon 46. J Clin Invest 1993; 91:2091-6.

First case of γ-thalassemia in association with a βS allele: a pitfall in the neonatal screening for sickle cell disease

Numerous deletional thalassemias have been reported so far, involving one or several globin genes in combination.[1] A deletion specifically targeted on the fetal β-like genes was first described in 1983 and was characterized as a deletion-fusion removing the 3' end of Gγ-gene and the 5' end of Aγ-gene.[2] This deletion was found after the identification of several newborns with an abnormally low Gγ/Aγ ratio, in the course of an Hb F expression study in newborns from various origins (Asian, European and African-American).[3] This γ-gene deletion is clinically silent either in heterozygous or in homozygous conditions and was found in 1.42% of the tested newborns (mainly from China, India, Japan and the former Yugoslavia). In the present investigation, we have identified the first case of such a fetal γ-gene deletion found in association with a Sickle β-globin gene (βS).

The proband is a newborn screened at birth, in the course of the French neonatal screening program for Sickle Cell Disease (SCD). He was suspected of having SCD because of the presence of HbS and a very low level of HbA detected by IEF and HPLC, on a dry blood sample collected after three days of life (Figure 1A). At six weeks of age, control hemoglobin analysis on a venous blood sample revealed the following Hb rates: HbF 55%, HbS 27% and HbA 15% suggesting a compound heterozygosity for β⁺-thalassemia and βS alleles. However, molecular analysis of the β-globin gene showed, as a single defect, heterozygosity for the prevalent sickle cell mutation transmitted by the father. The proband's mother showed no Hb abnormality and normal red blood cell indices.

Nonetheless, the baby was included in the prevention program and a regular clinical follow-up was defined. At eight months of age, a control Hb analysis revealed a typical profile of βS heterozygote with Hb S: 35%, Hb A: 55% in contradiction with the neonatal diagnosis.

In an attempt to further explore this peculiar phenotype, the whole β-globin locus was analyzed by means of MLPA technical procedures (Xservices, the Netherlands) that allowed us to evidence a heterozygous deletion of the region localized between the fetal globin genes, Aγ and Gγ (Figure 1B). This deletion was confirmed by genomic qPCR (Applied Biosystem 7500) (Figure 1C) and

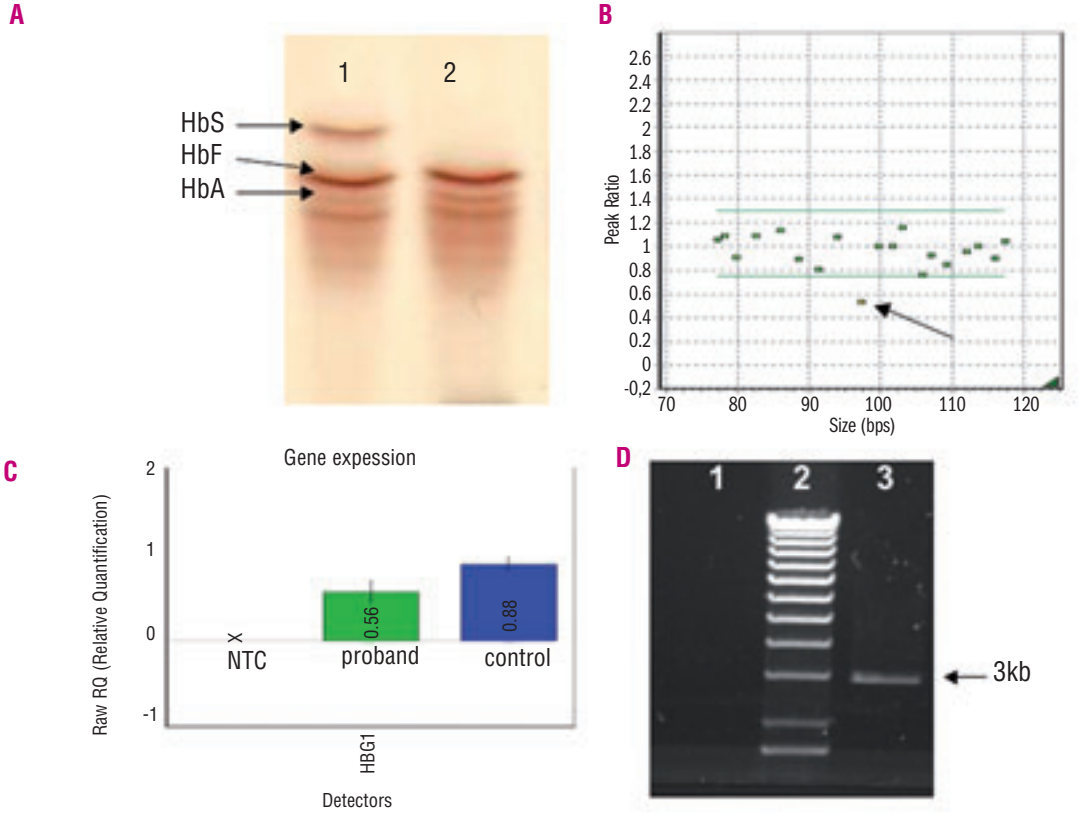

Figure 1. β-globin locus analysis. (A) Isoelectrofocalisation from 1:the proband's dried blood, 2: a normal newborn. (B) MLPA of the whole β-globin locus using the commercial kit from Xservices, the Netherlands (the arrow shows the probe located within the deletion). (C) Genomic Quantitative PCR with a set of primers located between G and Aγ genes (forward: aaatgtgtgtctttctggcctttt; reverse: ccttcatgatcctgtgtaaagcttata); NTC: No Template Control. (D) Standard PCR with primers flanking Gγ and Aγ genes: Forward 5'acaagtgtctttactgctttatttgct3' and Reverse 5'ccaaggtcatggatcgagtt3'; 1:Normal Control (no amplification); 2: Ladder 12kb (Invitrogen); 3: proband presenting a 3 kb amplicon.

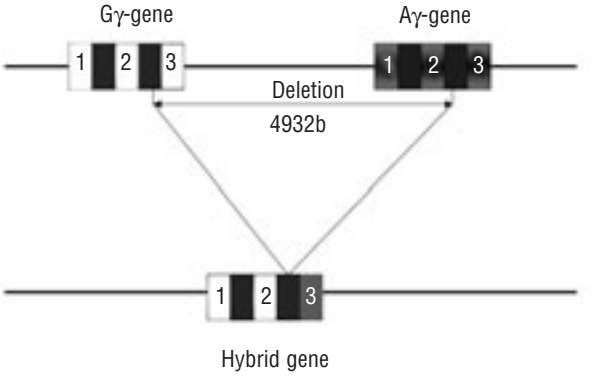

Figure 2. Schematic representation of the deletional γ-thalassemia in the β-globin locus; sequence co-ordinates: Gγ gene: 5232678-5230978, Ag 5227754-5226050.

by standard PCR (Figure 1D) through the amplification of an abnormal fragment 4.8 kb shorter than expected. The junction fragment was sequenced (Applied Biosystems 3130XL) and revealed the presence of a hybrid gene identical to Gγ-gene before the polymorphic TG repeat in IVS2[4] and identical to Aγ-gene after this repeat. As no abnormal sequence is created by the rearrangement, we assume that the breakpoint lies in this polymorphic sequence. The size of the deletion corresponds exactly to the size of DNA which separates the γ-genes (Figure 2). The hybrid gene produces an Aγ-globin chain under the control of the Gγ-gene promoter i.e. at a level normally seen for Gγ, explaining why the deletion is not symptomatic even in the homozygous condition. Such a deletion-fusion is also a common event occurring in α-globin locus: indeed, like the fetal γ-globin genes, α-globin genes present highly homologous flanking sequences which represent a potential target area for genetic rearrangements.[5] The resulting deletions, called type 2 α-thalassemia deletions, are very frequent in Asians, Africans and Mediterraneans and cause no clinical consequences.

Although we cannot confirm the presence of the deletion on the paternal chromosome which carries the βS allele, we hypothesize that the deletion is located in cis of the βS allele. Removing parts of the fetal coding region, it has led to premature interactions between LCR and the βS gene promoter, resulting in a high level of expression of the βS gene before birth. Thus, at birth, the proportion of HbS was higher than that of HbA, evocating SCD. In the weeks following birth, the switch on normal βA allele was processed, leading to the rise in the level of HbA, whereas the HbS level remained stable, and to the restoration of a typical heterozygous profile.

In conclusion, we show that a γ-thalassemia associated with a βS allele represents a pitfall in the neonatal screening for SCD.

Caroline Lacoste,[1] Nathalie Bonello-Palot,[1] Katia Gonnet,[1] Françoise Merono,[2] Nicolas Levy,[1,2,4] Isabelle Thuret,[3] and Catherine Badens[1,2,4]

[1]Laboratoire de Génétique Moléculaire, Hôpital d'enfants de la Timone, Marseille; [2]Centre d'Enseignement et de Recherche en Génétique Médicale, Faculté de Médecine, Université de la Méditerranée, Marseille; [3]Service d'Hématologie Pédiatrique, Centre de référence Maladies Rares «Syndromes Thalassémiques », Hôpital d'enfants de la Timone, Marseille; [4]Unité Inserm U910, Faculté de Médecine Marseille, France

Key words: deletional γ-thalassemia, neonatal screening for sickle cell disease.

Correspondence: Catherine Badens, Laboratoire de Génétique Moléculaire, Hôpital d'enfants de la Timone, 13385 Marseille, cedex 5 France. Phone: international +33.491387787. Fax: international +33.491384676. E-mail: catherine.badens@ap-hm.fr

Citation: Lacoste C, Bonello-Palot N, Gonnet K, Merono F, Levy N, Thuret I, Badens C. First case of γ-thalassemia in association with a βS allele: a pitfall in the neonatal screening for sickle cell disease. Haematologica 2008; 93:1754-1755. doi: 10.3324/haematol.13262

References

1. Kanavakis E, Traeger-Synodinos J. Molecular basis of thalassemia syndromes. In: Beaumont C, Beris P, Beuzard Y and Brugnara C, editors. Disorders of iron homeostasis, erythrocytes, erythropoiesis. Forum Service Editore; 2006. p. 211-27.
2. Sukumaran PK, Nakatsuji T, Gardiner MB, Reese AL, Gilman JG, Huisman TH. γ thalassemia resulting from the deletion of a γ-globin gene. Nucleic Acids Res 1983; 11:4635-43.
3. Huisman TH, Reese AL, Gardiner MB, Wilson JB, Lam H, Reynolds A, et al. The occurrence of different levels of G γ chain and of the A γ T variant of fetal hemoglobin in newborn babies from several countries. Am J Hematol 1983;14:133-48.
4. Lapoumeroulie C, Castiglia L, Ruberto C, Fichera M, Amata S, Labie D, et al. Genetic variations in human fetal globin gene microsatellites and their functional relevance. Hum Genet 1999;104:307-14.
5. Embury SH, Miller JA, Dozy AM, Kan YW, Chan V, Todd D. Two different molecular organizations account for the single α-globin gene of the α-thalassemia-2 genotype. J Clin Invest 1980;66:1319-25.

Long term biweekly 1 mg oral vitamin B_{12} ensures normal hematological parameters, but does not correct all other markers of vitamin B_{12} deficiency. A study in patients with inherited vitamin B_{12} deficiency

Parenteral vitamin B_{12} is considered the treatment of choice for vitamin B_{12} deficiency, but also treatment with 1-2 mg daily oral vitamin B_{12} is recommended.[1,2] Recently, alternative regimes have been proposed, such as an oral dose of 1 mg vitamin B_{12} daily for ten days, weekly for four weeks, and monthly or biweekly for life.[3,4] One short-term study assessed this regime employing hematologic markers and total plasma cobalamins.[4] It is unclear to what extent the patients included had a total or only a partial lack of active absorption of vitamin B_{12}.

In the present study, we examined the long-term efficacy of biweekly oral treatment with 1 mg vitamin B_{12} by studying hematologic parameters, cobalamins, methylmalonic acid (MMA), total homocysteine (tHcy), and cobalamin saturated transcobalamin (holo-TC) in patients with congenital vitamin B_{12} deficiency treated for at least five years, patients not following this treatment for at least one year, their biological parents, and healthy controls.

Patients (n=16) were unable to actively absorb vitamin B_{12} because of defects in the cubilin or amnionless receptors (n=12) or inherited lack of intrinsic factor (IF) (n=4). Genotyping was available for 11 patients.[5-7] Lack of vitamin B_{12} absorption was recently confirmed in all 16 patients.[7] After the initial diagnosis, all 16 patients except