A novel (εγδβ)°-thalassemia deletion associated with an α globin gene triplication leading to a severe transfusion dependant fetal thalassemic syndrome

(εγδβ)°-thalassemia is a very rare form of thalassemia only recognized in heterozygotes and caused by unique and sporadic deletions.[1-3] The α/non α–globin chain ratio is imbalanced like in β thalassemia patients but without elevation of HbF or HbA2.[4] They are characterized by a moderately severe neonatal hemolytic anemia with hypochromia but they distinguish from β-thalassemic patients because they may need red blood cell (RBC) transfusions for the first six months of life.[2,3] The phenotype usually improves spontaneously during the first year of life in correlation with the progressive increase of β-globin chain production. As in other β-thalassemic conditions,[5,6] the α-globin genotype may modify the phenotype of (εγδβ)°-thalassemia. We report, for the first time, a novel (εγδβ)°-thalassemia deletion, associated with an alpha globin gene triplication, leading to an undescribed fetal thalassemic syndrome responsible for hydrops foetalis syndrome requiring multiple intra uterine RBC transfusions.

The probant's mother (II. 2, Figure 1) was a 28-year-old Caucasian. She was transfused at birth because of a neonatal hemolysis with hypochromia. In adulthood her erythrocyte phenotype was: Hb: 10.7 g/dL; MCV: 61.0 fL; MCHC: 31 g/dL; MCH: 19.7 pg. During her first pregnancy, she presented with progressive worsening of her anemia (Hb: 5.8 g/dL) requiring RBC transfusions. The diagnosis of an (ε,γ,δ,β)°thal, removing 100 kb between the 3' of HS2 5'LCR region and the 5' of β-globin 3', was made after delivery by DNA analysis (Figure 2). Before her second pregnancy, Hb value was 10.5 g/dL and the husband's erythrocyte phenotype and electrophoresis of hemoglobin were normal (case II.3, Figure 1). During the first trimester of her second pregnancy, the mother was fine and the Hb value above 8.5/dL. The embryonic echography was normal. At week 20 of gestational age, the mother's Hb decreased to 8.3 g/dL without aggravating factors for anemia besides the (ε,γ,δ,β)°thal. The fetal echography at week 20 of gestational age revealed a very severe life-threatening hydrops fetalis related to severe anemia the fetus's Hb determined on umbilical cord blood sample was: 5.8g/dL. There were no other etiologies of hydrops fetalis (viral infection, immunization,

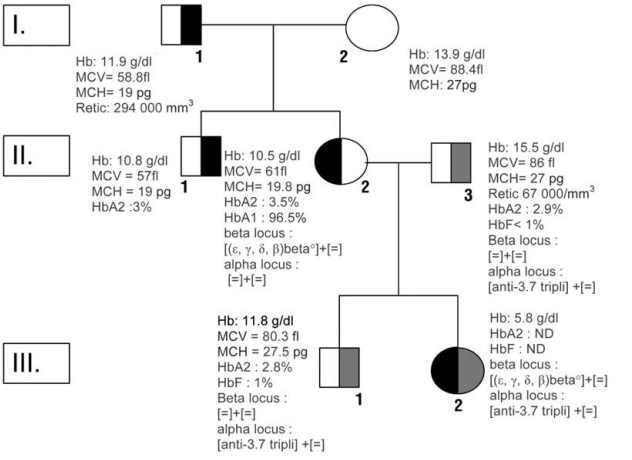
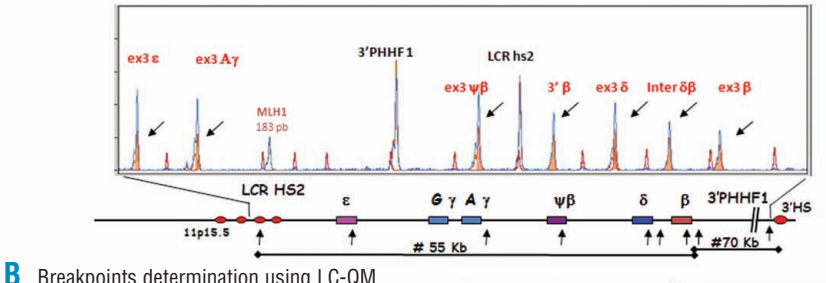


Figure 1. Family pedigree and hematologic data. Individuals indicated by half-black shaded symbol presented neonatal hemolytic anemia and microcytosis and those with a half grey shade symbol presented anti-3.7 triplication α gene. Erythrocyte parameters of patient III2 were determined during fetal life.

A Detection of the deletion using QMPSF



B Breakpoints determination using LC-QM

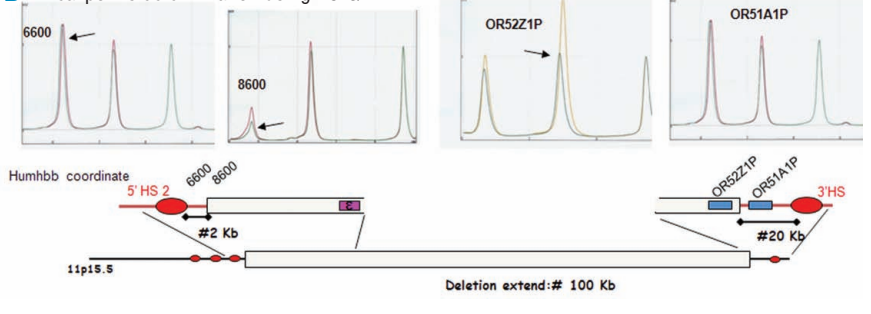


Figure 2. (A) QMPSF. The screening of deletion is done by a multiplex semi-quantitative PCR (sQ-PCR) with a set of 9 primers located on various positions covering the extent of the β-globin gene cluster, from the 5' locus control region (5'LCR) to a region approximately 90 kb 3' of the β-gene. The MLh1 gene is used as internal controls. Fluorescent chromatograms from PCR product obtained with patient and normal control are compared after normalization using MLh1. Deleted markers have a pic value roughly half of that of pic in normal control.[7] (B) LC-QM. In order to narrow the breakpoint location as much as possible, successive triplex sQ-PCR are tested using the same approach as QMPSF but with a DHPLC device.[8] Displayed chromatograms show the closest markers in both sides of the breakpoint. HUMHBB co-ordinates refer to the position of the sense primer set used in LC-QM steps within the β-globin cluster sequence (genbank ac code: HUMHBB). "6600" is located 5' to the 5'HS2 and 8600 is located 2kb downstream. *ORxxxxx* refers to olfactory receptor genes located downstream from β-globin cluster. Settings are available upon request.

organ dysfunction, autoimmunity). Genotyping of the globin loci revealed that the fetus had inherited the mother's (ε,γ,δ,β)° thal and an anti -3.7 α-triplication from the father (Figure 1). Two intrauterine RBC transfusions were performed at weeks 22 and 29. Hydrops did not recur and growth was satisfactory. The baby was born by caesarean section at 35 weeks and was well without hepatosplenomegaly; his Hb was 9.3 g/dL, reticulocyte count 356 10^6/L, bilirubin 40 mg/L. RBC transfusions have been necessary every three weeks to maintain Hb value above 9 g/dL, the baby now being four months old.

The severe hydrops foetalis was due in this case to inheritance of an (εγδβ)°-thalassemia and an anti -3.7 α-globin gene triplication. Typically, patients heterozygous for an (εγδβ)°-thalassemia deletion alone exhibit at birth a hypochromic anemia with various degrees of hemolysis. Blood transfusion in the neonatal period is sometimes necessary in (εγδβ)°-thalassemia like in the probant's mother's case II.2.[2,3] However contrasting phenotypes have been reported in one family.[1,9] The occurrence of early manifestations in this case is explained by the increasing imbalance between the α and non α-globin chains ratio during fetal life. In our case III.2, the association with the triplicated α-genes increased the imbalanced α/non α-globin ratio which explains the "fetal thalassemia intermedia" requiring blood transfusions during the intra-uterine period. *Thalassemia intermedia* is characterized by an unstable thalassemic erythropoiesis needing transfusion when an erytroid stress occurs. In our case, it is very likely that the transition from embryonic to fetal erythropoiesis was the "erythroid stress" causing a greater sensitivity to the effect of globin imbalance and the hydrops foetalis. Intra uterine transfusion in (εγδβ)°-thalassemia related to the presence of such a deletion has been previously cited twice; it could be hypothesized that triplication alpha might also be involved in these cases (α-triplication not evaluated).[2,3] The frequency of α-triplication varies according to population origin[10] and cannot be identified with routine parameters, as attested by the father's normal phenotype (II.3).[4] However the impact of such an association is extremely important and could threaten the fetus's life.

Our observation emphasizes the absolute necessity of systematically looking at α-gene status in partners of (εγδβ)°-thalassemia carriers before conception considering the severity of such an association. Careful follow-up of both the fetus and the mother, if carrier of the (εγδβ)°-thalassemia deletion, is mandatory.

Christian Rose,[1] Julien Rossignol,[1] Anne Lambilliotte,[2] Sandrine Depret,[3] Nathalie Le Metayer,[4] and Serge Pissard[4]

[1]Service d'Hématologie, Hôpital St Vincent de Paul, Université Catholique de Lille, Lille; [2]Service d'Hématologie Pédiatrique, CHU Lille; [3]Service de Gynécologie et d'Obstétrique, CHU Lille; [4]Service de Génétique et de Biochimie, CHU Hôpital Henri Mondor, Université Paris 12 Créteil, France

Correspondence: Christian Rose, Service d'Onco-Hématologie, Université Catholique de Lille, Boulevard de Belfort 59000 Lille, France. Phone: international +33.20874532. Fax: international +33.20874585. E-mail: Rose.Christian@ghicl.net

Key words: (εγδβ)°-thal, α-gene status, hydrops foetalis, β-thalassemia intermedia.

Citation: Rose C, Rossignol J, Lambilliotte A, Depret S, Le Metayer N, Pissard S. A novel (εγδβ)°-thalassemia deletion associated with an α globin gene triplication leading to a severe transfusion dependant fetal thalassemic syndrome. Haematologica 2009; 94:593-594. doi: 10.3324/haematol.2008.002675

References

1. Furuya C, Yamashiro Y, Hattori Y, Hino M, Nishioka H, Shimizu Y, et al. A novel epsilon gamma delta beta thalassemia of 1.4 Mb deletion found in a Japanese patient. Am J Hematol 2008;83:84-6.
2. Game L, Bergounioux J, Close JP, Marzouka BE, Thein SL. A novel deletion causing (epsilon gamma delta beta) degrees thalassaemia in a Chilean family. Br J Haematol 2003;123:154-9.
3. Harteveld CL, Osborne CS, Peters M, van der Werf S, Plug R, Fraser P, et al. Novel 112 kb (εγδ) δβ-thalassaemia deletion in a Dutch family. Br J Haematol 2003;122:855-8.
4. Giambona A, Passarello C, Vinciguerra M, Li Muli R, Teresi P, Anza M, et al. Significance of borderline hemoglobin A2 values in an Italian population with a high prevalence of β-thalassemia. Haematologica 2008;93:1380-4.
5. Rund D, Rachmilewitz E. β-thalassemia. N Engl J Med 2005;353:1135-46.
6. Thein SL. Genetic modifiers of the β-haemoglobinopathies. Br J Haematol 2008;141:357-66.
7. Casilli F, Di Rocco ZC, Gad S, Tournier I, Stoppa-Lyonnet D, Frebourg T, et al. Rapid detection of novel BRCA1 rearrangements in high-risk breast-ovarian cancer families using multiplex PCR of short fluorescent fragments. Hum Mutat 2002;20:218-26.
8. Houdayer C, Gauthier-Villars M, Lauge A, Pages-Berhouet S, Dehainault C, Caux-Moncoutier V, et al. Comprehensive screening for constitutional RB1 mutations by DHPLC and QMPSF. Hum Mutat 2004;23:193-202.
9. Trent RJ, Williams BG, Kearney A, Wilkinson T, Harris PC. Molecular and hematologic characterization of Scottish-Irish type (εγδβ)° thalassemia. Blood 1990;76:2132-8.
10. Goossens M, Dozy AM, Embury SH, Zachariades Z, Hadjiminas MG, Stamatoyannopoulos G, et al. Triplicated α-globin loci in humans. Proc Natl Acad Sci USA 1980; 77:518-21.

Successful unrelated donor stem cell transplantation for advanced myelofibrosis in an adult patient with history of orthotopic liver transplantation

Patriarca *et al.*[1] recently reported a significant improvement in outcome of patients with myelofibrosis (MF) after allogeneic stem cell transplantation (SCT). Some MF patients present to the transplant center with severe complications after a long disease history and their treatment may be very challenging.

In 1987, a 33-year old woman was diagnosed with polycythemia vera (PV) associated with portal and splenic vein thrombosis. A year later she developed acute Budd-Chiari syndrome and required orthotopic liver transplantation. In 2002, 15 years since diagnosis, PV progressed to MF. Molecular genetics revealed heterozygous V617F mutation in the Janus Kinase 2 (*JAK 2*) gene. In 2006, blood count showed: leukocytes 1.5×10^9/L, hemoglobin 8.1 g/dL, platelets 124×10^9/L and 13% circulating blasts. Blood transfusions were required every eight weeks. According to the Lille Scoring System the patient was at high risk of progression to acute leukemia. Since she had no siblings, we decided to start an unrelated donor search, although very few reports exist on allogeneic SCT in patients after preceding solid organ, respectively liver, transplantation. A compatible donor with a single human leukocyte antigen (HLA) Cw mismatch was identified. Patient and donor were mismatched for blood group and cytomegalovirus serology. In April 2007 allogeneic SCT following treosulfan (30