β thalassemia major due to acquired uniparental disomy in a previously healthy adolescent

β thalassemia major (β-TM) is an autosomal recessive disorder caused by the inheritance of null alleles (usually β^0 point mutations) in both *HBB* genes (chromosome 11p15.5). Typically, β-TM patients are asymptomatic at birth but, due to the decrease of the expression of γ-globin genes, during the first six months of life they become transfusion dependent. Late β-TM presentation is an extremely rare condition. To our knowledge only one case has been previously reported by Chang JG *et al.*[1] who described a patient with thalassemia major presenting at 28 years of age. β-TM late presentation was secondary to the loss of heterozigosity (LOH) due to acquired paternal uniparental disomy (UPD) involving the 11p15.5 chromosomal region. LOH can also be a consequence of acquired large deletions in the same region, and some cases have been described associated with late presentation of β-thalassemia intermedia.[2,3]

We describe a case of acquired β-TM in a young woman diagnosed in childhood as a β-thalassemia carrier. According to her clinical report, at the age of seven years, her hematologic parameters were: hemoglobin (Hb) 10.5 g/dL, mean cell volume (MCV) 60 fL, mean cell hemoglobin (MCH) 20 pg, and hemoglobin (HbA2) 5.2%, compatible with β-thalassemia trait. At the age of 15 years she started to complain of fatigue and her hemoglobin level progressively decreased to around 9-10 g/dL. By the age of 17 years she had starting suffering from fatigue that limited her daily routine. Her hemoglobin was 8-9 g/dL and required sporadic transfusions. Ultrasound examination showed that her spleen and liver had become slightly enlarged. She was started on hydroxyurea, which was discontinued due to thrombocytopenia. The intervals between transfusions became shorter, and at 21 years of age she started a regular transfusion program every 3-4 weeks. Due to severe hepatic iron overload, deferasirox (25 mg/Kg/day) was introduced.

Her father and sister are β-thalassemia carriers and her mother has normal hematologic parameters.

After obtaining informed consent from the patient and the family, total genomic DNA was isolated from white blood cells (WBC). From the patient, DNA was also extracted from oral mucosa epithelial cells and skin fibroblasts. *HBB* gene direct sequencing revealed heterozygosity for the HBB [c.48G>A] mutation [β^0 CD15 (TGG-TGA); p.W16X] in her father and sister. No muta-

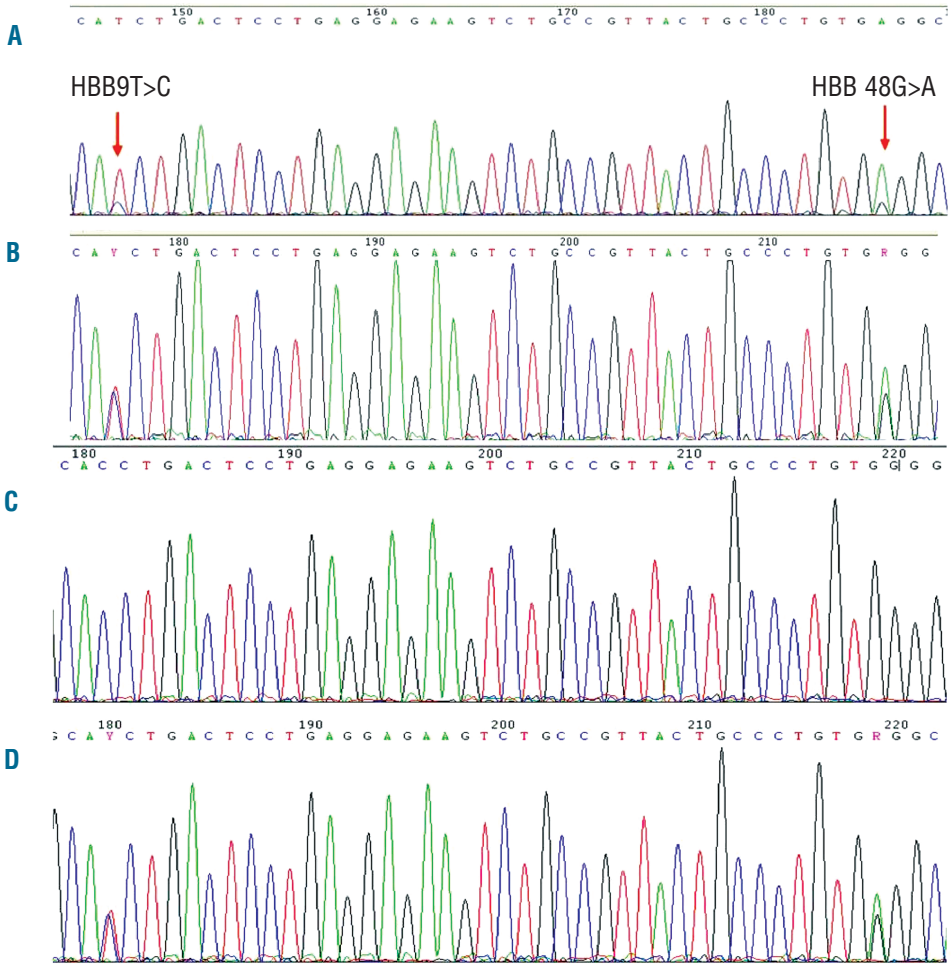

Figure 1. Sequence chromatograms of part of the first exon of the *HBB* gene. (A) Patient's DNA extracted from WBC harbors a normal peak corresponding to the HBB 48G>A mutated allele and a reduced peak corresponding to the normal allele. The same mosaic pattern can be observed in the silent mutation HBB9 T>C (maternally inherited). (B) Skin fibroblasts' DNA showing similar heights of the peaks for the normal and mutated allele. (C) DNA extracted from WBC of the mother, homozygous to the silent mutation HBB9 T>C and (D) the father, heterozygous to the HBB 48G>A mutation (and also to HBB9 T>C).

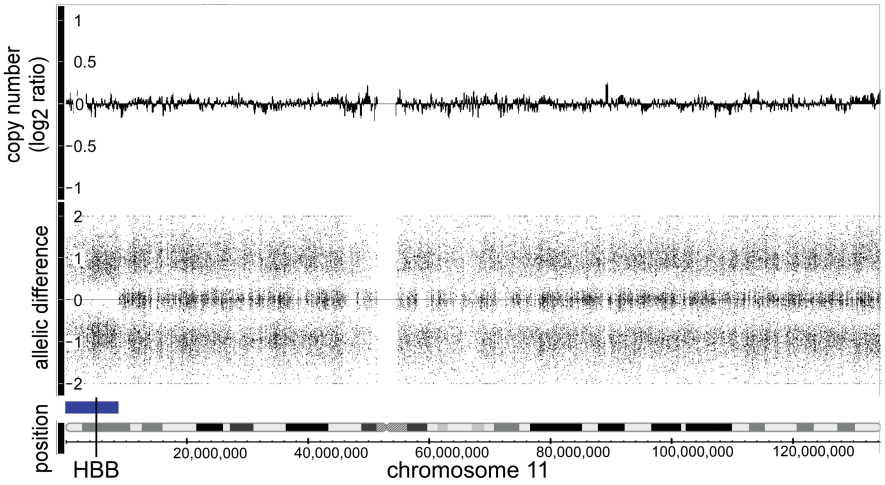

Figure 2. Acquired uniparental disomy (UPD) of chromosome 11p in the patient with acquired β thalassemia major. The copy number as well as allelic difference data from Genome-Wide Human SNP array 6.0 (Affymetrix) are shown in the output from the Genotyping Console Version 3.0.2. software. The position and size of the uniparental disomy is marked with a horizontal blue bar. The position of the *HBB* gene within the 11p region affected by the UPD is indicated.

tions were found in the mother's *HBB* genes.

Sequencing of the patient's DNA extracted from WBC and oral mucosa showed a strong peak due to the HBB 48G>A mutated allele and a very weak signal corresponding to the normal allele. On DNA extracted from skin fibroblasts, the patient is heterozygous for the HBB [c.48G>A] mutation, with a sequencing pattern similar to that observed in her father and her sister (Figure 1). These observations strongly suggest the occurrence of an acquired mutation affecting only the blood cell lineage. The fact that the homozygous pattern can be seen also in the DNA extracted from the oral mucosal was probably due to the contamination of leukocytes in the saliva.[4] In order to define the chromosomal aberration leading to homozygosity of the *HBB* gene mutation, we performed high-resolution genotyping and loss of heterozygosity mapping on the DNA sample from the patient using Affymetrix Genome-Wide Human SNP 6.0 arrays. We identified a 8.8Mb long acquired uniparental disomy of chromosome 11p, covering the position of the *HBB* gene (Figure 2). The patient has 2 copies of chromosome 11, however the 11p region shows an acquired loss of heterozygosity. Based on the allelic difference identified by the array we estimate that the clone carrying the UPD and the mutation represents more than 80% of cells in whole blood. The microarray did not detect any other copy number changes or losses of heterozygosity in the patient's genome.

HBB gene dosage analysis was confirmed by multiplex ligation-dependent probe amplification (MLPA) using the HBB SALSA MLPA kit (MRC-Holland, Amsterdam, The Netherlands). Fragment analysis was performed on the ABI Prism 3130. Data were analyzed using Coffalyser MLPA software. All peaks observed in WBC DNA were within the normal range.

We also performed the methylation analysis of H19 differentially methylated region (DMR) and KvDMR domains in the patient's DNA extracted from WBC and from skin fibroblasts. In a normal subject, H19DMR is hypermethylated in the paternal allele and KvDMR is hypermethylated in the maternal allele.[5] Methylation status analysis of H19DMR and KvDMR showed hypermethylation of the H19DMR and no methylation of the KvDMR in the patient's WBC DNA, and 50% methylation of both domains in the patient's skin fibroblast DNA (*data not shown*). These results prove the hypothesis that the acquired β-TM in this patient is due to paternal uniparental isodisomy and mosaicism of chromosomal region 11p14.3-11p15.[5] UPD, first reported and recently reviewed by Engel,[6,7] occurs frequently in various tumor tissues.[8] Beldjord C *et al.*[1] described a child with Wiedemann-Beckwith syndrome and β-TM due to uniparental isodisomy of paternal origin.[9] It has recently been shown that the acquired UPD can amplify germline variants to homozygosity affecting the disease course of hematologic malignancies.[10] LOH, with a homozyogous paternal hypermethylated H19MDR, would correlate with the activation and overexpression of the adjacent and reciprocally imprinted Insulin-like growth factor 2 (IGF2)[11] which could play a role in cellular proliferation, favoring its growth over the clone with the HBB [c.48G>A] mutation in heterozygosy. This clone's preferential proliferation would have given rise to the major hematopoietic cell clone in bone marrow, resulting in the β-TM phenotype.

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