A novel telomeric (285 kb) α-thalassemia deletion leading to a phenotypically unusual Hb H disease

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Many large deletions removing the entire α-globin gene cluster on the short arm of the human chromosome 16 (16p13.3) have been described (1-3). At the heterozygous state, the resulting phenotype consists in α-thalassemia (α-thal) for relatively short deletions (100 to 356 kb) while an α-thalassemia mental retardation syndrome (ATR-16 syndrome) is observed for larger deletions (> 1 Mb) which generally include the 16p telomere (4, 5). We report here a new large telomeric deletion (~285 kb) associated with the common alpha-thalassemia −α3.7 deletion in trans. This genotype led to a phenotypically unusual HbH disease.

The proband was a 14-year-old girl (French Caucasian mother and Algerian father) with a marked hypochromic and microcytic anemia (Hb: 9.2 g/dL; MCV: 55.0 fl; MCHC: 29.9 g/dL; MCH: 16.5 pg and reticulocytes count: 1.7%). Physical examination was normal (without hepatosplenomegaly or subicterus) at the exception of a marked scoliosis for which surgery was considered. She presented no developmental delay and had a normal school education. The presence of HbH (~8%) was detected at routine hemoglobin analysis using isoelectric focusing and cation-exchange liquid chromatography (Variant I, Bio-Rad). Unfortunately, a new blood sample to identify Heinz inclusion bodies could not be obtained. The search for the common α-thal deletions was carried out by multiplex PCR (6) and the common −α3.7 deletion was found at the homozygous state. This result could not be accepted for two reasons (i) the father carried the −α3.7 deletion at the heterozygous state but the mother did not, (ii) an homozygosity for the −α3.7 deletion is not associated with Hb disease. We thus performed an MLPA analysis (Salsa MLPA kit P140-B2 HBA, MRC Holland), which identified, for both the proband and her mother, a large deletion of the α-globin gene cluster (Figures 1-2).

CGH-array analysis was then carried out to gap the deletion which appeared to be approximately 285 kb in length, spanning from the telomeric region in 5’ to the AXIN1 gene in 3’ (Figure 1). We could finally determine, by semi-quantitative PCR assays (7), that
the deletion removes exons 5 to 10 of the AXIN1 gene but leaves exons 1 to 4 intact (the AXIN1 gene is orientated from 3' to 5' on the forward strand).

The exact α-globin genotype of our proband (– / –α^3.7etr) is in total accordance with her HbH disease. According to Horsley et al., monosomy for the 356 kb most telomeric region of the short arm of human chromosome 16 is not associated with the ATR-16 syndrome (3). As the deletion described in the present case report is shorter (~285 kb), it seems logical not to observe major dysmorphic features for our proband, but her very marked scoliosis remains unexplained. The AXIN1 gene has been involved in osteoclasts and osteoblasts regulation (8). Thus, the deleted AXIN1 gene could potentially encode a dominant negative protein for bone synthesis. This negative effect would be potentialized by HbH disease, explaining that no scoliosis was observed for the mother. AXIN1 is also a tumor suppressor gene involved in the development of embryo abnormalities and human cancers (9-11). A genetic counselling and a clinical follow-up are thus required for our proband as mutations, loss of heterozygosity or epigenetic inactivation on the unique functional AXIN1 gene could induce severe clinical consequences.

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Authorship and disclosures
PJ wrote the manuscript. PL and EB performed the MLPA and PCR assays. DS and AL performed and interpreted the CGH Array analysis. DS also had a few modifications to the manuscript. AF coordinated the entire study and approved the final manuscript. The authors reported no potential conflicts of interest.
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REFERENCES


**FIGURE 1**

CGH array analysis. Experiment was performed using an Agilent Technologies 180,000-oligonucleotide array (SurePrint G3 Human CGH 4X180K). The patient’s DNA and the reference DNA were digested with Rsal and AluI (Promega, Madison) respectively. The digestion products were labeled by random priming with Alexa Fluor 5 or Alexa Fluor 3 according to the Bioprime Total Labeling Kit protocol (Invitrogen, California). Each probe was purified by passage through a column, denatured and annealed with 50 µg of human Cot-1 DNA (Invitrogen, California). Hybridization was carried out at at 65°C for 24 hours. The array was then washed and analyzed with Feature Extraction® 10.5.1.1 software. The results were interpreted with the DNA Analytics® 4.0.85 software, with the following parameters: ADM-2, threshold: 6.0, window: 0.2 Mb. The control DNA consisted of DNA from two other patients presenting different diseases, in accordance with the loop model (12). A copy number variation was noted if at least 3 contiguous oligonucleotides presented an abnormal log₂ ratio (> + 0.5 or < -0.5 according the Alexa 5 deviation) with a mirror image. For our proband, a 16pter deletion was identified. The last deleted oligonucleotide has coordinates 289,205 pb to 289,264 pb and the first non-deleted one has coordinates 297,397 pb to 297,456 pb (NCBI reference sequence: NT_010393.16).
FIGURE 2

Schematic representation of the deletion. Genbank New Sequence Viewer of the 400 kb telomeric region of the short arm of the chromosome 16 (16p13.3) (NCBI reference sequence: NT_010393.16). MLPA analysis shows that the entire α-globin gene cluster is removed and, according to our CGH array protocol, the new deletion spans from the WASH4P gene to a part of the AXIN1 gene. The deletion breakpoints have been confirmed and more precisely defined by semi-quantitative PCR. In 5’, the WASH4P gene is entirely removed while the 3’ deletion breakpoint is located between exon 4 and exon 5 of the AXIN1 gene.
FIGURE 1
FIGURE 2