Citation: Bruno F, Bonalumi S, Camaschella C, Ferrari M and Cremonesi L. The -582a>g variant of the HAMP promoter is not associated with high serum ferritin levels in normal subjects. Haematologica. 2010;95:849-850. doi:10.3324/haematol.2009.018986

References

1. Andreani M, Radio FC, Testi M, De Bernardo C, Troiano M, Majore S, et al. Association of hepcidin promoter c.-582 A>G variant and iron overload in thalassemia major. Haematologica. 2009;94(9):1293-6.
2. http://www.ncbi.nlm.nih.gov/projects/SNP/.
3. Bayele HK, McArdle H, Srai SK. Cis and transregulation of hepcidin expression by upstream stimulatory factor. Blood. 2006;108(13):4237-45.
4. Bayele HK, Srai SK. Genetic variation in hepcidin expression and its implications for phenotypic differences in iron metabolism. Haematologica. 2009;94(9):1185-8.
5. De Gobbi M, D'Antico S, Castagno F, Testa D, Merlini R, Bondi A, et al. Screening selected blood donors with biochemical iron overload for hemochromatosis: a regional experience. Haematologica. 2004;89(10):1161-7.
6. Foglieni B, Cremonesi L, Travi M, Giambona A, Rosatelli MC, Perra C, et al. Beta-thalassemia microelectronic chip: a fast and accurate method for mutation detection. Clin Chem. 2004; 50(1):73-9.
7. Biasiotto G, Roetto A, Daraio F, Polotti A, Gerardi GM, Girelli D, et al. Identification of new mutations of hepcidin and hemojuvelin in patients with HFE C282Y allele. Blood Cells Mol Dis. 2004;33(3):338-43.

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

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 $-\alpha^{3.7}$ 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 reticulocyte count: 1.7%). Physical examination was normal (without hepatosplenomegaly or subicterus) except for 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 $-\alpha^{3.7}$ deletion was found at the homozygous state. This result could not be accepted for two reasons: (i) the father carried the $-\alpha^{3.7}$ deletion at the heterozygous state but the mother did not; (ii) a homozygosity for the $-\alpha^{3.7}$ 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 and 2). A 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,[8] that the deletion removes exons 5 to 10

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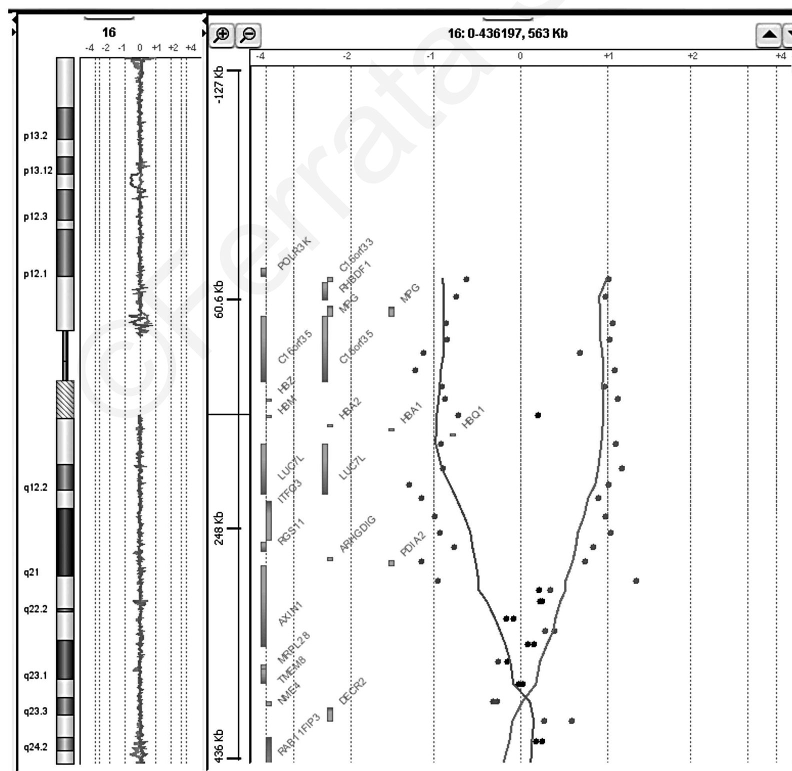

Figure 1. CGH array analysis. Experiment was performed using an Agilent Technologies 180,000-oligonucleotide array (SurePrint G3 Human CGH 4X180K). The patient's DNA and the reference DNA were digested with *Rsa*I and *Alu*I (Promega, Madison), respectively. The digestion products were labeled by random 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 2 other patients presenting different diseases, in accordance with the loop model.[7] A copy number variation was noted if at least 3 contiguous oligonucleotides presented an abnormal log_2 ratio (> + 0.5 or < -0.5 according to 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).

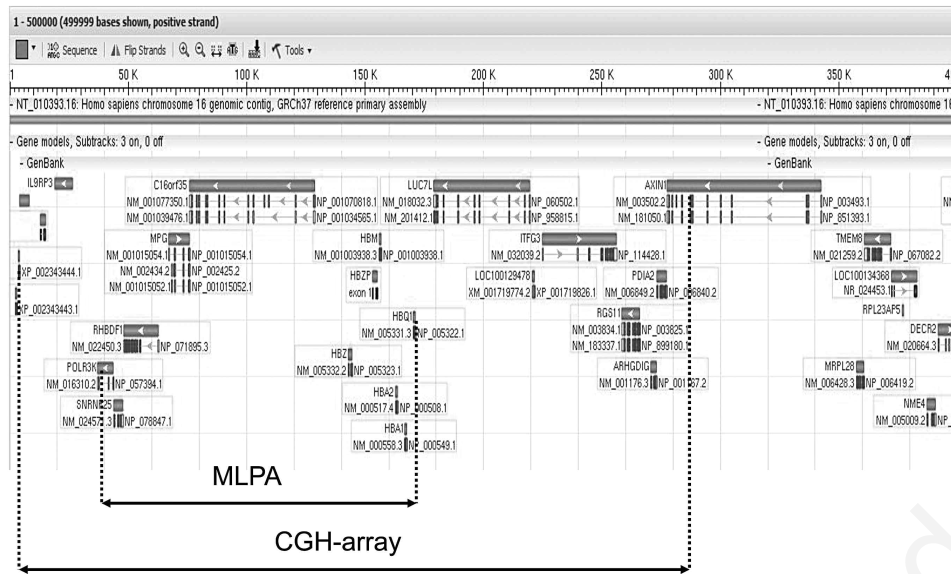

Figure 2. Schematic representation of the deletion. Genbank New Sequence Viewer of the 400 kb telomeric region of the short arm of the chromosome 16 (16p13.3) (NCBI reference sequence: NT_010393.16). MLPA analysis shows that the entire α-globin gene cluster is removed and, according to our CGH array 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.

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 ($--/-\alpha^{3.7}$) 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 osteoblast regulation.[9] 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 why no scoliosis was observed for the mother. *AXIN1* is also a tumor suppressor gene involved in the development of embryo abnormalities and human cancers.[10-12] Genetic counseling and a clinical follow-up are thus required for our proband as mutations, loss of heterozygosity or epigenetic inactivation on the unique functional *AXINI-1* gene could have severe clinical consequences.

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References

1. Higgs DR. The molecular basis of α-thalassemia, in Steinberg MH, Forget BG, Higgs DR, Weatherall DJ, eds. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. Cambridge University Press, Cambridge, UK, 2009, p.241-65.
2. Daniels RJ, Peden JF, Lloyd C, Horsley SW, Clark K, Tufarelli C, et al. Sequence, structure and pathology of the fully annotated terminal 2 Mb of the short arm of the human chromosome 16. Hum Mol Genet. 2001;10(4):339-52.
3. Horsley SW, Daniels RJ, Anguita E, Raynham HA, Peden JF, Villegas A, et al. Monosomy for the most telomeric, gene-rich region of the short arm of human chromosome 16 cause minimal phenotypic effects. Eur J Hum Genet. 2001;9(3):217-25.
4. Higgs DR, Buckle VJ, Gibbons RJ, Steensma D. Unsual types of α-thalassemia, in Steinberg MH, Forget BG, Higgs DR, Weatherall DJ, eds. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. Cambridge University Press, Cambridge, UK, 2009, p.296-320.
5. Harteveld CL, Kriek M, Bijlsma EK, Erjavec Z, Balak D, Phylipsen M, et al. Refinement of the genetic cause of ATR-16. Hum Genet. 2007;122(3-4):283-92.
6. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for common deletional determinants of α-thalassemia. Blood. 2000;95(1):360-2.
7. Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, et al. Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalities: a new series of 140 patients and review of published reports. J Med Genet. 2006;43(8):625-33.
8. Joly P, Lacan P, Garcia C, Couprie N, Francina A. Identification and molecular characterization of four new large deletions in the beta-globin gene cluster. Blood Cells Mol Dis. 2009;43(1):53-7.
9. Liu F, Kohlmeier S, Wang CY. Wt signaling and skeletal development. Cell Signal. 2008;20(6):999-1009.
10. Oates NA, Van Vliet J, Duffy DL, Kroes HY, Martin NG, Boomsma DI, et al. Increased DNA methylation at the AXIN1 gene in a monozygotic twin from a pair discordant for a caudal duplication anomaly. Am J Hum Genet. 2006;79(1):155-62.
11. Satoh S, Daigo Y, Furukawa Y, Kato T, Miwa N, Nishiwaki T, et al. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat Genet. 2000;24(3):245-50.
12. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009;17(1):9-26.