

Severe nondominant hereditary spherocytosis due to uniparental isodisomy at the *SPTA1* locus

Hereditary spherocytosis (HS) is the most common inherited hemolytic anemia in people of North European ancestry, although people of every ethnic background are affected. Inheritance is autosomal dominant in approximately two-thirds of cases, the remaining cases represent autosomal recessive inheritance or *de novo* mutations,¹ as shown in a survey of Italian HS patients with hematologi-

cally normal parents.^{1,2} Patients with nondominant HS (ndHS) are usually more severely affected than patients with typical, dominant HS, presenting in infancy with severe hemolytic anemia,³ some are transfusion-dependent. Erythrocytes from most ndHS patients are deficient in the membrane skeleton protein spectrin leading to destabilization of the lipid bilayer.¹ The degree of spectrin deficiency correlates with the degree of hemolysis and the clinical response to splenectomy. Although biochemical and genetic studies have implicated defects of α -spectrin in most ndHS patients, the genetic basis of α -spectrin defi-

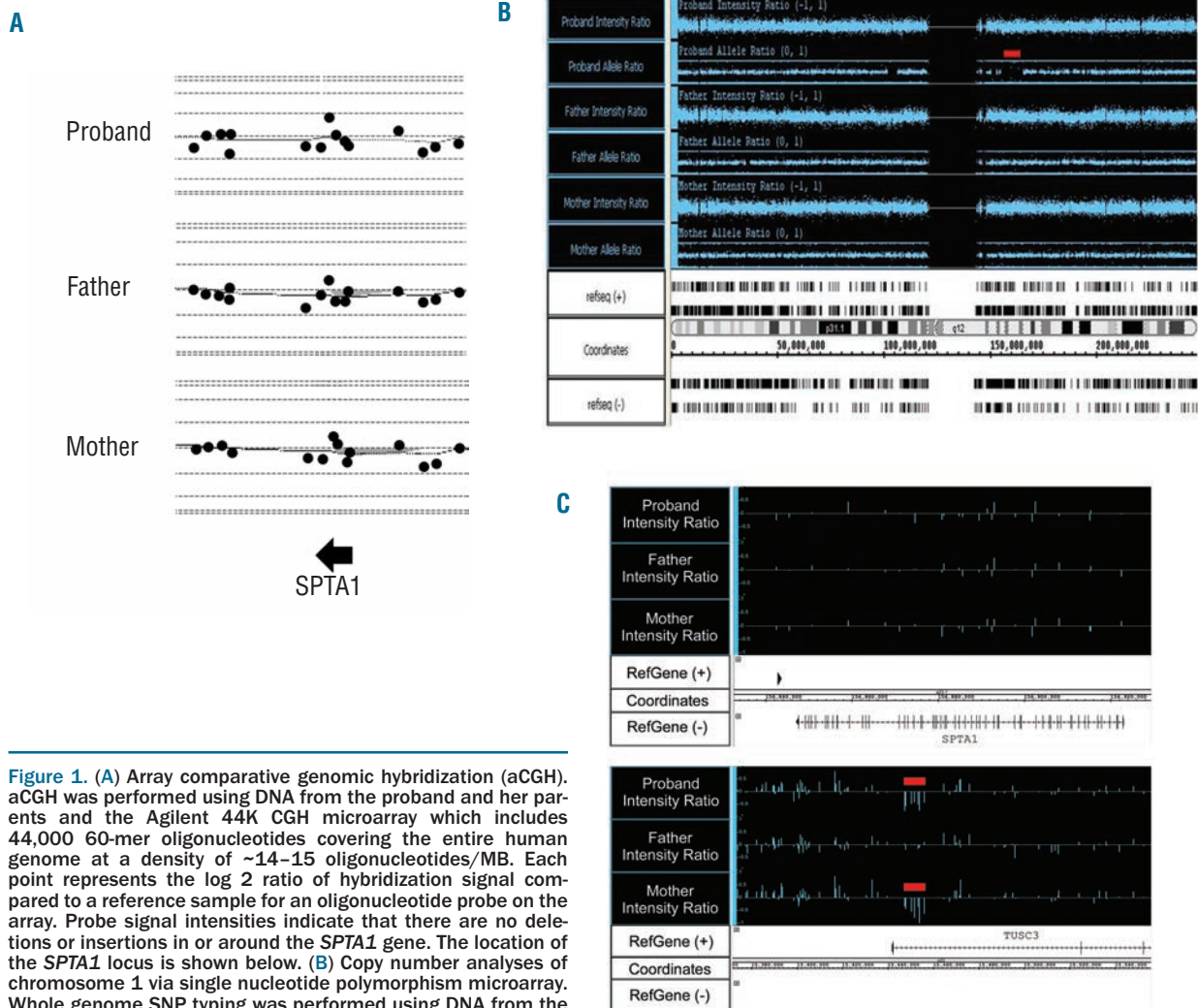


Figure 1. (A) Array comparative genomic hybridization (aCGH). aCGH was performed using DNA from the proband and her parents and the Agilent 44K CGH microarray which includes 44,000 60-mer oligonucleotides covering the entire human genome at a density of ~14–15 oligonucleotides/MB. Each point represents the log₂ ratio of hybridization signal compared to a reference sample for an oligonucleotide probe on the array. Probe signal intensities indicate that there are no deletions or insertions in or around the *SPTA1* gene. The location of the *SPTA1* locus is shown below. (B) Copy number analyses of chromosome 1 via single nucleotide polymorphism microarray. Whole genome SNP typing was performed using DNA from the proband and her parents and the Illumina HumanHap 550 Bead Chip array. The figure shows SNP genotyping from chromosome 1. For each individual, the top panel represents the Intensity Ratio and the bottom panel represents the Allele Ratio. Intensity Ratio is a measure of patient SNP log signal intensity compared to reference sample SNP intensity; 2 copies = intensity ratio of 0; 1 copy = intensity ratio of -1. Most values are centered around zero, indicating diploid copy number. Allele ratio is the ratio of the signal for one of two genotypes over the total signal. Homozygous SNPs localize to 0 or 1; heterozygous SNPs localize to 0.5. In the proband, genotyping data in the 1q24.2-16qter region demonstrate an extended block of homozygosity (Denoted by the red bar). Note that the intensity ratio in this region is unchanged, indicating the homozygosity is not caused by a large deletion. The lower panel shows, from top to bottom, (+) strand genes, cytogenetic bands, chromosome position, and (-) strand genes.

(C) Copy number analyses at the *SPTA1* locus via single nucleotide polymorphism microarray. Whole genome SNP typing was performed using DNA from the proband and her parents and the Illumina HumanHap 550 Bead Chip array. SNP genotyping Intensity Ratio data from the *SPTA1* (top) and *TUSC3* (control, bottom) gene loci. The Intensity Ratio is a measure of patient SNP log signal intensity compared to reference sample SNP intensity; 2 copies = intensity ratio of 0; 1 copy = intensity ratio of -1. Most values are centered around zero, indicating diploid copy number. The yellow bar indicates regions of heterozygous one copy deletion called by the QuantiSNP software. Note that the intensity ratio in the *SPTA1* region is unchanged, in contrast to the deletion seen in *TUSC3*. The lower panel shows, from top to bottom, (+) strand genes, cytogenetic bands, chromosome position, and (-) strand genes.

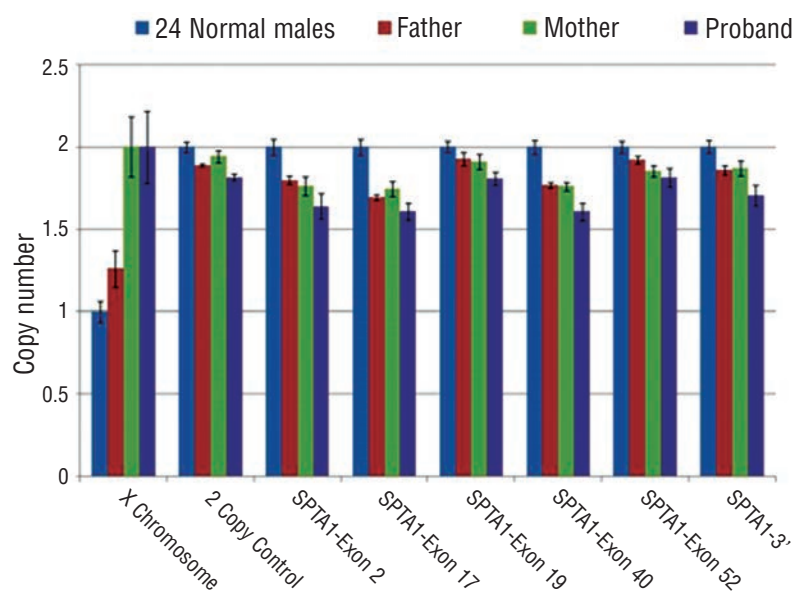


Figure 2. Quantitative copy number profiling. Quantitative copy number profiling using real-time quantitative PCR was performed using 6 probes in the *SPTA1* locus, including a probe in exon 19, the location of the R891X mutation, and both single and double copy controls. Copy number profiling revealed no evidence for intragenic microdeletion in the *SPTA1* gene locus.

ciency is unknown in most cases.³⁻⁵

We studied a girl with severe, transfusion-dependent hereditary spherocytosis. Initial clinical, laboratory, and biochemical characteristics of this patient have already been described.⁶ Severe α -spectrin deficiency was observed in membranes prepared from her marrow-derived cultured erythroid cells. Since this report, despite splenectomy at the age of two years, the proband remains transfusion-dependent.

To determine whether the molecular basis of the proband's transfusion-dependent hemolytic anemia was associated with a defect in α -spectrin, mutation analysis of the *SPTA1* gene was performed. Sanger sequencing of the 52 exons, flanking introns, and promoter region of the *SPTA1* gene identified a homozygous C to T mutation at nucleotide 2870 in exon 19 in DNA from the proband, changing arginine 891 to a stop codon. The patient was homozygous for 10 known coding region SNPs of the *SPTA1* gene, rs703116, rs59898431, rs11265044, rs6702042, rs443000, rs703121, rs3738791, rs35948326, rs3753068, and rs2251969. The mother was heterozygous for the R891X mutation. No *SPTA1* gene mutations were identified in the father.

Array comparative genomic hybridization (aCGH) was performed using the Agilent 44K-CGH microarray (G4426A) to determine if a deletion involving the *SPTA1* gene in trans was responsible for homozygosity of the nonsense mutation and the 10 *SPTA1* gene SNPs in the proband. This array includes 44,000 60-mer oligonucleotides covering the entire human genome at a density of approximately 14-15 oligonucleotides/MB. At the resolution of the aCGH array, no deletions or insertions in or around the *SPTA1* gene locus were found (Figure 1A). She was homozygous for 16 *SPTA1* SNPs on the array.

To interrogate the *SPTA1* gene locus at higher resolution, SNP typing of proband and parental genomic DNA was performed using the Illumina HumanHap550 SNP genotyping array. This array contains over 550,000 SNPs and includes 20 intragenic SNPs in the *SPTA1* gene locus. No genomic deletions or rearrangements at or around the *SPTA1* gene locus were identified in the proband or her

parents at 1q23.1 (Figure 1B). Further refining these observations, QuantiSNP software, which detects copy number changes using the B allele frequency and log R ratio data, did not detect any intragenic deletions in the *SPTA1* locus (Figure 1B).

SNP studies identified a large region of homozygosity at 1q21, approximately 10MB in length, from rs6657293 to rs6670426 (154,995,473-165,730,530,hg18) including the *SPTA1* gene and 158 other genes (Figure 1C). Seventeen homozygous SNPs were identified at the *SPTA1* locus in the proband, extending the observations of SNP homozygosity at the *SPTA1* locus observed from Sanger sequencing and aCGH.

Analyses of the SNP data also confirmed paternity and maternity, with a parent-parent-child heritability of 0.9997.

Quantitative copy number profiling was performed to exclude an intragenic microdeletion in the *SPTA1* gene locus not detectable by SNP arrays. Amplicons included exon 19 (R891X mutation), exons 2, 17, 40, 52 and the 3'UTR. The proband and both parents had 2 copies of the *SPTA1* gene at all sites examined (Figure 2). No deletions were identified, excluding the possibility that a microdeletion is the cause of homozygosity of exon 19 in the proband.

Together, these data indicate there is partial maternal isodisomy of chromosome 1 in the proband and that reduction to homozygosity of the 1q23 region containing the maternal *SPTA1* nonsense mutation is responsible for the nondominant HS phenotype.

Uniparental disomy (UPD) occurs when both copies of a chromosome or a segment of a chromosome are inherited from one parent.⁷ The degree of UPD can range from a small region of a single chromosome to UPD of entire chromosomes or to complex and segmental UPD of multiple chromosomes. UPD may be associated with heterodisomy-inheritance of a pair of non-identical chromosomal segments from one parent, or with isodisomy-inheritance of a pair of identical chromosomal segments, duplicated copies of a single parental allele.

In isodisomy, duplication of single alleles may lead to increased risk of reduction to homozygosity of deleterious

alleles, unmasking recessive mutations. In this case, maternal UPD of a region of chromosome 1 unmasked a deleterious *SPTA1* gene mutation resulting in the recessive spherocytosis phenotype. Unmasking recessive gene defects by reduction to homoallelism is the most common mechanism of UPD-associated disease.⁷

Other cases of inherited hemolytic anemia associated with UPD include pyruvate kinase deficiency, hemoglobin Bart's hydrops fetalis, and hereditary pyropoikilocytosis.⁸⁻¹¹ Acquired β -thalassemia major and sickle cell disease have been described in disease carriers with segmental UPD of the β -globin locus at 11p.¹²⁻¹⁵ In these cases, mosaic loss of heterozygosity at the β -globin locus due to 11p UPD has been implicated.¹⁴

Advances in genomic technologies are rapidly advancing our understanding of human disease. Studies like this report, the first to identify isodisomy as a pathogenetic mechanism of hereditary spherocytosis, demonstrate how utilization and integration of genomic-based datasets allow the localization and definition of critical events in disease pathogenesis. The synthesis of data obtained from complementary lines of investigation, i.e. clinical, laboratory, biochemical, genetic, and genomic, is now allowing us to unravel the complex mechanisms underlying hematologic disease. Integrative biology approaches hold great promise in dramatically enhancing our fundamental understanding of normal hematologic development, the environment and its influences, and disease pathogenesis and treatment.

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doi:10.3324/haematol.2014.110312

Key words: spectrin, membrane, spherocytosis, anemia, erythrocyte, uniparental disomy, isodisomy, chromosome 1q.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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