



Hereditary hyperferritinemia cataract syndrome: a *de novo* mutation in the iron responsive element of the L-ferritin gene

Sir,

Hereditary hyperferritinemia cataract syndrome (HHCS) is an autosomal dominant disorder characterized by high serum ferritin levels and early-onset of cataracts.¹ Molecular studies have shown the presence of mutations in the iron responsive element (IRE) sequence of the L-ferritin gene which compromise the iron-dependent regulation of mRNA translation leading to constitutive L-ferritin synthesis.²⁻⁵ We have studied a 21-year-old man with early-onset bilateral cataracts and marked hyperferritinemia (1138 µg/L) whose relatives have neither cataracts nor increased serum ferritin. Genomic DNA was isolated from peripheral leukocytes and the IRE L-ferritin region was amplified as described elsewhere;² the nucleotide sequence of both DNA strands was independently analyzed.

A mutation at the first position of the CAGUG loop (C39→T) was found in the heterozygous state in the proband⁶ while the other relatives had the wild-type sequence (Figure 1). The causal role of this mutation is supported by previous *in vitro* studies showing that this IRE-mutant has an 18-fold lower affinity for the iron regulatory protein than the native RNA.⁷ Family analysis of 14 microsatellite markers on 8 chromosomes showed that all alleles were

inherited in a Mendelian fashion consistent with the proband being the biological son of the parents. As far as we know, this is the first report of a *de novo* mutation in HHCS indicating that the disease should be searched for even in sporadic cases of early-onset cataract formation. Serum ferritin could be a simple screening marker of this disorder.

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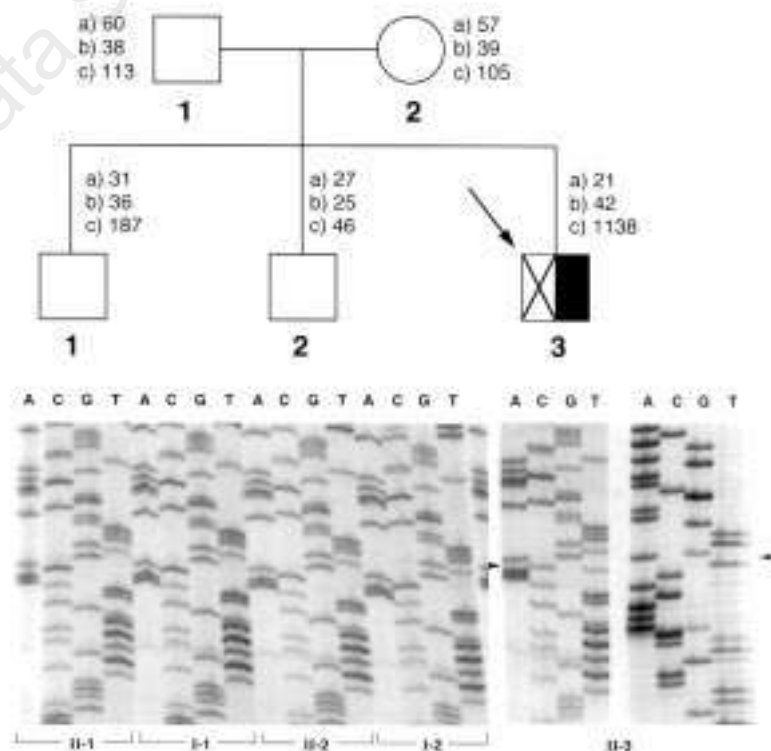


Figure 1. The family pedigree (above). The proband is indicated by the arrow. ■ and □ indicate the presence of hyperferritinemia and cataract, respectively. a) age (years), b) transferrin saturation (%) and c) serum ferritin (µg/L) at the time of our observation. Transferrin saturation and serum ferritin values were confirmed at subsequent analyses. Direct sequencing analysis of the region containing the IRE motif in each individual of the family (bottom). Arrows indicate the C39→T transition in the sense and antisense strands of the proband's DNA compared to the wild type sequence found in all the other members of the family.

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Enumeration of CD34⁺ cells in fresh and thawed/washed placental/umbilical cord blood units and thawed unit segments

Sir,

The current perspective of expanding the clinical use of human placental/umbilical cord blood (PB) for transplantation requires standardization of methods for its collection, processing, characterization and cryopreservation. In this regard, the flow cytometric enumeration of hematopoietic progenitor cells by detection of the CD34 antigen is a critical step. A number of assays to count CD34⁺ cells have been proposed. Most express the result as a percentage of total WBC or nucleated cells, which are usually counted with a hematology cell counter. This may be a cause of inaccuracy of the absolute CD34⁺ cell count, especially in thawed/washed samples.

Recently, new procedures which allow the determination of the absolute CD34⁺ cell count have been developed to overcome this problem. To our knowledge, limited information is available on the validity of these methods with fresh and frozen PB.

In this study we determined CD34⁺ cell counts in 10 routine PB units before and after thawing/washing,^{1,2} with three methods that do not require a separate

WBC count. We used, in parallel, the commercial IMAGN 2000 STELLer assay,³ (Biometric Imaging, Mountain View, CA, USA), the ProCOUNT system, (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) and our local flow-cytometric method.^{4,5} Our local method is a lyse and wash procedure in which, just before acquisition, a known number of Flow-Count™ fluorospheres (Coulter Corporation, Miami, FL, USA) are added to each sample in order to obtain the number of CD34⁺ cells/μL directly.

Moreover, we evaluated whether segments from the cryopreservation bag tube, which can be used for quality control before transplantation, allow an accurate estimation of the unit's CD34⁺ cell content and cell viability. The segments were stored in a pouch of the freezing bag and thawed at 37°C. Their content was slowly diluted with 10 mL of RPMI/25 percent fetal calf serum and centrifuged at 300 g x 10 min. The cell button was resuspended to 500 μL of RPMI/25 percent fetal calf serum and evaluated at the same time as the bag sample.

The CD34⁺ cell counts obtained with the three methods were compared with Friedman's test.⁶ *p* values below 0.05 were considered statistically significant. Furthermore, we performed Wilcoxon's test⁶ to determine whether, within each method, CD34⁺ cell counts and cell viability obtained in thawed/washed segments were different from those obtained in thawed/washed units.

Differences of CD34⁺ cell counts did not reach statistical significance when the methods were used on fresh PB samples. Conversely, differences between methods reached statistical significance when specimens from thawed/washed units and from segments were used (Table 1). Mean and SD cell viabilities of thawed/washed unit and segment specimens were 97±6 percent and 69±10 percent respectively. Cell viability and CD34⁺ cell counts computed from thawed/washed segment samples were significantly lower (*p*<0.05) than those found with each of the three methods in the corresponding thawed/washed units. These data suggest that the three methods pro-

Table 1. Median (and range) number of CD34⁺ cells/μL determined with 3 methods in 10 PB units before freezing (fresh units) and after freezing/thawing/washing (thawed units) and in 10 thawed/washed segments from the same units (thawed segments).

Method	STELLer	ProCOUNT	Local method	Friedman test
Fresh units	24 (8-81)	19 (4-83)	20 (4-81)	r = 0.95, p>0.05
Thawed units	20 (9-74)	18 (6-89)	15 (6-59)	r = 12.4, p<0.05
Thawed segments	11 (4-23)	4 (2-21)	5 (2-18)	r = 10.4, p<0.05