A Single Mutation Inside the NPA Motif of Aquaporin-1 Found in a Colton-Null Phenotype

To the Editor:

Major intrinsic protein (MIP) is a highly conserved superfamily of membrane proteins that regulate, in eukaryotes and prokaryotes, the transport of various small molecules. In all biological systems, water transport is a critical mechanism. Water crosses membranes by two pathways: either by diffusion or water-selective channels. Aquaporins (AQP) are members of the MIP superfamily that control osmolarity by limiting or promoting waterflow across cellular membranes. Nine distinct aquaporins have been cloned in mammals. Aquaporin monomer is a six-transmembrane domain protein of 28 kD. Both N- and C-terminal ends are located inside the cell.

The first water channel (AQP1) was discovered on the red blood cell membrane. AQP1 is also expressed in numerous tissues, particularly in kidney in proximal tubule and descending limb of Henle epithelia and in vasa recta endothelia. Disruption of the AQP1 expression in mice showed only a mild growth retardation, and these mice are lethargic. Studies of renal functions indicated that AQP1 is required for the formation of a concentrated urine. The Colton blood group antigens (Co/Co\(^b\)) represent a polymorphism on this protein resulting from an

754-bp DNA fragment including the 5' untranslated region, exons 1 and 2, as well as intron 1 and part of intron 2 was amplified by polymerase chain reaction (PCR). Amplification primers were based on the L-ferritin sequence published by Santoro et al\(^4\) (GenBank accession no. X03742). DNA fragments were subsequently cloned and more than 10 clones from each patient were sequenced by automated DNA sequencing. Sequence analysis identified a point mutation, C to T, at position 33, according to the numbering of Cazzola et al\(^5\), in a half of the analyzed clones of all patients as an average (GenBank accession no. AF117958). This nucleotide substitution is located at the consensus three nucleotide bulge structure, positions 31-33 (Fig 2).

Correlation between genotype and phenotype has been suggested for HHCS. Mutations placed at the conserved loop are associated to higher ferritin concentrations and more severe cataracts than those found in the three-nucleotide motif forming the IRE bulge. Furthermore, nucleotide changes located at the lower stem have been associated with asymptomatic cataract and serum ferritin levels in the range of 350 to 650 µg/L. Therefore, three nucleotide substitutions have been described at the IRE bulge motif. The mutation termed Pavia 1, G to A at position 32, was observed correlation between genotype and phenotype in HHCS and increase the genotypic diversity of this disease.

In conclusion, we have characterized a new mutation placed at the IRE sequence of the L-ferritin gene. This mutation is located at the bulge motif close to two different point substitutions previously described in three families that showed similar phenotypic characteristics to those found by us. These findings are in agreement with the observed correlation between genotype and phenotype in HHCS and increase the genotypic diversity of this disease.

Antonio Balas
Maria Jose Aviles
Felix Garcia-Sanchez
Jose L. Vicario

Histocompatibility and Molecular Biology Laboratory
Regional Transfusion Centre
Madrid, Spain

Paediatric Department
Mostoles General Hospital
Madrid, Spain

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Fig 2. Sequence and proposed structure of the ferritin L-subunit IRE mRNA. The mutation found in the 5' UGC bulge is indicated. Numbering is from the first transcribed nucleotide.
alanine to valine change at residue 45 in the extracellular loop A. The Colton-null or Co(a−b−) blood group corresponds to a lack of AQP1 expression. The frequency of this phenotype is extremely low and these patients appear clinically normal. Here we report a new example of a Co-null individual.

The patient (white female, born 1938) was first admitted in 1995 to the hospital with anemia (hemoglobin level, 7.8 g/dL) and rectal bleeding caused by hemorrhoids. Colon cancer was ruled out. She was not transfused and received only iron support. A potent antibody reacting with all cells from several panels and identified as anti-Co3 was discovered in her serum and she was shown to belong to the Co-null phenotype. In 1996, cararact surgery was performed. In February 1997, the patient was again admitted because of vaginal and rectal bleeding and a hemoglobin level of 4.2 g/dL. The patient was submitted to hemorrhoidectomy and hysterectomy with oophorectomy. A uterine high-grade uncommon sarcoma with ovarian metastasis was found. Although radiotherapy was performed, there was evolution of the gynecological disease and the patient had to be transfused with incompatible Co(a−b−) because of the shortage of Co-null blood. The direct antiglobulin test became positive and mild hemolytic reactions occurred. No renal impairment nor disseminated intravascular coagulation occurred. The patient died in December 1997.

The AQP1 cDNA from this patient was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) and the PCR product was directly sequenced. The patient was homozygous for the Coa allele (C at nucleotide 134). In addition, the comparison with the normal AQP1 cDNA sequence showed a homozygous C to A transition at nucleotide 614 (AQP1 sequence6: HUMCHIP28A from Genbank data). This mutation affects the codon 192, resulting in an N192K amino acid substitution (Fig 1A).

Fig 1. Identification and inheritance of the Co-null mutation. (A) Direct sequence analysis of normal AQP1 (top) and AQP1 Co-null (bottom) cDNAs displaying a mutation in the codon 192 of NPA motif. Primers used for PCR: 5'-TCGGGCCAGGGCTGGGCAT-3' (sense, nucleotides 441 to 460) and 5'-CTACAGTATGATTCCACCC-3' (anti-sense, 911 to 930). (B): Inheritance of the Co-null mutation in the propositus (arrow) family. Genomic DNA from blood cells of the different members of the Colton-null family were isolated and direct sequence analysis was performed with the primers: 5'-CCCTCCTCCTCTGTATTCTTCCC-3' (sense, intron B) and 5'-CTACAGTATG-GATTTCAACC-3' (anti-sense, 911 to 930 exon 4).
Inheritance of the Colton-null mutation was further assessed by direct PCR sequencing with genomic DNA from blood cells of two generations. This study confirmed the homozygoty of the propositus and indicated the heterozygoty of the kindred (Fig 1B). This novel mutation fits with Colton-null phenotype and should be essential for channel function: indeed, it occurred in the NPA (asparagine-proline-alanine) motif found in all MIP proteins. Two NPA motifs are found in each half of the protein; in the case of AQP1, the first one is found at residues 76-78 (cytoplasmic B loop) and the second at residues 192-194 (extracellular E loop) near cysteine 189, site of inhibition of osmotic water permeability by mercurials. It has been shown, in vitro, that conservative substitution inside the second NPA motif (N192D or N192Q amino acid substitutions) leads to reduced Pf, absence of oligomerization, and failure of the protein AQP1 to reach the membrane. An hourglass structural model mediated by these NPA motifs has been proposed by Agre. The presence of a mutation inside an NPA motif in a patient presenting a Colton-null phenotype shows the functional importance of this motif in vivo.

Stany Chrétién
Jean Pierre Cartron
INSERM U76 and INTS
Paris, France
Manuel de Figueiredo
Centro Hospitalar de Vila Nova de Gaia
Porto, Portugal

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Periodic Hematological Disorders

To the Editor:

In a recent issue of Blood, Haurie et al1 provided a detailed and historical review of cyclical neutropenia and other periodic phenomena in hematopoiesis. There are several points related to the review that need emphasis.

The feedback hypothesis as originally proposed postulated that hematopoiesis itself is an actual or potentially oscillatory system due to the presence of multiple and coupled negative feedback loops, each of which embodies a time delay and has nonlinear operating characteristics. This is essentially the conclusion drawn by Haurie et al. At the time it was presented, this hypothesis was a paradigm shift away from the concept that periodic phenomena in hematopoiesis were due to the action of external rhythms, such as hormonal rhythms. The feedback concept now appears to be taken as a given, because no other possibilities were even discussed in the article. However, while the feedback hypothesis can explain most oscillatory phenomena, it may not explain all. For example, the size of the peripheral lymphocyte pool and the length of lymphocyte life span are such that the oscillation that is occasionally seen in lymphocyte number is difficult to explain by the action of a feedback loop involving lymphocytes or by fluctuating input of lymphocytes from a cycling stem cell pool.

Stem cells may be involved in oscillatory phenomena in two ways, which are not mutually exclusive. Firstly, a stem cell defect may result in hematopoiesis moving into a region of actual or enhanced oscillation. Secondly, the stem cell compartment itself may be oscillatory. Both of these possibilities were postulated at the time that the feedback hypothesis was proposed and, contrary to a statement in the review, it was suggested that most cases of cyclical neutropenia result from a stem cell disorder. In my opinion, the distinction that is drawn between whether oscillation arises primarily as the result of a stem cell feedback loop or a peripheral feedback loop is artificial and hinders understanding. The system should be viewed as a whole, as a web of coupled feedback loops that interact with each other. Although a stem cell lesion is the most common disturbance in disease, oscillatory behavior may arise from disturbance at one of a number of points, as is shown by the situation in cyclical thrombocytopenia.

The control-systems aspects of hematopoiesis are very attractive to modellers. However, once the control-systems features of hematopoiesis are accepted, the ability to construct a model that shows oscillatory behavior, even if the model incorporates the latest advances in hematopoietic cell biology, really adds little new knowledge. Rather, the challenge to modellers would seem to be to provide detailed predictions for the input-output characteristics of the different parts of the various control systems so that these predictions can be tested by experimental hematologists and a truly quantitative description of hematopoiesis can emerge.

The oscillatory phenomena in chronic myeloid leukemia are occasionally of practical importance. If cycling is present but is not recognized, an increase of cytotoxic drug when the leukocyte count rises or a decrease when it falls may exacerbate the situation and be confusing.
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Stany Chrétien, Jean Pierre Cartron and Manuel de Figueiredo