Red blood cell phosphosphoglycerate mutase. Description of the first human BB isoenzyme mutation

Several human deficiencies have been described in phosphoglycerate mutase MM isoenzyme and in the related enzyme bisphosphoglycerate mutase. However, none had been previously detected in phosphoglycerate mutase BB isoenzyme, a ubiquitous enzyme form present in most mammalian cells, including red blood cells. In this work, an adult patient with phosphoglycerate mutase red blood cell BB isoenzyme deficiency was studied. By RT-PCR and sequencing analysis, we detected a point mutation (Guanine by Adenine) at position 690 which caused a substitution of methionine 230 by isoleucine. This mutation is located near the a-helix C-terminal end of the protein which is well conserved in all mammalian and yeast mutases and has been implicated in the reaction mechanism. Conformational studies showed that the mutation could produce destabilization of the helix and, as a consequence, a decrease in activity. This is the first report of a phosphoglycerate mutase BB isozyme deficiency.

Haematologica 2007; 88:(3)e30-e32

Phosphoglycerate mutase (PGAM, EC 5.4.2.1) is a glycolytic enzyme that catalyzes the interconversion of 3-phosphoglycerate and 2-phosphoglycerate. In vertebrates, the enzyme needs 2,3-bisphosphoglycerate (BPG) as a cofactor; the catalysis involves a phosphoenzyme as an intermediate and BPG is required to maintain the enzyme in its phosphorylated active form [1]. In mammals, three PGAM isozymes exist which result from the homo-and heterodimeric combinations of two subunits, M and B [2,3], coded by two different genes [4-7]. In adult mammals, skeletal muscle and mature sperm cells contain almost exclusively type PGAM-MM isozyme [2,3,8], whereas the type PGAM-BB isoenzyme is found in most other tissues and in red blood cells (RBC) [2,3,9]. Type PGAM-MB isoenzyme is present only in heart, a tissue which also contains PGAM-MM and PGAM-BB isoenzymes. Besides PGAM-BB, RBC contains both the bisphosphoglycerate mutase enzyme (BPGAM, EC 5.4.2.4) and the hybrid PGAM-BPGAM [10] which, in addition to the main BPG synthase activity, also possess BPG phosphatase and collateral PGAM activities. Moreover, the hybrid PGAM-BPGAM is also present in these cells [7].

Since 1964, several cases of BPGM deficiency have been described in human erythrocytes [11], but only one has been characterized at genetic level [12] and since 1981 several cases of type PGAM-MM deficiency in skeletal muscle have been reported [13], although only three different mutations have been detected [14]. However, to date, no type PGAM-BB deficiency has been found. Herein, we report the first case of PGAM-BB deficiency characterized at clinical and genetic levels.

A 34 year-old woman with moderate normocytic anaemia and markedly increased reticulocyte count ($300x10^9$/l) was referred to the Red Cell Pathology Unit (Hospital Clinic, University of Barcelona) for study. Physical examination showed only a mild scleral icterus with a tip of spleen but not liver enlargement. The patient reported dark urines on two different occasions. Red blood cell morphology revealed the existence of only few circulating spherocytes. Haemolytic tests performed demonstrated a negative direct antiglobulin test (DAT) with markedly decreased red blood cell osmotic fragility (before and after incubation) and the clinical diagnosis of Hereditary Spherocytosis (EH) was made. A battery of 18 RBC enzyme activities was also performed patient and a partial deficiency of about 50% of normal PGAM activity was found. The remaining enzyme activities were normal or slightly increased in accordance with the increased number of reticulocytes. Red cell PGAM deficiency was confirmed by repeating the test in three different patient's blood samples. Accordingy, coinheritance of a hereditary spherocytosis (HS) gene with the PGAM mutation was established.

Cell isolation and enzyme measurements

Venous blood samples from the propositus and 12 normal controls were filtered through a cellulose column to remove white cells and platelets [15]. RBC glycolytic and several non-glycolytic enzyme activities were measured in the hemolysates according to the methods recommended by the International Committee for Standarization in Haematology [16]. White blood cells were obtained from blood by centrifugation at 1000g at room temperature in a tube containing 3ml of Histopaque 1119 (Sigma).

Genetic analysis

RNA from controls and from the propositus was isolated from white cells using the TripureTM isolation reagent method (Boehringer Mannheim). RNA quantity and quality were checked spectrophotometrically and by agarose gel electrophoresis. The Retrotools cDNA/DNA Polymerase Kit (Biotools) two-step protocol was used for the RT-PCR amplification according to the manufacturer's instructions. The primers used were the following: primer 1 (sense): 5'ggtgccgcatccccagcccgcc3' and primer 2 (antisense): 5'aacaaagcactcgcaaaaac3'. Nested PCR was performed with primer 3 (sense): 5'ccgcatccccagcccgccgcca3' and primer 4 (antisense): 5'gaggggcagagggacaagac3'. The PCR product was purified with a "QIAquicTM" kit (Quiagen) following the manufacturer's instructions. The ABI Prism® BigDye TM Terminator Cycle Sequencing Ready Reaction kit was used for DNA sequencing reactions according to the manufacturer's manual. For the sequencing reactions, primers 3, 4, 5 (antisense): 5´gtatccttcagactctcaca3', 6: (sense): 5'cgaccatcctttctacagca3' and 7 (sense): 5´ tttctgggggatgaagagac3' were used. The restriction assay was performed with NlaIII (New England BioLabs Inc.) and electrophoresed through a 3.5% Sieve agarose and 1.5% agarose gel. One hundred healthy controls with normal red cell PGAM activity were screened by restriction assay to rule out the possibility that the mutation was a polymorphism; none presented the mutation.

PGAM modeling

The structure of the PGAM-B subunit was derived from the Saccharomices Cerevisae three-dimensional crystal structure [17] , the only one known to date. The homology between yeast and RBC PGAM was determined using the Blast and Fasta programs based on the sequences obtained from the Protein Data Bank. Graphic visualization was performed with the UNIX Turbo-frodo and Prepi programs and the C-terminal model was established with the Modeller and Turbo-frodo programs. The model was submitted to a refinament by energy minimization with the GROMOS program.

A partial deficiency of PGAM was found in a 34-year-old woman with moderate haemolytic anaemia due to HS. Since in some cases, the coexistence of HS with a

congenital red cell enzyme defect may increase the clinical manifestations of haemolysis [18], a battery of 18 different enzyme activities was also performed in this patient. Only PGAM activity was found to be reduced to around 50% of normal values whereas the remaining enzyme activities showed normal activity or slightly increased due to the reticulocytosis.

As the PGAM-B subunit gene has not yet been characterized, we performed RT-PCR from total RNA. Sequencing of the amplified products revealed a homozygous 690G-A transversion (Figure 1a) which leads to replacement of a methionine by an isoleucine at position 230 (Met230Ile). No other base changes were detected. Computer restriction analysis showed that several NlaIII restriction sites are present in the wild type PGAM-B cDNA, one of which affects the restiction site present at bases 687-693 (CATG to CATA). Restriction enzyme digestion revealed a lack of this restriction site in the propositus. Disruption of this NIaIII restriction enzyme recognition site confirmed the presence of the mutation in the propositus (Figure 1b). As the mutation was not found in any of the controls digested with NlaIII , the possibility of it being a polymorphism was ruled out.

Three mutations have been identified in patients with muscle PGAM-MM isoenzyme deficiency: a nonsense mutation and two missense mutations (Glu89Ala, Arg90Trp) which affect residues located in or near the active site [14]. A similar location had the only missense mutation (Arg89Cys) detected in patients with BPGAM deficiency [12]. A second mutation identified in these patients is a frameshift mutation caused by deletion of nucleotide C205 or C206 [11] The mutation detected by us in the PGAM-BB-deficient patient affects a residue far from the active site. However, it is located in a highly-conserved sequence (Figure 1c) that precedes the 14 residues of C-terminal region. In the yeast enzyme, this region, which adopts a mainly helical conformation, constitutes a flexible tail that has been implicated in the reaction mechanism, as confirmed by limited proteolysis experiments and mutational studies [17,19].

Bearing in mind the known three-dimensional structure of yeast PGAM subunit and considering the high degree of homology between yeast and mammalian PGAMs, we modeled and refined the structure of the human PGAM-B subunit using the programs abovementioned. As shown in Figure 2, the C-terminal region

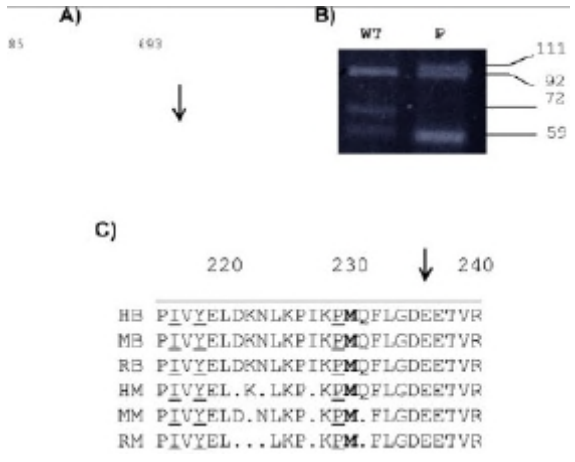

Figure 1.

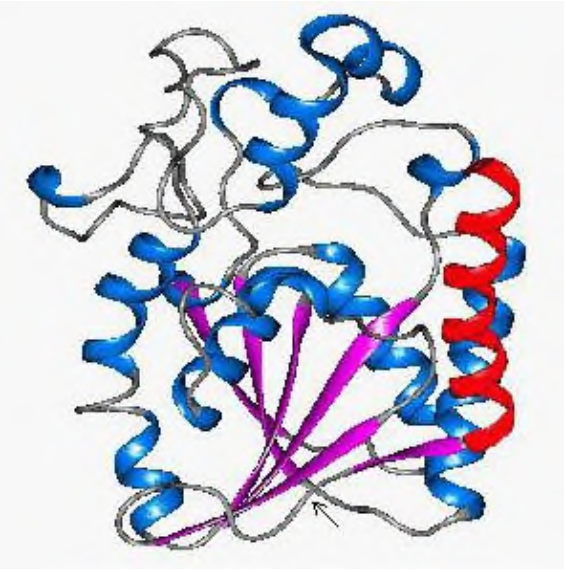

Figure 2.

of PGAM-B subunit could adopt a helical conformation which is similar to that of yeast enzyme [19]. The mutated methionine is located a few aminoacids before the start of the helix where it could interact with three residues (Ile-213, Tyr-215 and Phe-229). Substitution of methionine 230 by isoleucine would cause the disappearance of these interactions and, as a consequence, the functional properties of the C-terminal tail could be affected. Alternatively, a destabilization of PGAM conformation could result.

In conclusion, this work describes the first mutation found in PGAM-BB isoenzyme. The eventual influence of this enzymopathy on patient´s HS clinical manifestations remain unknown. Studies are underway to determine possible changes in thermal stability and kinetic parameters but since enzyme deficiency is only partial, it is dificult to accept a severe deleterious effect on red cell metabolism. The search of co-inheritance of HS with any other genetic defect of RBC seems of interest because it can improve our knowledge of clinical consequences of monogenic defects interactions. Unfortunately, patient´s family studies which could provide insight to individual HS and PGAM genes effect, were not available. However, we hope to be able to proceed to their analysis in the near future.

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