Molecular Immunology 90 (2017) 57–63

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

A founder mutation underlies a severe form of phosphoglutamase 3 (PGM3) deficiency in Tunisian patients

Leila Ben-Khemis[a,b], Najla Mekki[a,c], Imen Ben-Mustapha[a,c], Karen Rouault[d], Fethi Mellouli[e], Monia Khemiri[f], Mohamed Bejaoui[e], Leila Essaddam[g], Saayda Ben-Becher[g], Lamia Boughamoura[h], Saida Hassayoun[h], Meriem Ben-Ali[a,c,*], Mohamed-Ridha Barbouche[a,c]

[a] Laboratory of Transmission, Control and Immunobiology of Infections (LR11IPT02), Tunis, Tunisia
[b] University of Carthage, Sidi Bou Said, 1054 Carthage, Tunisia
[c] Université Tunis El Manar, 1068 Tunis, Tunisia
[d] Inserm, UMR 1078, Brest, France, Laboratoire de Génétique Moléculaire, CHRU Brest, Hôpital Morvan, Brest, France
[e] Department of Pediatrics, National Bone Marrow Transplantation Center, Tunis, Tunisia
[f] Department of Pediatrics, Bechir Hamza Children's Hospital, Tunis, Tunisia
[g] Department of Pediatrics PUC, Bechir Hamza, Children's Hospital, Tunis, Tunisia
[h] Department of Pediatrics, Farhat Hached Hospital, Sousse, Tunisia

ARTICLE INFO

Keywords:
PGM3 deficiency
Congenital disorder of glycosylation
Glu340del founder mutation
Consanguinity

ABSTRACT

Phosphoglucomutase 3 (PGM3) protein catalyzes the conversion of *N*-acetyl-D-glucosamine-6-phosphate (GlcNAc-6-P) to *N*-acetyl-D-glucosamine-1-phosphate (GlcNAc-1-P), which is required for the synthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) an important precursor for protein glycosylation. Mutations in *PGM3* gene have been recently shown to underlie a new congenital disorder of glycosylation often associated to elevated IgE. Herein, we report twelve PGM3 deficient patients. They belong to three highly consanguineous families, originating from a rural district in the west central Tunisia. The patient's clinical phenotype is characterized by severe respiratory and cutaneous infections as well as developmental delay and severe mental retardation. Fourteen patients died in early infancy before diagnosis supporting the severity of the clinical phenotype.

Laboratory findings revealed elevated IgE, CD4 lymphopenia and impaired T cell proliferation in most patients. Genetic analysis showed the presence, of a unique homozygous mutation (p.Glu340del) in *PGM3* gene leading to reduced PGM3 abundance. Segregating analysis using fifteen polymorphic markers overlapping *PGM3* gene showed that all patients inherited a common homozygous haplotype encompassing 10-Mb on chromosome 6. The founder mutational event was estimated to have occurred approximately 100 years ago.

To date, (p.Glu340del) mutation represents the first founder mutation identified in *PGM3* gene. These findings will facilitate the development of preventive approaches through genetic counselling and prenatal diagnosis in the affected families.

1. Introduction

Phosphoglutamase 3 (PGM3) deficiency, a congenital disorder of glycosylation (PGM3-CDG), is caused by hypomorphic mutations in *PGM3* gene. PGM3 protein, previously known as *N*-acetylglucosamine-phosphate mutase (AGM1), catalyzes the conversion of *N*-acetyl-D-glucosamine-6-phosphate (GlcNAc-6-P) into *N*-acetyl-D-glucosamine-1-phosphate (GlcNAc-1-P). The latter is required for the synthesis of Uridine Diphosphate *N*-Acetylglucosamine (UDP-GlcNAc), an important precursor for protein glycosylation (Freeze, 2013; Greig et al., 2007; Jaeken, 2012; Li et al., 2000; Pang et al., 2002). Indeed, UDP-GlcNAc is essential for N-linked and O-linked glycosylation, proteoglycan formation, glycosylphosphatidylinositol anchor synthesis and *O*-GlcNAc modification (Greig et al., 2007). Glycosylation defects have been associated with different immune disorders frequently showing neurologic impairment (Jaeken and Carchon, 2004; Scott et al., 2014). The lack of *Pgm3* is lethal in mice while hypomorphic and null alleles cause developmental and hematological defects (Greig et al., 2007).

In a previous study, we revealed that *PGM3* gene, segregated with the disease and followed a recessive mode of inheritance in two

* Corresponding author.
E-mail addresses: meriem.benali@pasteur.rns.tn, meriem_benali_nakti@yahoo.fr (M. Ben-Ali).

http://dx.doi.org/10.1016/j.molimm.2017.06.248
Received 8 April 2017; Received in revised form 18 June 2017; Accepted 28 June 2017

consanguineous Tunisian families with HIES-like phenotype. These patients had in addition a psychomotor retardation and developmental delay (Sassi et al., 2014). We identified two mutations that affect highly conserved amino acid residues, one substitution (p.Leu83Ser) and a single amino acid deletion (p.Glu340del). The latter, located in the sugar-binding domain of PGM3, blocks the formation of bi-antennary *N*-glycans and is associated with the most severe pathology as well as reduced PGM3 abundance (Sassi et al., 2014). Our results were supported by the study of Zhang et al., reporting three other *PGM3* mutations in eight patients presenting the same clinical phenotype (Zhang et al., 2014). Interestingly, Stray-Pedersen et al. reported three other PGM3 deficient patients with the classical features of congenital disorder of glycosylation (CGD), but with a severe combined immune deficiency (SCID). Only one patient had elevated IgE levels (Stray-Pedersen et al., 2014). Thus, PGM3 deficiency can be associated with a broad variety of symptoms which is not always associated with elevated serum IgE levels.

Almost all *PGM3* homozygous mutations have been identified in consanguineous families. All these families originated from the Middle East and the North African region (Tunisia, Morocco, Turkey, Egypt and Afghanistan) characterized by a high rate of consanguinity reaching 50% in some areas (Barbouche et al., 2011).

Herein, we report the first founder mutation in *PGM3* gene (p.Glu340del) in twelve Tunisian patients belonging to three consanguineous families originating from a rural district in west central Tunisia. Furthermore, we provide evidence that this mutation arose from a recent common ancestor.

2. Patients and methods

2.1. Patients

Twelve Tunisian patients were investigated and underwent clinical examination. They originated from the same rural area of the governorate of Kasserine located in west central Tunisia. Blood samples were collected for immunological and genetic investigation. Informed consent was obtained. A detailed history of all family members was obtained by personal interviews and pedigrees were constructed.

2.2. Proliferation assays

Peripheral blood mononuclear cells (PBMCs) (2×10^5 cells/well in 200 µl medium) were plated in triplicate in microtiter plates (Nunc, UK) and stimulated for 3 days with phytohemaglutinin (PHA; 5 µg/ml) or for 5–6 days with tuberculin purified protein derivative (PPD; 20 µg/ml) at 37 °C/5% CO_2. Cells were pulsed for 6 h with 1 µCi/well tritiated thymidine (Amersham, UK) and incorporated radioactivity was counted in a liquid scintillation counter.

2.3. TH17 and FOXP3$^+$CD4$^+$ T cells assessment

Standard flow cytometric methods were used for staining of cell surface markers. Freshly isolated PBMCs from patients and healthy control subjects were stained with APC-H7 conjugated anti-CD4 (BD Biosciences). TH17 cells were identified by means of intracellular staining of IL-17. Briefly, adherent monocytes were removed from the PBMC preparation by incubation 3 h at 37 °C, under an atmosphere containing 5% CO_2. 2.5×10^6 non-adherent cells were stimulated overnight with 40 ng/ml phorbol 12-myristate 13-acetate (Sigma-Aldrich) and 10^{-5} M ionomycin (Sigma-Aldrich) in the presence of a secretion inhibitor (1 µl/ml GolgiPlug BD Biosciences). After cell-surface staining of the CD4$^+$ T cells, cells were fixed, permeabilized (Cytofix/Cytoperm; BD Biosciences), and stained with PE conjugated mouse anti-IL-17A (BD Biosciences). As a control for cellular activation and intracellular staining, CD4$^+$ T cells were also evaluated IFN-γ production (Fluorescein isothiocyanate-conjugated anti-IFN-γ; BD Biosciences). CD4$^+$FOXP3$^+$ cells were identified by means of intracellular staining of CD4$^+$ T cells with PE conjugated mouse anti-FOXP3 (BD Biosciences). Stained cells were analyzed on a BD FACSCanto II Flow Cytometer and results were analyzed using Cell Quest Pro software (BD Biosciences).

2.4. Molecular analysis

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) or Epstein-Barr virus transformed lymphoblastoid B cell lines (EBV-LCLs) using the standard phenol-chloroform procedure. Cells were incubated in 5 ml of lysis buffer (100 mM NaCl, 10 mm Tris-HCL pH 7.4, 25 mM EDTA pH 8.0, 0.5% SDS) and incubated overnight at 37 °C in the presence of 20 µg/ml proteinase K. DNA was then extracted with phenol-chloroform, precipitated in ethanol, and suspended in TE 1X buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Polymerase chain reaction (PCR) amplification was carried out with Taq DNA Polymerase (Bioline). PGM3 Exon 8 was amplified by PCR using the following primers: Forward-5′AGATCAGGAGATACAGGCTTCTT3′ and Reverse-5′TGAGGGCAGTATCTTTCTCACA3′ under the following conditions: a denaturation step at 95 °C for 5 min followed by 35 cycles (95 °C, 30s/60 °C, 30s/72 °C, 1 min) and a final elongation step of 72 °C for 7 min. PCR products were purified using the EXO-SAP (Thermo Scientific) cleanup procedure and sequenced with the BigDye Terminator kit V3.1 (Applied Biosystems) using the PCR primers as sequencing primers. The sequencing was performed on an automated sequencer from Applied Biosystems (Applied Biosystems, Foster City, CA) and the data were analyzed with GENALYS software (CNG, France; http://software.cng.fr).

2.5. Western blot analysis

Total proteins were extracted from Epstein–Barr virus (EBV)-transformed B cell lines. 2×10^6 cells were harvested, washed briefly with ice-cold PBS and lysed in 200 µl 1× Laemmli buffer (12,5 mM TrisHCL, 4% glycerol, 0,4% SDS). Cell lysates were heated at 100 °C for 5 min, centrifuged at 12,000 rpm for 20 min and then supernatant was used for immunoblotting. Bicinchoninic Acid (BCA) Protein Assay (Sigma) was used for protein quantification. 25 µg of total protein per lane were separated on a 10% SDS–PAGE and electroblotted onto polyvinylidinedifluoride (PVDF) membranes (GE Healthcare Biosciences). The PVDF membrane was incubated in blocking buffer (TBS 1X, 0.4%Tween-20, 5% non-fat dry milk) for 1 h at room temperature then probed overnight at 4 °C with monoclonal rabbit anti-PGM3 (1:500, HPA029759, Sigma Aldrich), the membrane was washed three time (PBS 1×, 0.4% Tween-20) and incubated with the secondary HRP-conjugated (goat anti-rabbit IgG, 1:2000, #7074P2, Cell Signaling Technology). Rabbit anti-β-actin antibody (1:100, A2103, Sigma Aldrich) was used as an internal control probe. Protein detection was performed by enhanced chemiluminescent substrate kits: Pierce® ECL 2 Western Blotting Substrate (Thermo Scientific) for the detection of PGM3 and Pierce® ECL Western Blotting Substrate (Thermo Scientific) for the detection of β actin.

2.6. Haplotype analysis and mutation age estimation

Haplotype analysis was carried out using fifteen microsatellite markers, localized upstream (D6S257, D6S1619, D6S1596, D6S1589, D6S284, D6S460, D6S1707 and D6S1646) and downstream (D6S1609, D6S1627, D6S1652, D6S1595, D6S1613, D6S1570 and D6S1631) of the *PGM3* gene on the chromosome 6 and spanning a distance of approximately 35 Mb. Selection of the markers was performed using the UCSC Genome browser. Microsatellite markers were amplified by multiplex polymerase chain reaction (QIAGEN Multiplex PCR kit). One of the primers was fluorescently labelled with 6FAM or JOE markers. Capillary electrophoresis and pattern visualization was performed using an automated sequencer from Applied Biosystems (Applied Biosystems,