# Carbohydrate-deficient Glycoprotein Syndrome Type Ib

## Phosphomannose Isomerase Deficiency and Mannose Therapy

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## Abstract

**Phosphomannose isomerase (PMI) deficiency is the cause of a new type of carbohydrate-deficient glycoprotein syndrome (CDGS). The disorder is caused by mutations in the PMI1 gene. The clinical phenotype is characterized by protein-losing enteropathy, while neurological manifestations prevailing in other types of CDGS are absent. Using standard diagnostic procedures, the disorder is indistinguishable from CDGS type Ia (phosphomannomutase deficiency). Daily oral mannose administration is a successful therapy for this new type of CDG syndrome classified as CDGS type Ib. (*J. Clin. Invest.* 1998. 101:1414–1420.) Key words: CDG syndrome • phosphomannose isomerase • protein losing enteropathy • thrombosis • mannose**

## Introduction

Carbohydrate-deficient glycoprotein syndromes (CDGS)[1] are a group of hereditary multisystem disorders first recognized by Jaeken et al. (1). The clinical phenotype of all known CDG syndromes is dominated by severe psychomotor and mental retardation, as well as blood coagulation abnormalities presenting as thrombosis, bleedings, or stroke-like episodes.

The characteristic biochemical abnormality of CDG syndromes is the hypoglycosylation of glycoproteins. Depending on the type of CDGS, the carbohydrate side chains of glycoproteins are either truncated or completely missing from the protein core. The hypoglycosylation is routinely determined by isoelectric focusing (IEF) of serum transferrin. Affected glycoproteins have altered isoelectric focusing patterns compared with normal due to missing or truncated sugar chains. Based on the IEF patterns and the clinical symptoms, four different types have been classified so far (2–5). The most common form, CDGS type Ia, is caused by phosphomannomutase (PMM) deficiency (6).

In this paper, we describe the identification of a new type of CDG syndrome (CDGS type Ib). The clinical phenotype of the new disorder is fundamentally different from all other types of CDGS: no psychomotor or mental retardation is present. Instead, CDGS type Ib presents as a gastrointestinal disorder characterized by protein-losing enteropathy. Thrombosis as well as life-threatening bleeding can occur. We identified a deficiency of phosphomannose isomerase (PMI), a key enzyme in the metabolism of mannose, as the cause of the syndrome, analyzed the PMI1 gene for mutations and developed an effective therapy of oral administration of mannose. Mannose treatment corrected the clinical phenotype as well as the hypoglycosylation of serum glycoproteins.

## Methods

*Immunoprecipitation and SDS-PAGE.* Serum transferrin was precipitated in MNT buffer (20 mM 2-[*N*-morpholino]ethane sulfonic acid, 100 mM NaCl, 30 mM Tris-HCl, pH 7.5) including 1 mg/ml albumin, 1 mM Fe-(III)-citrate, protein A sepharose (10% wt/vol), protease inhibitors (10 μg/ml each of chymostatin, leupeptin, antipain, and pepstatin), and polyclonal rabbit anti–human transferrin IgG (A061; DAKOPATTS, Copenhagen, Denmark). Samples were rotated over night at 4°C, washed once with 0.05% TX-100, 0.1% SDS, 0.3 M NaCl, 0.02% $NaN_3$, 10 mM Tris, pH 8.6, and twice with MNT buffer. Concentrated sample buffer for SDS-PAGE was added to

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Primary authors R. Niehues and M. Hasilik made equal contributions to this paper. Secondary authors G. Alton and C. Körner contributed equally. H.K. Harms and K. Reiter took care of the patient, initiated the mannose therapy, and were instrumental in the cooperation with the other groups. M. Hasilik, R. Niehues, and T. Marquardt controlled mannose therapy by determination of the serum mannose levels, characterized the changes in the IEF, SDS-PAGE, and 2-dimensional patterns, and found the deficiency of phosphomannose isomerase activity. M. Schiebe-Sukumar and H.G. Koch found the Arg219 mutation and the imbalance of maternal and paternal alleles. K.P. Zimmer characterized the ultrastructural abnormalities. C. Körner and K. von Figura determined the mannose incorporation into glycoproteins and glycosylation intermediates. G. Alton and H. Freeze determined the PMI kinetic parameters, confirmed its deficiency in cells, analyzed the oligosaccharide structures, and, with R. Wu, expressed the Arg219 mutation in COS cells.

While this work was in progress, we learned that at the same time other investigators also had found a deficiency of phosphomannose isomerase in fibroblasts of four patients with CDG syndrome type I, including the patient described in the present study (Jaeken, J., G. Matthijs, J.M. Saudubray, C. Dionisi-Vici, E. Bertini, P. de Lonlay, H. Henri, H. Carchon, E. Schollen and E. Van Schaftingen, manuscript in preparation).

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1. *Abbreviations used in this paper:* 2D, two dimensional; CDGS, carbohydrate-deficient glycoprotein syndrome; IEF, isoelectric focusing; PMI, phosphomannose isomerase; PMM, phosphomannomutase.

each sample. Immunoprecipitated transferrin was run at 150 V on nonreducing 7.5% SDS-polyacrylamide minigels in an electrophoresis chamber (SE-250 Mighty Small II; Hoefer Scientific Instruments, San Francisco, CA). The gels were stained with Coomassie blue.

*Isoelectric focusing.* Iron-saturated serum was separated on 1% agarose gels on a Phast-System (Pharmacia Fine Chemicals, Uppsala, Sweden). Agarose gels consisting of agarose IEF and Ampholines with a pH range of 5.0–7.0 (Pharmacia Fine Chemicals) were cast on Gel-Fix for agarose plastic sheets (Serva Biochemicals, Paramus, NJ). To visualize the focused transferrin isoforms, rabbit anti–human transferrin IgG was applied directly to the gel for 15 min to precipitate transferrin isoforms inside the gel. Proteins not bound by the antibody were washed out and the gels were stained with Coomassie blue.

*Two-dimensional electrophoresis.* Two-dimensional (2D) protein separation was based on the immobiline system (Pharmacia Fine Chemicals). 75-μg proteins from serum samples were solubilized in a 500-μl solution containing 8 M urea, 4% CHAPS (3-[(3-cholamidepropyl)dimethylammonio]-1-propanesulfonate), 65 mM dithioerythritol, and 0.8% ampholytes (Pharmacia Fine Chemicals). Linear immobilized pH gradient (IPG) gels, pH 4–7, were reswollen overnight in the sample solution. Focusing was carried out for a total of 22,000 Vh. The strips were equilibrated for 15 min in 30% (vol/vol) glycerol, 2% SDS (wt/vol), 0.25% (wt/vol) DTT, and 50 mM Tris HCl, pH 6.8. A second equilibration step was performed in 30% (vol/vol) glycerol, 2% SDS (wt/vol), 25 mM iodoacetamide, 6 M urea, and 50 mM Tris-HCl, pH 6.8. The IPG gel strips were then applied onto a 12.5% SDS-Page (SE-250 Mighty Small II). The gels were stained with an ammoniac silver solution.

*Mannose measurements.* Serum mannose concentrations were measured enzymatically following a procedure described by Etchison and Freeze (7).

*Electron microscopy.* Thin frozen sections of a small bowel biopsy from the patient were labeled with a rat monoclonal antibody for BiP or polyclonal rabbit antibodies for sucrase-isomaltase, lactase, LAMP-1, and LAMP-2 as described elsewhere (8). Goat anti–rat or anti–rabbit antibodies conjugated with 12 nm gold particles were used as secondary antibodies. Epon sections of liver and small bowel tissue samples obtained from the patient were processed by standard techniques.

*Tissue culture and cell preparation.* Fibroblasts were grown in MEM supplemented with 10% fetal calf serum, 2 mM glutamine, 100 μg/ml penicillin, and streptomycin. In some experiments, the fetal calf serum was dialyzed to remove free mannose. The cells were washed with PBS and trypsinized. Trypsin was inactivated by addition of medium. The fibroblasts were pelleted and washed in PBS several times before they were suspended in 200 μl 0.9% NaCl and stored at −80°C.

*Phosphomannomutase assay.* PMM activity was assayed according to a procedure by van Schaftingen and Jaeken (6). The assay was modified by adding glucose-1,6-bisphosphate (100 μM) instead of mannose-1,6-bisphosphate as a cofactor (K. Paneerselvam, personal communication).

*PMI assay.* Fibroblasts were freshly prepared or stored at −80°C. Before the assay, the cells were lysed by sonication. 40 μg protein was used for the assay. PMI activity was determined in a buffer system containing 40 mM Tris-HCl, pH 7.4, 6 mM $MgCl_2$, 5 mM $Na_2HPO_4$/$KH_2PO_4$, 1 mM NADP, 100 mU phosphoglucose isomerase, and 500 mU glucose 6-phosphate dehydrogenase (G6PDH). The enzymatic reaction was initiated by the addition of mannose 6-phosphate (1 mM final concentration). The samples were incubated at room temperature and the OD at 340 nm was measured during the next 2 h (modified from reference 9).

*Molecular genetics.* The sequence of the human PMI cDNA (10) was used to design primers for PCR amplification. Total RNA was prepared from cultured fibroblasts using the RNeasy extraction procedure (Quiagen, Hilden, Germany). First strand cDNA was synthesized using AMV reverse transcriptase and oligo $p(dT)_{15}$ priming (Boehringer Mannheim, Mannheim, Germany). The nearly full length PMI coding region was PCR amplified using PMI-8F (5′-CTC-

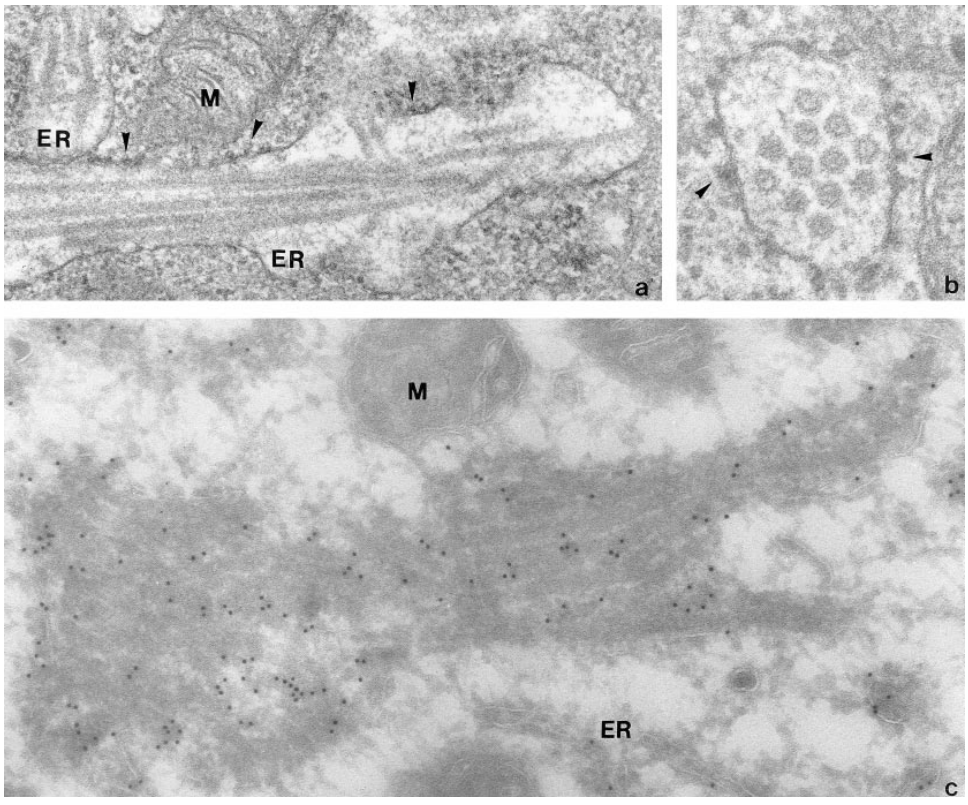


*Figure 1.* Electron microscopy. (*a*) Epon sections of the small bowel showed an enlarged rough endoplasmic reticulum (*ER*) with long tubular bundles inside. Ribosomes are present on the outside of the organelle (*arrowhead*). M, mitochondrium. 75,000×. (*b*) Cross section of the bundles within the ER. Arrows, ribosomes. 150,000×. (*c*) Thin-frozen section labeled by an antibody for BiP, a resident ER chaperone. The tubular bundles were surrounded by gold particles indicating binding sites of the antibody. The bundles might represent hypoglycosylated protein precipitates. They were not labeled by antibodies for major proteins synthesized by enterocytes (sucrase-isomaltase, lactase), nor by antibodies for heavily glycosylated lysosomal membrane proteins (LAMP-1, LAMP-2) (not shown). M, mitochondrium. 85,000×.

CGCGAGTATTCCCACTT; positions 8–27) and PMI-1321R (5′-CCCGAGGAGGTGAGGTTG; positions 1321–1338). Cycling conditions: (*a*) 94°C, 4 min (hot start). (*b*) 94°C, 1 min; 57°C, 1 min; 72°C, 1 min (10 cycles). (*c*) 94°C, 1 min; 68°C, 1 min (25 cycles). (*d*) 72°C, 10 min. The 1314-bp product was subsequently cloned into the pCR2.1 vector (Invitrogen Corp., De Schelp, The Netherlands) and sequenced using a DNA sequencer (LI-COR, Lincoln, NB).

*Transient transfection.* $2 \times 10^6$ COS-7 cells grown in DMEM medium with 10% FCS were transfected with 10 μg pcDNA3 plasmids by lipofectamin (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's instructions. The plasmids contained no insert, the normal human PMI coding sequence, or the $R_{219} \rightarrow Q$ PMI allele from the patient. The cells were harvested 48 h later, washed three times with cold PBS, and scraped into 50 μl of 50-mM Tris-HCl, pH 7.4, 250 mM sucrose for measurements of specific PMI activity. Equal transfection efficiency of each insert was determined by quantitative PCR using PMI-specific primers.

## Results

*Clinical phenotype.* The boy was born at term with a normal birth weight. Clinical symptoms began at the age of 11 mo with diarrhoea and vomiting. The leading symptom in the following years was protein-losing enteropathy. Ultrastructural analysis of a small bowel biopsy showed lysosomal inclusion bodies as well as a dilated rough endoplasmic reticulum filled with prominent tubular bundles (Fig. 1). In addition to protein-losing enteropathy, recurrent thrombotic events occured at variable locations. Antithrombotic prophylaxis with vitamin K antagonists was started when thrombosis in both legs occurred by the end of the fifth year of life. Within the next months, several episodes of severe life-threatening gastrointestinal bleedings of diffuse origin appeared that continued even after the prophylaxis was stopped.

Laboratory diagnostics repeatedly revealed severe hypoproteinemia, anemia, and markedly reduced antithrombin III (AT III) levels (10–30%). Low AT III levels initiated CDGS diagnostics (11). The IEF of serum transferrin, the standard diagnostic procedure for CDGS, showed the characteristic hypoglycosylation pattern of CDGS type Ia. The diagnosis of CDG syndrome, however, was considered unlikely as all known types of CDG syndrome show psychomotor and mental retardation, and these were absent in our patient. Further clinical details will be described elsewhere (Harms, H.K., K. Reiter, K.-P. Zimmer, K. Auberger, R.M. Bertele-Harms, S. Weidinger, H. Freeze, L. Niehues, M. Hasilik and T. Marquardt, manuscript in preparation).

*Carbohydrate structure of glycoproteins.* Since CDG syndrome seemed an unlikely diagnosis with regard to the clinical phenotype, a more detailed serum glycoprotein analysis was performed. The IEF pattern of serum transferrin of the patient was identical to the pattern observed in CDGS type Ia (Fig. 2 *A*, lane *2*). In CDGS type Ia, complete carbohydrate side chains are absent from the protein core. Since IEF analysis gives no information on the size of the oligosaccharides, SDS-PAGE was also used. In SDS-PAGE, even small truncations of the carbohydrate side chains of glycoproteins can be detected (12). The SDS-PAGE banding pattern of the patient was identical to CDGS type Ia (Fig. 2 *B*, lane *6*). It was concluded that the patient's transferrin lacked most or all of the complete carbohydrate chains.

The carbohydrate structures released from the glycoproteins of the patient's fibroblasts were analyzed in detail. In CDGS type Ia, a portion of the carbohydrates released from lipid-linked oligosaccharide precursors and newly synthesized glycoproteins are truncated, but subsequent oligosaccharide processing occurs normally (13–15). In the patient, oligosaccharides released by mild acid hydrolysis from lipid-linked precursors or by PNGase F from newly synthesized glycoproteins were identical to controls based on Concanavalin A-Sepharose lectin affinity chromatography, ion-exchange chromatography, or amine adsorption HPLC analysis (see Methods in references 16 and 17). No structural abnormality of the carbohydrates was detected.

*Mannose incorporation.* CDGS type Ia is a disorder of mannose metabolism. Mannose incorporation into newly synthesized glycoproteins is considerably decreased (13–15). When fibroblasts of the patient were labeled with [2-$^3$H]-mannose, the incorporation of mannose into newly synthesized glycoproteins was 4.4× the incorporation found in controls and 25.9× the incorporation found in CDGS type Ia cells. In addition, incorporation of the label into mannose derivatives used for the synthesis of the oligosaccharide precursor was also considerably higher than normal (Table I). One explanation for increased labeled mannose was that the size of the intracellular

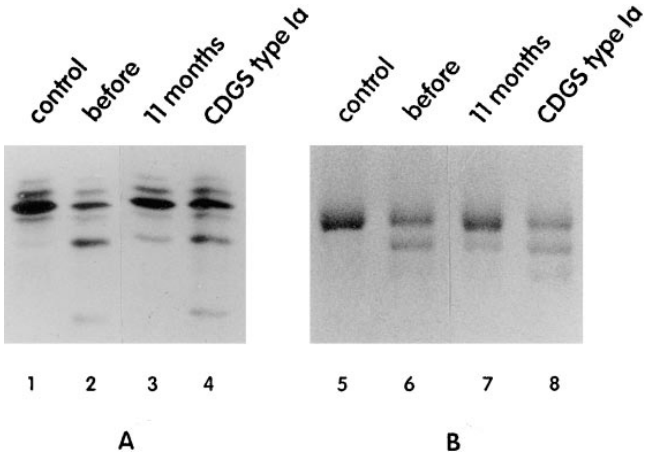


*Figure 2.* IEF and SDS-PAGE. Serum transferrin was analyzed by IEF (*A*) and SDS-PAGE (*B*) before mannose therapy was started. 11 mo later, the serum was reanalyzed to detect changes in transferrin glycosylation.

*Table I. Mannose Incorporation*

| | $^3$H-Man/$^{35}$S | Man 6-P | Man 1-P | GDP-Man and GDP-Fuc | Dol-P-Man |
|---|---|---|---|---|---|
| Control | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| CDGS type Ia | 17.2 | 133.1 | 36.7 | 16.3 | 12.2 |
| CDGS type Ib | 444.7 | 473.7 | 461.2 | 504.2 | 1447.1 |

Fibroblasts were labeled for 30 min with 20 μCi 2-$^3$H-mannose and 2 μCi $^{35}$S-methionine. Incorporation of the label into total protein and metabolites was determined as described earlier (17). All numbers are the mean of two experiments normalized per milligram protein and are given in percentage of normal. CDGS type Ib, patient; Man, mannose; Fuc, fucose; Dol, dolichol.

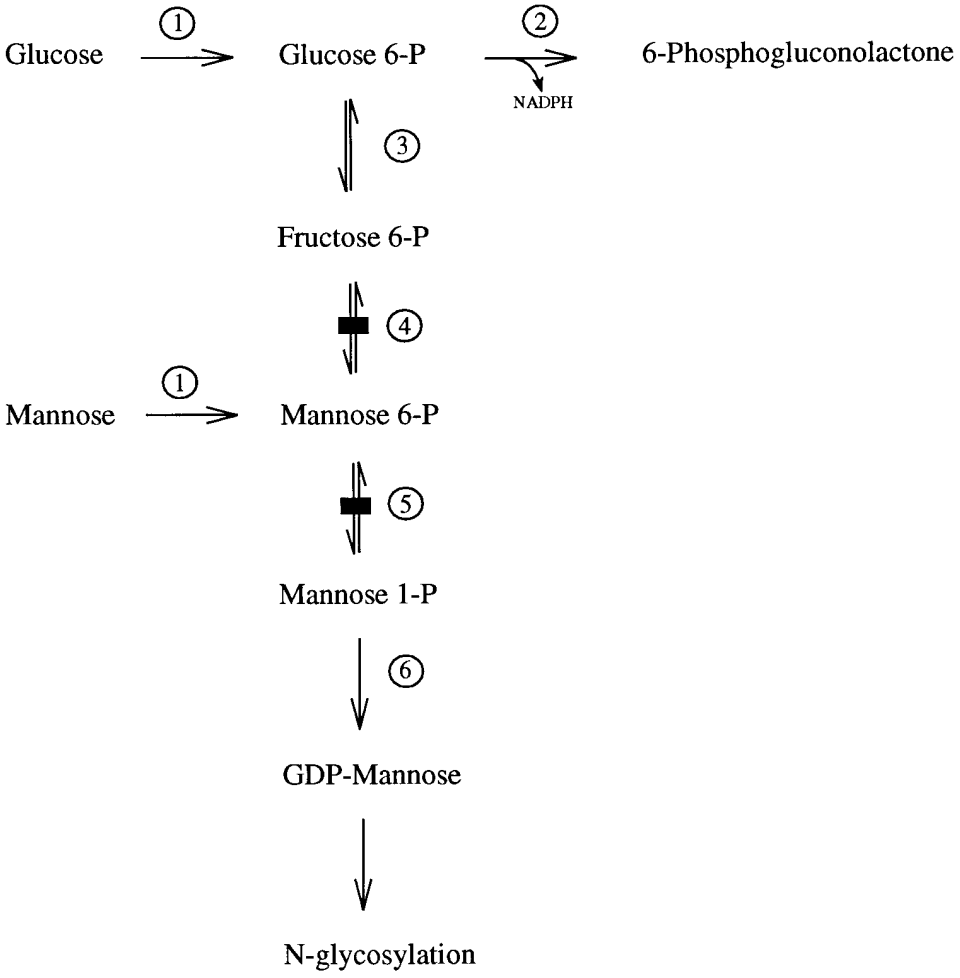


*Figure 3.* Metabolic pathways of mannose and principle of the enzymatic assay. (*1*) Hexokinase; (*2*) glucose 6-phosphate dehydrogenase; (*3*) phosphoglucose isomerase; (*4*) phosphomannose isomerase; (*5*) phosphomannomutase; (*6*) GDP-mannose synthase. In CDGS type Ib, mannose can bypass the enzymatic defect and contribute to the GDP-mannose pool that serves as the mannose donor for N-glycosylation. Horizontal bars indicate the enzymatic defects in CDGS types Ia and Ib.

pools was decreased, thus increasing the specific activity of those pools. A possible defect could be in the metabolic pathway providing mannose 6-phosphate from glucose (Fig. 3).

*Enzymatic defect.* A deficiency of phosphomannose isomerase was identified as the specific enzymatic defect. The specific activity of PMI in the patient's fibroblasts was reduced to 7.4% of normal (Fig. 4). In leukocytes, the PMI activity was 3.3% of normal. In mixed lysates from the patient and a control, the activities gave the expected sums. The father and mother of the patient had PMI activities of 53.0 and 33.7%, respectively, in their leukocytes, indicating their heterozygous status (Table II). The PMI activity in the healthy brother was 47%.

*Table II. PMI Activity in Fibroblasts and Leukocytes*

| | Fibroblasts | | | Leukocytes | | |
|---|---|---|---|---|---|---|
| | Range | Mean | Percent | Range | Mean | Percent |
| Controls | 14.7–34.7 | 21.9 | 100.0 | 16.6–20.4 | 18.1 | 100.0 |
| Patient | 1.4–1.8 | 1.6 | 7.4 | 0.5–0.6 | 0.6 | 3.3 |
| Father | | | | 7.7–11.5 | 9.6 | 53.0 |
| Mother | | | | 6.0–6.2 | 6.1 | 33.7 |

PMI activity from isolated blood leukocytes as well as from cultured fibroblasts was determined. Activities are given in nanomoles NADPH per milligram protein per minute; $n = 12$ for normal fibroblasts.

The in vitro results were supported by data obtained by labeling fibroblasts with [2-$^3$H]-mannose. This precursor is either used directly for N-glycosylation or catabolized to $^3$HOH via PMI. Intact CDGS type Ib cells catabolized only 15% of transported [2-$^3$H]-mannose to $^3$HOH, compared with 90% in controls (Table III).

When the PMI activity in fibroblast lysates was analyzed in more detail, it was found that the PMI had a 16-fold lower $V_{max}$ and the same $K_m$ for mannose 6-phosphate compared with controls (Table III). These data indicated that the catalytic competency of the enzyme was probably not affected. Instead, a reduced amount of PMI due to decreased transcription, translation, or protein instability probably accounts for these data. This is also supported by the finding that the patient's PMI activity was sensitive to freezing while that from control cells was stable.

*Molecular defect.* The sequence of human cDNA PMI (10) was used to design primers for PCR amplification. Reverse transcription–PCR-amplified PMI from mRNA from control fibroblasts and hepatoma cells, or from the patient and his parents, were sequenced. In the patient, a nucleotide exchange at the position 656 was found (G656 → A656). The nucleotide exchange caused an amino acid substitution from a basic to a neutral amino acid residue (Arg219 → Gln219). Homology

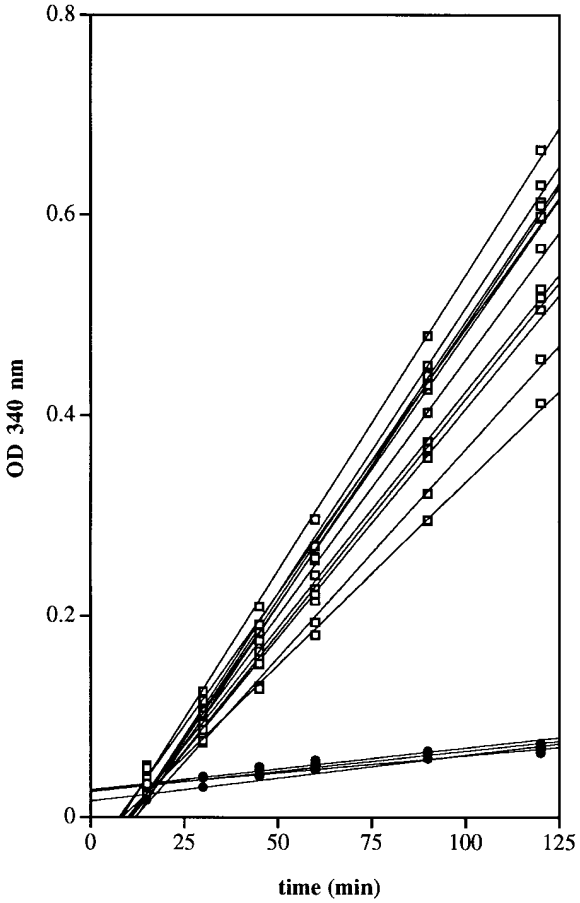


*Figure 4.* PMI assay. Fibroblast cell lysates were assayed for PMI activity as described. CDGS type Ib cell lysates (●) had 7.4% of the PMI activity found in controls (□) (controls: 14.7–34.7, mean 21.9; patient: 1.40–1.82, mean 1.63 nmol NADPH/mg protein per min). PMM activity in the patient was within the normal range (5.15; normal 2.36–6.18, mean 3.81; CDGS type Ia: 0.26–0.71, mean 0.40 nmol NADPH/mg protein per min). The same was true for phosphoglucose isomerase and hexokinase activities (not shown).

modeling of human PMI onto the crystal structure of *Candida albicans* PMI (18) showed that residue 219 is located on an α-helix at the surface of the molecule. Arg219 of this enzyme is conserved in *C. albicans*, *Caenorhabditus elegans* (10), and mouse (Wu and Freeze, unpublished results). To test whether the Arg219 mutation affected the enzyme activity, normal and

*Table III. In Vivo and In Vitro PMI Activity in Fibroblasts*

| | Percentage of transported $^3$H-Man converted to $^3$HOH | $K_m$ | $V_{max}$ |
|---|---|---|---|
| | | *μM* | *nM/min per mg* |
| Control | 90 | 250 | 8.0 |
| CDGS type Ib | 15 | 247 | 0.5 |

Cells were labeled with 20 μCi/ml of 2-$^3$H-mannose in medium containing 5 mM glucose for various times. The amount of label incorporated into cell-associated material and $^3$HOH was measured as described (16). $K_m$ and $V_{max}$ were determined in vitro as described (16).

*Table IV. PMI Activity in Transfected COS Cells*

| | Untransfected | pCDNA3 | PMI(R219)-pCDNA3 | PMI(Q219)-pCDNA3 |
|---|---|---|---|---|
| Activity | 16 | 7 | 150 | 9 |

COS cells were transfected with expression vectors containing no insert (pCDNA3), the wild-type PMI cDNA (PMI(R219)-pCDNA3), or the Gln219 mutation (PMI(Q219)-pCDNA3). PMI activities in the transfected cells were determined and are expressed in nanomoles NADPH per milligram protein per minute.

mutant cDNA in pcDNA3 were expressed in COS-7 cells. The PMI activity of cells expressing the mutant allele was undistinguishable from background while expression of normal PMI increased the specific activity ~10-fold compared with controls (Table IV).

The mutation at position 656 generated a unique BglII restriction site that gave two fragments of approximately equal size. When the patient's cDNA was cut with BglII, uncut cDNA as well as the two fragments were found, indicating the Arg219 mutation in one of the two alleles (not shown). Digestions of the father's cDNA gave the same result, whereas the mother's cDNA was not cut by the enzyme. In conclusion, the patient was heterozygous for the Arg219 mutation and this mutation was inherited from the father. PMI cDNA from the mother was sequenced and no mutation was detected. The cDNA gave the expected size so that no splice-site mutation could be present.

In the patient, a considerable disequilibrium of paternal and maternal alleles was found. The paternal Arg219 mutation was found in 22 of 24 clones. Assuming an equal expression of the maternal and paternal alleles, it is highly unlikely that this distribution would occur by chance (probability 0.0018%). Although no mutation was found in the maternal allele at the cDNA level, her heterozygous level of PMI activity and the unequal distribution of paternal and maternal transcripts could indicate a reduced transcription of the maternal allele or reduced stability of the transcripts.

*Therapy.* PMI deficiency causes a decreased synthesis of mannose 6-phosphate from glucose (Fig. 3). In this situation, mannose 6-phosphate can only be synthesized from external mannose originating from the diet or from glycoconjugate breakdown. Apparently, these pathways did not supply sufficient mannose. However, mammalian cells have mannose-specific transporters that under normal conditions provide the majority of mannose used for N-glycosylation (16). At the physiological mannose concentration in the blood (mean 55 μM) (7), this transporter operates at about half maximal rate. Therefore, increasing the extracellular mannose concentration should increase the amount of transported mannose available for N-glycosylation.

When the boy was 6 yr of age, recurrent severe gastrointestinal bleedings that were refractory to medical and surgical treatment caused a life-threatening condition. At this time, the PMI deficiency was unknown. Based on studies showing that mannose could correct abnormal glycosylation in CDGS type Ia fibroblasts (15), mannose therapy was initiated and followed up for 11 mo. In accordance with previous human ingestion studies (19), mannose was given orally in an initial dose of 100 mg/kg body wt three times a day. 8 mo later, the dose was

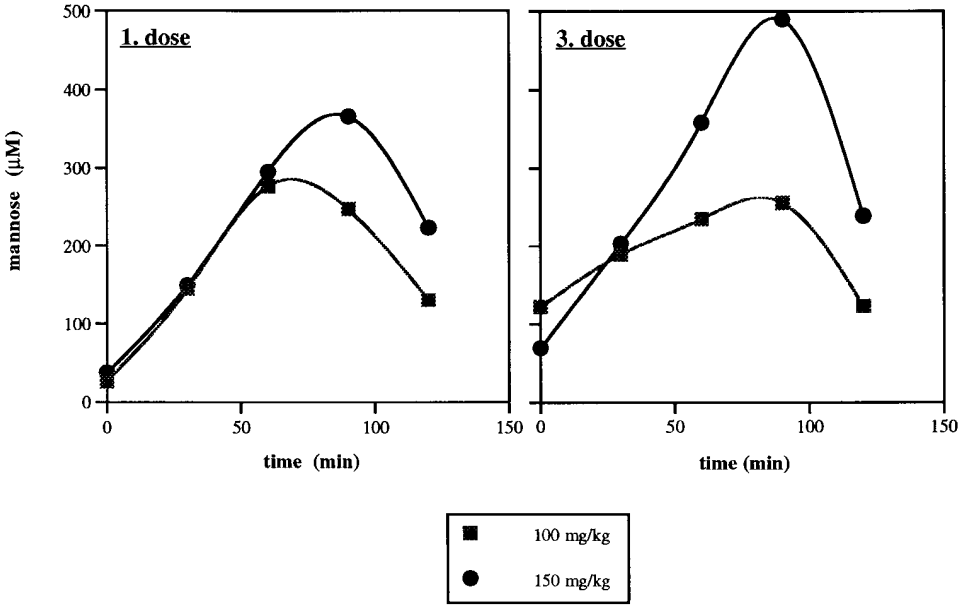


*Figure 5.* Serum mannose levels. Mannose was given orally five times a day at 100 or 150 mg/kg. Shown are the serum mannose levels after the first and third dose of the day. Serum mannose levels in the patient before mannose therapy were below normal (11–37 μM; normal 45–65 μM; reference 19).

increased to 150 mg/kg five times a day. Serum mannose levels reached a maximum of 490 μM 90 min after oral ingestion of 150 mg mannose per kg body wt (Fig. 5). Gastrointestinal bleeding and the chronic diarrhoea disappeared within the first few weeks and did not reappear. In the past, severe bleedings could not be stopped by medical treatment and surgery was necessary on several occasions. Total serum protein and antithrombin III rose to normal levels and stayed in the normal range without further changes in the dosing regimen. No side effects were observed.

After the initiation of mannose therapy, serum transferrin was reanalyzed several times by IEF, SDS-PAGE, and 2D electrophoresis. After the first 6 mo with a dose of three times daily 100 mg/kg, a partial correction in the IEF and SDS-PAGE patterns of serum transferrin was observed. The mannose dose was increased to achieve a more complete correction, and 11 mo after the initiation of therapy, a profound decrease of the abnormal isoforms and a shift towards a normal pattern were observed (Fig. 2 *A*, lanes *2–3*). The same result was seen in SDS-PAGE (Fig. 2 *B*, lanes *6–7*). 2D electrophoresis demonstrated that the improvement in the glycosylation of affected proteins was not restricted to transferrin (Fig. 6).

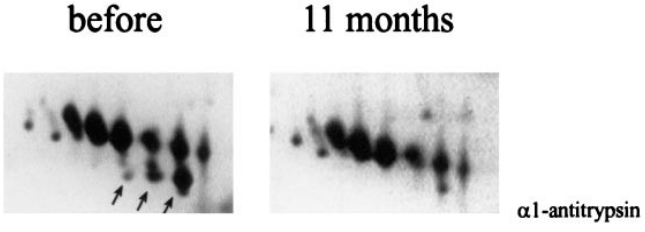


*Figure 6.* 2D gel electrophoresis. Total serum proteins were separated by 2D gel electrophoresis before and 11 mo after the initiation of mannose therapy. As shown for α1-antitrypsin, several glycoproteins showed changes of their isoform distribution with time. Hypoglycosylated isoforms (*arrows*) disappeared or were considerably reduced in their concentrations.

## Discussion

PMI deficiency represents a new enzyme defect in humans causing an additional variant of CDG syndrome. For historical reasons, the current classification of CDG syndromes is based on the isoelectric focusing patterns of serum glycoproteins instead of on the underlying enzymatic defects. Since the IEF patterns in PMI deficiency are indistinguishable from the most common form of CDGS, PMM deficiency (CDGS type Ia), PMI deficiency is classified as CDGS type Ib.

CDGS type Ib is caused by a genetic defect in the PMI1 gene. The Arg219 mutation of the paternal allele decreased the enzyme activity to a low level, but no mutation was detected in the maternal allele. Her low PMI activity (33% normal) suggests a decreased level of functional maternal transcripts. PMI deficiency abolishes or decreases the synthesis of mannose 6-phosphate from fructose 6-phosphate. Mannose provided by the diet or that released from cellular glycoconjugates becomes the only source of mannose 6-phosphate (Fig. 3). Whereas mannose uptake through a high affinity mannose-specific transporter has been demonstrated (16), the extent of reutilization is unknown. Mannose 6-phosphate supplies 9 of the 14 monosaccharides in the lipid-linked oligosaccharide (LLO) precursor required for protein N-glycosylation, can be a precursor for fucose, and is required for the synthesis of glycophospholipid-anchored glycoproteins. A decreased pool of mannose 6-phosphate results in a decreased amount of full-length LLO available for N-glycosylation.

Hypoglycosylation of liver-derived serum glycoproteins was a prominent biochemical feature in the PMI-deficient patient and was as severe as in CDGS type Ia. Little is known about the quantity or bioavailability of diet-derived mannose. A regular diet apparently did not provide sufficient mannose

for glycoprotein biosynthesis in the liver of our patient. By simply increasing the external supply of mannose, it was possible to correct the phenotype of PMI deficiency. The clinical symptoms disappeared within weeks after the initiation of mannose therapy and hypoglycosylation was nearly completely corrected after 11 mo. The apparently normal glycosylation of glycoproteins synthesized in cultured fibroblasts suggests that, under cell culture conditions in a medium lacking free mannose, the supply of mannose derived from breakdown of endogenous or internalized glycoconjugates is sufficient to ensure normal N-glycosylation. The same might be true for different organs in the patient.

Phenotypic correction of the PMI deficiency in yeast was achieved by supplying 5 mM mannose together with glucose (20). It has to be mentioned that excessive mannose doses can be potentially toxic in PMI deficiency. However, this so-called "honeybee syndrome" (21) has no relevance for the oral mannose therapy in PMI-deficient patients, since the uptake capacity of the gut is a natural protection. Single mannose doses of $>$ 200 mg/kg body wt induce osmotic diarrhea and limit the maximal serum concentrations that can be reached by oral uptake. The peak mannose concentrations in the patient's serum were 60-fold lower than the toxic dose in PMI-deficient tumor cells (22).

Based on the four known types of CDG syndrome, it was generally assumed that CDG syndromes present as disorders with severe psychomotor and mental retardation. For this reason, CDGS diagnostics was restricted to children with neurological impairment. With the detection of PMI deficiency, this restriction is no longer prudent. Although the metabolic block in PMI deficiency is localized in the same biosynthetic pathway as in PMM deficiency, the clinical presentation of PMI deficiency is fundamentally different from all other types of CDGS. PMI deficiency is dominated by severe gastrointestinal symptoms and no neurological impairment was present in the patient. Only the occurrence of thrombosis and bleeding was a common clinical feature. The reason for the different clinical phenotypes is unknown, but could be related to the fact that mannose uptake from both mannose and glucose are blocked in PMM deficiency, whereas the uptake of exogenous mannose is unaffected by PMI deficiency. The PMI deficiency demonstrates that the screening for carbohydrate-deficient glycoproteins should be considerably extended if the complete spectrum of hypoglycosylation disorders in humans is to be detected.

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