Mannose supplements induce embryonic lethality and blindness in phosphomannose isomerase hypomorphic mice

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ABSTRACT Patients with congenital disorder of glycosylation (CDG), type Ib (MPI-CDG or CDG-Ib) have mutations in phosphomannose isomerase (MPI) that impair glycosylation and lead to stunted growth, liver dysfunction, coagulopathy, hypoglycemia, and intestinal abnormalities. Mannose supplements correct hypoglycosylation and most symptoms by providing mannose-6-P (Man-6-P) via hexokinase. We generated viable Mpi hypomorphic mice with residual enzymatic activity comparable to that of patients, but surprisingly, these mice appeared completely normal except for modest (~15%) embryonic lethality. To overcome this lethality, pregnant dams were provided 1–2% mannose in their drinking water. However, mannose further reduced litter size and survival to weaning by 40 and 66%, respectively. Moreover, ~50% of survivors developed eye defects beginning around midgestation. Mannose started at birth also led to eye defects but had no effect when started after eye development was complete. Man-6-P and related metabolites accumulated in the affected adult eye and in developing embryos and placentas. Our results demonstrate that disturbing mannose metabolic flux in mice, especially during embryonic development, induces a highly specific, unanticipated pathological state. It is unknown whether mannose is harmful to human fetuses during gestation; however, mothers who are at risk for having MPI-CDG children and who consume mannose during pregnancy hoping to benefit an affected fetus in utero should be cautious.—Sharma, V., Nayak, J., DeRossi, C., Charbono, A., Ichikawa, M., Ng, B. G., Grajales-Esquível, E., Srivastava, A., Wang, L., He, P., Scott, D. A., Russell, J., Contreras, E., Guess, C. M., Krajewski, S., Del Rio-Tsonis, K., Freeze, H. H. Mannose supplements induce embryonic lethality and blindness in phosphomannose isomerase hypomorphic mice. FASEB J. 28, 1854–1869 (2014). www.fasebj.org

Key Words: congenital disorder of glycosylation • MPI-CDG • lens • eye defects

A potentially lethal form of rare congenital disorder of glycosylation (CDG), type Ib [CDG-Ib; or phosphomannose isomerase (MPI)-CDG] can be treated with mannose as a dietary supplement (1). Supplementation overcomes impaired glycosylation caused by hypomorphic mutations in MPI because mannose bypasses the impaired conversion of fructose-6-phosphate (Fru-6-P) to mannose-6-phosphate (Man-6-P), which is the major source of Man-6-P derived from glucose. Mannose alleviates patients’ stunted growth, hypoglycemia, liver dysfunction, coagulopathy, and protein-los-

Abbreviations: AAT, α-1 antitrypsin; AGA, aspartyl glucosaminidase; β-hex, β-hexosaminidase; BSA, bovine serum albumin; BSTFA, N,O-bis-(trimethylsilyl)trifluoroacetamide; CDG, congenital disorder of glycosylation; DMEM, Dulbecco’s modified Eagle’s medium; ECM, extracellular matrix; FACE, fluorophore-assisted carbohydrate electrophoresis; Fru-6-P, fructose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GC-MS, gas chromatography-mass spectrometry; Glc-6-P, glucose-6-phosphate; H&E, hematoxylin and eosin; HK, hexokinase; Man-6-P, mannose-6-phosphate; KO, knock-in; KO, knockout; mEF, mouse embryonic fibroblast; MPI, phosphomannose isomerase; PBS, phosphate-buffered saline; PGI, phosphoglucone isomerase; PLE, protein-losing enteropathy; PMM2, phosphomannomutase; RPE, retinal-pigmented epithelium; TCA, trichloroacetic acid; TNF-α, tumor necrosis factor; WT, wild type

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ing enteropathy (2). Exogenous mannose is converted to Man-6-P by hexokinase (HK), replenishing this deficient precursor needed for multiple glycosylation pathways, including the N-glycosylation pathway, via phosphomannomutase (PMM2); excess Man-6-P is catabolized by the residual MPI activity (Scheme 1). Patients on this therapy survive and lead a normal life without obvious side effects (2).

To model MPI-CDG and follow the effects of mannose therapy, we previously knocked out the single Mpi gene in mice, leading to death at embryonic day 11.5 (E11.5) due to abnormalities in both placenta and the embryo. Mannose could not rescue because Man-6-P accumulates to toxic levels, limiting ATP and inhibiting several glycolytic enzymes (3). However, because patients with MPI-CDG have residual enzymatic activity, hypomorphic mice would offer a more patient-relevant model than would a complete Mpi knockout (KO). Here, we describe a viable, hypomorphic mouse line containing a patient-derived mutation that reduced enzymatic activity and altered mannose metabolism, as predicted. While a minority of mutant embryos died \textit{in utero}, surprisingly, adolescent and adult mice had none of the expected symptoms reported in patients with MPI-CDG; these Mpi-deficient mice had a subclinical phenotype. However, if dams consumed mannose during pregnancy, most of the Mpi hypomorphic embryos died, and nearly half of the survivors were born with severe ocular defects. The combination of reduced enzymatic activity and the increased mannose load altered its metabolic flux, leading to Man-6-P accumulation in the eyes.

Mannose is widely used as a “natural” treatment for urinary tract infections; this seemingly innocuous sugar may have a negative effect for some pregnant women. While the frequency of MPI-CDG is unknown, women at risk for having subsequent MPI-CDG children who intend to take mannose as a “prenatal therapy” may inadvertently cause other side effects.

MATERIALS AND METHODS

Materials

Most of the reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco’s modified Eagle’s medium (DMEM) with 1 mg/ml glucose was purchased from Corning Cellgro (Manassas, VA, USA).

Fetal bovine serum (FBS) was obtained from Hyclone Laboratories (Logan, UT, USA). [2-\textsuperscript{3}H]-mannose was procured from Perkin Elmer (Boston, MA, USA). Protease inhibitor cocktail was purchased from Roche Diagnostics (Indianapolis, IN, USA). Carrier-free recombinant human tumor necrosis factor α (TNF-α) and recombinant mouse α-1 antitrypsin (AAT) were purchased from Cell Sciences (Canton, MA, USA), and ICL, Inc. (Portland, OR, USA), respectively. N-O-β-nitro-1-trifluoroacetamide (BSTFA) was procured from Thermo Scientific (Waltham, MA, USA). Z-fix and Bouin’s solution for tissue fixation were obtained from Anatech, Ltd. (Battle Creek, MI, USA) and Ricca Chemical (Arlington, TX, USA), respectively. Protein was measured by using the Pierce BCA protein estimation kit from Thermo Scientific.

Primers and probes

Primers were synthesized by Integrated DNA Technologies (San Diego, CA, USA). The following sequences were used. Primers: M14a-F1, GTA/GCGGAGT/GAATG/GCG/GCG; M14-F3, ATG/GCGGTTTTG/GTGA/GA/GCTCA; MoxP-R1, CCT/CAGCAGGACCC/CAATA; LoxP-F1, CCT/TAGCTCC/TT/GCCCGACTTG. Probes for Southern blot: 5’ probes P717_01, GCAGGGTGGCTGTGGA/GCTGAG; P717_02, CGTTGAGGCTGCCT/GATAGA; 3’ probes P717_03, GTGCGGCT/CATGTTCC/TA; P717_04, GATGTCCCC/GAACT/GATTGCTT.

Antibodies

Antibodies used in this study include antisera against AAT from Siemens (Marburg, Germany), chicken anti-mouse AAT IgG from ICL, and alkaline phosphatase-conjugated goat anti-chicken IgG IgG from Jackson ImmunoResearch (West Grove, PA, USA). The MPI-specific antibody was raised in our laboratory and has been described previously (4). The AP-2 antibody was obtained from the Developmental Studies Hybridoma Bank and was developed under the auspices of the U.S. National Institute of Child Health and Human Development and maintained by the University of Iowa Department of Biological Sciences (Iowa City, IA, USA). Antibodies against Brn3a, S-opsin, and M/L-opsin were purchased from Chemicon International, Inc. (Temecula, CA, USA). The anti-Pax6 antibody was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). The secondary antibodies were purchased from Molecular Probes, Inc. (Eugene, OR, USA).

Animals

The Sanford-Burnham Medical Research Institute (SBMRI) Institutional Animal Care and Use Committee approved all the animal studies. Mpi\textsuperscript{+/+} double knock-in (KI/KI) mice were created from C57BL/6J strain. Mpi\textsuperscript{−/−} wild-type (WT) animals obtained from heterozygous Mpi\textsuperscript{+/−} crosses were used as controls. KI/KI animals were then crossed with mice heterozygous for the Mpi-KO allele (Mpi\textsuperscript{+−}) on a mixed background (C57BL/6J and 129/SvEv) to create Mpi\textsuperscript{−/−} (KI/KO) mice. All the mice were maintained on a 12-h dark-light cycle.

Genotyping of mice

Genomic DNA was extracted from tail clips, and PCR was performed to confirm the genotype using primers M14a-F1
Isolation of murine embryonic fibroblasts (mEFs)

mEFs were isolated from KI/KI embryos at E11.5 as described previously (3). No additional mannose was required to propagate these mEFs.

3H-mannose injection

[2-3H]-mannose (50 µCi) was injected in the tail vein of 3 mice/group (WT, KI/KI, and KI/KO). Blood (20–30 µl) was collected at 5, 15, 30, 60, 90, 120, and 180 min, diluted with phosphate-buffered saline (PBS) and centrifuged at 1000 rpm. Diluted plasma was used to estimate the amount of 3H2O, [2-3H]-mannose, and trichloroacetic acid (TCA) precipitable labeled glycoproteins. After 3 h, the mice were humanely euthanized, and their organs were collected.

Analysis of plasma 3H2O

A 10-µl aliquot of plasma was used to determine radioactivity with and without evaporation to dryness. The difference was defined as the amount of 3H2O in 10 µl of blood (5).

Analysis of plasma [2-3H]-mannose

Free [2-3H]-mannose in the plasma was determined by using HK and recombinant human MPI to convert it to 3H2O, before evaporating it. The difference between the radioactivity level before and after evaporation was defined as the amount of [2-3H]-mannose in plasma (5).

Precipitation of plasma proteins

A 20-µl aliquot of plasma was diluted to 200 µl with PBS, and an equal volume of 20% TCA was added. The samples were incubated on ice for 1 h and centrifuged at 14,000 rpm. The protein pellet was washed once with acetone and resuspended in 0.2 N sodium hydroxide. The amount of 3H radiolabel in the precipitated protein was determined.

Stable isotope label

The cells were labeled with stable isotopes [1,2-13C]-glucose and [4-13C]-mannose, and isolated glycans were analyzed by gas-chromatography-mass spectroscopy (GC-MS) as described before (6).

Enzymatic activity assays

A small section of each organ to be tested was minced and disrupted by sonication in chilled 50 mM HEPES buffer (pH 7.4) containing protease inhibitors. Tissue lysate was centrifuged at 14,000 g for 10 min at 4°C. The supernatant was collected, and the amount of protein was measured by using the Pierce BCA protein estimation kit. A 15-µg aliquot of protein was used to estimate MPI activity. A standard coupled assay using phosphoglucose isomerase (PGI) and glucose-6-phosphate dehydrogenase (G6PD) with NADPH readout at 340 nm was used to estimate MPI activity in the organ lysate (7). The original protocol was modified to a final volume of 210 µl, and enzyme activity was measured in a 96-well plate for 2 h using a microplate reader (SpectraMax Plus384; Molecular Devices, Sunnyvale, CA, USA).

Western blot analysis

A total of 50 µg of organ lysate (as described above) was separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane. Unbound sites were blocked overnight at 4°C with PBS containing 5% bovine serum albumin (BSA). The membrane was probed with primary anti-mouse MPI diluted in PBS/2% BSA for 1 h at room temperature, followed by three 10-min washes, and then probed with horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody at 1:2000 dilution in PBS/2% BSA for 1 h at room temperature. After 3 washes, the blot was developed by using BCIP substrate for HRP.

Blood/serum analysis

Glucose levels were determined by using FreeStyle glucometer and glucose strips from Abbot (Alameda, CA, USA). Several comprehensive parameters of freshly drawn blood were measured by using the VetScan VS2 instrument (Abaxis, Union City, CA, USA). Hematocrit analysis was done by using a VetScan HM2 analyzer (Abaxis).

Fecal AAT estimation

Fecal extracts were prepared as described previously (8), and AAT was measured by noncompetitive ELISA. Briefly, 96-well plates were coated with 100 µl of diluted antisera against human AAT, blocked with 1% BSA, washed with PBS, and incubated with 100 µl of fecal extract (diluted 1:50 with PBS) or standards; all steps were performed for 1 h at 37°C. Wells were washed with PBS and incubated with 100 µl of 0.4 µg/ml primary chicken anti-mouse AAT IgY prepared in 1% BSA and 0.05% Tween 20 for 18–24 h at 4°C. After washes with 0.05% Tween in PBS, wells were incubated with 100 µl of 0.25 µg/ml alkaline phosphatase-conjugated goat anti-chicken IgY prepared in 1% BSA and 0.05% Tween 20 for 2 h at 37°C. This was followed by washes and color development using 4-nitrophenyl phosphate as substrate. Absorbance was measured at 405 nm using a microplate reader (SpectraMax Plus384; Molecular Devices). The amount of fecal AAT (ng/ml, feces extract) was derived from standard curve and reported as mean micrograms AAT per gram of dry stool.

Serum mannose determination by GC-MS

Mannose was estimated by using a previously described method (9). Briefly, 10-µl serum and mannose standards were derivatized by addition of 50 µl hydroxylamine hydrochloride (50 mg/ml) in 1-methyl imidazole and incubation at 65°C for 30 min. Then, 100 µl acetic anhydride was added. Extraction was done with 100 µl chloroform and 200 µl water. After vortexing and centrifuging the samples, the aqueous layer was removed and reextracted once more with water. The chloroform layer containing sugars was dried and solubilized in chloroform for GC-MS analysis (GCMS-QP2010 Plus; Shimadzu, Kyoto, Japan).

In vivo imaging of embryos in utero

Timed matings were set up for WT and KI/KI mice supplemented with 5% mannose in their drinking water. Females
were checked for the presence of a vaginal plug the following morning to confirm the mating. The Vevo 770 (VisualSonics, Toronto, Canada) in vivo microimaging system was used with the RMV scan heads 708 and 703 at a center frequency of 55 and 35 MHz, respectively, to visualize embryos in pregnant mice starting at E6.5.

Histological examination of the tissues

Freshly dissected tissues or embryos were immediately fixed in buffered zinc-formalin, Z-fix. Bouin’s solution was used to fix the entire head with eyes after birth. Paraffin-embedded sections (5 μm thick) were cut and then stained with hematoxylin and eosin (H&E) for structural analysis using the Aperio Scanscope slide-scanning system (Aperio Technologies, Inc., Vista, CA, USA). Morphometric measurements were performed with Aperio Imagescope software.

Immunohistochemistry

The sections were deparaffinized, and antigen retrieval was performed in 0.01 M sodium citrate for 30 min. Permeabilization was done with 1% saponin. The sections were blocked in 10% normal goat serum (for Brn3a, S-opsin, and M/L-opsin) or 10% donkey serum (for Pax6 and AP-2) and incubated overnight at 4°C with the following diluted primary antibodies: Brn3a (1:10), S-opsin and M/L-opsin (1:200), Pax6 (1:100), and AP-2 (1:10). Next, fluorescently tagged secondary antibody was added. After a thorough washing, Vectashield (Vector Laboratories, Burlingame, CA, USA) was added, and sections were sealed with a coverslip. Confocal images (size 1024×1024) were obtained sequentially on a Zeiss 710 Laser Scanning Confocal System (Carl Zeiss, Oberkochen, Germany) using either a ×20/0.80 NA WD 0.55 objective lens or EC Plan-Neofluor ×40/0.75 M27 objective lens. Results were confirmed using 3 different biological samples. Quantification consisted of using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA) to count the number of immunopositive cells in 2 different confocal images (1024×1024 taken at ×40) of the posterior region of 3 different eyes.

Man-6-P assay

The embryo, placenta, and eye extracts were prepared as previously published (10). The method for Man-6-P estimation was modified from a protocol originally designed for glucose-6-phosphate (Glc-6-P) estimation (11). We used a coupled enzyme assay, which uses MPI, PGI, and G6PD to convert Man-6-P to Glc-6-P, with a final fluorescence readout. This method was optimized and validated by estimating Man-6-P in the mixtures containing known amounts of Man-6-P, Fru-6-P, and Glc-6-P or adding known amounts of Man-6-P to tissue lysates. The values obtained by this method were comparable to the results obtained by using fluorophore-assisted carbohydrate electrophoresis (FACE; refs. 12, 13). Briefly, 20 μl tissue extract of each sample type was dispensed in different wells of a black assay plate. Depending on the number of samples, 2 sets of reaction mix were prepared containing 50 mM HEPES, 1 mM magnesium chloride, 100 μM NADP, 10 μM resazurin, and 0.25 U diaphorase in a total volume of 80 μl/sample. MPI, PGI, and G6PD (0.25 U/sample) were added to one set (Fb) and only G6PD and PGI (0.25 U/sample) were added to the other set (Fb). Another mix containing G6PD, but devoid of NADP, was prepared to determine the background (Fb) due to the endogenous NADP in each sample. An 80-μl aliquot of the mix was added to the wells containing 20 μl of the extract. After 15 min, fluorescence was recorded at excitation wavelength 530 nm and emission at 590 nm using Flexstation III (Molecular Devices). Background fluorescence (Fb) was subtracted from all the samples (Fb and Fb). Difference between the fluorescence values (Fb – Fb) was used to calculate picomols of Man-6-P using a standard curve generated with known amounts of Glc-6-P in 20 μl deionized water and 80 μl reaction mix containing G6PD.

GC-MS analysis of metabolites in the eyes

The eyes were crushed and washed once with cold PBS. Then, 100 μl chilled 0.1 M acetic acid with 3 nmol arabinol (internal standard) was added to the tissue, and the mixture was sonicated briefly on ice. The samples were centrifuged at 8000 g for 3 min in a cold centrifuge, and the supernatant was collected. Extraction was repeated once more, and the supernatants were combined. The extract was hydrolyzed, derivatized with hydroxylamine hydrochloride and BSTFA, and subjected to GC-MS (GCMS-QP2010 Plus; Shimadzu) according to a method as modified from Halket and Zaikin (14).

Vision test

Optokinetics was measured by using an optometer with motorized drum painted with black and white strips, which was built in-house at The Scripps Research Institute (La Jolla, CA, USA). The measurements were done under photopic conditions with light intensity of 150 lux. The mouse was placed on the wire mesh inside the drum and allowed to acclimate for 5 min without rotation and then for 30 s with rotation. The drum rotation (2 rotations/min) went clockwise for 1 min and then continued antclockwise for 1 min with an interval of 30 s between the rotations. The animals with normal vision followed the movement of the rotating drum, whereas the ones with impaired vision failed to track the drum rotations. The visual response was measured by recording the number of head turns during drum rotations.

RESULTS

Selection of MPI mutation

Since patients with MPI-CDG have 3–20% residual MPI activity (1), we wanted to make a hypomorphic mouse line with similar residual activity to model the disorder and test mannose therapy. To identify a candidate, 8 mutations from patients with confirmed MPI-CDG were introduced into the mouse Mpi gene to obtain the following amino acid changes: S102L, D131N, M138T, R152Q, R219Q, Y255C, I398T, and R418H. Subsequently, mutant MPI constructs were expressed in mpi-null yeast strain SEY6210delpmi40::URA3, and activity was determined (Table 1). Mutation Y255C (TAC→TGC) retained ~12% activity (Table 1), which was confirmed by expression in CHO, COS7, and Mpi+/− mEFs and consistently had 8–14% activity (data not shown). Y255C was selected to generate a KI mouse line, as described in Supplemental Fig. S1.

Hypomorphic mice have modest embryonic lethality

Both Mpi+/Y255C/Y255C (KI/KI) and Mpi+/Y255C−/− (KI/KO) mice were viable. Heterozygous crosses (KI/+ × KI/+)
showed a small, but significant ($P=0.004$), decrease of 16% below the expected number of KI/KI animals, indicating modest embryonic lethality (Table 2). Similarly, homozygous (KI/KI × KI/KI) crosses produced litters that were significantly ($P=0.01$) smaller than WT animals [WT: 8.6 (n=46) vs. KI/KI: 7.5 (n=33)]. KI/KI survivors had normal weight gain (Supplemental Fig. S2) and life span, showing no visible abnormalities in the organs as monitored by dissections at different times up to 13 mo (data not shown).

**Hypomorphic mice have reduced MPI activity and protein in various organs**

KI/KI mice were predicted to have 8–14% residual MPI activity and KI/KO mice 4–7% activity compared with WT. Table 3 shows the result of enzymatic assays of different organ lysates. In KI/KI mice, the enzyme activity was reduced to 18% in heart and nearly 2–3% in small intestine and liver (Fig. 1A). KI/KO small intestine and liver had 4- and 6-fold lower MPI activity, respectively, than those of KI/KI (Fig. 1A). Assays of mixed extracts of different organs showed no evidence for the presence of inhibitors or activators. Despite these unexpected low activities in liver and small intestine, all mutant animals appeared to be healthy, and none had any abnormal hepatointestinal pathological features on histochemical analysis (data not shown). Residual MPI protein antigen ranged from 25 to 45% in various tissues from KI/KI mice and 14 to 33% in KI/KO mice (Fig. 1B). Our results clearly demonstrate reduced MPI protein and enzyme activity in hypomorphic mice, similar to that in fibroblasts and leukocytes of patients with MPI-CDG (1).

**Reduced MPI alters mannose flux in hypomorphic mice**

Reduced MPI activity is predicted to increase mannose flux toward glycoprotein synthesis and decrease its catabolism (6). To confirm this, we labeled mice, organ cultures, and mEFs with [2-3H]-mannose and measured label incorporation into glycoproteins and catabolic production of $^3$H$_2$O ([2-3H]-mannose$\rightarrow$[2-3H]-Man-6-P$\rightarrow$Fru-6-P$\rightarrow$Fru-6-P$\rightarrow$H$_2$O). Reduced MPI should increase label incorporation into proteins and decrease catabolism of [2-3H]-mannose to $^3$H$_2$O (Scheme 1). As seen in Fig. 2A, KI/KI mEFs incorporated 5-fold more [2-3H]-mannose into glycoproteins than did WT, similar to the phenomena seen in the fibroblasts of patients with MPI-CDG (Fig. 2B). Compared with WT mice, KI/KI and KI/KO mice injected with 50 μCi [2-3H]-mannose in the tail vein had a reduced rate of production of $^3$H$_2$O in serum (Fig. 2C) together with a 5- to 6-fold increase in [2-3H]-mannose incorporation in serum glycoproteins (Fig. 2D). Labeling of organ explants ex vivo showed 2- to 6-fold higher incorporation of [2-3H]-mannose in glycoproteins from KI/KI organs (Fig. 2E), levels similar to those of mEFs and MPI-CDG patient fibroblasts (Fig. 2A, B). We also labeled mEFs from WT and KI/KI mice with the stable isotopes [1,2-13C]-glucose and [4-13C]-mannose under physiological conditions of 5 mM glucose and 50 μM mannose. Mannose directly contributed ~25% of mannose to WT N-glycans. This contribution increases by 2.3-fold to 57% in KI/KI mEFs with reduced MPI activity (data not shown). These results show that $Mpi$ hypomorphic mice clearly have altered mannose metabolic flux due to the decreased activity.

Since the biochemical difference between KI/KI and KI/KO mice was small, only KI/KI mice on the pure C57BL/6J background were used in subsequent experiments.

### Table 1. Heterozygous crosses [KI/+ × KI/+]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Genotype</th>
<th>WT (+/+</th>
<th>Het (KI/+)</th>
<th>KI/KI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice (n)</td>
<td>133</td>
<td>303</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Predicted (%)</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Observed (%)</td>
<td>24.1</td>
<td>54.9</td>
<td>20.8</td>
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</tr>
<tr>
<td>$P$</td>
<td>0.187</td>
<td>0.830</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

Data from 70 litters. Het, heterozygous.

### Table 2. Heterozygous crosses [KI/+ × KI/+]

<table>
<thead>
<tr>
<th>Organ</th>
<th>Activity (nmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo, E11.5</td>
<td>24.6 ± 1.6, 1.6 ± 0.32, ND</td>
</tr>
<tr>
<td>Placenta, E11.5</td>
<td>22.1 ± 6.9, 1.5 ± 0.15, ND</td>
</tr>
<tr>
<td>Small intestine</td>
<td>20.5 ± 0.6, 0.51 ± 0.29, 0.14 ± 0.20</td>
</tr>
<tr>
<td>Kidney</td>
<td>19.7 ± 0.9, 1.20 ± 0.14, 0.57 ± 0.08</td>
</tr>
<tr>
<td>Colon</td>
<td>16.8 ± 2.7, 1.46 ± 0.53, 0.92 ± 0.18</td>
</tr>
<tr>
<td>Heart</td>
<td>14.9 ± 2.1, 2.80 ± 0.6, 1.4 ± 0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>8.3 ± 0.9, 0.25 ± 0.03, 0.04 ± 0.005</td>
</tr>
<tr>
<td>Lung</td>
<td>8.4 ± 2.3, 0.68 ± 0.21, 0.32 ± 0.18</td>
</tr>
<tr>
<td>Spleen</td>
<td>7.5 ± 0.2, 0.42 ± 0.15, 0.28 ± 0.06</td>
</tr>
<tr>
<td>Eye</td>
<td>1.5 ± 0.2, 0.12 ± 0.02, ND</td>
</tr>
</tbody>
</table>

Data are means ± sd of $n=3$ mice. ND, not determined.
KI/KI mice do not mimic MPI-CDG symptoms despite altered mannose metabolism

Patients with MPI-CDG are often hypoglycemic and have elevated serum alanine transaminase (ALT) and aspartate aminotransferase (AST) indicating liver pathology. They also have hypoalbuninemia and low antithrombin III (ATIII) levels and liver fibrosis, and some have intestinal villus atrophy and protein-losing enteropathy (PLE).

We comprehensively analyzed serum from WT and KI/KI mice aged 3 and 10 mo. No major differences existed in serum parameters of WT and KI/KI mice at any age (data not shown). KI/KI mice have normal glucose and liver enzymes. Potassium, an indicator of renal function, was significantly (*P*=0.01) higher in KI/KI animals (7.5 mM) than in WT (7.05 mM) but was still within the normal range for mice. KI/KI mice had normal kidney function with no evidence of either proteinuria or increased blood urea nitrogen (BUN) concentration. The results of hematocrit analysis did not show any difference in the numbers of various blood cells, except for slight but significant (*P*=0.02) decrease in red blood cells of KI/KI mice (10.8±0.2×10¹²/L) from that in WT mice (11.06±0.1×10¹²/L). However, this decreased amount was within the normal range for mice. Lysosomal enzymes, asparyl glucosaminidase (AGA) and β-hexosaminidase (β-hex) are elevated in patients with CDG-I (15), but in Mpi hypomorphic mice, these activities (AGA: 9.7±0.77 nmol/h/ml; β-hex: 33.4±4.5 nmol/min/ml) were no different from WT (AGA: 10.5±0.73 nmol/h/ml; β-hex: 36.2±8.3 nmol/min/ml). Plasma proteins, such as transferrin and AAT, and coagulation factors, such as antithrombin III and factor XI, are affected in patients with MPI-CDG (16), but they were unaltered in KI/KI mice, based on Western blots (data not shown). Histological analysis of various organs, including liver and intestine at 3, 5, 7, and 13 mo, showed no morphological changes (data not shown), confirming that no visible pathology was present even in older mice. Because some patients with MPI-CDG have PLE (*i.e.*, loss of plasma proteins through small intestines), we also determined enteric protein loss in WT and KI/KI mice by measuring fecal AAT. KI/KI mice had neither enhanced levels of fecal AAT at basal state nor increased susceptibility to enteric protein loss when systematically challenged with TNF-α, which is known to increase intestinal permeability (ref. 17 and Supplemental Fig. S3). Our observations demonstrate that, despite having altered mannose metabolism and reduced MPI activity levels similar to those of patients with MPI-CDG, KI/KI mice failed to mimic any of the expected MPI-CDG symptoms.
effects for over 4–6 mo. They were healthy and grew normally, confirming that there were no toxic effects of mannose therapy, which is also consistent with results in human studies. However, providing mannose to dams was lethal to the developing embryos. Crossing heterozygous KI/H11001 mice did not produce any KI/KI progeny when dams drank water containing 5% mannose (Table 4). Crossing homozygous KI/KI mice gave the same results. Ultrasound analysis of the dams showed that implantation occurred, but embryos were resorbed over time (Fig. 3). Dissections at E13.5 revealed normal development of WT embryos (Fig. 4A, B) and abnormal hemorrhaged placenta and resorbed embryos for KI/KI (Fig. 4C). The results of histology analysis also showed a grossly disorganized placenta with a labyrinth layer devoid of blood vessels and embryonic erythrocytes, abnormally organized giant cells, and an expanded spongiotrophoblast (Fig. 4D, E).

Table 4. Heterozygous breedings with mannose supplementation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KI/+ × KI/+; no mannose</th>
<th>KI/+ × KI/+; 5% mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
<td>KI/+</td>
</tr>
<tr>
<td>Average litter size</td>
<td>8.0 ± 1.7, n = 14</td>
<td>6.0 ± 2.3, n = 6</td>
</tr>
<tr>
<td>Survival to weaning (%)</td>
<td>84.8</td>
<td>94.4</td>
</tr>
<tr>
<td>Progeny (n)</td>
<td>29</td>
<td>49</td>
</tr>
<tr>
<td>Progeny (%)</td>
<td>31</td>
<td>52</td>
</tr>
<tr>
<td>P</td>
<td>0.26</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Data are means ± sd; n = number of litters.
bryos (Fig. 5B, C). These affected embryos would eventually die before birth.

The results here clearly show adverse effects of mannose supplementation on both placentas and embryos. Dams continued nursing on 1 or 2% mannose during weaning, but pup survival showed a dramatic dose-dependent decrease vs. controls (Table 5). The causes of death could not be investigated, because most pups were reported as missing (most likely, cannibalized by the mother).

Man-6-P accumulates in KI/KI embryos and placentas from dams drinking mannose-supplemented water

KI/KI mice given 1 and 2% mannose supplements significantly increase plasma mannose by 1.3- to 1.5-fold compared with mannose levels of those given plain water (Fig. 6). WT mice also showed a 50% increase in mannose levels with 2% mannose, which is consistent with the results of earlier studies (18, 19).

At E11.5, MPI specific activities in placental and whole-embryo lysates of KI/KI mice were 6–7% of those in WT (Table 3). None of the organs from WT or KI/KI adult mice supplemented with mannose showed significant Man-6-P accumulation (data not shown). In contrast, baseline Man-6-P was higher in both KI/KI placentas and embryos compared with that in WT. There was an additional increase by ~2.5 fold on 1–2% mannose supplementation (Fig. 7). Man-6-P content in the embryos of WT dams supplemented with 2% mannose was comparable to that of KI/KI mice without mannose, and neither group showed any ill effects. Our previous studies on Mpi-null mice show that increased Man-6-P can cause ATP depletion and death (3).

However, ATP levels of E11.5 embryos and placentas from mannose-supplemented KI/KI and WT dams were similar (data not shown).

**Mannose-supplemented KI/KI mice have eye defects**

**Effect of mannose supplementation during conception and gestation**

Surviving KI/KI pups born to mannose-fed dams and subsequently maintained on mannose had normal growth and life span. There were no visible abnormal-
ities in any organ when KI/KI mice were dissected and compared with WT. The eyes were checked every week starting at wk 2, when the mice just opened their eyes, and continuing for up to 8 mo. Approximately 45% of mannose supplemented pups displayed severe morphological eye defects (Table 6) that were clearly evident 2–8 wk after birth. Most of the affected pups had either cloudy/opaque eyes (Fig. 8B) or no eyes that appeared as shuteye (Fig. 8C) compared with normal eyes (Fig. 8A).

Histological analysis was done on the adult eyes isolated at 8 mo. WT eyes showed normal development of all eye structures including lens, retinal-pigmented epithelium (RPE), and retina (Fig. 8D). KI/KI mice that developed obvious eye defects by 3 wk of age showed smaller eyecups, absence of the lens, disorganized retina, and clustering of pigmented cells inside the eyecup (Fig. 8E). Mice with shuteye developed much smaller eyecups and had extensive hemorrhage, with no eye structure remaining except for an eye socket-like structure containing pigmented tissue with vacuoles (Fig. 8F, G). The identity of these cells is unknown, but markers for RPE progenitors, Mitf and Otx2, failed to stain the pigmented mass (data not shown). One KI/KI mouse supplemented with mannose since conception had a defective right eye and a seemingly normal left eye. However, when euthanized at 8 mo, the left eye also had a degenerating lens (Fig. 8H).

**Effect of mannose on postnatal eye development in KI/KI mice**

Since eye development continues up to 4 wk postbirth, we supplemented KI/KI mice with 1 or 2% mannose after birth, starting at P1, where the development of the eye and lens is expected to be normal till birth. However, this set of mice also showed eye defects with 2% mannose supplementation, some of which were observed by 3 wk; those exposed to 1% mannose did not (Table 6). The histological examination at 8 mo showed an eye with disintegrated lens, excess extracellular matrix (ECM), distorted retina, and accumulated pigmented cells (Fig. 8J), suggesting ill effects of P1 mannose supplementation. Some mice with mannose exposure also developed cloudy eyes later at ∼3 mo, with clear signs of cataract and a deteriorating lens and retina (Fig. 8J). In this case, besides accumulation of ECM, the nucleus of the lens protruded out of the lens capsule and was vacuolated.

Vision of WT and KI/KI mice was checked by using an optometer with a motorized drum, painted with black and white strips. Mice with defective right eyes did not show any head turns during clockwise rotation, and the ones having defects in the left eye failed to show any head turns during counterclockwise rotation (Supplemental Table S1). Mice with both eyes affected did not show any head turns in either direction. These data confirmed that the eyes that developed defects either early on or later in life were functionally impaired. None of the KI/KI animals had eye defects when mannose was added to the drinking water at 6–8 wk of age, which is 2–4 wk after full eye development.

Our results here clearly suggest that mannose supplementation starting before conception or 1 d postbirth causes two separate, time-resolved types of eye

### Table 5. Homozygous breedings with mannose supplementation

<table>
<thead>
<tr>
<th>Mannose in drinking water (%)</th>
<th>Litters (n)</th>
<th>Average litter size (n)</th>
<th>Survival to weaning (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>KI/KI</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Survival to weaning (%)</td>
</tr>
<tr>
<td>0</td>
<td>46</td>
<td>33</td>
<td>8.6 ± 1.8</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>17</td>
<td>8.0 ± 1.3</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>9</td>
<td>9.6 ± 1.3</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>5</td>
<td>6.7 ± 1.2</td>
</tr>
</tbody>
</table>

Average litter size data are means ± sd.
pathology in KI/KI mice, which seemed to be rooted in altered mannose metabolism.

Mannose initiates ocular defects during embryogenesis

We could observe the eyes once mice opened them ~2 wk. Eye development starts around E8.5 and continues until 4 wk postbirth. We wanted to know whether eye deterioration starts at embryonic stages or postbirth in mice exposed to mannose in utero. We provided 2% mannose in KI/KI dams' drinking water during timed matings and monitored embryonic eye development at various stages by histology. At E10–E11, WT mice develop an appropriate lens pit (Fig. 9A), but KI/KI mice given mannose do not (Fig. 9B). Instead they form smaller lens vesicles at E12–E13 (Fig. 9D) or none at all (Fig. 9E). Wild-type mice had well-formed lenses at E12–E13 (Fig. 9C), and both WT and KI/KI mice formed a neuroepithelium and an outer layer of RPE. Some of the mannose-exposed eyes continued to develop with small lens vesicles or without any lens (E14–E15; Fig. 9G, H) when compared with WT (Fig. 9F). By E16–E17, WT eyes had a well-differentiated clear lens and a laminated retina with inner and outer layers (Fig. 9I). Eyes from mannose-exposed mice were much smaller and had a neuroepithelium that lagged behind in its differentiation and mesenchymal cells from the surrounding area, as well as blood cells that appeared to infiltrate the vacant area of the optic cup (Fig. 9J, K). By E18–E19, in some cases, the retina folded around in the vitreous space with an overproduction of retina cells and delayed differentiation. The optic cup of affected KI/KI eyes was significantly smaller than that of WT eyes at all embryonic stages (Fig. 10). Some of the mannose-supplemented mice were then born with eyes that had a small, deteriorating cataractous lens (Fig. 9P) or were devoid of lens (Fig. 9Q), and the optic cup was filled with different cell types, which further deteriorated with time (Fig. 8E, G).

We assessed the effects of mannose on retinal development by using a series of retinal cell type-specific antibodies. Pax6 served as an early marker for retinal progenitors during neurogenesis. After the onset of retinal differentiation, it indicates ganglion and amacrine cells. In addition, we could specifically detect ganglion cells (Brn-3a), amacrine cells (AP-2), and photoreceptors (S opsin and M/L opsin). We could detect ganglion cells present in the ganglion cell layer, amacrine cells present in the inner nuclear layer, and photoreceptors in the outer nuclear layer that expanded their outer segments next to the RPE at the back of the eye in WT and KI/KI eyes at P28 (Fig. 11A, B). The number of Brn-3a+ ganglion cells was not affected (Fig. 11B). However, there were significantly fewer AP-2-positive amacrine cells in the mature KI/KI eyes (Fig. 11A, D), as well as at embryonic stage E18.5 (Fig. 11C, D). Mannose may either partially impair differen-

Figure 6. Serum mannose analysis. Concentration of mannose in serum from WT and KI/KI mice was determined by GC-MS, as described in Materials and Methods. *P < 0.05.

Figure 7. Man-6-P determination. Embryos (A) and placentas (B) were isolated at E11.5 from ≥2 different dams with or without mannose supplementation, and Man-6-P was determined. Each symbol represents an individual embryo/placenta. *P < 0.05, **P < 0.005.
tiation of normal retina progenitor cells to amacrine cells in KI/KI embryonic eyes or lead to their apoptosis.

Our results clearly demonstrate that mannose-induced eye abnormalities in KI/KI embryos begin early in embryogenesis, starting with impaired lens formation and subsequently differentiation of some retinal cells required for further eye development. The results of our statistical analyses (Table 6) and histological examination of the eyes suggest that there is a variable penetrance of \( Mpi \) eye pathology.

Affected eyes have elevated mannose and Man-6-P levels

Eyes have the lowest MPI specific activity of all organs tested, and this activity was reduced by 92% to 0.124 ± 0.025 nmol/min/mg in KI/KI animals (Table 3). Low MPI activity might render eyes more susceptible to adverse effects of mannose supplementation by accumulating Man-6-P. The Man-6-P content of the eyes was similar in WT and KI/KI mice without mannose supplementation (Fig. 12A). However, it increased 1.3-fold in the normal appearing KI/KI eyes and by 2.0-fold in the visibly cloudy eyes in 1 or 2% mannose-supplemented KI/KI mice (Fig. 12A). We used GC-MS to check the levels of mannose, Man-6-P and mannitol in the affected and normal eyes of 1% mannose-supplemented KI/KI mice. Mannitol did not accumulate, but mannose and Man-6-P levels were significantly higher in the affected eyes than in the normal eyes (Fig. 12B). These results suggest that ongoing mannose consumption raises mannose and Man-6-P levels in KI/KI eyes and correlates with increased ocular abnormalities.

DISCUSSION

There are now 100 known types of human glycosylation disorders. The CDG group comprises most of them. One of the major hurdles in studying these disorders is the lack of animal model systems. Mouse and rat mammalian models are closely related to humans and are widely used to study inherited human disorders. A few attempts have been made in the past to create KO mouse models; however, the models for MPI-CDG (CDG-Ib), PMM2-CDG (CDG-Ia), SRD5A3-CDG (CDG-Iq), and DPAGT1-CDG (CDG-Ij) are embryonically lethal (3, 20–22), and those for MGAT2-CDG (CDG-IIa), SLC35C1-CDG (CDG-IIc), and \( \beta 4GalT1 \)-CDG (CDG-IId) have early postnatal lethality (23–26). These outcomes emphasize the importance of glycosylation for early human development, but they cannot be used to model most aspects of the disease. Hypomorphic lines are more likely to mimic patient phenotypes.

We chose to create a murine line containing a mutation that generates a Y255C transition in MPI because preliminary experiments in cell lines showed that this line retains 8–14% residual MPI activity. This amount of activity should ideally mimic residual activity seen in the patient who was a compound heterozygote for Y255C and I398T and retained 7% residual MPI activity. This patient had hypoglycemia, hyperinsulinemia, hepatomegaly, elevated transaminase levels, and reduced factor XI, antithrombin III, protein S, and protein C levels (27).

\( Mpi \) is an essential, nonredundant gene in mice. Surprisingly, both hypomorphic lines with very low residual MPI activity and those with altered mannose metabolism grew normally and failed to mimic any of the broad array of symptoms of patients with MPI-CDG. The activity was still sufficient for normal function in this strain. Limited outcrossing into a mixed 129/SV background also yielded no obvious pathological phenotype (data not shown).

We observed modest embryonic lethality in KI/KI mice that was presumably due to insufficient glycosylation. By analogy with mannose treatment of young patients with MPI-CDG, we assumed that mannose provided prenatally would correct the slight embryonic lethality. The results were opposite to our expectations. Mannose supplementation caused a dose-dependent lethality during gestation in KI/KI mice. This was likely due to high local mannose or Man-6-P concentration that alters glucose metabolism at a crucial time during development. The results of earlier studies showed that mannose provided at 5 mM slowed rat embryo development in culture and showed impaired neural tube morphogenesis (28). The teratogenic effect on rat

<table>
<thead>
<tr>
<th>Mannose supplementation</th>
<th>With eye defects (n)</th>
<th>Affected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>2%</td>
<td>88</td>
<td>1</td>
</tr>
<tr>
<td>Postbirth (P1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>2%</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>6 wk</td>
<td>1%</td>
<td>14</td>
</tr>
<tr>
<td>2%</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>
expression of the two receptors is spatially and tempo-
inhibit processes mediated by Man-6-P receptors, as Man-6-P accumulation (29). Alternatively, Man-6-P may was also found to be toxic to honeybees because of was also found to be toxic to honeybees because of...embryos was attributed to the impairment of glycolysis, and metabolite enzymes (39, 40). Hyperglycemia also causes cataracts to develop in those with diabetes (41). Aldol reductase, an enzyme with low specificity, causes polyol accumulation and is implicated in galactosemic and diabetic cataracts (42). Therefore, we expected an accumulation of mannose in KI/KI eyes with mannose supplementation. However, mannitol did not accumulate despite elevated mannose levels in the affected/cloudy KI/KI eyes. The adverse effects are at least partially explained by the accumulation of mannose and Man-6-P in the affected KI/KI eyes. We also observed increased Man-6-P in KI/KI embryos but were unable to measure it specifically in embryonic eyes. Our data clearly show adverse effects on lens and amacrine cells in the retina. Other cell types could also be affected via several different mechanisms. For example, phagocytosis of photoreceptor outer segments by retinal pigmented epithelium is mediated by mannose receptors on the apical side and is inhibited specifically by mannose and mannanos (43, 44). Also, transforming growth factor-β (TGF-β), which is critical for eye development (45) and is expressed in lens fibers around E14.5 through E17.5 (46), could be affected. TGF-β precursor activation is specifically inhibited by Man-6-P before binding to its receptor but not by Man-1-P (47).

KI/KI mice were continuously exposed to mannose during conception and gestation and after birth. Therefore, we do not know the time frame during...
early development when embryos are most susceptible to mannose. The first indications of mannose-induced embryo demise and impaired lens develop-
metabolic flux and steady state of Man-6-P (5). Imbalance of this ratio in Mpi hypomorphic embryos makes them incapable of coping with the increased mannose influx. Man-6-P was not reported in the Pmm2R137H/F118L embryos (19).

It is uncertain whether the results of mannose supplementation using Mpi in this study or Pmm2 hypomorphic mice (19) can be extrapolated to humans. Based on the published results that mannose supplementation rescues mpi-morpholino-mediated knock-

![Figure 11. Immunofluorescence of WT and KI/KI eye sections. A) staining with AP-2, an amacrine-specific cell marker (in red), in the inner nuclear layer and M/L opsin (for red and green opsins), a cone photoreceptor cell marker (in green), for outer segments on P28 WT and KI/KI eyes. Scale bar = 100 μm. B) Staining with Brn3a, a ganglion cell marker (in red), in the ganglion cell layer and S opsin, a blue opsin cone photoreceptor cell marker (in green), in the outer segments of P28 WT and KI/KI sections. Scale bar = 100 μm. C) Staining with Pax6, a ganglion and amacrine cell marker (in green), and AP-2 (in red) on E18.5 WT and KI/KI eyes. Right panels show enlarged views of the boxed areas in left panels. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segments. D) ImageJ software was used to count the number of AP2-immunopositive cells from the posterior region of 2 different ×40 confocal images from 3 different eyes and calculate means ± sd. **P < 0.005, ****P < 0.0001.

![Figure 12. Measurement of metabolites in the eyes. A) A coupled fluorescent assay was used to measure the concentration of Man-6-P in the eyes of WT and KI/KI mice supplemented with or without mannose. Status of the eye is represented by n = not affected. C = cloudy. B) Mannose, Man-6-P and mannitol concentrations were estimated by GC-MS of the normal and affected eyes of mannose-fed KI/KI mice. Bars represent means ± sem of 5 mice. ns, not significant. *P < 0.05, **P < 0.005, ***P < 0.001.](image-url)
down in zebrafish embryos (MPI-CGD model) and reverses lethality of Pmm2 hypomorphic mouse embryos (PMM2-CGD model) when started before conception and continued through gestation till birth, physicians might encourage mothers of children with MPI-CGD to take mannose during a subsequent pregnancy to prevent symptoms that might develop during gestation. However, on the basis of our limited results, we strongly advise against this action. Similarly, we regard the recommendation made by an earlier publication (19) that mothers at risk of having babies with PMM2-CGD consume mannose during pregnancy to overcome the symptoms of PMM2-CGD in utero as being highly premature. A narrow window of mannose metabolic flux may determine normal vs. pathological state during embryogenesis, as demonstrated in Fig. 7A, where there is a significant increase in Man-6-P levels even in WT mice supplemented with 2% mannose. A similar study to demonstrate toxicity in humans is not feasible.

Mannose is also widely sold as a urinary tract health supplement. It is known to competitively inhibit binding of infectious Escherichia coli to urinary tract of mice (48), and recently, the first human clinical trial showed mannose as an effective prophylactic agent with minimal side effects (49). The widespread use of mannose as a risk-free, Internet-available, “natural glyconutrient” remedy for urinary tract infections underscores the need for caution, especially during pregnancy, since the prevalence of MPI-CGD is unknown.

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REFERENCES


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