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# Incomplete synthesis of N-glycans in congenital dyserythropoietic anemia type II caused by a defect in the gene encoding α-mannosidase II

(glycosyltransferase/genetic disease/lactosaminoglycans/glycolipids/Ii antigens)

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ABSTRACT **Congenital dyserythropoietic anemia type II, or hereditary erythroblastic multinuclearity with a positive acidified-serum-lysis test (HEMPAS), is a genetic anemia in humans inherited by an autosomally recessive mode. The enzyme defect in most HEMPAS patients has previously been proposed as a lowered activity of *N*-acetylglucosaminyltransferase II, resulting in a lack of polylactosamine on proteins and leading to the accumulation of polylactosaminyl lipids. A recent HEMPAS case, G.C., has now been analyzed by cell-surface labeling, fast-atom-bombardment mass spectrometry of glycopeptides, and activity assay of glycosylation enzymes. Significantly decreased glycosylation of polylactosaminoglycan proteins and incompletely processed asparagine-linked oligosaccharides were detected in the erythrocyte membranes of G.C. In contrast to the earlier studied HEMPAS cases, G.C. cells are normal in *N*-acetylglucosaminyltransferase II activity but are low in α-mannosidase II (α-ManII) activity. Northern (RNA) analysis of poly(A)$^+$ mRNA from normal, G.C., and other unrelated HEMPAS cells all showed double bands at the 7.6-kilobase position, detected by an α-ManII cDNA probe, but expression of these bands in G.C. cells was substantially reduced (<10% of normal). In Southern analysis of G.C. and normal genomic DNA, the restriction fragment patterns detected by the α-ManII cDNA probe were indistinguishable. These results suggest that G.C. cells contain a mutation in α-ManII-encoding gene that results in inefficient expression of α-ManII mRNA, either through reduced transcription or message instability. This report demonstrates that HEMPAS is caused by a defective gene encoding an enzyme necessary for the synthesis of asparagine-linked oligosaccharides.**

Congenital dyserythropoietic anemia type II is a genetic anemia in humans inherited by an autosomally recessive mode (1, 2). This type of anemia has been characterized by hereditary erythroblastic multinuclearity associated with a positive acidified-serum-lysis test (HEMPAS) (3) and so has also been called HEMPAS. Earlier studies implicated plasma membrane abnormalities of erythrocytes and erythroblasts as the major cause of this disease (1–3).

Anselstetter *et al.* (4) first reported that band 3 glycoproteins from HEMPAS erythrocyte membranes migrate faster on NaDodSO$_4$/PAGE than those from normal membranes. Since then, the lower molecular weight of band 3 has been recognized as a specific molecular feature of HEMPAS (5–8). Decreased glycosylation of HEMPAS erythrocyte-membrane proteins was demonstrated by cell-surface labeling (7, 9) as well as by chemical carbohydrate analysis (10, 11). Thus, in HEMPAS, band 3 and band 4.5 appear to lack polylactosamines made of galactose and *N*-acetylglucosamine repeats (7, 9), whereas in normal erythrocytes, these glycoproteins contain large carbohydrates, polylactosaminoglycans (12, 13). Structural analysis of HEMPAS band 3 carbohydrate revealed a truncated trimannosyl hybrid-type oligosaccharide (14). The formation of such an unusual oligosaccharide agrees with the lowered activity of *N*-acetylglucosaminyltransferase II (GlcNAcT II) found in the same patient's cells (14). [Nomenclature of GlcNAcTs is patterned after Schachter *et al.* (15). UDP-GlcNAc:polylactosamine, β1→3 *N*-acetylglucosaminyltransferase, or polylactosamine extension enzyme is called GlcNAcT VIII in this paper.]

During analysis of additional HEMPAS cases, we noticed that some HEMPAS cells exhibited normal GlcNAcT II activity but were low in α-mannosidase II (α-ManII) activity. (Preliminary results indicate two HEMPAS cases, including G.C., that showed low α-ManII and normal GlcNAcT II activities.) This report describes a HEMPAS case in which expression of α-ManII mRNA is significantly reduced.

## MATERIAL AND METHODS

**Cells.** A peripheral blood sample obtained from HEMPAS patient G.C. was kept at 4°C for 2–4 days with anticoagulant before analysis. Other HEMPAS patients' blood samples have been provided by P. Scartezzini (Galliera Hospital, Genoa, Italy), P. Izzo (University of Bari, Bari, Italy), and G. F. Gaetani (University of Genoa). Lymphocytes were isolated from the peripheral blood and transformed by the Epstein–Barr virus (EBV) derived from culture supernatant of B95-8 cells (ATCC CRL-1612) and maintained in tissue culture.

**cDNA Probes.** Rat α-ManII cDNA has been isolated and sequenced as reported (16). Human α-ManII cDNA was cloned by screening a random-primed HepG2 cDNA prepared in a λZapII vector (Stratagene) library with rat α-ManII cDNA probe. The human α-ManII cDNA used for probe in this study is 1.3 kilobases (kb) long and covers nucleotide −5 to 1344 relative to the translation-start site (K.W.M., unpublished work).

Human galactosyltransferase (GalT) cDNA has been isolated and sequenced (17). The cDNA probe used in this study

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Abbreviations: α-ManII, α-mannosidase II; GlcNAcT, *N*-acetylglucosaminyltransferase; GalT, β1,4-galactosyltransferase; EBV, Epstein–Barr virus; FAB-MS, fast-atom-bombardment mass spectrometry; HEMPAS, hereditary erythroblastic multinuclearity with positive acidified-serum-lysis test.

is a ≈400-base-pair (bp) fragment excised by *Sma* I and covers the 5'-untranslated region plus coding sequence up to nucleotide 285.

**Cell-Surface Labeling.** Erythrocytes were incubated with galactose oxidase and then reduced with $NaB[^3H]_4$ so that the terminal galactose and *N*-acetylgalactosamine of glycoproteins and glycolipids were labeled with tritium (12). Surface-labeled erythrocytes were treated with endo-β-galactosidase from *Escherichia freundii*. Membranes were prepared and analyzed by $NaDodSO_4$/PAGE after which fluorography, as described (12), was done.

**Preparation of N-Glycans and Fast-Atom-Bombardment Mass Spectrometry (FAB-MS).** Erythrocyte membranes were prepared by hypotonic lysis of 20 ml of erythrocytes (as packed volume) from patient G.C. and were first extracted with 10 times the volume with chloroform/methanol, 2:1 (vol/vol), to remove lipids. The residues were extensively digested with Pronase, and glycopeptides were isolated by using gel filtration and affinity chromatography on Con A–Sepharose, as described (14). About 75% of the glycopeptides (judged by orcinol reaction) were bound to Con A. The bound materials were eluted with 100 mM methyl-α-mannoside. The affinity-purified glycopeptides were methylated and analyzed by FAB-MS as described (14).

**Glycosyltransferase and α-ManII Assay.** Mononucleated cells were obtained from the peripheral blood by Histopague centrifugation. The cells were homogenized, and microsome fractions were obtained as described (14). EBV-transformed and cultured B lymphoblasts were also homogenized, and microsomes were obtained. Assays of GlcNAcT I, GlcNAcT II, GlcNAcT VIII, and GalT were done according to described methods (14, 18). Synthetic oligosaccharides Manα1→6(Manα1→3)Manβ1→$O(CH_2)_8$COOMe and Manα1→6(GlcNAcβ1→2Manα1→3)4-deoxyManβ1→$O(CH_2)_8$-COOMe were provided by O. Hindsgaul (University of Alberta) and used as acceptors for GlcNAcT I and GlcNAcT II, respectively (19). GlcNAcT VIII was assayed using asialo-$\alpha_1$ acid glycoprotein as an acceptor, as described (14). GalT II was assayed by using di-*N*-acetylchitobiose as acceptor (18). α-ManII was assayed exactly as described (20). Cultured EBV lymphoblasts ($1 \times 10^6$ cells) were homogenized, and the nuclei and unbroken cells were collected by low-speed centrifugation. The supernatant was centrifuged at 160,000 × *g* for 1 hr. The pellet (designated total membrane fraction) was washed with buffer containing 0.3 M NaCl and was centrifuged to obtain salt-washed membranes. α-ManII activity was assayed by measuring hydrolysis of 4-methylumbelliferyl α-D-mannoside (Sigma). Fluorescence was measured by a Turner fluorometer model 112.

**Northern Analysis.** Total RNA was prepared from EBV-transformed B lymphoblasts ($1 \times 10^8$ cells) in culture using an RNA isolation kit (Stratagene). Poly(A)$^+$ mRNA was isolated by applying total RNA to an oligo-dT column as described (21, 22). Gel electrophoresis of total RNA (10 to ≈20 μg per lane) or poly(A)$^+$ mRNA (10 μg per lane) was done in a 1% agarose gel containing formaldehyde. RNAs were then blotted onto a nylon filter (Nytran filter, Schreicher & Schnell) and fixed by UV irradiation (totally 1200 μJ). Prehybridization was done at 42°C for 6 hr in 50% (wt/vol) formamide/5× Denhardt's solution (1×Denhardt's solution is 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 5× SSPE (1× SSPE is 0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA)/0.1% $NaDodSO_4$/fragmented and denatured herring sperm DNA at 150 μg/ml. Hybridization occurred at 42°C for 12 hr in a fresh solution similar to that described above but containing either $^{32}$P-labeled α-ManII cDNA probe or $^{32}$P-labeled GalT cDNA probe. The filter was washed twice in 1× SSPE/0.5% $NaDodSO_4$ at 37°C and once in the same buffer at 65°C for 30 min and was exposed to x-ray film at −70°C for 16 hr with an intensifying screen. The sizes of the mRNAs were determined by using a 0.24- to ≈9.5-kb RNA ladder (BRL).

**Southern Analysis.** Genomic DNA was isolated from cultured B lymphoblasts from HEMPAS G.C. and from normal according to a described procedure (23). The genomic DNA (10 μg each) was digested with *Bam*HI, *Eco*RI, *Hin*dIII, and *Pvu* II for 12 hr at 37°C followed by addition of fresh enzymes for another 12 hr. The enzyme-digested DNAs were separated by 0.7% agarose gel electrophoresis and then blotted onto a nylon filter (24). Prehybridization occurred at 42°C for 4 hr in 6× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/10× Denhardt's solution/0.5% $NaDodSO_4$/fragmented and denatured herring sperm DNA at 50 μg/ml. Hybridization was done at 42°C for 16 hr in the same solution with the addition of 50% formamide and $^{32}$P-labeled α-ManII cDNA probe and minus Denhardt's solution. After being washed three times with 1× SSPE/0.5% $NaDodSO_4$ at 42°C and once with 1× SSPE/0.5% $NaDodSO_4$ at 65°C for 30 min, the nylon filter was exposed to x-ray film at −70°C for 2 days with intensifying screen.

## RESULTS

**Cell-Surface Labeling of Erythrocyte Glycoconjugate.** Cell-surface labeling, with galactose oxidase/$NaB[^3H]_4$, efficiently labels polylactosamine glycoconjugates. In normal erythrocytes, band 3 and band 4.5 glycoproteins are labeled, and the labeled carbohydrates are susceptible to endo-β-galactosidase treatment (12). In HEMPAS erythrocytes, however, these glycoproteins are not labeled by the same procedure; polylactosaminylceramides appear as a diffuse band in the 20- to 30-kDa region upon $NaDodSO_4$/PAGE (7). Cell-surface labeling followed by endo-β-galactosidase digestion of G.C. erythrocytes shows the characteristic glycoconjugate pattern typical of HEMPAS (Fig. 1). Thus, polylactosamines appear to shift from protein acceptors to lipid acceptors as described (7, 11) in other HEMPAS cases.

**Structural Analysis of N-Glycans.** To determine the carbohydrate structures, glycopeptides were prepared from G.C. erythrocyte membranes. The FAB-MS (Fig. 2) shows the relatively prominent peak *m/z* 2090 for $NeuNAc_1 \cdot Hex_6 \cdot HexNAc_2^+$ (Neu, neuraminic acid; NAc, *N*-acetyl; Hex,

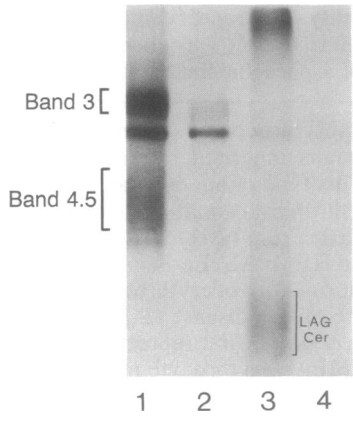
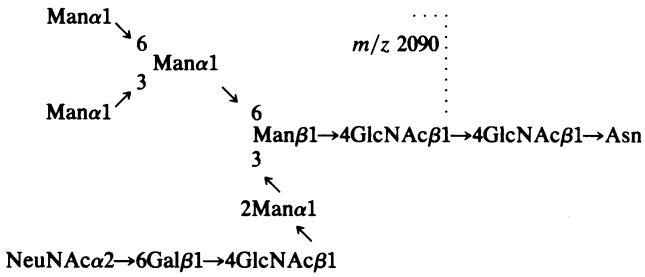



FIG. 1. Fluorogram of surface-labeled erythrocyte membrane components. Erythrocytes were treated with galactose oxidase followed by $NaB[^3H]_4$. Surface-labeled erythrocytes were incubated with or without endo-β-galactosidase. Erythrocyte membranes were then prepared, dissolved in $NaDodSO_4$ sample buffer, and analyzed by $NaDodSO_4$/PAGE followed by fluorography. Fluorograms of normal (lanes 1 and 2) and HEMPAS G.C. (lanes 3 and 4) membranes have been treated with (lanes 2 and 4) or without (lane 1 and 3) endo-β-galactosidase. LAG-Cer, polylactosaminylceramides.

hexose), indicative of the following hybrid structure:




There are also signals assignable to the structure of trimannosyl hybrid type ($m/z$ 1682), high-mannose type ($m/z$ 1280 and 1484), biantennary complex type ($m/z$ 2131, 2494), and polylactosamine repeats ($m/z$ 913, 1362, and 1811) (Fig. 2). In contrast, the FAB spectra of normal band 3 are dominated by signals derived from polylactosamine repeats (13). The FAB spectra of glycopeptides from G.C. also differ from spectra obtained on previously reported HEMPAS cases, which showed strong signals for hybrid-type oligosaccharides, particularly for the $m/z$ 1682 trimannosyl hybrid structure (14).

**Golgi Glycosylation Enzyme Activities.** To examine enzyme activities involved in N-glycan synthesis in G.C. cells, glycosyltransferases as well as α-ManII were assayed (Table 1 and Fig. 3). α-ManII was included because the structure described above suggested a deficiency of this enzyme in G.C. cells. Table 1 shows that analyses using both peripheral blood cells and cultured lymphoblasts exhibit normal GlcNAcT II activity in G.C., in contrast to the previously analyzed HEMPAS cases. Because of interference by the lysosomal α-mannosidase, a total membrane fraction and salt-washed membranes were prepared from control and G.C. cultured lymphoblast cells. pH profiles of the total membrane fraction from both sources revealed a prominent peak at pH 4.5–4.75 for the lysosomal α-mannosidase with a shoulder in the control cells at pH 5.75–6.0 for α-ManII (cf., pH 5.5 for rat liver α-ManII). A hypertonic wash of the membranes to release the lysosomal α-mannosidase activity confirmed the presence of α-ManII activity in control membranes, but negligible activity was detected in G.C. membranes. In a separate experiment, using total membrane fraction prepared from peripheral blood mononuclear cells, low α-ManII activity in G.C. was also suggested (data not shown).

**Northern Analysis.** Because cultured B lymphoblasts from the HEMPAS G.C. patient showed low α-ManII activity, we examined the mRNA encoding α-ManII by Northern blot analysis with a human α-ManII cDNA probe. Total RNA was isolated from cultured B lymphoblasts of HEMPAS G.C. and normal. Northern analysis of total RNA showed apparently reduced α-ManII message at 7.6 kb in HEMPAS G.C. (Fig. 4). Variation of the α-ManII signal (the relative intensity of G.C. α-ManII compared with normal varied between 10 and 70%) did not allow a decision whether α-ManII mRNA was reduced in G.C. However, the results clearly showed no increase in larger α-ManII mRNA species, suggesting that the splicing and the termination coupled to polyadenylylation occurred normally in G.C. cells.

Northern analysis of poly(A)$^+$ mRNA isolated from normal, HEMPAS G.C., and two other unrelated HEMPAS cases (B.R. and B.S.) demonstrated a double band at 7.6 kb in all cases, but the expression level of these bands in HEMPAS G.C. cells was substantially reduced (<10% of normal) (Fig. 5*A*). The same filter was stripped from the α-ManII probe and rehybridized with GalT cDNA probe. Fig. 5*B* shows that all normal and HEMPAS RNA samples exhibit a comparable level of GalT mRNA at 4.1 kb. These results

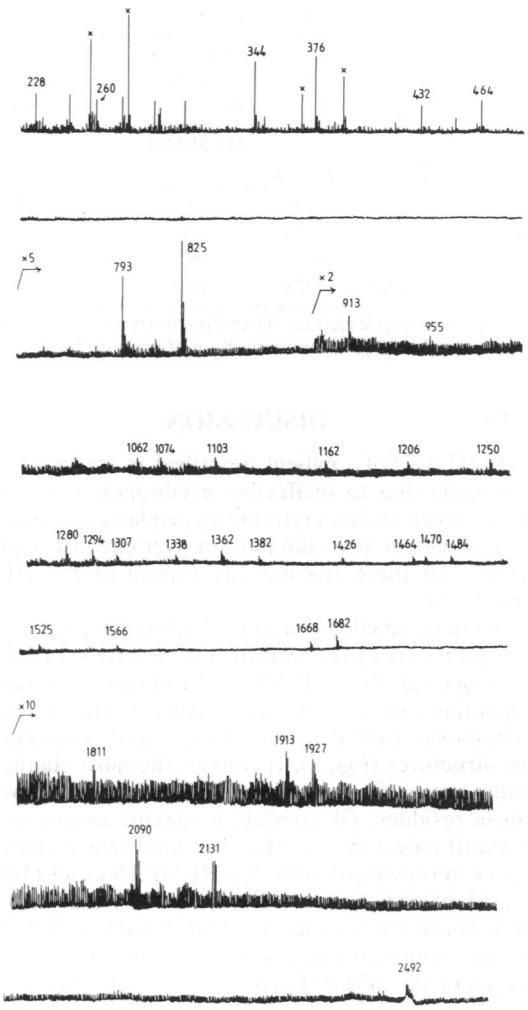



FIG. 2. FAB mass spectrum of permethylated glycopeptides of HEMPAS G.C. erythrocyte membranes. Signals attributable to high-mannose oligosaccharides are present at $m/z$ 1280 for $Hex_5{\cdot}HexNAc_1^+$ and $m/z$ 1484 for $Hex_6{\cdot}HexNAc_1^+$ (where Hex is hexose). The hybrid structure affords $m/z$ 1682 for $NeuNAc_2{\cdot}Hex_4{\cdot}HexNAc_1^+$ and $m/z$ 2090 for $NeuNAc_1{\cdot}Hex_6{\cdot}HexNAc_2^+$. The complex oligosaccharides afford $m/z$ 2131 for $NeuNAc_1{\cdot}Hex_5{\cdot}HexNAc_3^+$ and $m/z$ 2492 for $NeuNAc_2{\cdot}Hex_5{\cdot}HexNAc_3^+$. The presence of a small amount of lactosamine repeats is shown by ions $m/z$ 913 for $Hex_2{\cdot}HexNAc_2^+$, $m/z$ 1362 for $Hex_3{\cdot}HexNAc_3^+$, and $m/z$ 1811 for $Hex_4{\cdot}HexNAc_4^+$. Other signals are assigned as follows: $m/z$ 376 and $m/z$ 344 (376 − 32) for $NeuNAc^+$; $m/z$ 825 and $m/z$ 793 (825 − 32) for $NeuNAc_1{\cdot}Hex_1{\cdot}HexNAc_1^+$; and $m/z$ 1062 for the β-cleavage ion of $(OH)Hex_4{\cdot}HexNAc_1^+$. The series of signals 44 mass units apart at $m/z$ 1074, 1118, 1162, 1206, 1250, 1294, 1338, 1382, 1426, and 1470 arise from a trace amount of detergent impurity.

indicate that HEMPAS G.C. cells express abnormally low levels of α-ManII mRNA. The results also suggest the existence of the α-ManII gene in G.C. because a weak, but detectable, amount of the 7.6-kb mRNA is seen (Fig. 4, lane 1, and Fig. 5*A*).

**Southern Analysis.** To examine whether genomic DNA encoding α-ManII is altered in G.C., genomic DNA was isolated from cultured B lymphoblasts, digested with restriction enzymes, and analyzed by Southern blot. Fig. 6 shows that restriction fragments of the genomic DNA of G.C. were indistinguishable from those of normal genomic DNA.

This result indicates that, within the resolution of the restriction mapping described here, the mutation in the α-ManII gene in G.C. cells is not a consequence of a deletion or recombination event.

Table 1. Activities of glycosyltransferases

| | Peripheral mononucleated cells | | Cultured B lymphoblasts | |
|---|---|---|---|---|
| | Normal | HEMPAS G.C. | Normal | HEMPAS G.C. |
| GlcNAcT I (1 hr) | 595 | 783 | 1391 | 2104 |
| GlcNAcT II (4 hr) | 134 | 189 | 219 | 311 |
| GalT (1 hr) | 4039 | 4085 | 5303 | 6590 |
| GlcNAcT VIII (4 hr) | 728 | 880 | 704 | 857 |

Numbers are expressed as $^3$H radioactivity in cpm incorporated into acceptors by 10 μl of enzyme (8 μg of protein) for the indicated time.

## DISCUSSION

The HEMPAS G.C. patient described in this paper suffers from anemia due to ineffective erythropoiesis in his bone marrow, which shows erythroid hyperplasia and multinuclearity. The patient also suffers from liver cirrhosis and hemosiderosis. All these features are typical of the HEMPAS disease (2, 3).

Cell-surface labeling and endo-β-galactosidase digestion of G.C. erythrocytes gave a profile characteristic of HEMPAS (Fig. 1) (see ref. 7). FAB-MS of the erythrocyte membrane glycopeptides showed diverse oligosaccharides—including high-mannose, hybrid, complex-type, and polylactosamine repeat structures (Fig. 2). However, the most characteristic structure present in this patient is a hybrid structure with five mannose residues. Glycosylation enzyme assays suggested an α-ManII defect in G.C. (Fig. 3) rather than a GlcNAcT II defect as in previously reported HEMPAS cases (14). Low-level α-ManII in G.C. is confirmed by RNA analysis showing much reduced expression of α-ManII mRNA (Fig. 5). Furthermore, Southern analysis of genomic DNA shows the existence of the α-ManII gene in G.C. cells (Fig. 6).

These results indicate that the most likely gene defect in HEMPAS G.C. lies in the 5′ regulatory region, resulting in ineffective transcription of α-ManII mRNA because <10% of α-ManII mRNA is detected. We cannot, however, rule out the possibility that the mutation results in an instability of the α-ManII mRNA (25, 26).

We have previously reported HEMPAS cases that showed accumulation of trimannosyl hybrid oligosaccharide and low GlcNAcT II activity (14). The previous studies on HEMPAS were, however, limited to phenotype analysis. It was uncertain whether HEMPAS was primarily caused by a gene defect in the glycosylation enzyme or whether another genetic factor such as defective Golgi apparatus influenced glycosylation in HEMPAS. This report demonstrates that HEMPAS is caused by a defective gene encoding an enzyme necessary for the maturation of asparagine-linked oligosaccharides. The combined evidence also suggests that HEMPAS is a genetically heterogenous collection of diseases caused by glycosylation deficiencies.

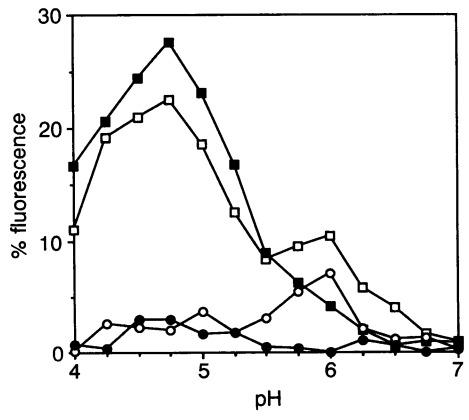



FIG. 3. pH profiles of α-mannosidase activity in cultured lymphoblast membrane fractions. Enzyme assay was done as described (20) at the indicated pH. Plots show the relative intensity of fluorescence as compared with substrate control. Plots are extracts from normal (□) and G.C. (■) total membranes and from normal (○) and G.C. (●) salt-washed membranes.

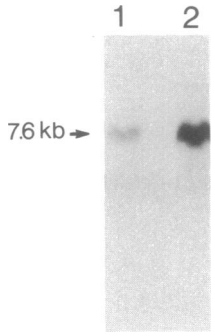



FIG. 4. Northern analysis for α-ManII mRNA in HEMPAS G.C. and normal membranes. Total RNA was isolated from EBV-transformed B lymphocytes of HEMPAS G.C. and normal membranes. Approximately 20 μg of total RNA was loaded in each lane. The filter was hybridized with $^{32}$P-labeled human α-ManII cDNA probe. Lanes: 1, HEMPAS G.C.; 2, normal.

Clinical reports on HEMPAS show variation among patients in the magnitude of anemia and in the association of other illness (1–3, 27, 28). Analysis of each HEMPAS case at the gene level with the cDNA probes for α-ManII and GlcNAcT II is needed to clarify the genetic defects and to understand the clinical variations.

Erythrocyte polylactosaminoglycans are unusual because they have a biantennary core structure (13). Polylactosamines are preferentially attached to the GlcNAcβ1→ 2Manα1→6Manβ1→ arm (13). Lowered activity of α-ManII results in the drastic failure of polylactosaminoglycan formation in erythrocyte glycoproteins, as seen in HEMPAS G.C. (Fig. 1). In the biosynthesis pathway, α-ManII hydrolyzes the high-mannose oligosaccharide, producing a substrate for GlcNAcT II (29). A defect of α-ManII might alter N-glycan synthesis, which may resemble a GlcNAcT II defect. In contrast, polylactosamines in granulocytes (30), teratocarcinoma cells (31), and lymphoma cells (32) have tri- and tetraantennary core structures. In these cells, low α-ManII (or GlcNAcT II) activity does not necessarily inhibit polylactosamine formation, as polylactosaminyl side chains

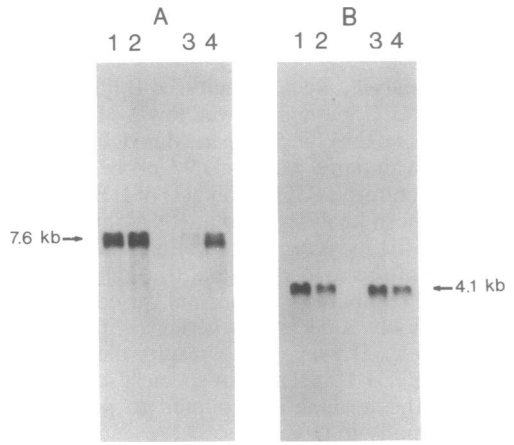



FIG. 5. Northern analysis for α-ManII and GalT mRNA in normal and HEMPAS cells. Poly(A)$^+$ mRNA was isolated from EBV-transformed B lymphocytes of normal, HEMPAS G.C., and two other unrelated HEMPAS patients. Approximately 10 μg of poly(A)$^+$ RNA was loaded in each lane. (A) Autoradiogram of the filter hybridized with $^{32}$P-labeled human α-ManII cDNA probe. (B) Same filter in *A* after removal of the α-ManII probe and hybridization with $^{32}$P-labeled human GalT cDNA probe. Lanes: 1, HEMPAS B.S.; 2, HEMPAS B.R.; 3, HEMPAS G.C.; and 4, normal.

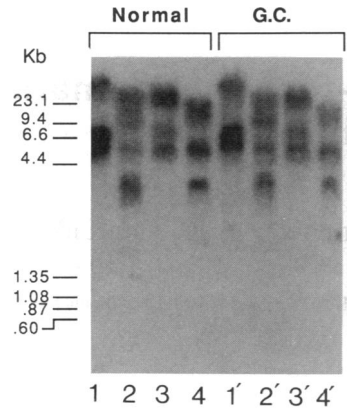



FIG. 6. Southern blot analysis of genomic DNA encoding α-ManII. Genomic DNAs from normal and HEMPAS G.C. were isolated from B lymphoblasts and were digested extensively with restriction enzymes. Each lane contains digested DNA (10 μg) with *Bam*HI (lanes 1 and 1'), *Eco*RI (lanes 2 and 2'), *Hin*dIII (lanes 3 and 3'), and *Pvu* II (lanes 4 and 4'). $^{32}$P-labeled α-ManII cDNA probe was used.

also arise from the Manα1→3Manβ1→ arm. Such difference in core structure must be responsible for the apparently erythroid-specific phenotype in HEMPAS defect.

In HEMPAS erythrocytes, band 3 and band 4.5, which are normally glycosylated by polylactosamines, virtually lack a large carbohydrate moiety. This lack increases total hydrophobicity of these glycoproteins and subsequently induces clustering of band 3 (33), which causes abnormal distribution of band 3-associated proteins and lipids; this may result in the morphologically visible membrane abnormality.

During differentiation of erythroid cells, the expression of polylactosamines is greatly increased at the erythroblast stage (34). The HEMPAS membrane abnormality is seen in erythroblasts but not in earlier precursor cells (35). The membrane glycoproteins in HEMPAS erythroblasts could be clustered or abnormally distributed due to the lack of polylactosamines, resulting in disturbance of membrane architecture. It is plausible that the single gene defect in α-ManII (or GlcNAcT II) could result in ineffective erythropoiesis in the HEMPAS patient's bone marrow.

Besides anemia, HEMPAS is associated with liver cirrhosis/hemosiderosis and secondary tissue siderosis (1–3). The α-ManII gene defect probably affects glycosylation in liver cells. In fact, incompletely processed N-glycans have been detected in HEMPAS patients' serum glycoproteins (M.N.F., A. Dell, P. Izzo, G. E. Gaetani, and P. Scartezzini, unpublished data) of which most are synthesized in hepatocytes and secreted into the circulation. Immature N-glycans of the circulating glycoproteins should be recognized by lectin-like receptors in hepatocytes, Kupffer cells in the liver, and macrophages in the reticuloendothelial system (36). The quantity of serum glycoproteins to be cleared from the circulation is enormous and could eventually cause cirrosis and siderosis in HEMPAS patients.

High incidence of diabetes, gall stones, mental, and sensory abnormalities have been reported in HEMPAS patients (2, 3). The primary gene defect in one of the glycosylation enzymes must be directly or indirectly responsible for each of these clinical symptoms. Future studies should attempt to define the link between the primary gene defect and the above-mentioned symptoms in HEMPAS.

On the other hand, many tissues and organs are apparently unaffected by HEMPAS, despite the fact that α-ManII is normally present in all types of cells. Thus in most tissues and organs, altered N-glycan synthesis may not necessarily cause ill effects, as exemplified in the α-ManII-deficient BHK mutant cell line (37) or isozyme forms could exist. Fetal-type α-ManII isozyme (and GlcNAcT II isozyme) could be present because HEMPAS patients have developed normally throughout embryonic stages. The GalT isozyme, GalT 2 (38), is expressed in some tumor cells and in the human fetus (39). Further studies on the α-ManII gene, as well as possibly defective genes encoding glycosyltransferases in HEMPAS and its variant, will allow us to explore the fundamental roles of glycosylation enzymes in human cells.

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