Glucose 6-phosphate transport in fibroblast microsomes from glycogen storage disease type 1b patients: evidence for multiple glucose 6-phosphate transport systems

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In liver endoplasmic reticulum the intraluminal glucose-6-phosphatase activity requires the operation of a glucose 6-phosphate transporter (G6PT1). Mutations in the gene encoding G6PT1 cause glycogen storage disease type 1b, which is characterized by a loss of glucose-6-phosphatase activity and impaired glucose homeostasis. We describe a novel glucose 6-phosphate (G6P) transport activity in microsomes from human fibroblasts and HeLa cells. This transport activity is unrelated to G6PT1 since: (i) it was similar in microsomes of skin fibroblasts from glycogen storage disease type 1b patients homozygous for mutations of the *G6PT1* gene, and in microsomes from human control

subjects; (ii) it was insensitive to the G6PT1 inhibitor chlorogenic acid; and (iii) it was equally active towards G6P and glucose 1-phosphate, whereas G6PT1 is highly selective for G6P. Taken together, our results provide evidence for the presence of multiple transporters for G6P (and other hexose phosphoesters) in the endoplasmic reticulum.

Key words: endoplasmic reticulum, glucose-6-phosphatase system, glucose-6-phosphate translocase, hexose phosphate transport system, von Gierke's disease.

INTRODUCTION

During the last 20 years glucose 6-phosphate (G6P) transport has been well characterized in liver microsomal fractions [1–3]. This transport system is required for the access of the substrate to the glucose-6-phosphatase (G6Pase; EC 3.1.3.9) enzyme, which is situated in the hepatic endoplasmic reticulum (ER) lumen ([3] and references therein). The genetic deficiency of this G6P transport activity causes a severe metabolic disease termed type 1b glycogen storage disease (GSD1b) [4]. A gene encoding a putative liver ER transporter for G6P (G6PT1) was cloned [5] and shown to be mutated in patients suffering from GSD1b [5–13]. In this disease the major tissue damage is present in liver and kidney, consistent with the high representation of the G6Pase system in these organs [14]. In addition, many GSD1b patients also have defects in neutrophils/monocytes [4,9]. By contrast, the G6Pase enzyme/activity is not present in these blood cells, which suggests another role for the G6P transport protein in addition to supplying the substrate to the G6Pase enzyme.

A G6P transport activity has been occasionally reported in ER fractions from non-hepatic tissues [15–18], but it has not been extensively characterized. Anti-peptide antibodies against the liver G6PT1 have been raised by several labs ([19,20] and A. Benedetti and A. Burchell, unpublished work), but they are either poorly reactive or react with more than one band on Western blots. Two different G6PT1 mRNAs, arising by differential splicing of the same gene, have been detected by Northern blotting: the predominant liver form contains eight exons and a major brain form contains nine exons [21]. Northern-blot analysis also revealed that G6PT1 messages are present in a variety of extrahepatic tissues and cells [22], including leukocytes [22,23], but their sequences have not been determined. In

addition, G6P transport has not been characterized in microsomal fractions of non-hepatic tissues from GSD1b patients.

In the present study we have evaluated the G6P transport activity in microsomal fractions of fibroblasts from control humans and GSD1b patients. Both control and patient fibroblast microsomes possess very similar G6P transport activities, which exhibit different properties to liver microsomal G6P transport. Our results provide the first evidence for the existence of ER G6P transport activities unrelated to (liver) G6PT1 and encoded by gene(s) other than the *GSD1b* gene.

EXPERIMENTAL

Cell cultures

Skin fibroblast cultures were established from punch biopsies of two GSD1b patients and two healthy donors with parental consent. Fibroblasts between passages 5–25 were grown in monolayer culture in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal-calf serum, 4 mM sodium lactate, 100 units/ml penicillin and 100 µg/ml streptomycin. HeLa cells were cultured as fibroblasts except that no sodium lactate and 2 mM glutamine were added to the medium. Fibroblasts from GSD1b patients showed the same growth rate, cell shape and morphology as fibroblasts from human control subjects.

Mutational analysis

The *G6PT1* gene mutations were determined as reported in [6,9].

Reverse transcription (RT)-PCR assay of G6PT1

RNA from human fibroblasts and HeLa cells was isolated

Abbreviations used: G6P, glucose 6-phosphate; G6Pase, glucose-6-phosphatase; ER, endoplasmic reticulum; GSD1b, glycogen storage disease type 1b; G6PT1, glucose 6-phosphate transporter; G1P, glucose 1-phosphate; RT, reverse transcription.

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according to the SV Total RNA isolation procedure (Promega). A 5 µg portion of total RNA was reverse transcribed at 37 °C for 50 min in a volume of 25 µl with 50 mM Tris/HCl, pH 8.3, 50 mM KCl, 10 mM MgCl₂, 0.5 mM spermidine, 10 mM dithiothreitol, 200 µM each dNTPs, 40 units of Rnasin Ribonuclease Inhibitor (Promega), 0.5 µg random hexamers and 2.5 units of avian myeloblastosis virus reverse transcriptase (Promega). The enzyme was inactivated at 90 °C for 5 min, and 10 µl of the cDNA was then combined with 10 mM Tris/HCl, pH 8.8, 50 mM KCl, 0.08 % Nonidet P40, 1.5 mM MgCl₂, 200 µM each dNTPs, 0.2 µM forward primer (5'-TGCTGTTCATGATGGCTGGC-AT-3'), 0.2 µM reverse primer (5'-CTATGACTCCAAACAG-GGCAAT-3') and 2.5 units of *Taq* Polymerase (Helena Biosciences, London, U.K.). The two primers are located respectively in exon 6 and exon 8 of the human G6PT1 [21]. PCR was carried out as follows: cycle 1, 95 °C (5 min), 50 °C (2 min), 72 °C (1 min); cycles 2–30, 95 °C (1 min), 50 °C (2 min), 72 °C (1 min), and elongation step, 72 °C (10 min). A 20 µl portion of the final mixture containing the PCR products (out of a total volume of 100 µl) were analysed on a 12 % acrylamide gel. The gel (8 cm × 10 cm) was run at 100 volts and then silver stained (protocol modified from [24]). The identity of the RT-PCR products was confirmed by DNA sequencing.

Preparation of microsomal fractions

Cultured HeLa cells and fibroblasts (90 % confluence) were harvested by plate scraping and washed twice with PBS. Cell pellets were resuspended (20 %, w/v) in 0.3 M sucrose, 10 mM Hepes (pH 7.2) including a cocktail of protease inhibitors (1 mM PMSF, 1 µM pepstatin A, 1 µM leupeptin and 1 µg/ml of aprotinin). Microsomes were then prepared from the suspensions of human fibroblasts and HeLa cells, as well from the liver of male Sprague–Dawley rats (180–230 g) according to [3]. Microsomal fractions were resuspended in 100 mM KCl, 20 mM NaCl, 1 mM MgCl₂ and 20 mM Mops (pH 7.2). The suspensions were rapidly frozen and maintained under liquid N₂ until used.

Intactness of liver microsomes was inferred from the latency of mannose-6-phosphatase activity [3], which was found to be greater than 90 % in all the preparations assayed. Due to the negligible G6Pase activity in fibroblast and HeLa cell microsomes, mannose-6-phosphatase activity could not be used to evaluate intactness. We therefore evaluated the permeability of fibroblast and HeLa microsomes towards the poorly permeant compound sucrose [2,25]. To this end, microsomes were incubated in the presence of 1 mM sucrose plus [¹⁴C]sucrose (1 µCi/ml) at 22 °C, as detailed in [26]. Steady-state levels of sucrose uptake were reached at incubation times ≥ 3 h in all the fractions employed, which was consistent with a low permeability to sucrose of microsomal membranes [2,3,25,26].

The total intravesicular space was evaluated by measuring the sucrose-accessible space of microsomes incubated for 6 h in the presence of 1 mM sucrose plus [¹⁴C]sucrose (1 µCi/ml) at 22 °C [26]. The sucrose-accessible space was 1.91 ± 0.63, 1.81 ± 0.70, 2.07 ± 0.52, 1.80 ± 0.59, 1.71 ± 0.38 and 3.43 ± 0.42 µl/mg microsomal protein (means ± S.D.; *n* = 3) in fibroblasts from GSD1b patient no. 1 and no. 2, fibroblasts from human control no. 1 and no. 2, and HeLa cells and rat liver respectively.

Uptake measurements

G6P and glucose 1-phosphate (G1P) microsomal uptake were measured as reported previously [3]. Briefly, microsomes (100–200 µg of protein/ml) were incubated in the KCl/Mops buffer (see above) in the presence G6P (1 mM, 10 mM or 30 mM) plus

[¹⁴C]G6P (8–10 µCi/ml) or G1P (1 mM) plus [¹⁴C]G1P (8–10 µCi/ml). When required, the pore-forming antibiotic alamethicin (0.02 mg/ml) was added to the incubation mixture to distinguish the intravesicular and the bound radioactivity [3,26]. The alamethicin-releasable portion of radioactivity was regarded as intravesicular [3]. At the indicated time intervals, aliquots (0.1 ml) were rapidly withdrawn and filtered through cellulose acetate/nitrate filters (pore size 0.22 µm) and were washed with 4 ml of Hepes (20 mM) buffer, pH 7.2, containing 250 mM sucrose. During the measurement of G6P uptake, parallel filters were treated after wash with ZnSO₄/Ba(OH)₂ to separate intravesicular glucose from G6P, as described in [3]. The radioactivity associated to microsomes retained by filters was measured by liquid-scintillation counting.

Assay of G6Pase

G6Pase activity was measured in native and alamethicin-permeabilized microsomes at 5 min of incubation in the KCl/Mops buffer at 22 °C on the basis of [¹⁴C]glucose production from [¹⁴C]G6P, as detailed in [3].

RESULTS

Fibroblasts were derived from two GSD1b patients who had the classic symptoms of the disease, including neutropenia. The mutations of the *GSD1b* gene in one of the patients (GSD1b no. 1) had been previously described by us [6], and consisted of a deletion of glycine-1094, resulting in reading frame shift after methionine-308 and a stop codon at position 311 in one allele, as well as a glycine-to-alanine change at position 1068, resulting in an arginine-to-histidine change at position 300, in the other allele. Single-strand conformation polymorphism analysis and DNA sequencing of the coding region and exon–intron boundaries of the *GSD1b* gene revealed that the other patient (GSD1b no. 2) had a maternally inherited deletion of glycine-1094, resulting in reading frame shift after methionine-308 and a stop codon at position 311. The paternally inherited mutation was

Figure 1 RT-PCR analysis of G6PT1 in human fibroblasts and HeLa cells

Analysis of the RT-PCR products of a fragment (see the Experimental section) of the human G6PT1 from GSD1b patient no. 1 (lane 3), GSD1b patient no. 2 (lane 4), control subject no. 1 (lane 5), HeLa cells (lane 6), human (liver) exon 8 G6PT1 147 bp marker (lane 7) and human (brain) exon 9 G6PT1 213 bp marker (lane 8). PCR products were analysed on a 12 % acrylamide gel, which was then silver stained (see the Experimental section). In lane 1, molecular-mass-markers of 500 bp (upper band) and 250 bp DNA (lower band) are shown. In lane 2 molecular-mass-markers of 400 bp, 300 bp, 200 bp, 100 bp and 80 bp DNA (from the top to the bottom of the gel) are shown.

Figure 2 G6P uptake in microsomes isolated from human fibroblasts and HeLa cells

Microsomes obtained from fibroblasts and HeLa cells were assayed for G6P uptake upon addition of 1 mM G6P by a rapid filtration technique (see the Experimental section). (A–D) G6P uptake in fibroblast microsomes from control subject no. 1, control subject no. 2, GSD1b patient no. 1 and GSD1b patient no. 2 respectively. (E) Uptake of G6P in HeLa cell microsomes. (□) The total amount of G6P associated with microsomal vesicles. Where indicated by the arrow, the pore-forming antibiotic alamethicin was added to allow distinction between intravesicular and bound hexose phosphate [3,26]. (■) Incubation systems including alamethicin from time zero. Data are the means $\pm$ S.D. of 3–5 separate experiments. The time course of intravesicular accumulation of G6P is represented by (○) and continuous lines; values were calculated as the difference of microsomal-associated radioactivity in the absence and in the presence of alamethicin. The total intravesicular volume of microsomes estimated as sucrose-accessible space (see the Experimental section) is indicated by broken lines.

a threonine-to-cysteine change at position 716, resulting in a cysteine-to-arginine change at position 183.

To determine whether the G6PT1 mRNAs are expressed in fibroblasts, fragments of G6PT1 were amplified by RT-PCR from human fibroblast RNA from GSD1b patients and a control subject, and also from HeLa cell RNA. The primers were located in exons 6 and 8 of G6PT1, allowing amplification of either exon 6 and exon 8 (G6PT1 liver isoform, 147 bp) or exons 6, 7 and 8 (G6PT1 brain isoform, 213 bp). Figure 1 shows that both isoforms are expressed in human fibroblasts (lanes 3 and 4, GSD1b no. 1 and no. 2 respectively; lane 5, control no. 1) and also in HeLa cells (lane 6). However, in all cases, the results indicate that the

Figure 3 G1P uptake in microsomes isolated from human fibroblasts and HeLa cells

Microsomes obtained from fibroblasts and HeLa cells were assayed for G1P uptake upon addition of 1 mM G1P by a rapid filtration technique (see the Experimental section). (A–D) G1P uptake in fibroblast microsomes from control subject no. 1, control subject no. 2, GSD1b patient no. 1 and GSD1b patient no. 2 respectively. (E) Uptake of G1P in HeLa cell microsomes. (□) The total amount of G1P associated with microsomal vesicles. Where indicated by the arrow, the pore-forming antibiotic alamethicin was added to allow distinction between intravesicular and bound hexose phosphate [3,26]. (■) Incubation systems including alamethicin from time zero. Data are the means $\pm$ S.D. of 3–5 separate experiments. The time course of intravesicular accumulation of G1P is represented by (○) and continuous lines; values were calculated as the difference of microsomal-associated radioactivity in the absence and in the presence of alamethicin. The total intravesicular volume of microsomes estimated as sucrose-accessible space (see the Experimental section) is indicated by broken lines.

nine-exon G6PT1 'brain' isoform [21] is expressed at a lower level than the eight-exon 'liver' isoform.

Figure 2 illustrates that G6P uptake into microsomal vesicles is present in fibroblasts. The total amount of G6P that becomes associated with microsomes along the incubation time is shown in Figure 2 (open squares). The major portion of this G6P was released upon membrane permeabilization (on addition of alamethicin [3]) in all cases, indicating that it was indeed intraluminal. A relatively low amount of G6P was also associated with microsomes which have been permeabilized with alamethicin prior to the start of the uptake assay (Figure 2, filled squares). The intravesicular accumulation of G6P (open circles) was similar

Table 1 Effect of chlorogenic acid on the intravesicular accumulation of G6P and glucose, and G6Pase activity in microsomal vesicles

Intravesicular accumulation of G6P and glucose were measured after 5 min of incubation in the presence of 1 mM G6P by a rapid filtration technique, as described in the Experimental section. G6Pase activity was also measured with 1 mM G6P as substrate. Where indicated, microsomes were preincubated for 5 min (at 22 °C) with 2 mM chlorogenic acid before initiating assays, and the inhibitor (2 mM) was also present in the assay media. Where indicated, liver microsomes were preincubated at pH 5 to inactivate specific G6Pase enzyme activity as detailed in [3]. NM, non-measurable. Results are means $\pm$ S.D. of 3–5 experiments.

	Intravesicular accumulation (nmol/mg protein)		G6Pase activity (nmol/min per mg of protein)	
	G6P	Glucose	Intact	Permeabilized
Liver microsomes	0.40 $\pm$ 0.12	4.41 $\pm$ 0.90	34 $\pm$ 6	53 $\pm$ 9
Liver microsomes preincubated at pH 5	1.80 $\pm$ 0.32	0.56 $\pm$ 0.17	4 $\pm$ 1	6 $\pm$ 2
Liver microsomes + chlorogenic acid	0.20 $\pm$ 0.08	0.81 $\pm$ 0.35	8 $\pm$ 3	46 $\pm$ 8
Fibroblast microsomes, control no. 1	1.00 $\pm$ 0.24	NM	< 0.5	< 0.5
Fibroblast microsomes, control no. 1 + chlorogenic acid	0.92 $\pm$ 0.20	NM	< 0.5	< 0.5
Fibroblast microsomes, GSD1b no. 1	0.91 $\pm$ 0.19	NM	< 0.5	< 0.5
Fibroblast microsomes, GSD1b no. 1 + chlorogenic acid	0.89 $\pm$ 0.23	NM	< 0.5	< 0.5
Fibroblast microsomes, GSD1b no. 2	0.87 $\pm$ 0.20	NM	< 0.5	< 0.5
Fibroblast microsomes, GSD1b no. 2 + chlorogenic acid	0.89 $\pm$ 0.30	NM	< 0.5	< 0.5
HeLa microsomes	0.57 $\pm$ 0.16	NM	< 0.5	< 0.5
HeLa microsomes + chlorogenic acid	0.51 $\pm$ 0.09	NM	< 0.5	< 0.5

in the control subjects and the GSD1b patients (compare Figures 2A and 2B with 2C and 2D), with respect to both the steady-state uptake and the initial rate of transport.

Figure 3 illustrates that G1P uptake into microsomal vesicles is also present in fibroblasts. Again, the uptake of this hexose phosphate was similar in GSD1b patients and controls (compare Figures 3C and 3D with 3A and 3B).

Both G6P and G1P were also transported into microsomes isolated from HeLa cells (Figures 2E and 3E respectively). The level of accumulation of both hexose phosphates in this case was lower than in microsomes from fibroblasts. The time course of the microsomal uptake was similar for G6P and G1P, both in human fibroblasts (independently of G6PT1 mutations) and HeLa cells. Intravesicular steady-state levels of both hexose phosphates accounted only for a portion of the total intravesicular volume estimated as sucrose-accessible space (Figures 2 and 3, right-hand columns, values indicated by dotted lines).

The initial rate of G6P/G1P transport cannot be easily evaluated. At 10 s (the shortest detection time with the method applied) approx. 70% of the steady-state level has already been taken up due to the limited intravesicular volume and the high rate of transport (Figures 2 and 3). Similarly, the efflux of G6P/G1P provoked by the dilution of the medium (including microsomes pre-loaded with the phosphoesters) with G6P/G1P-free buffer resulted in the loss of 70% of the intravesicular phosphoesters within 10 s (results not shown).

Table 1 shows some features of intravesicular accumulation of G6P in rat liver microsomes, human fibroblast microsomes and HeLa cell microsomes. In liver microsomes the G6P taken into the lumen can be converted into glucose (and P_i) by the intralumenal G6Pase activity. In transport assays using [^{14} C]G6P, the radioactivity accumulated into microsomal lumen is therefore a mixture of [^{14} C]glucose and [^{14} C]G6P [3]. In liver microsomes luminal [^{14} C]glucose exceeds [^{14} C]G6P by approx. 10:1 because of the high hydrolytic rate of the G6Pase enzyme (Table 1) and the relatively low rate of release of glucose [3,27]. The block of the enzyme activity by preincubation of liver microsomes at pH 5 (Table 1) resulted in a dramatic change in the luminal ratio, with [^{14} C]G6P accumulation now exceeding that of [^{14} C]glucose. The decrease in G6Pase activity after preincubation at pH 5 (Table 1) also resulted in an increase (approx. 3-fold) of the intravesicular

accumulation of G6P. Treatment of liver microsomes by the G6PT1 inhibitor chlorogenic acid [28] resulted in a decrease of the accumulation of both [^{14} C]G6P and [^{14} C]glucose, and in a significant inhibition of G6Pase activity in intact, non-permeabilized microsomes (Table 1).

In fibroblast microsomes from both GSD1b patients and controls [^{14} C]G6P, but not [^{14} C]glucose, was accumulated (Table 1). This is consistent with the negligible level of G6Pase activity found in fibroblast microsomes (Table 1). Similar results were obtained with HeLa cell microsomes (Table 1). Chlorogenic acid had no effect on G6P uptake in either fibroblast or HeLa cell microsomes (Table 1). Chlorogenic acid had also no effect on G1P transport in fibroblast microsomes from both control and GSD1b subjects, as well as from HeLa cells (results not shown).

DISCUSSION

Both the patients investigated in this study presented classical symptoms of type 1 GSD, including hypoglycaemia. There was no doubt that in both cases liver glucose production was seriously impaired. In addition, the mutations found in the *GSD1b* gene are consistent with non-functional G6PT1 protein in both cases, i.e. both have stop mutations in one allele and one of the point mutations (arginine-to-histidine change at position 300) has recently been shown to produce a non-functional protein in transfected COS cells [12]. In spite of this defect, microsomes isolated from the fibroblasts of these patients are still able to transport G6P. The simplest explanation for this observation is that transporter(s) other than the liver G6PT1 are expressed in the ER membrane and mediate the entry of the phosphoester. Obviously, this novel G6P transport system(s) must be encoded by other gene(s). The lack of inhibition of the fibroblast ER G6P transport by chlorogenic acid, a well-established inhibitor of liver G6PT1 [28], is also consistent with the existence of different transport proteins. Another difference between the fibroblast transport system(s) and liver G6PT1 is that the former equally transports G6P and G1P (Figure 2), whereas the latter is highly specific for G6P [26]. To avoid confusion with G6PT1, we propose that the novel transport activity measured in fibroblasts be called glucose phosphate transporter (GPT).

HeLa microsomes have a similar transport activity to that of fibroblast. In fact, they transport both G6P and G1P at a very similar rate and levels of steady-state accumulation (Figure 2). Moreover the G6P transport in HeLa microsomes is insensitive to the G6PT1 inhibitor chlorogenic acid (Table 1). Previous reports show that G6P can enter microsomes from a variety of extrahepatic tissues, including brain [15,18], heart [18], skeletal muscle [16,25] and adrenal glands [17]. Neither G1P transport nor chlorogenic acid inhibition was studied in these reports; however, the transport systems described appeared less specific than in liver microsomes. Other phosphoesters, e.g. mannose 6-phosphate and 2-deoxyglucose 6-phosphate, entered brain and muscle microsomes [15,16,18]. It is therefore possible that GPT is also present in tissues other than those investigated here.

GPT may also be operative in liver microsomal vesicles at a relatively low level compared with G6PT1 since there is some G6P uptake and G6Pase activity in intact microsomes that is not inhibited by chlorogenic acid (Table 1). Consistent with this possibility, we previously observed [26] that G1P is also taken up by liver microsomes, and that its uptake results in negligible glucose accumulation. The lack of glucose accumulation indicates that G1P is transported into vesicles lacking G6Pase enzyme activity, since G1P is a good substrate for the G6Pase enzyme [26]. Classic reports on the localization of the G6Pase enzyme activity in liver ER *in situ* [29], and in isolated liver microsomal vesicles [30], indicated that the enzyme is not present in all vesicles, even though it is evenly distributed throughout the entire ER network. In liver, GPT activity is therefore probably not involved in G6P hydrolysis by the G-6-Pase enzyme.

It is possible that G6PT1 is also present in relatively low levels compared with the GPT in the fibroblast (and HeLa) microsomes. The fact that RT-PCR indicates the message for both the eight- and nine-exon forms of G6PT1 to be present in these cells (Figure 1) is consistent with this possibility. Northern-blot analysis has also shown the presence of G6PT1 messages in a variety of tissues, including HeLa cells [10]. The relative contribution of the two systems to the total G6P transport cannot be evaluated because of the high rate of uptake. However, the G6PT1-mediated transport cannot be dominant since no significant differences were observed between fibroblast microsomes isolated from GSD1b patients and control subjects (Figures 2 and 3), and there was no significant inhibition of G6P transport by chlorogenic acid in fibroblast microsomes from patients and controls (Table 1). In addition, the pathology in GSD1b does not indicate any fibroblast impairment.

The existence of a second microsomal G6P transport system raises the possibility that in some circumstances it may (partially) compensate for the loss of G6PT1 in GSD1b patients. For example some GSD1b patients present with no apparent leucocyte defect [6,7,13].

The physiological role of GPT is unknown. One possibility is that it supplies hexose phosphates to hexose-phosphate dehydrogenase, which is present in the lumen of the ER [31–33]. Alternatively, it may serve as a G6P sensor in non-gluconeogenic cells, as speculated in [10].

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REFERENCES

- Arion, W. J., Lange, A. J., Walls, H. E. and Ballas, L. M. (1980) Evidence for the participation of independent translocases for phosphate and glucose 6-phosphate in the microsomal glucose-6-phosphatase system. *J. Biol. Chem.* **255**, 10396–10406
- Fulceri, R., Gamberucci, A., Scott, H. M., Giunti, R., Burchell, A. and Benedetti, A. (1995) Fatty acyl-CoA esters inhibit glucose-6-phosphatase in rat liver microsomes. *Biochem. J.* **307**, 391–397
- Bánhegyi, G., Marcolongo, P., Fulceri, R., Hinds, C., Burchell, A. and Benedetti, A. (1997) Demonstration of a metabolically active glucose-6-phosphate pool in the lumen of liver microsomal vesicles. *J. Biol. Chem.* **272**, 13584–13590
- Chen, Y. T. and Burchell, A. (1995) Glycogen storage diseases. In *The Metabolic Basis of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, W. S. and Valle, D., eds.), pp. 935–965, McGraw-Hill, New York
- Gerin, I., Veiga-da-Cunha, M., Achouri, Y., Collet, J. F. and Van Schaftingen, E. (1997) Sequence of a putative glucose 6-phosphate translocase, mutated in glycogen storage disease type Ib. *FEBS Lett.* **419**, 235–238
- Marcolongo, P., Barone, V., Priori, G., Pirolo, B., Giglio, S., Biasucci, G., Zammarchi, E., Parenti, G., Burchell, A., Benedetti, A. and Sorrentino, V. (1998) Mutations in the glucose-6-phosphate transporter (G6PT) gene in patients with glycogen storage diseases type 1b and 1c. *FEBS Lett.* **436**, 247–250
- Veiga-da-Cunha, M., Gerin, I., Chen, Y. T., de Barys, T., de Lonay, P., Dionisi-Vinci, C., Fenske, C. D., Leonard, J. V., MacConkie-Rossel, A., Schweitzer, S. et al. (1998) A gene on chromosome 11q23 coding for a putative glucose-6-phosphate translocase is mutated in glycogen storage disease types 1b and 1c. *Am. J. Hum. Genet.* **63**, 976–983
- Kure, S., Suzuki, Y. and Matsubara, Y. (1998) Molecular analysis of glycogen storage disease type 1b: identification of a prevalent mutation among Japanese patients and assignment of a putative glucose-6-phosphate translocase gene to chromosome 11. *Biochem. Biophys. Res. Commun.* **248**, 426–431
- Galli, L., Orrico, A., Marcolongo, P., Fulceri, R., Burchell, A., Melis, D., Parini, R., Gatti, R., Lam, C. W., Benedetti, A. and Sorrentino, V. (1999) Mutations in the glucose-6-phosphate transporter (G6PT) gene in patients with glycogen storage diseases type 1b and 1c. *FEBS Lett.* **459**, 255–258
- Hiraiwa, H., Pan, C. J., Lin, B., Moses, S. W. and Chou, J. Y. (1999) Inactivation of the glucose 6-phosphate transporter causes glycogen storage disease type 1b. *J. Biol. Chem.* **274**, 5532–5536
- Veiga-da-Cunha, M., Gerin, I., Chen, Y. T., Lee, P. J., Leonard, J. V., Maire, I., Wendel, U., Vikkula, M. and Van Schaftingen, E. (1999) The putative glucose 6-phosphate translocase gene is mutated in essentially all cases of glycogen storage disease type I non-a. *Eur. J. Hum. Genet.* **7**, 717–723
- Chen, L. Y., Lin, B., Pan, C. J., Hiraiwa, H. and Chou, J. Y. (2000) Structural requirements for the stability and microsomal transport activity of the human glucose 6-phosphate transporter. *J. Biol. Chem.* **275**, 34280–34286
- Kure, S., Hou, D. C., Suzuki, Y., Yamagishi, A., Hiratsuka, M., Fukuda, T., Sugie, H., Kondo, N., Matsubara, Y. and Narisawa, K. (2000) Glycogen storage disease type 1b without neutropenia. *J. Pediatr.* **137**, 253–256
- Nordlie, R. C., Foster, J. D. and Lange, A. J. (1999) Regulation of glucose production by the liver. *Annu. Rev. Nutr.* **19**, 379–406
- Forsyth, R. J., Bartlett, K., Burchell, A., Scott, H. M. and Eyre, J. (1993) Astrocytic glucose 6-phosphatase and the permeability of brain microsomes to glucose-6-phosphate. *Biochem. J.* **294**, 145–151
- Gamberucci, A., Marcolongo, P., Fulceri, R., Giunti, R., Watkins, S. L., Waddell, I. D., Burchell, A. and Benedetti, A. (1996) Low levels of glucose-6-phosphate hydrolysis in the sarcoplasmic reticulum of skeletal muscle: involvement of glucose-6-phosphatase. *Mol. Membr. Biol.* **13**, 103–108
- Hume, R., Voice, M., Pazouki, S., Giunti, R., Benedetti, A. and Burchell, A. (1995) The human adrenal microsomal glucose-6-phosphatase system. *J. Clin. Endocrinol. Metab.* **80**, 1960–1966
- Chen, P. Y., Csutora, P., Veyna-Burke, N. A. and Marchase, R. B. (1998) Glucose-6-phosphate and Ca^{2+} sequestration are mutually enhanced in microsomes from liver, brain and heart. *Diabetes* **47**, 874–881
- Kramer, W., Burger, H. J., Arion, W. J., Corsiero, D., Girbig, F., Weyland, C., Hemmerle, H., Petry, S., Haberman, P. and Herling, A. (1999) Identification of protein components of the microsomal glucose 6-phosphate transporter by photoaffinity labelling. *Biochem. J.* **339**, 629–638
- Li, Y., Mechin, M. C. and van de Werve, G. (1999) Diabetes affects similarly the catalytic subunit and putative glucose-6-phosphate translocase of glucose-6-phosphatase. *J. Biol. Chem.* **274**, 33866–33868
- Middleditch, C., Clottes, E. and Burchell, A. (1998) A different isoform of the transport protein mutated in the glycogen storage disease 1b is expressed in brain. *FEBS Lett.* **433**, 33–36
- Lin, B., Annabi, B., Hiraiwa, H., Pan, C. J. and Chou, J. Y. (1998) Cloning and characterization of cDNAs encoding a candidate glycogen storage disease type 1b protein in rodents. *J. Biol. Chem.* **273**, 31656–31660
- Gerin, I., Veiga-da-Cunha, M., Noel, G. and Van Schaftingen, E. (1999) Structure of the gene mutated in glycogen storage disease type Ib. *Gene* **227**, 189–195

- 24 Morrissey, J. L. (1981) Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Anal. Biochem.* **117**, 307–310
- 25 Meissner, G. and Allen, R. (1981) Evidence for two types of rat liver microsomes with different permeability to glucose and other small molecules. *J. Biol. Chem.* **256**, 6413–6422
- 26 Puskas, F., Marcolongo, P., Watkins, S. L., Mandl, J., Allan, B. B., Houston, P., Burchell, A., Benedetti, A. and Banhegyi, G. (1999) Conformational change of the catalytic subunit of glucose-6-phosphatase in rat liver during the fetal-to-neonatal transition. *J. Biol. Chem.* **274**, 117–122
- 27 Bánhegyi, G., Marcolongo, P., Burchell, A. and Benedetti, A. (1998) Heterogeneity of glucose transport in rat liver microsomal vesicles. *Arch. Biochem. Biophys.* **359**, 133–138
- 28 Arion, W. J., Canfield, W. K., Ramos, F. C., Schindler, P. W., Burger, H. J., Hemmerle, H., Schubert, G., Below, P. and Herling, A. W. (1997) Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose 6-phosphatase. *Arch. Biochem. Biophys.* **339**, 315–322
- 29 Leskes, A., Siekevitz, P. and Palade, G. E. (1971) Differentiation of endoplasmic reticulum in epatocytes. I. Glucose-6-phosphatase distribution *in situ*. *J. Cell Biol.* **49**, 264–287
- 30 Leskes, A., Siekevitz, P. and Palade, G. E. (1971) Differentiation of endoplasmic reticulum in epatocytes. II. Glucose-6-phosphatase in rough microsomes. *J. Cell Biol.* **49**, 288–302
- 31 Hino, J. and Minakami, S. (1982) Hexose-6-phosphate and 6-phosphogluconate dehydrogenases of rat liver microsomes. Involvement in NADPH and carbon dioxide generation in the luminal space of microsomal vesicles. *J. Biochem. (Tokyo)* **92**, 547–557
- 32 Bublitz, C. and Stevenson, S. (1988) The pentose phosphate pathway in the endoplasmic reticulum. *J. Biol. Chem.* **263**, 12849–12853
- 33 Collard, F., Collet, J. F., Gerin, I., Veiga da Cunha, M. and Van Schaftingen, E. (1999) Identification of the cDNA encoding human 6-phosphogluconolactonase, the enzyme catalyzing the second step of the pentose phosphate pathway. *FEBS Lett.* **459**, 223–226

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