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ARTICLE

The Putative glucose 6-phosphate translocase gene is mutated in essentially all cases of glycogen storage disease type I non-a

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The purpose of this work was to test the hypothesis that mutations in the putative glucose 6-phosphate translocase gene would account for most of the cases of GSD I that are not explained by mutations in the phosphohydrolase gene, ie that are not type Ia. Twenty-three additional families diagnosed as having GSD I non-a (GSD Ib, Ic or Id) have now been analysed. The 9 exons of the gene were amplified by PCR and mutations searched both by SSCP and heteroduplex analysis. Except for one family in which only one mutation was found, all patients had two allelic mutations in the gene encoding the putative glucose 6-phosphate translocase. Sixteen of the mutations are new and they are all predicted to lead to non-functional proteins. All investigated patients had some degree of neutropenia or neutrophil dysfunction and the clinical phenotype of the four new patients who had been diagnosed as GSD Ic and the one diagnosed as GSD Id was no different from the GSD Ib patients. Since these patients, and the four type Ic patients from two families previously studied, shared several mutations with GSD Ib patients, we conclude that their basic defect is in the putative glucose 6-phosphate translocase and that they should be reclassified as GSD Ib. Isolated defects in microsomal Pi transporter or in microsomal glucose transporter must be very rare or have phenotypes that are not recognised as GSD I, so that in practice there are only two subtypes of GSD I (GSD Ia and GSD Ib).

Keywords: glucose 6-phosphate; translocase; mutation; GSD Ib, GSD Ic; GSD Id; neutropenia; polymorphonuclear neutrophils; hypoglycaemia

Introduction

Glycogen storage disease type I (GSD I) are recessive disorders resulting from defects in the microsomal glucose-6-phosphatase (G6Pase) enzyme system (see Chen YT, Burchell A[1] for review), which produces free glucose in the liver and in kidneys. According to the substrate-transport model for the G6Pase system, glucose 6-phosphate (G6P) is transported by a specific transporter into the endoplasmic reticulum where it is hydrolysed by G6Pase, and the products, glucose and inorganic phosphate (Pi), are in turn transported out into the cytoplasm. Even though none of the

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transporters has been isolated, the cDNAs of the G6Pase[2] and of a putative G6P transporter[3] have both been cloned. The existence of the Pi[4,5] and the glucose[6] transporters remains to be shown at the molecular level.

GSD Ia (MIM 232200), which accounts for about 80 % of the GSD I cases, is caused by mutations in the *G6Pase* gene. The gene was mapped to chromosome 17 and more than 20 mutations have already been reported.[7] The remaining 20 % of the GSD I patients have been diagnosed as GSD Ib (MIM 232220), Ic and even Id, whenever the G6P,[8] Pi[9] or glucose[1] transporters were thought to be deficient, based on the enzymatic assays performed in liver extracts or in liver microsomes. In addition to the classical symptoms of GSD I (ie hepatomegaly, hypoglycaemia, hyperlactataemia and hyperuricaemia), the patients from the second group are often susceptible to infection as well as having neutropenia and neutrophil dysfunction.[10]

Gerin and co-workers[3] were the first to show mutations in the putative G6P transporter in two patients with GSD Ib, a finding that was later confirmed by Kure *et al*.[11] The cloning of the gene[11,12] opened the possibility of finding mutations at the genomic level by SSCP analysis and sequencing.[13,14] In the most extensive study yet published,[14] we reported 20 different mutations in patients from 20 families with GSD Ib and also from two families with GSD Ic. This suggested that GSD Ic is also due to a defect in the gene encoding the putative G6P translocase.

The purpose of the present work was to test the hypothesis that mutations in the *GSD Ib* gene would account for most if not all cases of GSD I that are not explained by mutations in the phosphohydrolase gene, ie for all type I non-a cases.

Patients and Methods

Patients and Diagnosis of GSD Ib, Ic and Id

The criterion for including patients in this study was that they had typical signs of GSD type I (elevated liver glycogen levels, hepatomegaly, hypoglycaemia, lactic acidosis) and normal total G6Pase activity in frozen or disrupted liver homogenates. All of them were eventually found to have neutropenia or neutrophil dysfunction although this was not a criterion for including patients in this study. The diagnosis of GSD Ib was based on the existence of a significant latency (more than 80 %) of G6Pase. Latency is defined as the ratio of enzyme activity in microsomes or extracts to that of the total activity in disrupted samples using detergents or freezing/thawing.[8,15] Assays were carried out on sucrose homogenates prepared either from unfrozen liver biopsies or from liver samples that had been frozen immediately after being collected.[16]

Three of the patients reported in Table 2 (nos 41, 42 and 44) had been diagnosed as GSD Ic on the basis of

(1) normal total G6Pase activity;

(2) normal or marginally increased G6Pase latency; and

(3) a complete latency of inorganic pyrophosphatase in microsomes (as compared with about 80 % latency in controls or GSD Ib patients).[9]

Patient 43 had an abnormal G6Pase latency and was thought to be GSD Ic because he had apparently no neutropenia. The patient diagnosed as GSD Id (no 45) is the one described in a report by Burchell, Scott, Waddell and Leonard that is quoted in the review by Chen and Burchell.[1] G6Pase in intact microsomes was clearly latent and the patient was thought to have deficient microsomal glucose transporter on the basis that the residual G6Pase activity in intact microsomes was not inhibited by glucose.

Template Preparation

Leukocytes from 5 ml of EDTA blood were collected by centrifugation (500 × *g*, 15 min). Genomic DNA was isolated from these leukocytes using the QIAmp Blood Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. For the preparation of cDNA, leukocytes were freed from red blood cells by addition of an erythrocyte lysis solution (5 mM $MgCl_2$, 10 mM NaCl, 10 mM Tris-HCl, pH 7.0) followed by centrifugation (5 min at 500 × *g*). The leukocyte pellet was washed three times, or until all the red blood cells were removed, with the same solution and finally resuspended in 50 µl diethyl pyrocarbonate-treated water. Total RNA was isolated from these leukocytes with the Trizol reagent (GIBCO-BRL, Basel, Switzerland) and the final pellet resuspended in 40 µl diethyl pyrocarbonate-treated water. To obtain the G6P transporter cDNA of patients 29, 40a and 40b, 5 µg of RNA were reverse transcribed, PCR-amplified and subcloned in the *Eco*RV restriction site of pBlueScript as described in Gerin *et al*.[3]

Mutation Detection

Single-strand conformation polymorphisms (SSCP)[17] and heteroduplex[18] analysis were used to screen all 9 exons, the flanking intronic sequences, part of the presumed promoter region (up to –559 with respect to the ATG codon) and intron 1. PCR reactions were performed on 10–20 ng of genomic DNA in 10 µl volumes as previously described.[14] The primers used to amplify exons 1 to 9 and the 5' region of exon 1 have been reported.[14] Additional primers were used to amplify:

the 'upstream promoter' region (from –177 to –559 with respect to the ATG codon; 5'TGGTGCGGTGGTGGTGAGTGG3' and

5'TCCAAGGGGACCCAGTGTCC3'); the 5' region of intron 1 (5'GGAAGAGATCCCTTTGGACAAGG3' and

5'CTCCACATGCACCCACACAGG3'); the middle region of intron 1 (5'AGTGTTTGAGGGGCTTCTGAGC3' and

5'GGAGCAGGACGTGGCAGTTCC3'); and the 3' region of intron 1 (5'TGAGGTGGGAAATCCCATATGC3' and

5'CATAAGCTGCCGACTGGCTGC3').

Of the 10 µl reaction volume, 4 µl were mixed with 20 µl of formamide buffer and analysed by SSCP.[14] For the heteroduplex analysis, the remaining 6 µl were mixed with 1 µl of 35 mM EDTA, heated for 4 min at 95°C and cooled to 30°C at 1°C/min. Renaturation of the DNA in heteroduplexes/homoduplexes was best achieved if a PCR machine was used to control the cooling of the samples. Once the sample reached room temperature 15 µl of gel loading buffer [9.6 % (w/v) sucrose, 0.06 % (w/v) bromophenol blue, 0.06 % (w/v) xylene cyanol] were added to it. For each sample 5 µl were loaded on to a 1 × MDE (FMC Bioproducts, Rockland, Maine, USA), 0.6 × TBE, polyacrilamide gel containing 15% (w/v) urea. The gels were run at a constant voltage of 20 V/cm (in our case about 700 V) for 14 to 16 h (200–300 bp fragments) at room temperature before drying and autoradiography.

The G339C mutation, which is not detectable in SSCP gels, but is well in heteroduplex, was confirmed by allele-specific PCR[3] using the conditions previously described.[14]

Genomic DNA and cDNA Sequencing

Direct sequencing of PCR products showing a shift in SSCP or heterduplex analysis was performed on both strands using the dsDNA Cycle Sequencing System (GIBCO-BRL) with [γ-^{33}P] end-labelled primers following the manufacturer's protocol. The PCR products were purified before sequencing using the QIAquick PCR Purification Kit (QIAGEN). The 5' and 3' halves of the open-reading frame of G6P transporter cloned in pBlueScript were analysed on a LICOR automated sequencer.[3]

Results and Discussion

Detection of New Mutations

This study includes all patients, none analysed previously,[14] who were reported to us as having the clinical features of GSD I non-a as already defined. These patients, from 23 different families, had been variously diagnosed as GSD Ib, GSD Ic or even GSD Id. DNA was isolated from leukocytes and amplified by PCR using intronic and, when necessary, exonic PCR primers. Mutation screening was performed by both SSCP and heteroduplex analysis to optimise the detection procedure, enabling us to find 15 new mutations (Table 1), as well as nine that had been previously

Table 1 New mutations identified in patients with glycogen storage disease type Ib, Ic and Id

Location	*Nucleotide change*	*Effect on protein*	*Amino acids at same position in bacterial transporters*	*Mutation detected by* SSCP	Heteroduplex	*Patient no. Table 2*
	Substitutions					
Exon 1	A170G	M1V: mutation at start codon		–	+	26, 41
Exon 2	G317C	G50 R	Only G	+	+	32
Exon 3	A567C	Q133P	E, K	+	(+)	43
Exon 3	T695C	C176R	A	+	(+)	26
Exon 3	T716C	C183R	G, V, A, L	+	+	27a–b, 29
Exon 6	C1067T	R300C	Only R	+	nd	45
Exon 9	G1295A	G376S	Only G	+	+	43
	Nonsense mutations					
Exon 2	G403A	W78X		–	+	34
Exon 9	C1412T	R415X		–	+	40a–b
	Deletions/Insertions					
Exon 2	528–529insC	Change in RF after P120, Stop at 130		–	+	36
Exon 5	1013–1029del	Change in RF after G281, Stop at 319		+	+	28
	Splice site mutations					
Exon 2/Intron 2	c.550+1(G→T)	Truncated protein?		–	(+)	25, 27a–b
Exon 2/Intron 2	c.550+2(T→G)	Skipping of Exon 2		–	–	29
Intron 3/Exon 4	c.795–1(G→A)	Truncated protein?		+	+	24
Intron 7/Exon 8	c.1154–2(A→G)	Truncated protein?		+	+	24
Exon 8/Intron 8	c.1292+1–1292+4delGTAA	Truncated protein?		+	+	38

The position of the mutated nucleotides refers to the cDNA sequence (EMBL : Y15409). For the amino acid substitutions, the residues found in the same position in the sequences of bacterial organophosphate transporters[3] are shown. RF: reading frame; nd: not determined; –: no shift; +: clear shift; (+): very small shift.

described.[11,14] Ten of the new mutations were detectable by SSCP alone and the remaining five were found by heteroduplex analysis.

An additional new splice site mutation (550 + 2T- > G, Table 1) in patient 29 could not be identified by heteroduplex or SSCP analysis but was found by analysis of reverse-transcribed RNA from leukocytes. Separate amplifications of the 5' and 3' halves of the coding sequence yielded one product of the expected size for the 3' half but two products for the 5' half, one with the expected size (700 bp) and one about 250 bp shorter. This shorter product was found, after cloning and sequencing, to lack exon 2. Sequencing of genomic DNA disclosed the presence of a splice site mutation at the exon2/intron2 junction.

In the case of family 40 (Table 2), only one mutation was found (R415X) in the heterozygous state. Since heteroduplex and SSCP analysis of all exons, of intron 1 and of the promoter (500 bp) did not identify the presence of a second mutation, leukocyte cDNA was amplified by PCR as described above. The 5' and 3' fragments obtained had the expected sizes and were cloned and sequenced. No mutation was found in any of the four clones corresponding to the 5' half. All seven clones corresponding to the 3' half contained the R415X mutation. These results indicated that the mRNA that did not contain the R415X mutation was under-represented. We speculate that this is due to the presence of a mutation that greatly reduces the expression of this copy of the gene.

Table 2 Distribution of mutations in the new GSD Ib, Ic and Id patients investigated

Patient (sex)	*Allele 1*	*Allele 2*	*Consanguinity*	*Origin*	*Diagnosis (GSD type)*
23 (M)	E355X	R28C	No	France	Ib
24 (M)	**∑In7/Ex8**	**∑In3/Ex4**	No	Italy	Ib
25 (M)	ΔRF A347	**∑Ex2/In2**	No	United States	Ib
26 (M)	**C176R**	**M1V**	No	United States	Ib
27a (F)	**∑Ex2/In2**[a] G1393A (Silent)	**C183R**[b]	No	United States	Ib
27b (M)	**∑Ex2/In2**[a] G1393A (Silent)	**C183R**[b]	No	United States	Ib
28 (F)	**ΔRF G281**[a]	ΔRF A347[b]	No	United States	Ib
29 (F)	**∑Ex2/In2**[a]	**C183R**[b]	No	France	Ib
30 (M)	ΔRF A347	G339C[b]	No	France	Ib
31a (M)	ΔRF A347[a]	ΔRF A347[b]	No	Poland	Ib
31b (M)	ΔRF A347[a]	ΔRF A347[b]	No	Poland	Ib
32 (M)	G339C	**G50R**[b]	No	France	Ib
33 (M)	ΔRF M308[a]	ΔRF M308[b]	No	Japan	Ib
34 (M)	G339C	**W78X**	No	Australia	Ib
35a (M)	ΔRF A347 G1393A (Silent)	W96X	No	Australia	Ib
35b (F)	ΔRF A347 G1393A (Silent)	W96X	No	Australia	Ib
36 (F)	ΔRF A347[a] G1393A (Silent)	**ΔRF P120**[b]	No	Germany	Ib
37 (F)	Q248X[a]	ΔRF A347[b]	No	France	Ib
38 (M)	**∑Ex8/In8**	**∑Ex8/In8**	Yes	Mexico	Ib
39 (F)	ΔRF A347	ΔRF A347	No	Poland	Ib
40a (F)	**R415X**		No	Afro-Caribbean	Ib
40b (M)	**R415X**		No	Afro-Caribbean	Ib
41 (M)	**M1V**[a]	G339C[b] G1393A (Silent)	No	United Kingdom	Ic
42 (F)	G292P; L293X	ΔRF A347	No	United Kingdom	Ic
43 (F)	**Q133P**	**G376S**	No	Germany	Ic
44 (F)	ΔRF T312	ΔRF T312	Yes	Pakistan	Ic
45 (M)	**R300C**	**R300C**	Yes	Mexico	Id

When available, the DNA from the parents was analyzed by SSCP and/or heteroduplex to identify parental origin of the mutation. The new mutations are in **bold** characters and the other mutations have been reported previously.[11,14] ΔRF = change in reading frame, and ∑ = splice-site mutation. [a]paternal allele; [b]maternal allele.

Description of New Mutations

Among the 16 new mutations found, seven were substitutions. Remarkably, one of these, found in two different families, replaced the initiator methionine by a valine. The use of the next ATG codon (Met17), which is in a good Kozak consensus sequence,[19] would result in the synthesis of a protein that is amputated of its first 16 amino acids. Since this region includes part of the predicted first helix,[3] the amputated protein is most likely non-functional.

Three of the other substitutions (G50R, R300C, G376S) we have now found resulted in replacement of one of the 30 residues that are strictly conserved in the organophosphate transporter family.[3] Two other substitutions replaced a cysteine by an arginine in a hydrophobic stretch of residues predicted to be helix 6 by comparison with the sequence analysis of UhpC and UhpT,[20] whilst in the Q133P mutation a hydrophilic residue is replaced by a more constrained, hydrophobic one. These six substitutions are almost certain to impair folding or activity of the protein in a significant way.

Of the two new nonsense mutations that were found, one (R415X) resulted in the amputation of only 15 amino acids at the C-terminal end. The effect of this mutation is to remove the retention signal for the endoplasmic reticulum.[21] Two other nucleotide changes resulted in a frameshift either due to insertion of 1 bp or deletion of 2 bp. Five new splice site mutations (at the exon 2/intron 2, intron 3/exon 4, intron7/exon8 and exon8/intron8 junctions) were also found; they all modified at least one of the highly conserved nucleotides in the splice site motifs and presumably result in exon skipping (Table 1). As mentioned above, this was experimentally confirmed for one of the two mutations found at the exon 2/intron 2 junction.

Figure 1 shows all 43 mutations that have been found to date.[11–14] It is remarkable that the majority of them are expected to disrupt the protein structure and therefore to abrogate its function. This suggests that a complete lack of activity of the G6P transporter is not lethal. Although mutations can be identified in all exons (except exon 7, which is not expressed in liver, kidney and leukocytes[22]), there seems to be a hot spot for insertions or deletions between nucleotides 1094 and 1105 where four out of 10 frame-shifting mutations were found. Remarkably, no amino acid substitutions have yet been found in exons 4 and 5.

Distribution of Mutations in Patients

Table 2 shows the distribution of the mutations among all GSD I non-a patients we analysed in the present study. With the exception of the patients from family 40 described above, all were found to have two mutations that we describe now or that had been described previously.[11,14] As suggested earlier, two mutations are relatively frequent in populations of European origin.[14] The 2bp deletion (1211–1212delCT) which results in a change in the reading frame after A347 accounts for 11 of the 46 expected mutated alleles (Table 2) and actually for 22 % of the mutated alleles considering all patients (mostly of European origin) who have been analysed so far. The G339C mutation is the next most common mutation, accounting for three of the alleles in the present series and for 13 % considering all the analysed patients. Interestingly, a Japanese patient and a Pakistani patient were found to be homozygous for

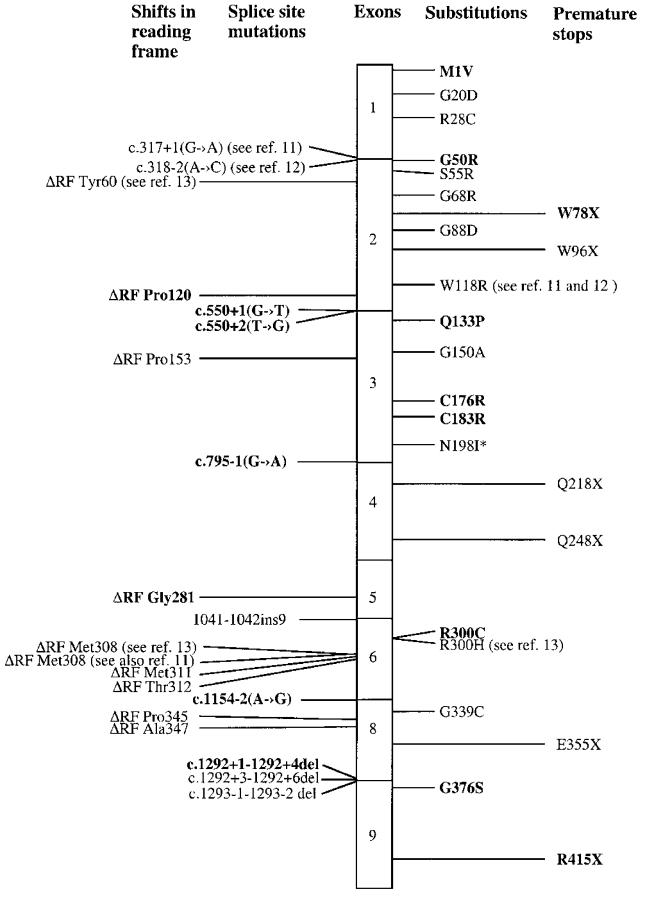

Figure 1 *Mutations found in the putative glucose 6-phosphate translocase.*
The non-translated regions of exon 1 and 9 are not shown. Exon 7, which is not present in liver, kidney and leukocyte cDNA and in which no mutations have been found, is also not shown. When references are not indicated, mutations are taken from Veiga-da-Cunha *et al*[14] (normal characters) or from this paper (bold characters). ΔRF: change in reading frame starting after the indicated residue; *this mutation may be a polymorphism.[14]

mutations (1094delGCTG/insTC and 1105–1106insA, respectively) that had already been found in patients from the same ethnic groups.[11,14] This suggests that these mutations may be relatively frequent in the Japanese and Pakistani populations, respectively.

Presence of Mutations in the Putative G6P Transporter in GSD Ic and GSD Id

From the analysis of Table 2 it is clear that the patients reported as GSD Ic or GSD Id have mutations in the *GSD Ib* gene and are therefore GSD Ib patients. One may object that the putative G6P transporter could transport both G6P and Pi, like its bacterial homologue[23] and that different mutations could differently affect the two functions, resulting in two different phenotypes. However, eight of the 11 mutations that have been found in the GSD Ic and Id patients were also present in GSD Ib patients. Furthermore, one of the remaining three mutations (R300C), found in the GSD Id patient, concerns the same (extremely conserved) residue as the mutation R300H found by Marcolongo *et al* in a GSD Ib patient.[13]

Another argument in favour of all these patients belonging to a single group is the fact that they all have neutropenia or neutrophil dysfunction in addition to the classic symptoms of GSD Ia. Interestingly the patient (no 43) who was initially reported as having normal neutrophil counts and a lack of susceptibility to infection was found, after the genetic diagnosis of GSD Ib was made, to have a severely reduced oxygen radical production of the granulocytes. This confirms a previous report that neutropenia is not always present in GSD Ib.[24] Reciprocally, one should be cautious not to base the diagnosis of GSD Ib simply on the association of GSD Ia phenotype and neutropenia. In one case of GSD Ia with neutropenia, screening showed the presence of mutations in the phosphohydrolase gene but not in the putative G6P translocase gene (results not shown). In this case, the neutropenia is probably unrelated to the dysfunction of the G6Pase system.

Therefore most, if not all, patients with GSD Ic and GSD Id who have been described so far have GSD Ib, having been misdiagnosed probably because the tests used to differentiate these subtypes are not reliable. Misdiagnosis of GSD Ic patients would explain also why the '*GSD Ic*' gene[25] has been localised in the same region as the *GSD Ib* gene.[26] This does not mean that deficiencies in the Pi translocase or in the glucose translocase do not exist, but they are probably extremely rare, or lethal, or they give rise to a markedly different phenotype that is not recognised as GSD I.

Conclusion

Since the patients mentioned here and in our previous paper[14] represent all GSD I non-a cases we have studied, we conclude that patients with the classic symptoms of GSD I are either type Ia or type Ib, except perhaps in very rare cases, but this has still to be demonstrated. Thus in the investigation of GSD I patients, enzyme studies can be simplified. It is only necessary to measure G6Pase activity in a microsomal preparation before and after disruption. Diagnosis can then be confirmed by mutation analysis of the *GSD Ia* and *GSD Ib* genes. A second conclusion of our work is that there is a great diversity of mutations in the *GSD Ib* gene and that almost all of them can be detected by a combination of SSCP and heteroduplex analysis.

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References

1 Chen YT, Burchell A: Glycogen storage disease. In: Scriver CR, Beaudet AL, Sly WS and Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*. 7th edn. McGraw-Hill: 1995, pp 935–967.
2 Lei KJ, Shelly LL, Pan CJ, Sidbury JB, Chou JY: Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type Ia. *Science* 1993; **262**: 580–583.
3 Gerin I, Veiga-da-Cunha M, Achouri Y, Collet JF, Van Schaftingen E: Sequence of a putative glucose 6-phosphate translocase mutated in glycogen storage disease type Ib. *FEBS Lett* 1997; **419**: 235–238.

4 Arion WJ, Wallin BK, Lange AJ, Ballas LM: On the involvement of a glucose 6-phosphate transport system in the function of microsomal glucose 6-phosphatase. *Mol Cell Biochem* 1975; **6**: 75–83.
5 Arion WJ, Lange AJ, Walls HE, Ballas LM: Evidence for the participation of independent translocases for phosphate and glucose 6-phosphate in the microsomal glucose-6-phosphatase system. *J Biol Chem* 1980; **255**: 10396–10406.
6 Burchell A: A re-evaluation of GLUT 7. *Biochem J* 1998; **331**: 973.
7 Lei KJ, Pan C-J, Shelly LL, Liu J-L, Chou JY: Identification of mutations in the gene for glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type Ia. *J Clin Invest* 1994; **93**: 1994–1999.
8 Narisawa K, Igarashi Y, Otomo H, Tada K: A new variant of glycogen storage disease type I probably due to a defect in the glucose-6-phosphate transport system. *Biochem Biophys Res Commun* 1978; **83**: 1360–1364.
9 Nordlie RC, Sukalski KA, Munoz JM, Baldwin JJ: Type Ic, a novel glycogenosis. Underlying mechanism. *J Biol Chem* 1983; **258**: 9739–9744.
10 Gitzelmann R, Bosshard NU: Defective neutrophil and monocyte functions in glycogen storage disease type Ib: a literature review. *Eur J Pediatr* 1993; **152**: S33–S38.
11 Kure S, Suzuki Y, Matsubara Y *et al*: Molecular analysis of glycogen storage disease type Ib: 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* 1998; **248**: 426–431.
12 Ihara K, Kuromaru R, Hara T: Genomic structure of the human glucose 6-phosphate translocase gene and novel mutations in the gene of a Japanese patient with glycogen storage disease type Ib. *Hum Genet* 1998; **103**: 493–496.
13 Marcolongo P, Barone V, Priori G *et al*: Structure and mutation analysis of the glycogen storage disease type Ib gene. *FEBS Lett* 1998; **436**: 247–250.
14 Veiga-da-Cunha M, Gerin I, Chen Y-T *et al*: A gene on chromosome 11q23 coding for a putative glucose-6-phosphate translocase is mutated in glycogen-storage disease types Ib and Ic. *Am J Hum Genet* 1998; **63**: 976–983.
15 Lange AJ, Arion WJ, Beaudet AL: Type Ib glycogen storage disease is caused by a defect in the glucose-6-phosphate translocase of the microsomal glucose-6-phosphatase system. *J Biol Chem* 1980; **255**: 8381–8384.
16 Brown BI: Diagnosis of glycogen storage disease. In: Wapnir PA (ed.). *Congenital Metabolic Myopathy.* Dekker: New York, 1985, pp 227–250.
17 Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T: Detection of polymorphisms of human single-strand conformation polymorphisms. *Proc Natl Acad Sci USA* 1989; **86**: 2766–2770.
18 Nagamine CM, Chan K, Lau Y-FC: A PCR artefact: generation of heteroduplexes. *Am J Hum Genet* 1989; **45**: 337–339.
19 Kozak M: An analysis of the 5'-non-coding sequences from 669 vertebrate messenger RNAs. *Nucleic Acids Res* 1987; **15**: 8125–8132.
20 Island MD, Wei BY, Kadner RJ: Structure and function of the *uhp* genes for the sugar phosphate transport system in *Escherichia coli* and *Salmonella typhimurium*. *J Bacteriol* 1992; **174**: 2754–2762.
21 Jackson MR, Nilsson T, Peterson PA: Identification of a consensus motif for retention in the endoplasmic reticulum. *EMBO J* 1990; **9**: 3153–3162.
22 Gerin I, Veiga-da-Cunha M, Noël G, Van Schaftingen E: Structure of the gene mutated in glycogen storage disease type Ib. *Gene* 1999; **227**: 189–195.
23 Maloney PC, Ambudkar SV, Anantharan V, Sonna LA, Varadhachary A: Anion-exchange mechanisms in bacteria. *Microbiol Rev* 1990; **54**: 1–17.
24 Narisawa K, Ishizawa S, Okumura H, Tada K, Kuzuya T: Neutrophil metabolic dysfunction in genetically heterogeneous patients with glycogen storage disease type Ib. *J Inher Metab Dis* 1986; **9**: 297–300.
25 Fenske CD, Jeffery S, Weber JL, Houlston RS, Leonard JV, Lee P: Localisation of the gene for glycogen storage disease type Ic by homozygosity mapping to 11q. *J Med Genet* 1998; **35**: 269–272.
26 Annabi B, Hiraiwa H, Mansfield BC *et al*: The gene for glycogen-storage disease type Ib maps to chromosome 11q23. *Am J Hum Genet* 1998; **62**: 400–405.