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The Gene for Glycogen-Storage Disease Type 1b Maps to Chromosome 11q23

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Summary

Glycogen-storage disease type 1 (GSD-1), also known as "von Gierke disease," is caused by a deficiency in microsomal glucose-6-phosphatase (G6Pase) activity. There are four distinct subgroups of this autosomal recessive disorder: 1a, 1b, 1c, and 1d. All share the same clinical manifestations, which are caused by abnormalities in the metabolism of glucose-6-phosphate (G6P). However, only GSD-1b patients suffer infectious complications, which are due to both the heritable neutropenia and the functional deficiencies of neutrophils and monocytes. Whereas G6Pase deficiency in GSD-1a patients arises from mutations in the G6Pase gene, this gene is normal in GSD-1b patients, indicating a separate locus for the disorder in the 1b subgroup. We now report the linkage of the GSD-1b locus to genetic markers spanning a 3-cM region on chromosome 11q23. Eventual molecular characterization of this disease will provide new insights into the genetic bases of G6P metabolism and neutrophil-monocyte dysfunction.

Introduction

Neutrophil dysfunction and neutropenia contribute to increased susceptibility to bacterial infection in a number of clinical conditions, including the 1b subgroup of glycogen-storage disease type 1 (GSD-1b; MIM 232220 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?232220]), mannosidosis, and Chediak-Higashi, Schwachman-Diamond, hyperimmunoglobulin E, and periodontitis syndromes (Gallin 1992; Anderson et al. 1995). For these autosomal recessive diseases, an underlying genetic defect in neutrophils has been implicated, but the molecular mechanism is unknown. GSD-1b, one of the four distinct subgroups of glycogen-storage disease type 1 (GSD-1), offers a potential model for exploring such mechanisms. GSD-1, also known as "von Gierke disease," is caused by a deficiency in microsomal glucose-6-phosphatase (G6Pase) activity (Moses 1990; Chen and Burchell 1995). In addition to GSD-1b, three other GSD-1 subgroups—1a, 1c, and 1d—have been described (Narisawa et al. 1978; Beaudet et al. 1980; Nordlie et al. 1983; Chen and Burchell 1995). GSD-1a, which represents 80%–90% of GSD-1 cases, is caused by defects in the G6Pase gene, which is localized on chromosome 17 (Lei et al. 1993, 1994). GSD-1b is the second most prevalent form of GSD-1, and it is the most severe (Narisawa et al. 1978; Beaudet et al. 1980; Chen and Burchell 1995). It presents with the clinical manifestations of GSD-1, including fasting hypoglycemia, hepatomegaly, growth retardation, lactic acidemia, hyperlipidemia, and hyperuricemia, that directly reflect the defects in metabolism of glucose-6-phosphate (G6P) (Chen and Burchell 1995). However, unlike other GSD-1 subgroups, GSD-1b patients suffer additional infectious complications that are due to the heritable neutropenia and the functional deficiencies of neutrophils and monocytes (Beaudet et al. 1980; Gitzelmann and Bosshard 1993). These complications are not readily attributable to the G6Pase deficiency. Identification of the gene responsible for GSD-1b should, therefore, provide insights into the genetic basis of neutrophil-monocyte dysfunction and its link to G6P metabolism.

GSD-1b is inherited as an autosomal recessive disorder, with no obvious genetic heterogeneity (Chen and Burchell 1995). Current treatments for GSD-1b consist of the combination of dietary therapy (Fernandes et al. 1988; Chen and Burchell 1995), to correct symptoms of

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G6Pase deficiency, and human granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy (Roe et al. 1992; Wendel et al. 1993; Spiekermann et al. 1997). GM-CSFs are humoral regulators of hematopoiesis that increase both the proliferation of myeloid precursors and the functional capacity of neutrophils (Golde and Baldwin 1992). In GSD-1b patients, GM-CSF treatment increases neutrophil counts and reduces the frequency of infection and inflammation (Roe et al. 1992; Wendel et al. 1993; Spiekermann et al. 1997), complications that are not manageable by dietary therapy alone. This suggests that neutrophil dysfunction associated with GSD-1b is distinct from the defect in G6P metabolism.

In this study, we report a genetic analysis of the gene that is deficient in GSD-1b patients. Highly informative microsatellite markers map the GSD-1b locus to the long arm of chromosome 11.

Subjects and Methods

Families

Eight consanguineous families (1–8) and one nonconsanguineous family (9) (fig. 1) were included in this study. The ethnic origins of these families are Bedouin (families 1, 2, and 4), North African (family 3), Pakistani (family 5), Iranian Jewish (family 6), Israeli Arab (families 7 and 8), and European (family 9). These families were chosen because all patients presented with the typical GSD-1 clinical manifestations: fasting hypoglycemia, hepatomegaly, and growth retardation. All were diagnosed as having GSD-1b on the basis of additional symptoms: neutropenia, neutrophil dysfunction, recurrent bacterial infections, and the absence of a genetic defect in the G6Pase gene.

Genomic DNA preparations were extracted from blood samples by means of a Nucleon II kit (Scotlab Bioscience). All peripheral blood samples were obtained with the informed consent of the patients and/or their parents.

Linkage Analysis

The high-density microsatellite markers kit, spanning 10–20-cM intervals of the genome (Human Linkage Screening Set, version 8, from the Cooperative Human Linkage Center), and additional, chromosome 11 markers were all obtained from Research Genetics. For initial genomewide screening, two generations (IV and V) of family 1, which has two affected siblings, were chosen. Markers that were homozygous for the two affected individuals were then used to genotype the last two generations of the other eight families. DNA polymorphisms were analyzed by means of PCR amplification of simple sequence repeats. Reaction mixtures (20 μl) contained 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 1.5–2.0 mM $MgCl_2$; 200 μM each of dATP, dGTP, and dTTP; 20 μM dCTP; 2.5 μCi [^{32}P]-dCTP (3,000 Ci/mmol); 1 μM each primer; 100 ng genomic DNA; and 1 U *Taq* DNA polymerase. The PCR was performed for a total of 30 cycles by use of a Perkin-Elmer 9600 Thermal Cycler. The amplified fragments were analyzed by electrophoresis through standard DNA-sequencing gels.

Two-point linkage analysis was performed with MLINK, and multipoint linkage analysis was performed with LINKMAP (Lathrop et al. 1984; Dracopoli et al. 1995). The LOD score calculations assumed an autosomal recessive mode of inheritance with full penetrance, no sex difference, no interference, a mutant gene frequency of 1/100,000, and equal allele frequencies for each marker.

Results

A total of 366 high-density microsatellite markers, spaced at 10–20 cM and covering chromosomes 1–22, were initially used to screen two generations (IV and V) of consanguineous family 1 (fig. 1). Patients from consanguineous matings are expected to be homozygous for the disease locus and for its linked markers. Therefore, markers that exhibited homozygosity were used to analyze the other families. Marker D11S1998 on chromosome 11q23 showed homozygosity across all pedigrees and yielded a LOD score of 6.882 at a recombination fraction of $\theta = .00$ (table 1). Additional markers mapping to this region supported the assignment of the GSD-1b locus to chromosome 11q23 (table 1). Pairwise linkage analysis of all available members in the nine families yielded LOD scores between 2.948 and 10.015, at $\theta = .00$, for markers D11S1340, D11S1998, D11S939, D11S1356, D11S4104, D11S1341, D11S976, D11S614, and D11S4129 (table 1).

The GSD-1b locus was identified, by use of the haplotype data, in a genetic region of ~3.6 cM, between markers D11S1340 and D11S4129. We were able to achieve the localization by examining for lack of homozygosity in affected individuals from consanguineous families. Lack of homozygosity was observed in individuals V-4 and V-5, in family 1, for marker D11S1340 (fig. 1). Since the parents of V-4 and V-5 do not share an allele for D11S1340, this recombination is considered a historic one that must have occurred in previous generations. The distal recombination is again a historic one and is observed in individual IV-3, in family 2, for marker D11S4129 (fig. 1). The chromosomal order of the markers used was adopted from the Genetic Location Database (http://cedar.genetics.soton.ac.uk/public_html), provided by Collins et al. (1996). The estimated distances (in cM) between the analyzed markers are given in table 1 and are shown schematically in figure 2. The haplotypes for the nine markers of the available

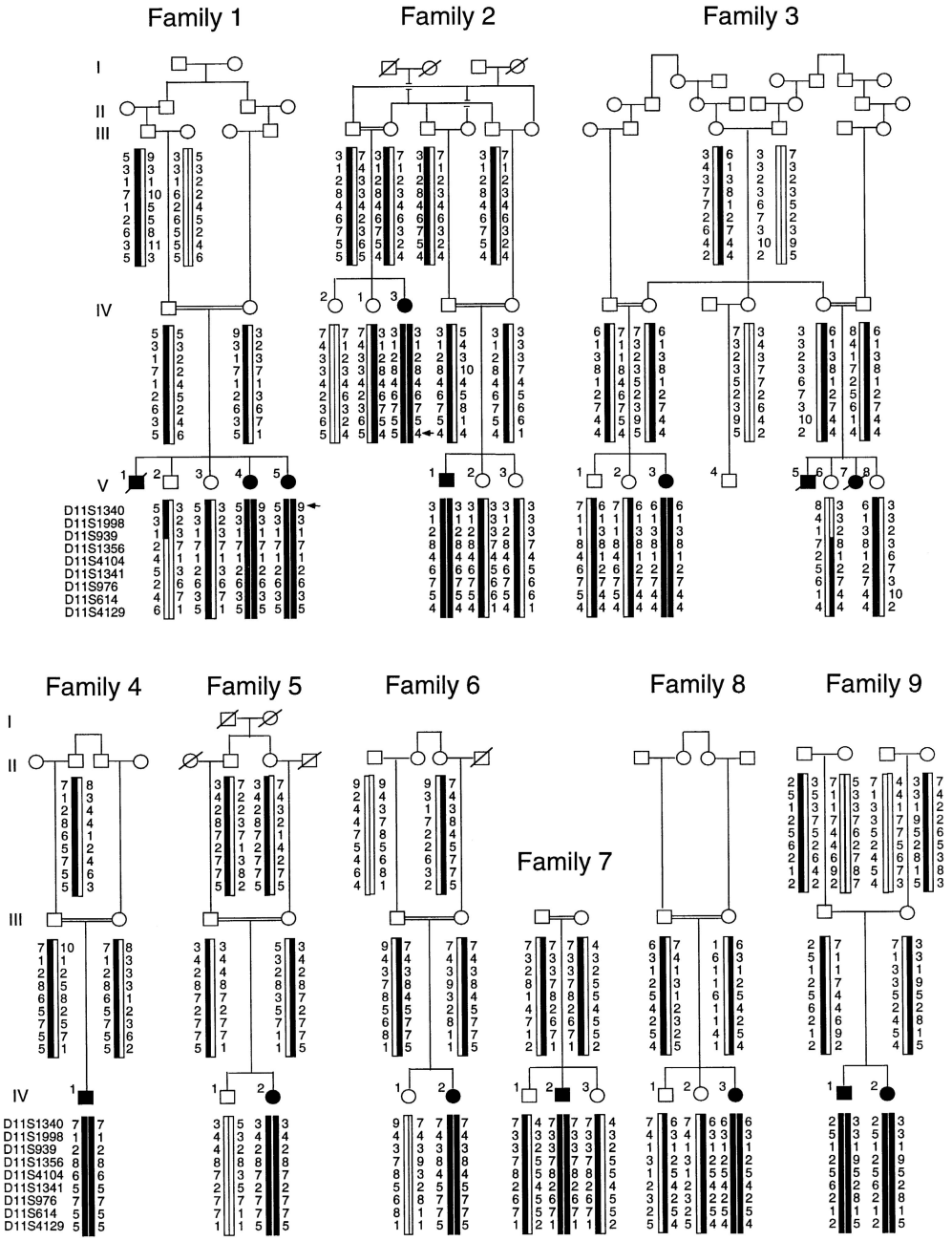

Figure 1 Pedigrees of the nine GSD-1b families used in the linkage study. The haplotypes in loci D11S1340, D11S1998, D11S939, D11S1356, D11S4104, D11S1341, D11S976, D11S614, and D11S4129 are given for each genotyped individual. Blackened symbols represent affected individuals, and unblackened symbols represent unaffected individuals. Lack of homozygosity—observed in individuals V-4 and V-5, in family 1, for marker D11S1340, and in individual IV-3, in family 2, for marker D11S4129—is indicated by an arrow.

family members are displayed in figure 1. A recombination in the genome of individual V-2, in family 1, and individual V-6, in family 3 (fig. 1), placed the GSD-1b locus distal to D11S939, refining its position to a 3.0-cM region on chromosome 11q23, between markers D11S939 and D11S4129 (fig. 2). The results are consistent with transmission of GSD-1b as an autosomal recessive trait (Chen and Burchell 1995) at a single locus.

Multipoint LOD-score analysis between markers D11S938, D11S1340, D11S1998, D11S976, D11S614, D11S4129, and D11S4107 and the disease locus placed the GSD-1b gene between markers D11S1340 and

Table 1

Two-Point LOD Scores between the GSD-1b Locus and Chromosome 11 Polymorphic Markers, for Nine GSD-1b Families

		LOD Score at θ =						
Marker Locus	cM	.00	.001	.01	.05	.1	.2	.3
D11S938	115.491	∼∞	−.430	1.486	2.425	2.362	1.615	.820
D11S1340	120.857	2.948	4.673	5.516	5.485	4.807	3.164	1.619
D11S1998	121.291	6.882	6.860	6.663	5.796	4.743	2.834	1.372
D11S939	121.378	6.094	6.073	5.881	5.042	4.038	2.280	1.018
D11S1356	121.900	8.713	8.688	8.459	7.442	6.186	3.831	1.932
D11S4104	123.222	7.052	7.031	6.851	6.047	5.056	3.207	1.704
D11S1341	123.363	7.962	7.937	7.712	6.716	5.502	3.289	1.586
D11S976	123.936	8.325	8.300	8.074	7.705	5.845	3.566	1.765
D11S614	124.114	10.015	9.987	9.735	8.612	7.217	4.568	2.368
D11S4129	124.421	7.068	7.046	6.849	5.980	4.922	2.987	1.459
D11S925	126.399	1.983	4.799	5.622	5.531	4.839	3.221	1.736
D11S4107	126.772	∼∞	4.517	5.369	5.333	4.644	3.000	1.536

Note.—Linkage analysis was performed with consanguinity loops included for all markers, the allele frequencies were kept equal, and the LOD scores were calculated under the assumption of an autosomal recessive trait of inheritance, with 100% penetrance.

D11S4129, centered around D11S976, with a maximum LOD score of 14.110 at θ = .00 (data not shown). It is worth noting that the LINKMAP multipoint analysis failed to exclude the positions of markers D11S1340 and D11S4129 as candidate locations for the GSD-1b locus because the recombinations are historic and are not seen when only the parental haplotypes are examined.

Discussion

Using one nonconsanguineous and eight consanguineous GSD-1b families, we have mapped the GSD-1b gene locus to chromosome 11q23, between markers DS11S939 and D11S4129, which define an interval of ∼3 cM. The results are consistent with transmission of GSD-1b as an autosomal recessive trait (Chen and Burchell 1995) at a single locus.

G6Pase is a membrane-spanning protein (Lei et al. 1993) localized in the endoplasmic reticulum (ER), with the active site apparently facing into the lumen (Waddell and Burchell 1991). Despite the functional G6Pase deficiency in GSD-1b patients, the G6Pase gene in these patients is normal (Lei et al. 1995), and high G6P hydrolytic activity is observed when the microsomal membranes are disrupted (Narisawa et al. 1978; Lange et al. 1980). This observation, along with kinetic (Narisawa et al. 1978; Arion et al. 1980; Lange et al. 1980) and G6P-transport (Igarashi et al. 1984) studies, has implied that the GSD-1b defect prevents uptake of G6P by the intact microsomal membrane. In earlier work, we demonstrated that the G6Pase gene, localized on chromosome 17 (Lei et al. 1994), is defective in GSD-1a patients (Lei et al. 1993). Using G6Pase-deficient mice, we showed that there is a tight functional dependence of G6P transport on G6Pase activity (Lei et al. 1996). These observations, taken in conjunction with the lack of genetic heterogeneity in GSD-1b (Chen and Burchell 1995), strongly suggest that (1) transport and hydrolysis of G6P involve only two proteins, (2) one of these components is the G6Pase catalytic unit, and (3) the second component, encoded by the GSD-1b gene, is an ER

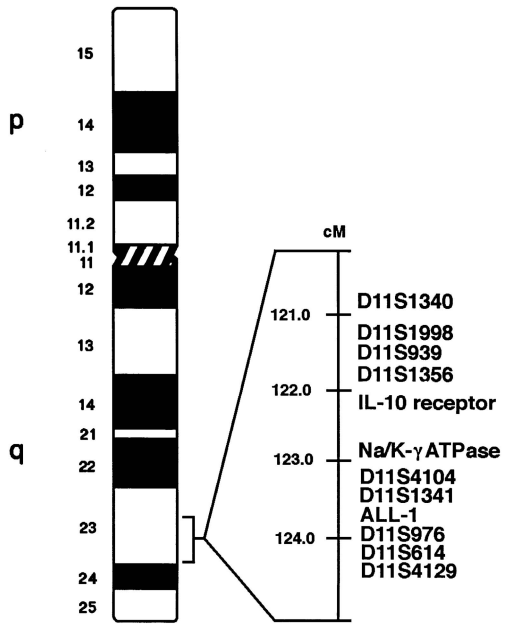

Figure 2 The best-supported order and sex-averaged distances (in cM) between the markers showing linkage to the GSD-1b locus. The IL-10 receptor, Na/K-γATPase, and ALL-1 genes mapping to this region are shown.

membrane–associated protein that either transports G6P into the lumen or transduces a signal from the cytoplasm into the lumen, triggering G6Pase to transport G6P.

How a G6P metabolic defect in GSD-1b can impair chemotaxis, mobility, and respiratory-burst activity in patients' polymorphonuclear leukocytes (Kilpatrick et al. 1990; Gitzelmann and Bosshard 1993), as well as their ability to mobilize Ca^{2+} in response to physiological stimuli in neutrophils and monocytes, is unclear (Kilpatrick et al. 1990). The G6Pase gene is expressed primarily in the liver, kidney, intestine, and pancreas, but it is not expressed in myeloid cells (Foster et al. 1997). Indeed, liver allografts that correct the apparent G6P metabolic deficiencies do not correct neutrophil dysfunction (Lachaux et al. 1993). Therefore, the defect must be intrinsic to the cells of the myeloid lineage. Although G6P has been shown to enhance Ca^{2+} uptake by microsomes (Benedetti et al. 1988) and although a role for G6P in regulating microsomal Ca^{2+} sequestration and release has been suggested, Ca^{2+}-mediated events cannot explain all respiratory-burst defects in patients with GSD-1b (Kilpatrick et al. 1990). One possible explanation lies in the ER location of the GSD-gene products. Although the signal transduced by the GSD-1b protein in the liver controls G6Pase activity, it may also be used by the myeloid cells to modify the processing of proteins passing through the ER, leading to the observed dysfunction. It is interesting to note that chronic infusion of GM-CSF, which increases neutrophil and monocyte counts, improves the neutrophil-monocyte response in GSD-1b patients (Roe et al. 1992; Wendel et al. 1993; Spiekermann et al. 1997).

A survey of the expressed-sequence–tag database describes >60 transcribed sequences in the GSD-1b region of interest. The genes of known function in this region include the ALL-1 gene and those for Na/K-γ-ATPase and the interleukin-10 receptor (Gu et al. 1992) (fig. 2). Since none of these genes encode proteins that are localized to the ER, are involved in carbohydrate metabolism, or are involved in signaling, the GSD-1b defect must be an uncharacterized gene in this region. The linkage of the GSD-1b locus to genetic markers on chromosome 11q23 facilitates the positional cloning of the gene, which should lead both to the elucidation of its biological role and to an understanding of the interrelationship between G6P metabolism and neutrophil function.

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References

Anderson DC, Kishimoto TK, Smith CW (1995) Leukocyte adhesion deficiency and other disorders of leukocyte adherence and mobility. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw-Hill, New York, pp 3955–3994

Arion WJ, Lange AJ, Walls HE, Ballas LM (1980) Evidence of the participation of independent translocases for phosphate and glucose-6-phosphate in the microsomal glucose-6-phosphatase system. J Biol Chem 255:10396–10406

Beaudet AL, Anderson DC, Michels VV, Arion WJ, Lange AJ (1980) Neutropenia and impaired neutrophil migration in type 1B glycogen storage disease. J Pediatr 97:906–910

Benedetti A, Fulceri R, Romani A, Comporti M (1988) MgATP-dependent glucose-6-phosphate-stimulated calcium accumulation in liver microsomal fractions. J Biol Chem 263:3466–3473

Chen Y-T, Burchell A (1995) Glycogen storage diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw-Hill, New York, pp 935–965

Collins A, Frezal J, Teague J, Morton NE (1996) A metric map of humans: 23,500 loci in 850 bands. Proc Natl Acad Sci USA 93:14771–14775

Dracopoli NC, Haines JL, Korf BR, Moir DT, Morton CC, Seidman CE, Seidman JG, et al (1995) Use of linkage programs for linkage analysis. In: Current protocols in human genetics, John Wiley & Sons, Inc., unit 1.7, New York

Fernandes J, Leonard JV, Moses SW, Odievre M, di Rocco M, Schaub J, Smit GPA, et al (1988) Glycogen storage disease: recommendations for treatment. Eur J Pediatr 147:226–228

Foster JD, Pederson BA, Nordlie RC (1997) Glucose-6-phosphatases structure, regulation, and function: an update. Proc Soc Exp Biol Med 215:314–332

Gallin JI (1992) Disorders of phagocytic cells. In: Gallin JI, Goldstein IM, Snyderman R (eds) Inflammation: basic principles and clinical correlates, 2d ed. Raven Press, New York, pp 859–874

Gitzelmann R, Bosshard NU (1993) Defective neutrophil and monocyte functions in glycogen storage disease type 1b: a literature review. Eur J Pediatr 152, suppl 1:S33–S38

Golde DW, Baldwin GC (1992) Myeloid growth factors. In: Gallin JI, Goldstein IM, Snyderman R (eds) Inflammation: basic principles and clinical correlates, 2d ed. Raven Press, New York, pp 291–301

Gu Y, Nakamura H, Alder H, Prasad R, Canaani O, Cimino G, Croce CM, et al (1992) The t(4:11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to *Drosophila trithorax*, to the AF-4 gene. Cell 71: 701–708

Igarashi Y, Kato S, Narisawa K, Tada K (1984) A direct evidence for defect in glucose-6-phosphatase transport system in hepatic microsomal membrane of glycogen storage disease type 1b. Biochem Biophys Res Commun 119:593–597

Kilpatrick L, Garty B-Z, Lundquist KF, Hunter K, Stanley CA, Baker L, Douglas SD, et al (1990) Impaired metabolic function and signalling defects in phagocytic cells in glycogen storage disease type 1b. J Clin Invest 86:196–202

Lachaux A, Boillot O, Stamm D, Canterino I, Dumontet C,

Regnier F, Floret D, et al (1993) Treatment with lenograstim (glycosylated recombinant human granulocyte colony-stimulating factor) and orthotopic liver transplantation for glycogen storage disease type Ib. J Pediatr 123:1005–1008

Lange AJ, Arion WJ, Beaudet AL (1980) Type 1b glycogen storage disease is caused by a defect in the glucose-6-phosphate translocase of the microsomal glucose-6-phosphatase system. J Biol Chem 255:8381–8384

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lei K-J, Chen Y-T, Pan C-J, Ward JM, Mosinger B, Lee EJ, Westphal H, et al (1996) Glucose-6-phosphatase dependent substrate transport in the glycogen storage disease type 1a mouse. Nat Genet 13:203–209

Lei K-J, Pan C-J, Shelly LL, Liu J-L, Chou JY (1994) Identification of mutations in the gene for glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1a. J Clin Invest 93:1994–1999

Lei K-J, Shelly LL, Lin B, Sidbury JB, Chen Y-T, Nordlie RC, Chou JY (1995) Mutations in the glucose-6-phosphatase gene are associated with glycogen storage disease type 1a and 1aSP but not 1b and 1c. J Clin Invest 95:234–240

Lei K-J, Shelly LL, Pan C-J, Sidbury JB, Chou JY (1993) Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. Science 262:580–583

Moses SW (1990) Pathophysiology and dietary treatment of the glycogen storage diseases. J Pediatr Gastroenterol Nutr 11:156–174

Narisawa K, Igarashi Y, Otomo H, Tada K (1978) 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 83:1360–1364

Nordlie RC, Sukalski KA, Munoz JM, Baldwin JJ (1983) Type 1c, a novel glycogenosis. J Biol Chem 258:9739–9744

Roe TF, Coates TD, Thomas DW, Miller JH, Gilsanz V (1992) Treatment of chronic inflammatory bowel disease in glycogen storage disease type Ib with colony-stimulating factors. N Engl J Med 326:1666–1669

Spiekermann K, Roesler J, Emmendoerffer A, Elsner J, Welte K (1997) Functional features of neutrophils induced by G-CSF and GM-CSF treatment: differential effects and clinical implications. Leukemia 11:466–478

Waddell ID, Burchell A (1991) Transverse topology of glucose-6-phosphatase in rat hepatic endoplasmic reticulum. Biochem J 275:133–137

Wendel U, Schroten H, Burdach S, Wahn V (1993) Glycogen storage disease type Ib: infectious complications and measures for prevention. Eur J Pediatr 152, suppl 1:S49–S51