Glycogen storage disease type II (GSD II/glycogenosis type II/Pompe's disease/acid maltase deficiency) is caused by the deficiency of lysosomal α-glucosidase resulting in lysosomal accumulation of glycogen. The disease is inherited as an autosomal recessive trait and is clinically heterogeneous. Early and late onset phenotypes are distinguished. Insight in the molecular nature of the lysosomal α-glucosidase deficiency and the underlying genetic defect has increased significantly during the past decade. This minireview on GSD II was written at the occasion of The International Symposium on Glycolytic and Mitochondrial Defects in Muscle and Nerve, held in Osaka, Japan, July 1994. It is an update of current literature, but also includes original data from the collaborating authors on mutations occurring in the lysosomal α-glucosidase gene and on prenatal diagnosis by chorionic villus sampling. The genotype–phenotype correlation and the prospects for therapy are addressed.
Key words: glucosidase • glycogenosis • lysosomal • prenatal diagnosis • chorionic villi • therapy • acid maltase

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GLYCOGENOSIS TYPE II (ACID MALTASE DEFICIENCY)

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Glycogenosis type II (GSD II) is one of the more than ten glycogen storage diseases recognized at present as separate genetic entities.[28] Characteristic for this disease is the organelle bound, lysosomal, accumulation of glycogen as opposed to the cytoplasmic accumulation of glycogen in the other glycogenoses. The disease was described first in 1932 by the Dutch pathologist, J.C. Pompe, from whom it lends its name Pompe's disease.[34] The name "acid maltase deficiency" was introduced in 1963 after identification of the missing enzyme.[14] That publication by H.G. Hers on the primary defect in Pompe's disease has accelerated the subsequent discovery of lysosomal enzyme deficiencies in more than 30 other lysosomal storage diseases. In addition, deficiencies of nonenzymic lysosomal proteins were discovered.

Since the discovery of the primary lysosomal α-glucosidase deficiency in GSD II important new information has been acquired on the structure and function of the enzyme, and progress has been made with elucidating the underlying genetic defect in case of α-glucosidase deficiency. Enzyme therapy is under development.

This minireview starts with a brief description of the clinical phenotypes of GSD II and a review of recent findings pertaining to the structure and function of lysosomal α-glucosidase. The genotype–phenotype correlation is addressed with reference to the mutations discovered so far and the residual α-glucosidase activity in cultured fibroblasts from a large series of patients. Original data on the prenatal diagnosis by chorionic villus sampling in 42 pregnancies at risk are presented, and the prospects for enzyme therapy are discussed.

THE CLINICAL PHENOTYPE

Among patients with GSD II a pronounced variation is observed with regard to age of onset and

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clinical presentation. This has led to the distinction of three subtypes of the disease, an infantile, juvenile, and adult variant.[7,28] In patients with the infantile form of GSD II symptoms become apparent within the first 3 months of life. Mostly, poor motor development and failure to thrive are noticed first. On clinical examination there is generalized hypotonia with muscle wasting, increased respiration rate with sternal retractions, moderate enlargement of the liver, and protrusion of the tongue. Ultrasound examination of the heart shows a progressive hypertrophic cardiomyopathy, eventually leading to insufficient cardiac output. The ECG is characterized by marked left axis deviation, a short PR interval, large QRS complexes, inverted T waves, and ST depressions. The disease shows a rapidly progressive course leading to cardiorespiratory failure within the first year of life. On histological examination at autopsy lysosomal glycogen storage is observed in various tissues, most pronounced in heart and skeletal muscle.

Patients with the adult form of GSD II may not experience symptoms within the first two decades of life. In this clinical subtype only skeletal muscles are involved with predilection of those of the limb girdle, the trunk and the diaphragm. Difficulty in climbing stairs is often the initial complaint. The respiratory impairment varies considerably. It can dominate the clinical picture, or it is not experienced by the patient until late in life.[7] The eldest patient recorded at the University Hospital Rotterdam was referred at the age of 53, and died at the age of 72 because of respiratory insufficiency, which is the main cause of death in late onset GSD II.

Besides infantile and adult variants an intermediate juvenile subtype is distinguished. In these patients symptoms usually become apparent in the first decade of life. As in adult GSD II, skeletal muscle weakness is the major problem, cardiac involvement does not occur. In many cases nightly ventilatory support is ultimately needed. Pulmonary infections in combination with wasting of the respiratory muscles are life threatening and mostly become fatal before the third decade. On autopsy in late onset (juvenile and adult) cases glycogen storage is solely encountered in skeletal muscles. However, in some rare cases storage of glycogen has been described in vascular smooth muscle cells, leading to an aneurysm of the basilar or other intracranial arteries.[21,27]

In practice, the clinical appearance of Pompe's disease is a spectrum with an early onset and rapidly progressive phenotype with cardiac involvement at one end and a late onset slowly progressive phenotype at the other.[7] The clinical histories of 20 patients diagnosed by our colleague, Dr. M.C.B. Loonen (neurologist, University Hospital Rotterdam), are depicted in Figure 1.

LYSOSOMAL α-GLUCOSIDASE (ACID MALTASE)

Lysosomal or acid α-glucosidase is the best name for this enzyme since the substrate specificity is not limited to maltose, and the catalytic activity not to hydrolysis of the 1,4-glycosidic bond. The natural substrate is glycogen. When engulfed by the lysosomal system,[6] glycogen is degraded to glucose whereby both the α-1,4 and α-1,6 linkages are cleaved. Also, activity for α-1,2 and α-1,3 diglycosidic bonds has been reported,[26] which implies that carbohydrate structures other than glycogen may serve as natural substrate.

The enzyme consists of 952 amino acids as deduced from the cDNA sequence and shows structural similarity with the intestinal enzymes sucrase and isomaltase.[15] It is assumed that the three enzymes have arisen from a common ancestral gene.[15,20] The newly synthesized lysosomal α-glucosidase precursor has a signal peptide at the N-terminal end for guiding the nascent protein to the lumen of the endoplasmic reticulum[15] where N-linked glycosylation occurs at all the seven avail-

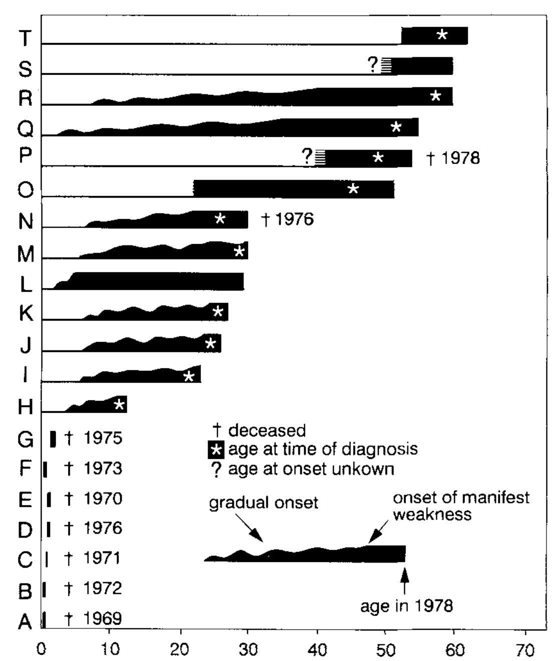

FIGURE 1. The clinical spectrum of Pompe's disease: onset and course of the disease in 20 random cases. This figure was taken with permission from the academic thesis of Dr. M.C.B. Loonen (Erasmus University Rotterdam, 1979), and was made by analogy to the figure published by Engel AG, et al.: *Neurology* 1973;23:95–106.

able positions. Phosphorylation (mannose 6-phosphate) occurs at two sites minimally.[13] The relative molecular mass of this precursor is 110 kd.[31,36] Posttranslational processing during transport through the Golgi complex and *trans*-Golgi network, and in the lysosomes, leads to a stepwise mass reduction from 110 kd via 95 kd to 76 kd and finally 70 kd.[9,31,36,50] The posttranslational modification is complex and involves the proteolytic removal of both N- and C-terminal peptides in fixed order, and further, a partial alteration of the initial carbohydrate structures. The conformational changes are essential for the development of catalytic activity.[18,50] Figure 2 shows schematically the location of the seven glycosylation sites (G1–G7) and the places of proteolytic cleavage. Also summarized in this figure are the amino acid substitutions that have been found in GSD II patients (discussed separately).

NATURE OF THE LYSOSOMAL α-GLUCOSIDASE DEFICIENCY IN GSD II

Activity. The discovery of acid maltase deficiency in a severe case of infantile Pompe's disease has been the first important step in elucidating the molecular basis of GSD II.[14] The "same" enzyme deficiency was later also discovered in late onset variants of the disease.[53] The cause of this clinical heterogeneity remained obscure. The hypothesis of a coexistent deficiency of acid and neutral maltase activities in the severe form of GSD II as compared to a single acid α-glucosidase deficiency in the milder clinical forms was raised but could not be substantiated.[44] An alternative presumption that patients with a milder form of the disease would have a higher residual α-glucosidase activity was made,[17,29,35,38,41,43] but left unexplained the mild clinical phenotype of some adult patients with an exceptionally low residual activity.[3,36,38]

To reevaluate the latter presumption we have recently compared the level of residual α-glucosidase activity in cultured fibroblasts from patients with different clinical phenotypes. These cells are for several reasons more suitable for this type of study than muscle biopsy specimens or leukocytes: (i) fibroblast cell lines can be established and collected over the years and can be stored frozen in liquid nitrogen without loss of viability and enzyme activity, ensuring a large sample size; (ii) the culture conditions are well controlled and the condition of the cells can be monitored by visual inspection and by recording the growth rate; (iii) the lysosomal glycogen accumulation in cultured fibroblasts is moderate compared to muscle fibers[35] and does not induce cellular damage with concurrent effects on lysosomal enzyme activities,[5,49] moreover, the pathological changes in muscle tissue vary locally;[7] and (iv) leukocyte preparations, which are the preferred material for the routine assay of many lysosomal enzymes, are not ideal for α-glucosidase assay, probably because of high and variable levels of neutral maltase activities. The influence of neutral maltase activities in fibroblasts is negligible, provided that the assay is performed at low pH.

The data obtained with fibroblasts were collected in our diagnostic department over a 15-year period, and are summarized in Figure 3. The activity of each fibroblast cell line is depicted separately. The control series includes 84 cell lines from healthy individuals and ranges from 42 to 160 nmol/h per milligram protein with an average activity of 97.5. Twenty of the 24 cell lines from patients with adult GSD II have activities ranging

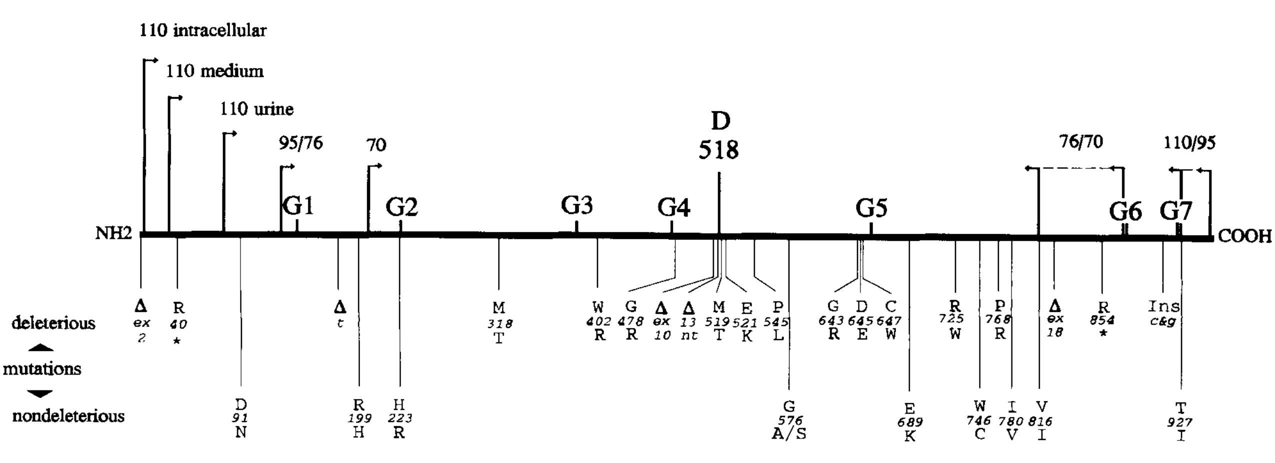

FIGURE 2. A schematic representation of lysosomal α-glucosidase. The beginning and end of the various molecular forms of the enzyme are indicated, as well as the positions of the catalytic carboxylate Asp518 (D518) and the seven glycosylation sites (G1–7). Also summarized are the deleterious and nondeleterious amino acid substitutions known to us.

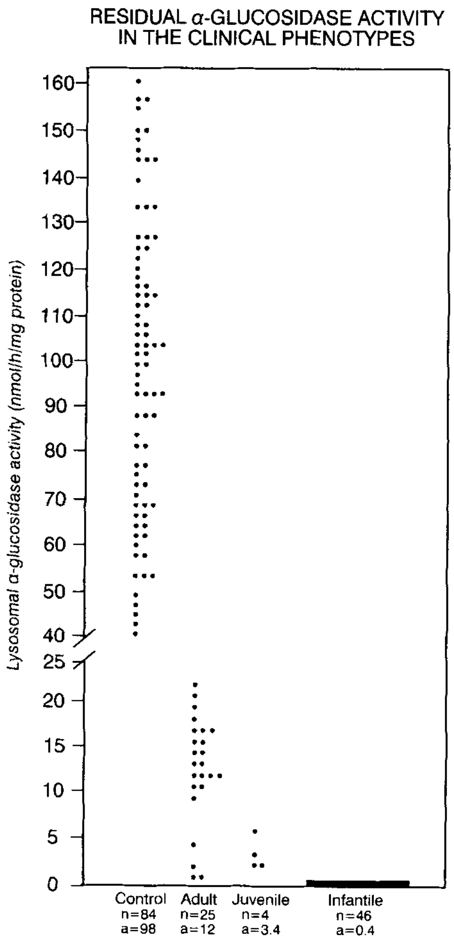

FIGURE 3. Correlation between clinical phenotype and residual α-glucosidase activity. The lysosomal α-glucosidase activity was measured in homogenates of cultured fibroblasts from control individuals and patients with different clinical forms of GSD II. 4-Methylumbelliferyl-α-D-glucopyranoside was used as substrate at pH 4.0. The fibroblasts were cultured in Ham's F10 medium supplemented with 10% fetal calf serum and antibiotics, and harvested 4 days after having reached confluence.

from 7 to 22 nmol/h per milligram (i.e., 7–23% of the average control activity), and four cell lines have significantly lower activities. Four cell lines were available from patients with juvenile GSD II, all with a low but significant activity (2–6%). Patients with severe infantile GSD II form the most homogeneous population in that none of the cell lines exhibits more than 1% residual activity.

Thus, Figure 3 shows a logical correlation between the level of residual activity and the course of the disease. A similar correlation has been observed in a small series of muscle cell cultures.[43] This correlation can be taken as a starting point for understanding the cause of clinical diversity. Effort can then be devoted to finding an explanation for the relatively mild clinical phenotype of some adult patients with an exceptionally low α-glucosidase activity. We have done so in one particular case of adult GSD II with 4% residual activity and noticed that α-glucosidase was normally synthesized, but disappeared during the subsequent formation of mature enzyme. Immunocytochemistry at the ultrastructural level suggested that the deficiency of lysosomal α-glucosidase activity was compensated in part by expansion and activation of the lysosomal system: a phenomenon seen in the affected but not in the unaffected muscle fibers.[49]

Mutations. Since the stepwise cloning and characterization of the α-glucosidase gene, tools have become available to analyze GSD II at the DNA level.[15,16,23–25] Mutation analysis performed by several groups has resulted in the identification of a variety of molecular lesions. All mutations known to us at present are listed in Table 1, and the resulting amino acid substitutions are indicated by position in Figure 1. References to the publications in which these mutations were reported are given in the footnote of Table 1. Also included are mutations discovered in our laboratory that remained as yet unpublished. The genetic heterogeneity in GSD II is immediately evident. The mutations are spread over most of the exons and are of different nature. Single base-pair deletions, (double) insertions, and larger deletions occur besides missense and nonsense mutations. Also, splice defects with loss of coding sequences have been reported. Frequent mutations are the exon 18 deletion, which occurs in the Dutch patient population with an allele frequency of 0.13 among infants and adults, and the exon 2 deletion in adults (allele frequency 0.35). Also ΔT525 and Pro545Leu seem to be more common mutations (see Table 1 for references).

Some mutations are predictably deleterious by creating a stop codon at the (very) 5′ end of the coding sequence (e.g., ΔT525 and Arg40Stop) or by leading to substantial loss of coding sequence (e.g., the deletions of exon 18, exon 2, and nt 1456–1468). Other mutations are obviously harmless because they do not result in an amino acid substitution or are also found in healthy individuals (polymorphism). Caution has to be taken with predicting the effect of most other mutations. Evolutionary conservative amino acid substitutions such as Asp645Glu can be deleterious. Very drastic alterations such as Glu→Lys remain without effect when occurring at position 689, but are deleterious at position 521, adjacent to the catalytic carboxylate at Asp518.[12] When doubt remains about the

Table 1. Deleterious and nondeleterious mutations in the lysosomal α-glucosidase gene.

Exon	Mutation	aa Change	Ref.
A. Deleterious			
Intron 1	T(−13)G [Δexon 2]	Δ182aa 1→	a,b
2	C118T	Arg40Stop	UR
	ΔT525	Thr175→Shift	c
5	T953C	Met318Thr	d
8	C1204T	Trp402Arg	e,f
9	G1432A	Gly478Arg	UR
10	Δ13nt1456→	Thr485→Shift	g
Intron 10	G(+1)C [Δexon 10]	Δ38aa 480→	b
11	T1556C	Met519Thr	UR
	G1561A	Glu521Lys	h
	C1634T	Pro545Leu	c
14	G1927A	Gly643Arg	i
	C1935A	Asp645Glu	j,k,l
	C1941G	Cys647Trp	g
15	C2173T	Arg725Trp	i
16	C2303G	Pro768Arg	UR
18	Δexon 18	Δ55aa 828→	m,g,n
	C2560T	Arg854Stop	j
19	insC2741/insG2743	Pro913→Shift	UR
B. Nondeleterious			
	G(−82)C	Noncoding	f
	C(−79)G	Noncoding	f
2	G271A	Asp91Asn	o
	C324T	Cys108	e,f
3	G596A	Arg199His	j,e,f
	C642T	Ser214	i,f
	A668G	His223Arg	UR
5	A921T	Ala307	h
8	A1203G	Gln401	j,c,f
9	C1374T	Tyr458	i,j
11	A1581G	Arg527	h,i,j,e,f
12	G1726A	Gly576Ser	UR
	C1727G	Gly576Ala	f
14	G1917A	Val639	UR
15	G2065A	Glu689Lys	g
	A2133G	Thr711	i
16	G2238C	Trp746Cys	g
17	A2338G	Ile780Val	f
	G2446A	Val816Ile	j,k
18	A2553G	Gly815	j,e,f
19	C2780T	Thr927Ile	j,k
20	C2808T	Asp936	UR
	G2862A	Noncoding	j,k
	ΔG2998/ΔG2999	Noncoding	e
	C3002T	Noncoding	f
	C3082T	Noncoding	f
	G3086C	Noncoding	e,f
	T3277C	Noncoding	e,f

[a]*Boerkoel N, et al.: The International Symposium on Glycolytic and Mitochondrial Defects in Muscle and Nerve, Osaka, Japan, July 7–8, 1994;* [b]*Huie ML, et al.: Hum Mol Genet 1994;3:2231–2236,* [c]*Hermans MMP, et al.: Hum Mol Genet 1994;3:2213–2218;* [d]*Zhong N, et al.: Am J Hum Genet 1991;49:635–645;* [e]*Hoefsloot LH, et al.: Biochem J 1990;272:493–497;* [f]*Martiniuk F, et al.: DNA Cell Biol 1990;9:85–94;* [g]*Huie ML, et al.: Hum Mol Genet 1994;3:1081–1087;* [h]*Hermans, MMP et al.: Biochem Biophys Res Comm 1991;179:919–926;* [i]*Hermans MMP, et al.: Hum Mutat 1993;2:268–273;* [j]*Hermans MMP, et al.: Biochem J 1993;289:687–693;* [k]*Hermans MMP, et al.: Genomics 1993;16:300–301;* [l]*Shie JJ, et al: J Inher Metabol Dis 1994;17:145–148;* [m]*Boerkoel N, et al.: Am J Hum Genet 1992;51:1367;* [n]*Van der Kraan M, et al.: Biochem Biophys Res Comm 1994;203:1535–1541;* [o]*Martiniuk F, et al.: Am J Hum Genet 1990;47:440–445; UR: authors' unpublished result*

significance of a mutation, it is necessary to perform a detailed analysis by site-directed mutagenesis of the "wild-type" cDNA followed by expression in eukaryotic cells.

To correlate genotype and phenotype it is essential to know the effect of a given mutation exactly. The frequent occurrence of compound heterozygotes is a complicating factor because the two mutant allele products have a combined effect. To investigate the genotype–phenotype correlation in GSD II we have expressed the mutant allele products transiently in COS cells. The residual activity in the COS cell expression system appeared to correlate percentagewise with the residual activity in the patients' fibroblasts.[10,11] Moreover, similar defects in synthesis and posttranslational modification were observed in COS cells as in fibroblasts. Thus, mutation analysis via the COS cell system demonstrates the causal relation between mutation, defective enzyme biosynthesis, and loss of enzymatic function. However, this sophisticated assay is not per se more informative about the cause of clinical diversity than the level of α-glucosidase activity realized (in fibroblasts) by the two mutant allele products in natural coexistence. Mutation analysis has the advantage that it provides the means to diagnose with certainty carriers in families at risk, which may be problematic by enzyme assay. Furthermore, insight in the genotype–phenotype correlation and better understanding of enzyme structure and function, obtained by mutation analysis, will help to design a strategy for therapy.

PRENATAL DIAGNOSIS OF GSD II

Prenatal diagnosis for the infantile type of GSD II is reliably possible by chorionic villus sampling (CVS) or amniocentesis. We have performed prenatal analysis in 114 pregnancies at risk for GSD II and diagnosed 23 affected fetuses. The first 5 cases in this series, concerning the assay of α-glucosidase in cultured amniotic fluid cells, were reported nearly 20 years ago.[30] The diagnostic test is complicated by the generally low activity of α-glucosidase in first-passage amniotic fluid cells (10–65 nmol/h per milligram protein) compared to 42–160 nmol/h per milligram in cultured skin fibroblasts. Activities as low as 3–4 nmol/h per milligram have been measured in a few pregnancies with an unaffected heterozygous fetus. However, with optimal accuracy and sufficient experience, these low carrier levels can be distinguished reliably from the virtually complete deficiency of α-glucosidase in amniocytes from affected fetuses (i.e., <1 nmol/h per milligram).

The introduction of CVS has strongly improved the prenatal diagnosis of GSD II. The most important advantage of CVS obviously is that it is done at a much earlier gestational age (10–12 weeks) than amniocentesis (15–16 weeks). Moreover, the enzyme analysis can be carried out directly on the chorionic biopsy making the diagnosis available within a day. The use of chorionic villi (CV) has also facilitated the diagnosis of GSD II, because the activity of α-glucosidase in normal CV is much higher than in amniotic fluid cells, which results in a better separation of values indicating a carrier or an affected fetus, respectively.

A definitive and correct diagnosis could be made by direct analysis of the CV in 41 of the 42 pregnancies (Table 2). In 10 cases an almost complete deficiency of α-glucosidase left no doubt about the diagnosis of an affected fetus. In the remaining cases the activity was normal ($n = 20$), moderately reduced as expected for heterozygotes ($n = 11$), or strongly reduced in 1 case ($n = 1$). In this exceptional case amniocentesis also indicated heterozygosity.

Previous reports on small series of first-trimester diagnoses for GSD II have emphasized the risk of maternal tissue contamination of CV samples,[4] or have anticipated the possible presence of neutral α-glucosidase activity in CV and have used an immunoprecipitation method[8] or maltose as substrate to prevent such interfering activity.[32] We have used a simple and rapid assay with artificial 4-methylumbelliferyl α-D-glucopyranoside substrate at pH 4.0. The absence of activity in the CV of 10 affected pregnancies indicates that neither interfering enzyme activity nor the presence

Table 2. Prenatal diagnosis in 42 pregnancies at risk for glycogenosis type II by chorionic villus sampling.

Chorionic villi	α-Glucosidase activity*	Diagnosis†
Pregnancies at risk		
$n = 10$	0.7–2.4	(+) homozygous
$n = 1$	13	(−) heterozygous
$n = 11$	33–77	(−) heterozygous
$n = 20$	90–258	(−) heterozygous or normal
Controls		
$n = 45$	90–260	

*Nanomoles per hour per milligram protein.
†(+) = affected; all pregnancies were terminated and the diagnoses were confirmed by enzyme assay in cultured CV cells or fetal skin fibroblasts or both. (−) = Unaffected; the outcome of all pregnancies was an unaffected child.

of maternal tissue or enzyme has played a role in these cases.

THERAPY

The most exciting progress in the development of therapy for lysosomal storage diseases is the effectiveness of enzyme therapy in type 1 Gaucher's disease.[1,33,52] The rationale of the approach is that (macro)molecular compounds are able to enter cells by endocytosis and reach the lysosomal system.[6] Cell surface receptors can function as efficient mediators in this process.[42] The first attempt at enzyme therapy for lysosomal diseases dates from 1965 when a patient suffering from infantile GSD II received intravenous injections of α-glucosidase from *Aspergillus niger*.[2] A 5-month-old infant was treated for 116 days with a similar enzyme preparation.[19] An increase of enzyme activity in the liver was recorded and a clearance of lysosomal glycogen was noted. However, the positive effects could not be maintained. The treatment was stopped after 4 months because of a developing immune nephritis. The patient died of cardiorespiratory insufficiency. At postmortem examination the liver and spleen pathology was typical for advanced GSD II. Also, other attempts at therapy with α-glucosidase from human placenta were unsuccessful. Without present-day knowledge, these early trials were bound to fail, because of insufficient quality and quantity of the administered enzyme. Moreover, in these trials it was not attempted to facilitate α-glucosidase uptake via cell surface receptors. The protocol for treatment of Gaucher's disease with enzyme therapy prescribes the use of placental glucocerebrosidase with modified carbohydrate side chains so that the enzyme binds to mannose receptors on the cell surface of affected macrophages in liver (Kupffer cells), spleen, and bone marrow.[40] Enzyme therapy is at present applied almost as a routine for the treatment of type 1 Gaucher's disease and appears to be effective although questions remain about the exact mechanism of action.

Following the same basic principles we started to develop models for (re)testing the potential effect of α-glucosidase administration to patients with GSD II. The most essential information obtained so far is that enzyme administered to cultured fibroblasts and muscle cells is taken up in an efficient manner when containing mannose 6-phosphate groups, enabling the enzyme to bind to the mannose 6-phosphate receptor.[37,43,45,47,48] Evidence for lysosomal targeting and function was obtained most notably by the resulting breakdown of stored glycogen. The relatively long half-life of the ingested enzyme (approximately 6–9 days) is favorable for successful application. In a more realistic model system, preparations of human placental and bovine testis α-glucosidase were administered intravenously to healthy mice in a dose of 180 μg per animal. The enzyme was recovered in various organs up to 9 days after injection. Most of it appeared to be taken up by liver and spleen, virtually independent of the mannose 6-phosphate content. The enzyme was not found in brain indicating that the blood–brain barrier is not passed. Most importantly, however, uptake was obtained also in heart and skeletal muscle, the target organs in GSD II. The increase of α-glucosidase activity obtained with bovine testis α-glucosidase containing mannose 6-phosphate residues was higher than with placental enzyme lacking this recognition signal.[46] In theory, the 40% increase obtained should be sufficient to supplement the enzyme deficiency of patients, under the assumption that cellular pathology does not ensue at activity levels above 30% of the average control level (according to the data in Fig. 2).

Although these results are promising, the ultimate effect of enzyme therapy in GSD II can only be tested in clinical trails in humans, preferably preceded by tests in animal models. With respect to the latter, the development of a mouse model of GSD II via mutagenesis of the murine α-glucosidase gene in embryonic stem cells is in progress. Animal models of lysosomal storage diseases have already proven their value. For instance, mice with a natural β-glucuronidase deficiency (mucopolysaccharidosis type VII/Sly syndrome in humans) have been used for investigating the effect of bone marrow transplantation, enzyme infusion, and implantation of a neo-organ with glucuronidase-producing fibroblasts.[22,39,51] A prerequisite to enzyme therapy in lysosomal storage diseases including GSD II is the realization of large scale production of (recombinant) enzyme. Production in genetically modified CHO cells is an option already applied for the production of glucocerebrosidase. An alternative approach we are exploring is production of human recombinant α-glucosidase in the milk of transgenic mammals. There are still obstacles toward therapy for GSD II, but the goal is challenging.

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