

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/24033>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

Short Communication

Homozygous deletion of exon 18 leads to degradation of the lysosomal α -glucosidase precursor and to the infantile form of glycogen storage disease type II

Ausems MGEM, Kroos MA, Van der Kraan M, Smeitink JAM, Kleijer WJ, Ploos van Amstel HK, Reuser AJJ. Homozygous deletion of exon 18 leads to degradation of the lysosomal α -glucosidase precursor and to the infantile form of glycogen storage disease type II.
Clin Genet 1996; 49: 325–328. © Munksgaard, 1996

We describe two unrelated Dutch patients with typical symptoms of infantile glycogen storage disease type II (GSD II) and virtual absence of acid α -glucosidase activity in leukocytes and cultured skin fibroblasts. The patients were identified as homozygotes for a deletion of exon 18 of the acid α -glucosidase gene (GAA). The in-frame deletion manifests at the protein level in a characteristic way: the enzyme precursor is smaller than normal and degraded in the endoplasmic reticulum or Golgi complex. These cases present an evident example of a genotype-phenotype correlation in glycogen storage disease type II.

**Margreet G. E. M. Ausems¹,
Marian A. Kroos²,
Magna Van der Kraan²,
Jan A. M. Smeitink³,
Wim J. Kleijer²,
Hans Kristian Ploos van Amstel¹
and Arnold J. J. Reuser²**

¹Clinical Genetics Centre Utrecht, Utrecht, ²Department of Clinical Genetics, Erasmus University, Rotterdam, and ³Department of Metabolic Diseases, University Children's Hospital "Het Wilhelmina Kinderziekenhuis", Utrecht, The Netherlands

Key words: acid maltase – glucosidase – lysosomal storage disease

Arnold J. J. Reuser, Dept. of Clinical Genetics, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands
Tel: -31-10-408.7153
Fax: -31-10-436.2536

Received 6 June 1995, revised version received 8 January, accepted for publication 17 January 1996

Glycogen storage disease type II (GSD II; Pompe disease; acid maltase deficiency) is an autosomal recessive lysosomal storage disorder caused by deficiency of acid α -glucosidase (McKusick 1994). The disease is characterized by lysosomal accumulation of glycogen, affecting muscle function and leading to muscle wasting. Early- and late-onset phenotypes are distinguished. The major difference is cardiac involvement and early demise in the first phenotype, and slowly progressive muscle degeneration in the second (Hirschhorn 1995).

One of the aims of current research on GSD II is to establish a genotype-phenotype correlation. The

gene for acid α -glucosidase (GAA) has a length of over 20 kb (Hoefsloot et al. 1990, Martiniuk et al. 1991) and is composed of 20 exons coding for a protein of 952 amino acids (Hoefsloot et al. 1988, Martiniuk et al. 1990). Several gene defects have been reported leading to acid α -glucosidase deficiency (Reuser et al. 1995, Raben et al. 1995). Patients often have rare mutations and are compound heterozygotes, which complicates the assessment of a genotype-phenotype correlation. It is only recently that relatively frequent mutations have been identified, enabling the search for defined combinations of genotype and phenotype. These mutations are the

-13T→G transversion in intron 1, promoting aberrant splicing and loss of exon 2 (Huie et al. 1994b, Boerkoel et al. 1995), the single base-pair deletion ΔT525 (Hermans et al. 1994), and the deletion of exon 18 (Boerkoel et al. 1992, Huie et al. 1994a, Van der Kraan et al. 1994, Boerkoel et al. 1995).

In this paper we describe two unrelated patients with infantile onset GSD II, who are both homozygotes for the exon 18 deletion and show the direct effect of this mutation on acid α-glucosidase synthesis and function.

Case reports

Patient A (cell line 93RD148) was the second male child of healthy, non-consanguineous Dutch Caucasian parents. He presented with prolonged jaundice 3 weeks after birth. At age 2.5 months, hypotonia, tachypnea and a cardiac murmur were noticed. Ultrasound of the heart demonstrated a hypertrophic cardiomyopathy. A severe deficiency of acid α-glucosidase was demonstrated in leukocytes (not shown) and cultured fibroblasts (Table 1). The boy died at the age of 4 months.

Patient B (cell line 80RD156) was the third male child of healthy, non-consanguineous Dutch Caucasian parents. By history he was hypotonic from the age of 6 weeks. At presentation at age 5 months, severe hypotonia and areflexia were noticed. There was a hypertrophic cardiomyopathy. Also, the acid α-glucosidase activity of this patient was practically absent in fibroblasts (Table 1). The patient died at the age of 10 months. At autopsy, glycogen storage was demonstrated in heart, muscle, liver, kidneys and peripheral nerves.

Material and methods

DNA analysis

High molecular weight DNA was isolated from cultured fibroblasts according to established procedures. The essential parts of the GAA gene were amplified using the polymerase chain reaction (Saiki et al. 1985). The exon 18 deletion was analyzed by amplification of exon 17 and 18 in tandem using the primers POM17F (TGGTTCCTGAGGACAGCATG) (forward) and POM18R (AGTGGCAGGTAGCCATCGGTG) (reverse). The amplification was performed in 33 cycles, each cycle consisting of 1 min denaturation at 94°C, 1 min annealing at 55°C and 2 min extension at 72°C. The reaction mixture contained 400 ng of high molecular weight DNA, 400 ng of each primer; 1.5 mM of each dNTP; 100 μg/ml bovine serum albumin; 67 mM Tris-HCl (pH 8.8); 6.7 mM MgCl₂; 10 mM β-mercaptoethanol; 6.7

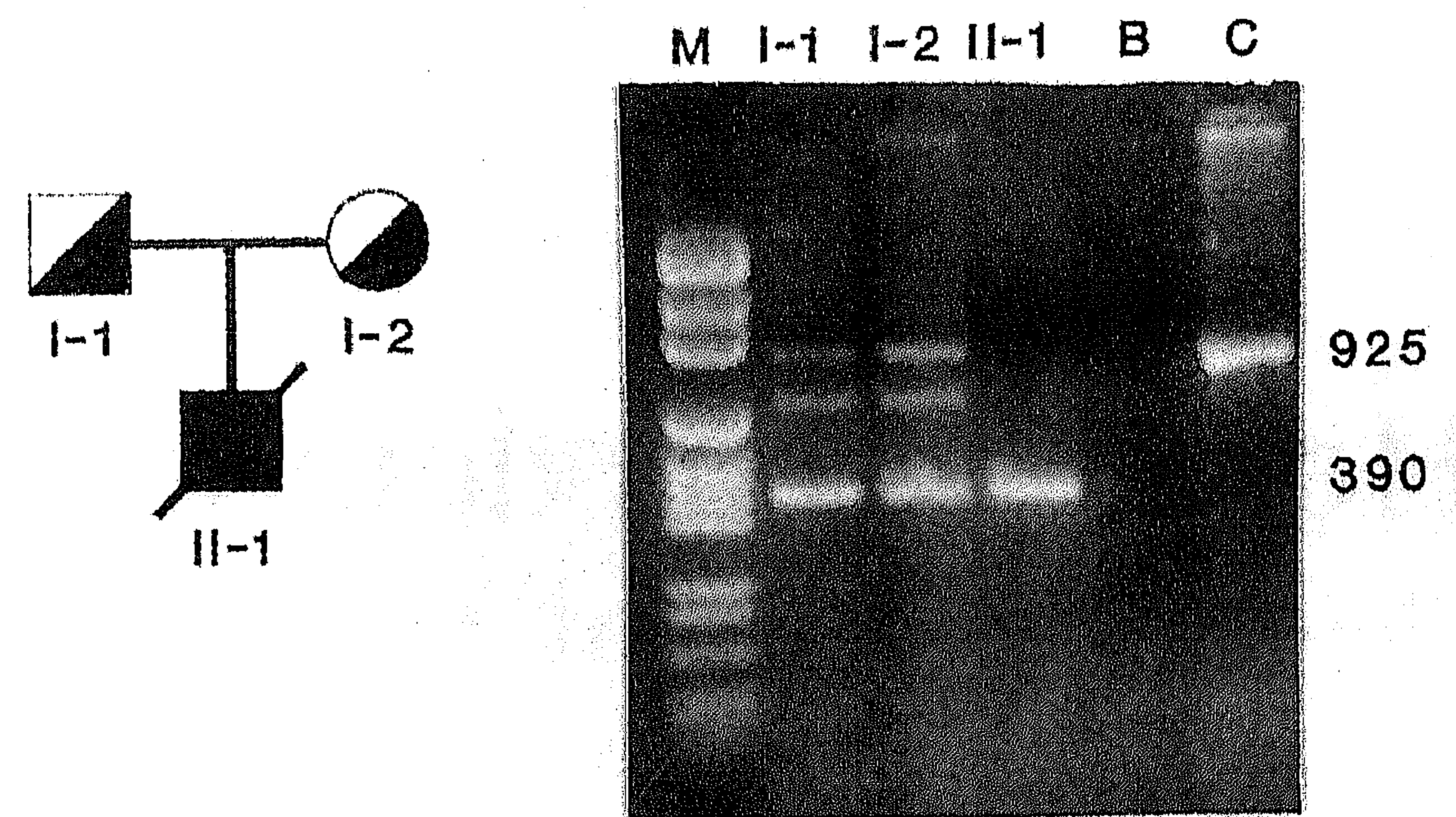


Fig. 1. Detection of the exon 18 deletion in patient A by PCR and agarose gel electrophoresis. Control individuals (C) have a fragment of 925 bp. Patient A (II-1) has a smaller fragment of 390 bp. His parents (I-1 and I-2) have both the smaller (390 bp) and the normal (925 bp) fragments. The additional band with an intermediate migration was identified by PCR analysis as a heteroduplex of normal and mutant fragments. M is a marker and B is a blank.

μM EDTA; 16.6 mM (NH₄)₂SO₄; 10% dimethylsulfoxide and 40 U/ml of Taq-DNA polymerase (Cetus Corp, Emeryville, CA, USA).

Protein analysis

The biosynthesis of acid α-glucosidase in fibroblasts was monitored by pulse-chase labelling with ³⁵S methionine (TranS, 1200 Ci/mmol, ICN) followed by immunoprecipitation and SDS-polyacrylamide gel electrophoresis, as described by Hermans et al. (1994). In particular experiments, Brefeldin A

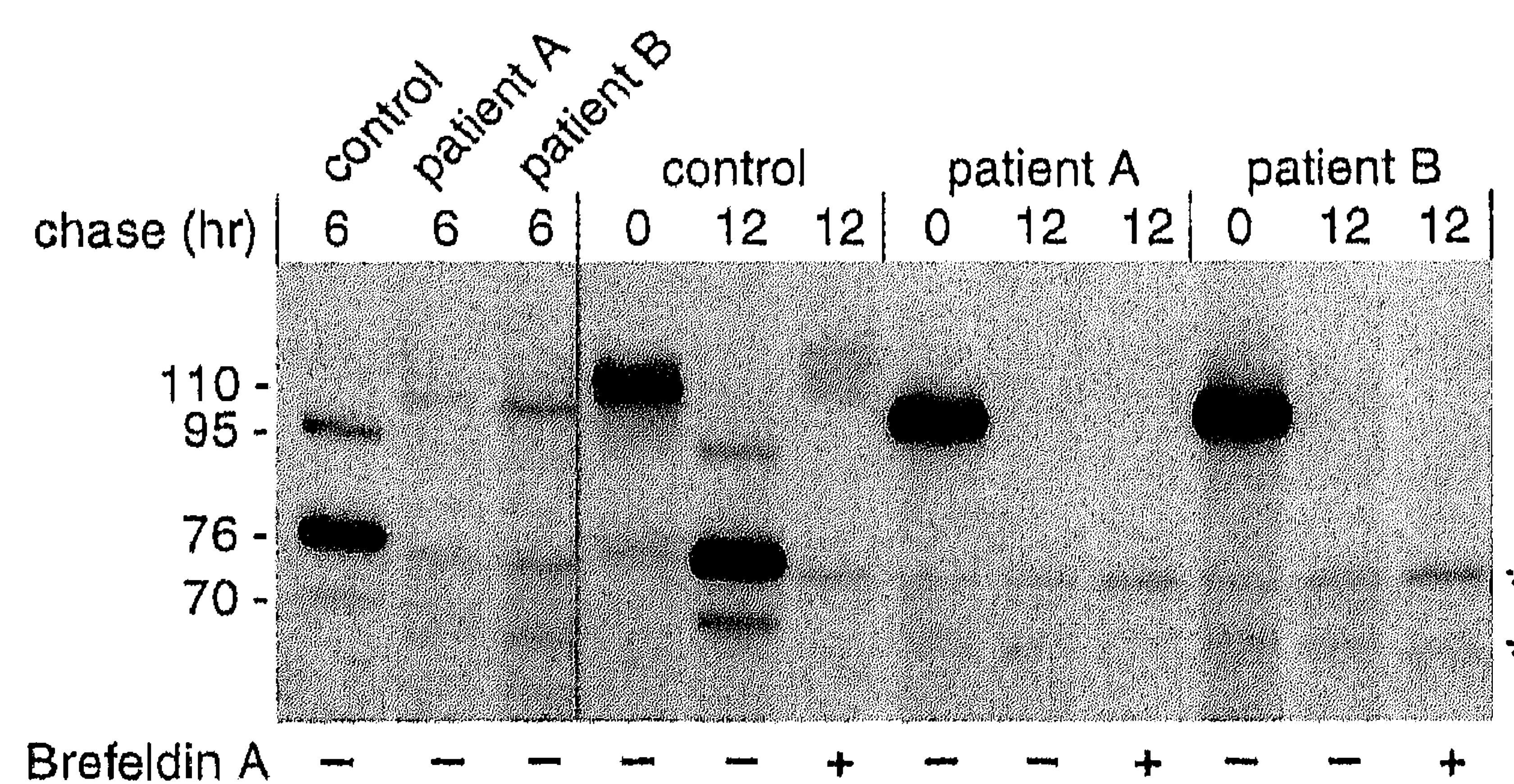


Fig. 2. Synthesis and processing of acid α-glucosidase in cultured fibroblasts of patients A and B. Acid α-glucosidase was metabolically labelled for 3 h with ³⁵S methionine. The cells were harvested after 0, 6 and 12 h of chase. Where indicated (+) Brefeldin A was present during the pulse-chase period. Acid α-glucosidase was immuno-precipitated from the cell homogenates and analyzed by SDS-PAGE. The molecular mass of the various forms of acid α-glucosidase is indicated. The bands marked with an asterisk (*) are non-specific precipitation products which are not related to acid α-glucosidase synthesis and processing.

Table 1. α -Glucosidase activities in two families with exon 18 deletion

	Enzyme activity ^a in fibroblasts	Δ exon 18 ^b
Family A		
Patient	0.2	+/+
Father	47	+/-
Mother	61	+/-
Family B		
Patient	0.4	+/+
Normal controls	40-160 n=84	-/-

^a The substrate used for the assay of acid α -glucosidase activity in fibroblasts is 4-methylumbelliferyl- α -D-glucopyranoside according to Hermans et al. (1994). The activity is expressed in nmol/h per mg protein;

^b Positive (+) and negative (-) identification of the Δ exon 18 alleles is indicated.

was added to the culture medium in a concentration of 10 μ g/ml from 1 h prior to labelling till harvest.

Results and Discussion

Exon 17 and 18 of the lysosomal acid α -glucosidase gene (GAA) of the two patients with infantile GSD II were PCR amplified in tandem to possibly identify the frequently occurring exon 18 deletion. Fig. 1 shows that abnormally small PCR fragments indicative for the deletion were found, and that patient A is homozygous for the deletion. The amplified DNA fragment was directly sequenced, and this revealed the deletion to be identical to the one previously described by Huie et al. (1994a), Van der Kraan et al. (1994) and Boerkoel et al. (1995). Homozygosity for the exon 18 deletion was also detected in patient B (data not shown).

The effect of the exon 18 deletion on acid α -glucosidase synthesis and function was studied by pulse-chase labelling of cultured fibroblasts. Fig. 2 shows that deletion of exon 18 (coding for 55 amino acids) results in synthesis of a mutant acid α -glucosidase precursor with a reduced molecular mass (less than the normal 110 kD) (0 h of chase). After 6 h of chase the wild-type precursor had been converted proteolytically to 95 kD and 76 kD species. The mutant precursor had not matured, but had almost disappeared. After 12 h of chase the mutant precursor was no longer detectable. When Brefeldin A is added (preventing exit of glycoproteins from the endoplasmic reticulum and Golgi complex), the normal precursor is not proteolytically processed but gains apparent molecular mass (Fig. 2) by acquisition of sialic acid residues (Wisselaar et al. 1993). The mutant precursor is not detectable under these conditions, which is indicative for degradation in the endoplasmic reticulum or Golgi complex.

This view is supported by two additional findings: the mutant precursor is not secreted and is not immunologically detectable in lysosomes (results not shown). Since degradation of lysosomal glycogen requires the formation of 76 kD acid α -glucosidase, it is understandable that homozygosity for the exon 18 deletion results in a complete loss of catalytic function (Table 1). The severe clinical phenotype of the patients is thereby explained.

These studies demonstrate the importance of frequent mutations with well-defined functional effects for the establishment of a genotype-phenotype correlation.

Acknowledgements

We thank Tom de Vries Lentsch and Ruud Koppenol for photographic artwork and Jeanette Lokker for secretarial assistance.

References

- Boerkoel C, Raben N, Martiniuk F, Miller F, Plotz P. Identification of a deletion common to adult and infantile acid α -glucosidase deficiency. *Am J Hum Genet* 1992; 51: 1367.
- Boerkoel CF, Exelbert R, Nicastrì C, Nichols RC, Miller FW, Plotz PH, Raben N. Leaky splicing mutation in the acid maltase gene is associated with delayed onset of glycogenosis type II. *Am J Hum Genet* 1995; 56: 887-897.
- Hermans MMP, De Graaff E, Kroos MA, Mohkamsing S, Eussen BJ, Joosse M, Willemsen R, Kleijer WJ, Oostra BA, Reuser AJJ. The effect of a single base pair deletion (Δ T525) and a C1634T missense mutation (Pro545Leu) on the expression of lysosomal α -glucosidase in patients with glycogen storage disease type II. *Hum Mol Genet* 1994; 3: 2213-2218.
- Hirschhorn R. Glycogen storage disease type II: Acid α -Glucosidase (Acid Maltase) deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*, 7th edn. New York: McGraw-Hill, 1995: 2443-2464.
- Hoefsloot LH, Hoogeveen-Westerveld M, Kroos MA, Van Beeumen J, Reuser AJJ, Oostra BA. Primary structure and processing of lysosomal alpha-glucosidase; homology with the intestinal sucrase-isomaltase complex. *EMBO J* 1988; 7: 1697-1704.
- Hoefsloot LH, Hoogeveen-Westerveld M, Reuser AJJ, Oostra BA. Characterization of the human lysosomal alpha-glucosidase gene. *Biochem J* 1990; 272: 493-497.
- Huie ML, Chen AS, Sklower Brooks S, Grix A, Hirschhorn R. A *de novo* 13 nt deletion, a newly identified C647W missense mutation and a deletion of exon 18 in infantile onset glycogen storage disease type II (GSD II). *Hum Mol Genet* 1994a; 3: 1081-1087.
- Huie ML, Chen AS, Tsujino S, Shanske S, DiMauro S, Engel AG, Hirschhorn R. Aberrant splicing in adult onset glycogen storage disease type II (AMD): molecular identification of an IVS1 (-13T→G) mutation in a majority of patients and a novel IVS10 (+1GT→CT) mutation. *Hum Mol Genet* 1994b; 3: 2231-2236.
- Martiniuk M, Bodkin M, Tzall S, Hirschhorn R. Isolation and partial characterization of the structural gene for human acid alpha glucosidase. *DNA Cell Biol* 1991; 83: 283-292.

- Martiniuk F, Mehler M, Tzall S, Meredith G, Hirschhorn R. Sequence of the cDNA and 5' flanking region of human acid α -glucosidase, detection of an intron in the 5' untranslated leader sequence, definition of 18-bp polymorphisms, and differences with previous cDNA and amino acid sequences. *DNA and Cell Biol* 1990; 9: 85-94.
- McKusick VA. Glycogen storage disease II. In: Mendelian inheritance in man, 11th edn. Baltimore and London: The John Hopkins University Press, 1994: 1861-1864.
- Raben N, Nichols RC, Boerkoel C, Plotz P. Genetic defects in patients with glycogenosis type II (acid maltase deficiency). *Muscle and Nerve* 1995; Suppl 3: S70-S74.
- Reuser AJJ, Kroos MA, Hermans MMP, Bijvoet AGA, Verbeet MP, Van Diggelen OP, Kleijer WJ, Van der Ploeg AT. Glycogenosis type II (acid maltase deficiency). *Muscle and Nerve* 1995; Suppl 3: S61-S69.
- Van der Kraan M, Kroos MA, Joesse M, Bijvoet AGA, Verbeet MPh, Kleijer WJ, Reuser AJJ. Deletion of exon 18 is a frequent mutation in glycogen storage disease type II. *Biochem Biophys Res Commun* 1994; 203: 1535-1541.
- Saiki RK, Sharf S, Faloona F, Mullis KB, Horn GT, Ehrlich MA, Arnheim N. Enzymatic amplification of β -globin genomic sequence and restriction site analysis for diagnosis of sickle cell anaemia. *Science* 1985; 230: 1350-1354.
- Wisselaar HA, Kroos MA, Hermans MMP, Van Beeumen J, Reuser AJJ. Structural and functional changes of lysosomal acid α -glucosidase during intracellular transport and maturation. *J Biol Chem* 1993; 268: 2223-2231.