EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 33, No. 1, 1-7, March 2001

Recent Progress in Lysosomal α-Mannosidase and Its Deficiency

Huaichang Sun[1] and John H. Wolfe[2,3]

[1] Department of Veterinary Medicine, College of Animal Science and Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu 225009, P.R.C.
[2] Center for Comparative Medical Genetics, School of Veterinary Medicine, University of Pennsylvania and the Children's Hospital of Philadelphia, 3416 Civic Center Blvd, Philadelphia, PA 19104, U.S.A.
[3] Corresponding author: Tel,+1-215-590-3779; Fax, +1-215-590-3779; E-mail: jhwolfe@vet.upenn.cdu

Accepted 12 January, 2001

Abstract

Lysosomal α-mannosidase (EC 3.2.1.24) is a major exoglycosidase in the glycoprotein degradation pathway. A deficiency of this enzyme causes the lysosomal storage disease, α-mannosidosis, which has been described in humans, cattle, domestic cats and guinea pigs. Recently, great progress has been made in studying the enzyme and its deficiency. This includes cloning of the gene encoding the enzyme, characterization of mutations related to the disease, establishment of valuable animal models, and encouraging results from bone marrow transplantation experiments.

Keywords: α-mannosidase, α-mannosidosis

Lysosomal α-mannosidase

Biochemical studies

Lysosomal α-mannosidase (HGMW-approved symbol MANB) is a major exoglycosidase that cleaves α-D-mannoside linkages during ordered degradation of N-linked oligosaccharides. The predominant activity in most tissues has an acidic pH optimum of 3.5-4.5, is relatively stable, is activated by Zn^{2+}, and is located in the lysosomal fraction of cells. The substrate specificity of MANB from human, rat, bovine and feline liver has been studied *in vitro*. All the activities can hydrolyze α1,2-, α1,3- and α1,6-mannosidic linkages in oligosaccharides derived from N-linked glycans of the glycoproteins, albeit at different rates. The substrates are hydrolyzed in a non-random manner, which is essentially the same in all species (for reviews see Daniel *et al.*, 1994; Thomas and Beaudet, 1995).

MANB is ubiquitous in eukaryotic cells. In all tissues investigated, it exists as two major forms, A and B, which can be separated by ion-exchange chromatography although they are immunologically identical. The proportions of the two forms vary from tissue to tissue. The form B, but not A, can be taken up into cells by receptor-mediated endocytosis, indicating that the two different forms of the enzyme result from differences in post-translational modifications of a common precursor. It is not known, however, whether the existence of the two different forms has any physiological significance (for reviews see Daniel *et al.*, 1994; Thomas and Beaudet, 1995).

Biosynthesis

Like other lysosomal enzymes, biosynthesis of MANB involves a high molecular weight precursor that undergoes specific post-translational modifications including addition of a mannose-6-phosphate (m6p), a recognition marker for lysosomal targeting. The precursor is subsequently transported to the lysosome, where the fully processed enzyme accumulates. Metabolic labeling of human fibroblasts shows that MANB is synthesized as a 110 kD precursor, which is partly secreted into the culture media of cells. Intracellularly, the enzyme is represented by several polypeptides with molecular weights ranging from 40 to 67 kD (Pholmann and Hasilik, 1983). Purification and expression studies, however, have shown considerable discrepancy in number and composition of the subunits of the enzyme. For example, the form A purified from human liver has a native molecular mass of 280 ± 10 kD and is composed of equimolar proportions of subunits of 62 and 26 kD. Whereas the form B has a molecular mass of 260 ± 10 kD with a mixture of subunits of 58 and 62 kD, as well as the 26 kD subunit (Cheng *et al.*, 1986). Another study shows that MANB purified from human placenta is synthesized as a single-chain precursor, which is processed into three glycopeptides of 70, 42 and 15 kD. The 70 kD peptide is further partially proteolyzed into three more peptides joined together by disulfide bridges (Tsuji and Suzuki, 1987). These differences in the molecular mass and composition of the enzyme are likely to be the result of using different tissues and purification methods.

MANB in other animal species is also synthesized as a single-chain precursor followed by proteolysis into subunits. For example, bovine MANB is originally synthesized as a single-chain precursor of 949 amino acids,

which is cleaved into five peptides of 35/38 (peptide a), 11/13 (peptide b), 22 (peptide c), 38 (peptide d) and 13/15 kD (peptide e). The peptides a, b and c are disulfide-linked. Variation in the degree of N-glycosylation accounts for molecular mass heterogeneity of peptides a, b and e (Tollersrud *et al*., 1997). Feline liver MANB consists of three polypeptides with molecular weights of 72, 41 and 12 kD (Berg *et al*., 1997a).

The precursor of normal human MANB is partly secreted and taken up by neighboring and distant cells. The uptake can be greatly inhibited in the presence of mannose 6-phosphate, indicating that the process is mainly mediated by m6p receptor-mediated endocytosis (Pholmann *et al*., 1983). Such secretion and uptake are the basis of the so-called cross-correction, the main rationale for lysosomal enzyme replacement therapy.

In addition, direct enzyme transfer by cell-to-cell contact has also been described for MANB, of which efficacy appears to be superior to that of receptor-mediated transfer. This was shown by co-culturing human lymphocytes with α-mannosidosis fibroblasts (Olsen *et al*., 1988). When co-cultured with fibroblasts from an α-mannosidosis patient, MANB activity increases markedly in the lymphocytes. Cell-to-cell contact is obligatory for such increase in activity, which requires new protein synthesis. The enzyme induced in the co-cultured lymphocytes is the precursor form of MANB, which is directly transferred to the deficient fibroblasts. Even low levels of such contact-transferred MANB activity are functionally effective in correction of the metabolic defect in α-mannosidosis cells (Abraham *et al*., 1985). These data suggest that direct contact with lymphocytes could play an important role in enzyme replacement therapy by bone marrow transplantation or gene therapy.

Genomic organization

The MANB gene is located on human chromosome 19p13.2-q12 (Kaneda *et al*., 1987), spans about 22.0 kb of genomic DNA, and contains 24 exons (Wakamatsu *et al*., 1997; Riise *et al*., 1997). The mouse MANB gene is also encoded by 24 exons, but spans approximately16 kb of genomic DNA (Tollersrud *et al*., 1997, Gonzalez *et al*. 1999).

Transcription

The major transcription initiation sites of the human gene map to positions -309, -196, and -191 relative to the first in-frame ATG codon. No CAAT or TATA sequences have been identified within 134 bp upstream of the transcription initiation site, but the 5'-flanking region contains several GC-rich regions with putative binding sites for transcription factors SP-1, AP-2, and ETF (Riise *et al*., 1997). Promoter analysis using deletion constructs of the 5'-flanking region fused to the bacterial CAT gene shows that 150 bp of the 5'-sequence can drive expression of the human MANB gene in COS-7 cells. RACE analysis of the 5'-sequence of human MANB mRNA shows that transcription is initiated from a cluster of sites centered -28 and -20 bp from the first in-frame ATG codon (Wakamatsu *et al*., 1997).

The processed mRNA contains 3443 bp from the most upstream transcription initiation site to the RNA cleavage site, based on the genomic organization of MANB gene (Riise *et al*., 1997). Northern blotting detects one main transcript of approximate 3.0 kb in most tissues investigated, but one commercial Northern blot of different human tissues shows smear bands of 2.0 to 3.6 kb, indicating that human pre-mRNA is alternatively spliced to generate different transcripts (Liao *et al*., 1996). This is consistent with the fact that a 3.6 kb alternatively spliced cDNA is isolated from human spleen (Liao *et al*., 1996). RT-PCR analysis of HeLa and HepG2 cells also suggests that alternative splicing is involved in human MANB pre-mRNA processing, in which at least three minor bands ranging from 2.3 to 2.8 kb, as well as the 3.0 kb main message, are detected (Sun *et al*., 2000).

The 5'-flanking region of the murine MANB gene contains GC-rich regions, TATA and CAAT elements, and putative binding sites for the transcription factors Sp1, AP2, and PEA3. The PEA3 transcription factor is thought to participate in the transcriptional control of the MANB expression in the mouse epididymis, based on the findings that PEA3 is spatially and temporally expressed in the mouse epididymis and that its accumulation is controlled by androgens and testicular factors. A 1279-bp fragment from the initiation codon has the strongest promoter activity, and three different transcription start sites have been identified at positions -131, -149, and -174 (Stinchi *et al.,* 1998).

cDNA cloning and expression

Several cDNAs for human MANB have been reported, but only one expresses high MANB activity in α-mannosidosis cells. The first was a composite cDNA isolated from human retina and muscle libraries by a PCR-based technique, which encodes a polypeptide of 961 amino acids (Nebes and Schmidt, 1994). Transfection of the cDNA into α-mannosidosis human fibroblasts shows a 43% or 91% increase in MANB activity over the background (Wang *et al*., 1996). The significance of such low levels of expression is arguable since they are within the normal variation ranges of MANB activity in human cells of different densities.

The second cDNA for human MANB was isolated by RT-PCR of human spleen, which encodes a polypeptide of 988 amino acids (Liao *et al*., 1996). The cDNA expresses detectable MANB activity in methylotropic yeast (*Pichia*). The expressed enzyme migrates as a diffuse

band with an apparent molecular weight of 210 kD on SDS-PAGE. The treatment of the purified enzyme under denaturing conditions with endo H results in decrease in molecular mass to ~150 kD, which is still larger than that predicted from the cDNA sequence and was proposed to result from O-linked glycosylation. The expressed enzyme shows catalytic activity toward both synthetic substrates and high mannose oligosaccharides accumulated in α-mannosidosis cells. The *Pichia*-expressed enzyme is inhibited by swainsonine and has a pH optimum, K_m and V_{max} characteristic of the enzyme purified from human liver. However, whether the cDNA could express active enzyme in α-mannosidosis cells remains to be tested.

The third cDNA encoding human MANB was assembled from overlapping fragments obtained by PCR on human fibroblast and lung cDNAs (Nilssen *et al*., 1997). The coding region is nearly identical to that of the spleen cDNA (Liao *et al*., 1996), but more 5'-sequence encoding the putative signal peptide and 3'-untranslated sequence were obtained. As with the spleen cDNA, whether the chimeric cDNA could express the active enzyme in α-mannosidosis is not known. This cDNA contains an in-frame ATG that is 63 bp upstream of the ATG identified in the previously characterized clones and was proposed to be the actual translation start codon. This would results in a predicted signal peptide of 48 amino acids followed by a peptide of 962 amino acids. However, more recent expression experiments have shown that the second ATG is sufficient as the translation start codon to generate active enzyme (Sun *et al*., 1999a). This start site is more consistent with the expected size of the leader sequence of other lysosomal enzymes.

A cDNA expressing high levels of MANB activity was isolated by RT-PCR from HeLa cells (Sun *et al*., 1999a). An amphotropic recombinant virus expressing the cDNA was generated and shown to efficiently transduce α-mannosidosis cells. An expected transcript and very high levels of MANB activity are detected in both human and feline α-mannosidosis fibroblasts after transduction with the virus vector. Biochemical properties of the recombinant enzyme are essentially same as that of normal human MANB purified from liver (Daniel *et al*., 1994). The recombinant enzyme is present in the lysosome of the transduced cells and partially secreted into the culture medium, which is taken up by uninfected cells. Such uptake is significantly inhibited in the presence of mannose 6-phosphate, showing that the recombinant enzyme has the correct modifications for lysosomal targeting. Expression of the cDNA corrects the secondary elevation of other lysosomal enzymes in affected cells, which is reported in most lysosomal storage diseases. When transplanted into α-mannosidosis cats, neo-organs containing the vector-corrected cells express detectable MANB activity *in vivo* (Sun *et al*., 1999b). These data demonstrate the authenticity and usefulness of the cDNA for development of gene therapy strategy for the human disease.

Among the reported cDNAs for human MANB, the retina/muscle composite cDNA (Nebes and Schmidt, 1994) is relatively distinct from the others (Liao *et al*., 1996; Nilssen *et al*., 1997; Sun *et al*., 1999a), with an identity of only 90% in terms of predicted amino acid sequence. The differences include a 69 bp deletion as well as a number of other smaller deletions/insertions, which cause several frame shifts and significant difference in the predicted amino acid sequences. The other three cDNAs for human MANB differ by only 3 or 4 single bases over the 2967-bp coding region. The cDNA expressing high activity MANB in α-mannosidosis fibroblasts differs from the consensus sequence by a single T to C conversion at position 2325 (Sun *et al*., 1999a).

Cloning of MANB cDNAs in other animal species have also been reported. A full-length cDNA for MANB has been isolated from a mouse macrophage cDNA library, of which deduced amino acid sequence is 75% identical with the human cDNA. Expression of the cDNA in COS cells shows that both the isoenzymes A and B can arise from a single transcript (Beccari *et al*., 1997). Another murine cDNA for MANB encodes a polypeptide of 992 amino acids and its deduced amino acid sequence is 76.5% identical to the human MANB. Expression of the cDNA in *Pichia pastoris* results in the secretion of the MANB activity in the culture medium. The recombinant enzyme has characteristics highly similar to the enzyme purified from mammalian sources (Merkle *et al*., 1997). The cDNA encoding bovine MANB has been isolated from kidney. The deduced amino acid sequence contains a putative signal peptide of 50 amino acids, followed by a polypeptide of 949 amino acids, which is cleaved into five subunits in the mature enzyme (Tollersrud *et al*., 1997). The cDNA sequence of feline MANB has been determined from RT-PCR products obtained from skin fibroblast mRNA. The deduced amino acid sequence consists of a putative signal peptide of 50 amino acids and a polypeptide of 957 amino acids. The deduced amino acid sequence is 81.1% and 83.2% identical with the human and bovine MANB sequences, respectively (Berg *et al*., 1997a).

α-mannosidosis

A deficiency of MANB causes the lysosomal storage disease, α-mannosidosis, which is an autosomal recessive disorder in humans (Ockerman, 1967), cattle (Whittem *et al*., 1957), domestic cats (Burditt *et al*., 1980) and guinea pigs (Crawley *et al*., 1999). The inherited disorder is characterized by massive accumulation of undegraded oligosaccharides, resulting in varied neural,

skeletal and immune defects, and extension of the lysosomes in most cell types.

Clinical manifestations

The human disease can be divided into two types, the infantile phenotype (or type I) and the juvenile-adult phenotype (or type II) according to its clinical manifestations. Virtually all patients have psycomotor retardation, facial coarsening and some degrees of dysostosis multiplex. Frequent clinical findings include recurrent bacterial infections, deafness, hepatomegaly, and lenticular or corneal opacities. The more severe infantile phenotype includes rapid mental deterioration, obvious hepatosplenomegaly, more severe dysostosis multiplex, and often death before age of 12. The milder juvenile-adult phenotype is characterized by more normal early development, followed by gradual appearance of mental retardation. Hearing loss is particularly prominent in type II patients (for review see Thomas and Beaudet, 1995).

Animal models

Naturally occurring α-mannosidosis has also been described in cattle (Red Angus, Murray Grey, Galloway, and short horns), domestic cats (Persian and shorthaired), and guinea pigs. About 10% of New Zealand stocks of Aberdeen Angus cattle are heterogeneous carriers of an abnormal MANB gene. Homozygous cattle have reduced levels of MANB activity and show a similar progressive ataxic disease to that seen in humans (Whittem and Walker, 1957).

Feline α-mannosidosis shows similar clinical finding, including multiple skeletal deformities, retarded growth, ataxia, intention tremors and deficiency in the acidic MANB activity (Burditt *et al*., 1980; Walkley *et al*., 1981; Vandevelde *et al*., 1982). In addition, the affected cats share similar phenotypic heterogeneity with the human patients, suggesting that the cats are suitable model system for studying the human disease (Raghavan *et al*., 1988). Recent studies using magnetic resonance imaging techniques have revealed significant amounts of dysmyelination in both the central and peripheral nervous systems (Vite *et al*., 2001).

Guinea pigs with stunted growth, progressive mental dullness, behavioral abnormalities, and a deficiency of MANB activity in variety of tissues have been described recently (Crawley *et al*., 1999). Thin layer chromatography of urine and tissue extracts shows a pattern of excreted and stored oligosaccharides almost identical to that of urine from a human α-mannosidosis patient. Systematic pathological experiments show that α-mannosidosis in the guinea pigs closely resembles the human disease.

An α-mannosidosis mouse model has been generated by targeted disruption of the gene for MANB (Stinchi *et al*., 1999). Homozygous mutant mice exhibit enzymatic deficiency and elevated urinary secretion of mannose-containing oligosaccharides. The morphological lesions and their distribution, as well as the biochemical changes, closely resemble those reported for human α-mannosidosis. This mouse model will be a valuable tool for studying the pathogenesis of the inherited disease and for evaluating therapeutic approaches for lysosomal storage diseases.

Enzymatic defect

Generally, patients with α-mannosidosis and affected animals lack or have severely decreased (about 2-5%) MANB activity. This is further supported by a recent study, in which no significant MANB activity was detected in any of the fibroblast cultures, though there are clinical variations among 43 patients from 39 families (Berg *et al*., 1999). However, it has also been reported that some patients have high residual MANB activity, which appears to have increased heat lability and a marked increase in the K_m against artificial substrates. The culture medium of some α-mannosidosis human fibroblasts accumulate acidic α-mannosidase activity. This was interpreted as indicating that normal synthesis and partial secretion of the enzyme occurred, and that the intracellular deficiency resulted from a defect that manifests itself only after delivery of the enzyme to the lysosome. However, later experiments showed that the acidic α-mannosidase activity secreted by deficient fibroblasts was immunologically unrelated to MANB (for review see Thomas and Beaudet, 1995).

Causative Mutations

Mutations related to α-mannosidosis are expected to be highly heterogeneous. Recently, a variety of missense and nonsense mutations related to α-mannosidosis have been described. One study analyzed 24 exons and intron/exon boundaries of the MANB gene from five patients using PCR followed by either SSCP analysis or direct sequencing (Gotoda *et al*., 1998). Two amino acid and two nonsense mutations were identified in four type II patients. One amino acid substitution was identified in exon 8 from a type I patient. This patient and three of the type II patients had homozygous mutations, while one type II patient was a compound heterozygote of two missense mutations. Two affected siblings of Palestinian origin were homozygous for a mutation that causes a His to Leu replacement at a position which is conserved among class II α-mannosidases from several species (Berg *et al*., 1999). In a follow-up study, 21 novel mutations and four polymorphisms were identified by screening 43 patients from 39 families. Causative mutations were identified in 72% of the alleles, including eight splicing, six missense, and three nonsense mutations, as well as two small insertions and two small deletions. Most of the mutations are private or occur in two or

three families, except for a missense mutation found in 13 patients from different European countries. No correlation between the types of mutations and the clinical manifestations is evident (Berg *et al*., 1999).

Causative mutations related to α-mannosidosis in other animal species have also been reported. A T to C transition, resulting in a Phe to Leu substitution, has been identified in a cDNA cloned from α-mannosidosis Angus cattle. In another affected breed, Galloway cattle, a G to A transition causes an Arg to His substitution. These substitutions are likely to be disease-causing since they occur in the conserved regions of class II α-mannosidases (Berg *et al*., 1997b). In the cat, a 4-bp deletion has been identified in an affected Persian cat by sequencing of RT-PCR product, which causes a frame-shift and a premature stop codon. A domestic longhaired cat expressing a milder α-mannosidosis phenotype than the Persian cat does not possess the deletion (Berg *et al*., 1997a).

Diagnosis

The clinical diagnosis for α-mannosidosis in children is likely to be neglected or misjudged due to its low rate of occurrence and heterogeneous phenotypes. Some pathological findings are indicative, which include vacuolated lymphocytes, a granular or foamy cytoplasm in the hepatocytes, and marked and widespread ballooning of the nerve cells. Based on these pathological findings, thin-layer chromotography and high-performance chromotography can be utilized to demonstrate accumulated or elevated amounts of oligosaccharides in urine and fibroblasts. Once a urinary oligosaccharide pattern suggestive of α-mannosidosis is demonstrated, the diagnosis can be confirmed or excluded by direct measurement of MANB activity in white blood cells, fibroblasts or cultured amniotic fluid cells.

There are several reports of the successful prenatal diagnosis of α-mannosidosis, including demonstration of MANB activity in trophoblast biopsy material and a trimester diagnosis using chorionic villi and cultured amniotic fluid cells (for review see Thomas and Beaudet, 1995).

In cattle, DNA tests based on polymerase chain reaction (PCR) have been developed for detection of two breed-specific mutations responsible for bovine α-mannosidosis. The tests involve separate amplification of two exons of the MANB gene, followed by restriction enzyme digestion of the amplicons. Using these methods, one of the mutations, the 662 G to A transition, has been demonstrated to be responsible for α-mannosidosis in Galloway cattle. The other mutation, the 961 T to C transition, is associated with α-mannosidosis in Angus, Murray Grey, and Brangus cattle. Of 39 animals classified as heterozygotes on the basis of biochemical assays, all were heterozygous for one of the two mutations and none of the 102 animals classified as homozygous normal on the basis of biochemical assays had the mutations (Berg *et al*., 1997b).

Treatment

Several potential therapeutic strategies for α-mannosidosis have been proposed, which include enzyme replacement therapy, bone marrow transplantation and gene therapy. An early attempt at enzyme replacement therapy using purified protein in cattle failed to normalize the bovine α-mannosidosis (Jolly *et al*., 1976).

One human patient with α-mannosidosis accepted bone marrow transplantation but died 18 weeks later. However, there was some evidence of somatic correction at autopsy (Will *et al*., 1987). In α-mannosidosis cats treated by bone marrow transplantation at an early age, increased MANB activity in the brain was found and little or no progression of neurological signs 1-2 years after transplant (Walkley *et al*., 1994). This encouraging result led to a successful bone marrow transplant in a child with a severe form of α-mannosidosis (type I), with complete resolution of the recurrent sinopulmonary disease and organomegaly, improvement in the bony disease, and stabilization of neurocognitive function (Wall *et al*., 1998). Even more encouragingly, the MANB genotype of the patients leukocytes is identical to that of the donor one year after transplantation, indicating colonization of the donor bone marrow cells in the patient (Frostad-Riise *et al*., 1999).

Success of the above experiments warrants further development of therapeutic strategies for α-mannosidosis by combination of bone marrow transplantation with gene therapy, which is facilitated by a number of particularities of α-mannosidosis. These include secretion (about 10%) of normal MANB and its uptake by adjacent or distant cells, highly efficient direct enzyme transfer by cell-to-cell contact, and requirement for low levels of enzymatic activity to correct the defect. Presently, feline and guinea pig colonies with the naturally occurring disease have been established and an α-mannosidosis mouse model has been generated by targeted disruption of the gene for MANB (Sinchi *et al*., 1999). In addition, a high activity human MANB cDNA has been cloned and shown to express authentic enzyme in both affected human and cat cells (Sun *et al*., 1999a). A recombinant retrovirus vector expressing the cDNA efficiently transduced primary fibroblasts from diseased cats and neo-organs containing the vector-infected cells express detectable levels of MANB activity (Sun *et al*., 1999b). All these will greatly facilitate development of the proposed combination strategy for treating the human disease.

Acknowledgements

Work in our laboratory was supported by grants from the U.S. National Institutes of Health (DK42707, DK46637,

DK54481, NS29390, NS38690, and RR02512), the Lucille P. Markey Charitable Trust, the Kleberg Foundation, and the Mrs. Cheever Porter Foundation.

References

Abraham, D., Muir, H., Olsen, H. and Winchester, B. (1985) Direct enzyme transfer from lymphocytes corrects a lysosomal storage disease. *Biochem. Biophys. Res. Commun.* 129: 417-425

Bach, G., Kokn, G., Lash, E. E., El Massri, M., Ornoy, A., Sekels, E., Legum, C. and Cohen, M. M. (1978) A new variant of mannosidosis with increased residual enzymatic activity and mild clinical manifestation. *Pediatr. Res.* 12: 1010-1015

Beccari, T., Apolloni, M. G., Costanzi, E., Stinchi, S., Stirling, J. L., Della Fazia, M. A., Servillo, G., Viola, M. P. and Orlacchio, A. (1997) Lysosomal α-mannosidases of mouse tissues: characteristics of the isoenzymes, and cloning and expression of a full-length cDNA. *Biochem. J.* 327: 45-49

Berg, T., Healy, P. J., Tollersrud, O. K. and Nilssen, O. (1997) Molecular heterogeneity for bovine alpha-mannosidosis: PCR based assay for detection of breed-specific mutations. *Res. Vet. Sci.* 63: 279-282

Berg, T., Tollersrud, O. K., Walkley, S. U., Siegel, D. and Nilssen, O. (1997) Purification of feline lysosomal alpha-mannosidase, determination of its cDNA sequence and identification of a mutation causing-mannosidosis in Persian cats. *Biochem. J.* 328: 863-870

Berg, T., Riise, H. M., Hensen, G. M., Malm, D., Tranebjaerg, L., Tollersrud, O. K. and Nilssen, O. (1999) Spectrum of mutations in alpha-manosidosis. *Am. J. Hum. Genet.* 64: 77-88

Burditt, L. J., Chotai, K., Hirani, S., Nugent, P. G., Winchester, B. G. and Blakemore, W. F. (1980) Biochemical studies on a case of feline mannosidosis. *Biochem. J.* 189: 467-473

Cheng, S. H., Molcolm, S., Pemble, S. and Winchester, B. G. (1986) Purification and comparison of the structures of human liver acidic α-D-mannosidase A and B. *Biochem. J.* 233: 65-72

Crawley, A. C., Jones, M. Z., Bonning, L. E., Finnie, J. W. and Hopwood, J. J. (1999) Alpha-mannosidosis in the guinea pig: a new animal model for lysosomal storage disorders. *Pediatr. Res.* 46: 501-509

Deniel, P. F., Winchester, B. and Warren, C. D. (1994) Mammalian α-mannosidases-multiple forms but a common purpose? *Glycobiology.* 4: 551-566

Frostad Riise, H. M., Hensen, G. M., Tollersrud, O. K. and Nilssen, O. (1999) Characterization of a novel alpha-mannosidosis-causing mutation and its use in leukocyte genotyping after bone marrow transplantation. *Hum. Genet.* 104: 106-107

Gonzalez, D. S., Kagawa, Y. and Moremen, K. W. (1999) Isolation and characterization of the gene encoding the mouse broad specificity lysosomal alpha-mannosidase. *Biochem. Biophys. Acta.* 1445: 177-183

Gotoda, Y., Wakamatsu, N., Kawai, H., Nishida, Y. and Matsumoto, T. (1998) Missense and nonsense mutations in the lysosomal alpha-mannosidase gene (MANB) in severe and mild forms of alpha-mannosidosis. *Am. J. Hum. Genet.* 63: 1015-1024

Jolly, R. D., Thompson, K. G., Murphy, C. E., Manktelow, B. W., Bruere, A. N. and Winchester, B. G. (1976) Enzyme replacement therapy-an experiment of nature in a chimeric mannosidosis calf. *Pediatr. Res.* 10: 219-224

Keneda, Y., Hayes, H., Uchida, T., Yoshida, M. C. and Okada, Y. (1987) Regional assignment of five genes on human chromosome 19. *Chromosoma.* 95: 8-12

Liao, Y. F., Lal, A. and Moremen, K. W. (1996) Cloning, expression, purification, and characterization of the human broad specificity lysosomal acid α-mannosidase. *J. Biol. Chem.* 271: 28348-28358

Merkle, R. K., Zhang, Y., Ruest, P. J., Lal, A., Liao, Y. F. and Moremen, K. W. (1997) Cloning, expression, purification, and characterization of the murine lysosomal acid alpha-mannosidase. *Biochem. Biophys. Acta.* 1336: 132-146

Nebes, V. L. and Schmidt, M. C. (1994) Human lysosomal alpha-mannosidase: isolation and nucleotide sequence of the full-length cDNA. *Biochem. Biophys. Res. Commun.* 200: 239-245

Nilssen, O., Berg, T., Riise, H. M., Ramachandran, U., Evjen, G., Hansen, G. M., Malm, D., Tranebjaerg, L. and Tollersrud, O. K. (1997) Alpha-mannosidosis: functional cloning of the lysosomal alpha-mannosidase cDNA and identification of a mutation in two affected siblings. *Hum. Mol. Genet.* 6: 717-726

Ockerman, P. A. (1967) A generalised storage disorder resembling Hurlers syndrome. *Lancet.* 2: 239

Olsen, I., Abraham, D., Shelton, I., Bou-Gharios, G., Muir, H. and Winchester, B. (1988) Cell contact induces the synthesis of a lysosomal enzyme precursor in lymphocytes and its direct transfer to fibroblasts. *Biochem. Biophys. Acta.* 968: 312-322

Pholmann, R., Hasilik, A., Cheng, S., Pemble, S., Winchester, B. and von Figura, K. (1983) Synthesis of lysosomal α-mannosidase in normal and mannosidosis fibroblasts. *Biochem. Biophys. Res. Comm.* 115: 1083-1089

Raghavan, S., Stuer, G., Riviere, L., Alroy, J. and Kolodny, E. H. (1988) Characterization of α-mannosidase in feline mannosidosis. *J. Inherit. Metab. Dis.* 11: 3-16

Riise, H. M., Berg, T., Nilssen, O., Romeo, G., Tollersrud, O. K. and Ceccherini, I. (1997) Genomic structure of the human lysosomal alpha-mannosidase gene (MANB). *Genomics.* 42: 200-207

Stinchi, S., Orlacchio, A., Costanzi, E., Stirling, J. L., Menghini, A. R., Orlacchi, A. and Beccari, T. (1998) Promoter characterization and structure of the gene encoding mouse lysosomal alpha-mannosidase. *Mamm. Genome.* 9: 869-873

Stinchi, S., Lullmann-Rauch, R., Hartmann, D., Coenen, R., Beccari, T., Orlacchi, A., von Figura, K. and Saftig, P. (1999) Targeted disruption of the lysosomal α-mannosidase gene results in mice resembling a milder form of human alpha-mannosidosis. *Hum. Mol. Genet.* 8: 1365-1372

Sun, H., Yang, M., Haskins, M. E., Patterson, D. F. and Wolfe, J. H. (1999a) Retrovirus vector-mediated correction and cross-correction of lysosomal α-mannosidase deficiency in human and feline fibroblasts. *Hum. Gene. Ther.* 10: 1311-1319

Sun, H., Haskins, M. E., Patterson, D. F. and Wolfe, J. H. (1999b) Preliminary studies on gene therapy for lysosomal alpha-mannosidosis. *Chin. J. Med. Genet*. 16: 205-207

Sun, H. and Wolfe, J. H. (2000) Characterization of an unusual variant mRNA of human lysosomal alpha-mannosidase. *Exp. Mol. Med.*, 32: 187-192.

Thomas, G. H. and Beaudet, A. L. (1995) Disorders of glycoprotein degradation and structure: α-mannosidosis, β-mannosidosis, sialidosis, aspartylglucosaminuria, and carbohydrate-deficient glycoprotein syndrome. In Metabolic Basis of Inherited Disease, 7th Ed. C.R. Scriver, A.L. Beaudet, W.S. Sly, and D. Valle, eds. (McGraw Hill, New York) 2529-2561

Tollersrud, O. K., Berg, T., Healy, P., Evjen, G., Ramachandran, U. and Nilssen, O. (1997) Purification of bovine lysosomal alpha-mannosidase, characterization of its gene and determination of two mutations that cause alpha-mannosidosis. *Eur. J. Biochem*. 246: 410-419

Tsuji, A. and Suzuki, Y. (1987) Purification of human placental acid α-mannosidase by an immunological method. *Biochem. Int*. 15: 483-489

Vandevelde, M., Fankhauser, R., Bichsel, P., Wiesmann, V. and Herschkowitz, N. (1982) Hereditary neurovisceral mannosidosis associated with α-mannosidase deficiency in a family of Persian cats. *Acta. Neuropathol*, 58: 64-68

Vite, C. H., McGown, J. C., Braun, K. G., Drobatz, K. J., Wolfe, J. H. and Haskins, M. E. (2001) Magnetic resonance imaging, electrodiagnostic testing and histopathology show significant central and peripheral nervous system myelin abnormalities in the cat model of alpha-mannosidosis. Submitted

Wakamatsu, N., Gotoda, Y., Saito, S. and Kawai, H. (1997) Characterization of the human MANB gene encoding lysosomal alpha-mannosidase. *Gene*. 198: 351-357

Walkley, S. U., Blakemore, W. F. and Purpura, D. P. (1981) Alterations in neuron morphology in feline mannosidosis. A Golgi study. *Acta. Neuropathol.* 53: 75-79

Wall, D. A., Grange, D. K., Goulding, P., Daines, M., Luisiri, A. and Kotagal, S. (1998) Bone marrow transplantation for the treatment of alpha-mannosidosis. *J. Pediatr.* 133: 282-285

Wang, W., Nebes, V. L., Schmidt, M. C. and Barranger, J. A. (1996) Expression of human lysosomal alpha-mannosidase activity in transfected murine cells and human alpha-mannosidase deficient fibroblasts. *Biochem. Biophys. Res. Commun.* 224: 619-624

Whittem, J. M. and Walker, D. (1957) "Neuronopathy" and "pseudolipidosis" in Aberdeen Angus calves. *J. Pathol. Bacteriol*. 74: 281

Will, A., Cooper, A., Hatton, C., Sardharwalla, I. B., Evans, D. I. and Stevens, R. F. (1987) Bone marrow transplantation in the treatment of α-mannosidosis. *Arch. Dis. Child.* 62: 1044-1049