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Mutations and Polymorphisms in GUSB Gene in Mucopolysaccharidosis VII (Sly Syndrome)

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Abstract

Mucopolysaccharidosis VII (MPS VII; Sly syndrome) is an autosomal recessive disorder caused by a deficiency of β-glucuronidase (GUS, EC 3.2.1.31; GUSB). GUS is required to degrade glycosaminoglycans (GAGs), including heparan sulfate (HS), dermatan sulfate (DS), and chondroitin-4,6-sulfate (CS). Accumulation of undegraded GAGs in lysosomes of affected tissues leads to mental retardation, short stature, hepatosplenomegaly, bone dysplasia, and hydrops fetalis. We summarize information on the 49 unique, disease-causing mutations determined so far in the GUS gene, including nine novel mutations (eight missense and one splice-site). This heterogeneity in GUS gene mutations contributes to the extensive clinical variability among patients with MPS VII. One pseudodeficiency allele, one polymorphism causing an amino acid change, and one silent variant in the coding region are also described. Among the 103 analyzed mutant alleles, missense mutations accounted for 78.6%; nonsense mutations, 12.6%; deletions, 5.8%; and splice-site mutations, 2.9%. Transitional mutations at CpG dinucleotides made up 40.8% of all the described mutations. The five most frequent mutations (accounting for 44/103 alleles) were exonic point mutations, p.L176F, p.R357X, p.P408S, p.P415L, and p.A619 V. Genotype/phenotype correlation was attempted by correlating the effects of certain missense mutations or enzyme activity and stability within phenotypes. These were in turn correlated with the location of the mutation in the tertiary structure of GUS. A total of seven murine, one feline, and one canine model of MPS VII have been characterized for phenotype and genotype.

Keywords

GUS; GUSB; mucopolysaccharidosis VII; MPS VII; Sly syndrome; alignment

Introduction

Mucopolysaccharidosis VII (Sly Syndrome; MPS VII) is an autosomal recessive disease classified in the group of mucopoly-saccharide storage diseases. MPS VII (MIM 253220) is characterized by the deficiency of activity of the enzyme β-glucuronidase (GUS: β-D-glucuronoside glucuronosohydrolase, EC 3.2.1.31; GUSB; MIM 611499) [Sly et al., 1973]. It is one of a class of diseases due to a deficiency of one of the dozen enzymes involved in

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the stepwise degradation of glycosaminoglycans (GAGs). In the absence of GUS, chondroitin sulfate (CS), dermatan sulfate (DS), and heparan sulfate (HS) are only partially degraded and accumulate in the lysosomes of many tissues, eventually leading to cellular and organ dysfunction. MPS VII is a rare disorder, and precise epidemiologic data are scarce. MPS VII causes mental retardation, hepatosplenomegaly, and skeletal dysplasia. MPS VII patients displayed a wide range of clinical variability, from the most severe type with hydrops fetalis to a milder phenotype with later onset and normal intelligence. MPS VII patients with the most severe phenotype have hydrops fetalis at birth and often do not survive beyond a few months. Patients with mild manifestations of MPS VII have survived into the fifth decade of life. MPS VII has also been reported in canine, feline, and murine species [Haskins et al., 1984; Birkenmeier et al., 1989; Sands and Birkenmeier, 1993; Gitzelmann et al., 1994; Gwynn et al., 1998; Ray et al., 1998; Fyfe et al., 1999; Sly et al., 2001; Vogler et al., 2001; Tomatsu et al., 2002b, 2003]. The initially described, natural MPS VII mice ($gus^{mps/mps}$) have a 1-bp deletion in exon 10 and have similar morphologic, genetic, and biochemical characteristics to human MPS VII patients, showing degenerative disease with progressive disability, widespread organ dysfunction, facial dysmorphism, growth retardation, deafness, behavioral deficits, and shortened lifespan [Birkenmeier et al., 1989; Sands and Birkenmeier, 1993]. We produced mL175F (corresponding to p.L176F, the most common human mutation), mE536A, and mE536Q (active site nucleophile replacements, corresponding to p.E540A and p.E540Q in humans) knock-in mice [Tomatsu et al., 2002b]. These models reflect the various clinical phenotypes of human MPS VII (Sly syndrome). Advanced treatments such as enzyme replacement therapy (ERT) and gene therapy for MPS VII are currently being developed using these models. We have recently created MPS VII mouse models tolerant to infused human GUS enzyme to test various treatment protocols using the human gene product [Sly et al., 2001; Tomatsu et al., 2003].

Characterization of GUS protein by X-ray crystallography and homology comparisons among several species of GUS and bacterial β-galactosidases suggested R382, E451, and E540 asactive site residues [Jain et al., 1996; Islam et al., 1999]. These three residues of human GUS are conserved among GUS and β-galactosidase proteins from bacterial species [Henrissat, 1991]. E540 was identified experimentally as the active site nucleophile of the human enzyme [Wong et al., 1998].

Isolation and characterization of the human cDNA and genomic gene made investigation of molecular lesions in the GUS gene of MPS VII patients feasible [Oshima et al., 1987; Miller et al., 1990; Shipley et al., 1991]. The GUS gene is located on chromosome arm 7 [Speleman et al., 1996] and spans approximately 20 kb containing 11 introns and 12 exons. The 1,953-bp GUS mRNA encodes a 651–amino acid precursor. After cleavage of a 22–amino acid N-terminal signal peptide and glycosylation, the 78-kDa monomer is transported to lysosomes and cleaved in the lysosome to become the 60-kDa and 18-kDa subunits of the mature active enzyme [Brot et al., 1978; Oshima et al., 1987].

MPS VII Mutations and their Biological Relevance

To date, 49 different mutations including nine novel mutations in the GUS gene have been found in MPS VII patients. These mutations have been identified in 103 mutant alleles in a total group of 56 patients by a variety of molecular techniques (92.0% of total investigated alleles) (Table 1). The numbers for the nucleotide changes are reported in accordance with GenBank entry NM_010368.1. Three nonpathogenic variants within the coding sequence of the GUS gene have been also identified (one pseudodeficiency, one benign amino acid change, one silent change) (Table 2). The DNA mutation numbering is based on cDNA sequence. For cDNA numbering, +1 corresponds to the A of the ATG translation initiation

codon in the reference sequence. The MPS VII patients were defined as attenuated if they did not have hydrops fetalis and severe mental retardation leading to death within a year.

The mutations are distributed along the whole gene and all types of mutations except insertion and rearrangement were found. The total of 49 mutations includes 36 missense mutations, six nonsense, two splice site mutations, and five deletions. The number of each type of mutation in a total of 103 mutant alleles was 81 alleles for missense mutations (78.6%), 13 for nonsense (12.6%), six for deletions (5.8%), and three for splice-site mutations (2.9%). Thus, missense mutations are the most prevalent among GUS mutations. The five most frequent mutations (Table 1) are represented by single nucleotide changes. Together, they make up 36.9% of all described mutant alleles. The remaining 63.1% of mutations each occur less than four times in the mutant population, indicating extensive molecular heterogeneity in GUS mutations.

Relation Between Transitions at CpG Sites and the Methylation Status of CpG Sites in the GUSB Gene

The variety, frequency, and location of point mutations causing human genetic disease are highly nonrandom. One important factor contributing to the nonrandomness at the DNA level is the local DNA sequence environment, especially CpG dinucleotides. DNA methylation at the cytosine residue of CpG dinucleotides produces 5-methylcytosine, which results in a C-to-T transitional change following deamination. The importance of CpG methylation in the etiology of genetic diseases was deduced from the evidence that 10 to 60% of point mutations causing human diseases in different genes result from transitions at CpG dinucleotides [Krawczak et al., 1998; Antonarakis et al., 2001].

There are 17 transitional mutations at CpG sites in the GUS gene. Transitions at CpG dinucleotides account for 40.8% of described mutant alleles and 44.7% of exonic point mutations that cause MPS VII. This percentage is higher than that compiled from many genes described previously [Krawczak et al., 1998; Antonarakis et al., 2001] and represents around a 30-fold higher probability of a transitional mutation at a CpG dinucleotide than expected. These findings explain why many transitional mutations at CpG sites are recurrent. No transitional mutation at CpG sites has been detected in exon 1. To explain this discrepancy, we analyzed the methylation pattern of the GUS coding region by a sensitive bisulfite-based technique [Tomatsu et al., 2002a]. We found that methylation of the 67 individual CpG cytosines within exons 2 to 12 was extensive while 24 CpG cytosines in exon 1 were completely unmethylated. All of the 17 transitional mutations at CpG sites out of the 42 exonic point mutations were located between exons 2 and 12, demonstrating the correlation of nonmethylation of exon 1 with the absence of transitional mutations at CpG sites in exon 1 and the reverse for exons 2 to 12. One pseudodeficiency allele (p.D152N) and one benign polymorphism allele (p.P649L), both of which change an amino acid residue, are also derived from G-to-A or C-to-T transition at CpG dinucleotides, respectively [Tomatsu et al., 1991; Vervoort et al., 1995].

Missense Mutations

This is the most frequent group of GUS mutations, with 36 changes including eight novel amino acid substitutions reported here (Tables 1 and 3; Fig. 1). Correlation of individual mutation with disease severity is based on phenotype of the homozygotes, predicted change of tertiary structure of the protein, and the observed level of enzyme activity on in vitro expression.

Several mutations are recurrent. Among the recurrent mutations, the most prevalent are: c. 526C>T (p.L176F), c.1244C>T (p.P415L), c.1222C>T (p.P408S), c.1856C>T (p.A619 V),

c.646C>T (p.R216W), c.1144C>T (p.R382C), and c.1429C>T (p.R477W), accounting for 20.4, 4.9, 4.9, 4.9, 3.9, 3.9, and 3.9%, respectively [Tomatsu et al., [1990],1991;Fukuda et al., 1991;Vervoort et al., 1993;Wu et al., 1994; Islam et al., [1996],1998; Vervoort et al., 1996,1997;Schwartz et al., 2003]. The p.L176F mutation has been identified in diverse ethnic populations while the p.P415L mutation and p.P415L/P408S double mutation, and the p.A619 V mutation have been detected only in Mexican and Japanese populations, respectively.

The most prevalent c.526C>T transitional mutation (p.L176F), originally found in two Mennonite siblings, was identified in 21 alleles of 11 patients from American (Caucasian), Brazilian, British, Chilean, French, Mexican, Polish, Spanish, and Turkish origins [Wu et al., 1994; Vervoort et al., 1996; Schwartz et al., 2003] (Sly, unpublished results). A total of 10 of 11 patients were homozygous for the mutation. Those homozygous patients developed an attenuated type of MPS VII with similar clinical symptoms and signs. The p.L176F conservative amino acid change generates a subtle structural alteration of GUS protein [Wu et al., 1994]. Although the cultured fibroblasts homozygous with p.L176F contained only 1.5 to 2.2% of normal GUS activity, overexpression of the p.L176F cDNA in COS cells produced 84% as much enzyme as the wild-type control cDNA. These findings suggested that overexpression can drive the folding reaction or the self-association of mutant monomers to formactive tetramers [Wu et al., 1994]. The mouse model corresponding with p.L176F was established and also showed an attenuated phenotype [Tomatsu et al., 2002b].

The p.P415L/p.P408S double point mutation and the p.A619 V mutation are of great interest since these mutations were specific to Mexican and Japanese populations, respectively [Tomatsu et al.,1990,1991; Islam et al., 1996,1998] (Sly, unpublished results). Both founder mutations are associated with an attenuated phenotype. The double mutant allele containing two C-to-T transitions resulting in p.P408S and p.P415L alterations was present in homozygous state in one Mexican patient and in heterozygous state in four. Expression of either of the mutations individually showed only modest effects on the properties of the enzyme. However, expression of the doubly mutant allele resulted in markedly reduced activity and rapid degradation in an early biosynthetic compartment (Table 4) [Islam et al., 1996]. Neither p.P408S nor p.P415L mutation was present alone in a normal Mexican population [Islam et al., 1998]. The p.A619 V mutation expressed 9.1% of normal cDNA in transfected COS cells. The residual activity of these expressed mutant proteins correlated with the attenuated phenotype for those mutations.

The X-ray structure of the homotetrameric human GUS (332,000 Mr) was determined at 2.6-Å resolution [Jain et al., 1996]. The tetramer had approximate dihedral symmetry and each protomer consisted of three structural domains with topologies similar to a jelly roll barrel, an immunoglobulin constant domain and a triosephosphateisomerase (TIM) barrel, respectively. Residues 179–204 formed a beta-hairpin motif similar to the putative lysosomal targeting motif of cathepsin D. The active site of the enzyme was formed from a large cleft at the interface of two monomers. Residues Glu 451, Tyr 504, and Glu 540 were shown to be important for catalysis.

Using homology modeling among different species of GUS and β-galactosidase proteins, the potential effect of missense mutations on the GUS tertiary structure was estimated and the localization of the mutation site was correlated with the residual activity and the clinical phenotype (Fig. 4). Among 12 missense mutations with a severe phenotype, 10 of these mutations involve destruction of the hydrophobic core or modification of the packing (p.S52F, p.P148S, p.E150 K, p.R216W, p.Y320S, p.H351Y, p.R435P, p.R477W, p.Y495C, p.G572D, p.K606N, and p.R611W). On the other hand, 5 out of 7 mutations located on the surface of the GUS protein (p.C38G, p.P415L, p.Y508C, p.R577L, and p.W627C) were

associated with attenuated phenotypes. However, two mutations on the surface (p.S52F and p.Y495C) were associated with severe phenotypes. These two mutations are nonconservative amino acid changes that, despite their location on the surface, disrupt the tertiary structure and result in a severe phenotype. On the other hand, mutations such as p.D152N, p.L176F, p.A354 V, p.R382 H, p.P408S, p.A619 V, and p.Y626 H are located on either the hydrophobic core or involve a salt bridge [Jain et al., 1996], and represent conservative or semiconservative amino acid changes that lead to attenuated phenotypes.

A total of 28 mutations, one pseudodeficiency, and one benign polymorphism were analyzed by in vitro transient overexpression. We used COS cells for 23 mutations, MPS VII fibroblasts for three mutations, and BHK cells for two mutations (Table 4). GUS activity was determined using 4-methylumbelliferyl-β-glucuronide as a substrate. A total of 8 out of 11 mutants associated with the severe phenotype had under 3% of normal cDNA GUS activity (mean, 1.5%) while 13 out of 14 mutants found in patients with the attenuated phenotype had higher than 3% of normal activity (3–112% of wild-type GUS activity; mean, 32.8%), indicating a positive correlation between the transient expression level and the clinical phenotype [Tomatsu et al., [1990],1991;Shipley et al., 1993;Wu and Sly, 1993; Vervoort et al., [1995],1996,1998a;Yamada et al.,1995;Storch et al., 2003]. One patient with an attenuated phenotype had 2.3% of wild-type activity in COS cells.

The G-to-A transition (c.454G>A) in the coding region of the GUS gene, which resulted in an aspartic-acid-to-asparagine substitution at amino acid position 152 (p.D152N), produced a pseudodeficiency allele that leads to greatly reduced levels of GUS activity in vitro without apparent deleterious consequences [Vervoort et al., 1998a]. The c.454G>A mutation was found initially in the pseudodeficient mother of a child with MPS VII, but it was not on her disease-causing allele, which carried the p.L176F mutation. Screening of 100 unrelated normal individuals for the c.454G>A mutation with a PCR method detected one carrier (a rough estimate of frequency: 0.5%). Reduced GUS activity following transfection of COS cells with the p.D152N cDNA supported the causal relationship between the p.D152N allele and pseudodeficiency. The mutation reduced the fraction of expressed enzyme that was secreted. Pulse-chase experiments indicated that the reduced activity in COS cells was due to accelerated intracellular turnover of the p.D152N enzyme [Vervoort et al., 1998a]. The presence of the p.D152N mutation in combination with certain other MPS VII mutations might be more deleterious.

Nonsense Mutations

A total of six nonsense mutations have been reported: c.328C>T (p.R110X), c.935C>G (p.S312X), c.1069C>T (p.R357X), c.1337G>A (p.W446X), c.1520G>A (p.W507X), and c. 1521G>A (p.W507X) (Table 1) [Shipley et al., 1993;Yamada et al., 1995; Vervoort et al., 1996,1997,1998a] (Sly, unpublished results). All of them should result in synthesis of truncated proteins without catalytic activity, predicting a severe phenotype in MPS VII. The second most frequent p.R357X mutation derived from a C-to-T transition at a CpG site occurred in diverse ethnic backgrounds suggesting a true recurrent mutation [Shipley et al., 1993; Vervoort et al., 1996,1997] (Sly, unpublished results). Other nonsense mutations were sporadic and observed in only one patient.

Splice-Site Mutations

Two splice-site mutations including one novel mutation at the donor site of intron 3 (c. 581+1G>A) were identified in the GUS gene (Table 1). One (c.1244+1G>A) is a homozygous mutation while the other one is in a compound heterozygote. Both splicing-site mutations in the GUS gene disrupt the consensus sequence between exon and intron. Both mutations at the acceptor site cause complete deletion of the following exons. Skipping an

exon would result in a frameshift and the appearance of a premature stop codon (p.K194fsX22, p.P415fsX1) and/or absence of a GUS active catalytic site (E540).

In accordance with this prediction, a patient homozygous for the C1244+1 G-to-A splice-site mutation developed a severe form of MPS VII and showed a complete loss of GUS activity in fibroblasts [Vervoort et al., 1997] (Sly unpublished results).

Deletions

A total of five deletions were identified so far in the GUS gene (Table 1) (c. 1081_1107del27, c.1454_1457del4, c.1616_1653del38, c.1775delT, and c. 1874_1875delGA). Four deletions cause frameshifts (c.1454_1457del4, c.1616_1653del38, c.1775delT, and c.1874_1875delGA) and the appearance of premature truncation codons (p.S485fsX13, p.S539RfsX7, p.F592SfsX2, and p.R625IfsX6, respectively), probably leading to nonsense-mediated decay of the mRNA and a complete loss of GUS activity in the affected cells. Patients homozygous for the c.1081_1107del27 in-frame mutation manifested a severe form of MPS VII [Vervoort et al., 1997]. The mechanism of the c. 1616_1653del38 deletion was unique. The patient was a compound heterozygote of p.W507X and a 38-bp deletion at position 1616–1653 in exon 10. The 38-bp deletion was caused by a C-to-T transition in exon 10 that generates a new, premature 5′ splice-site. The resulting nucleotide sequence AGA/GTGAGT has a close homology to the 5′ splice consensus sequence (A or C) AG/GT (A or G) AGT. This alteration interferes with normal splicing of the GUS gene transcript by forming a novel 5′ splice-site.

Slipped mispairing can in principle account for the generation of 4 out of 5 deletions because of a run of identical bases or direct repeat (2 bp or more) (c.1081_1107del27, c. 1454_1457del4, c.1775delT, and c.1874_1875delGA).

Vervoort et al. [1998b] reported a patient with an attenuated phenotype whose paternal allele (IVS8+0.6kbdelTC) (Table 3) was claimed to create a new donor splice-site that activated a cryptic exon in an Alu-element of the GUS gene and led to skipping of exon 9. This allele confers the alternate phenotype, since the maternal allele (p.W446X) is a null allele (Table 1).

No insertions were identified so far in the GUS gene. The GUS gene spans approximately 20 kb, in addition to the 1-kb promoterregion located at 7q11.21, and contains 37 *Alu* repeats [Miller et al., 1990; Shipley et al., 1991; Speleman et al., 1996]. Neither large deletions nor rearrangement were identified. *Alu* repeats represented around 45% of the entire GUS gene (8.8 kb of 19.5 kb in total length), showing an extremely high percentage of *Alu* elements compared with the human genome (representing 6–12%). In addition, over 20 pseudogenes of GUS gene were observed in the entire human genome. Nevertheless, no large rearrangement has been reported so far.

Polymorphisms

A total of two benign genetic variants in the coding regions of the GUS gene have been reported (Table 2) [Tomatsu et al., 1991;Wu et al., 1994; Vervoort et al., 1995,1998a]. One polymorphism changing an amino acid residue was identified in the normal population (c. 1946C>T, p.P649L). An in vitro expression study showed that this polymorphism provides 88.3% of normal GUS cDNA activity.

Relations Among Genotypes and Phenotypes

The genotype/phenotype correlation for each of 38 single-nucleotide alterations has been examined based upon the following four factors (Tables 1, 3, and 4): 1) the phenotype of the

patient homozygous for the mutation; 2) the level of activity by in vitro expression study; 3) prediction of the likely change in the protein structure; and 4) the presence of a second allele permitting residual enzyme activity, which would be dominant over an allele permitting no activity. In all, 15 mutations were associated with a severe phenotype, 14 mutations were associated with an attenuated phenotype, and one with a normal phenotype (pseudodeficiency allele). The other seven mutations were not defined by the current information. A total of 5 out of 6 nonsense mutations and 4 out of 5 deletions were associated with severe phenotypes. One splicing site mutation was associated with a severe phenotype and the other was not defined.

Clinical and Diagnostic Relevance

Clinical diagnosis is based on findings typical of an MPS disorder, including developmental delay and mental retardation, dysostosis multiplex, hepatosplenomegaly, and short stature. Biochemical diagnosis is based on demonstrating a deficiency of GUS in serum, leukocyte lysates, or cultured fibroblasts [Glaser and Sly, 1973]. This assay is included in the diagnostic panel of most biochemical genetics laboratories. Genetests.org lists 14 laboratories that offer diagnostic testing, three of which also offer sequence analysis of the coding region. Molecular diagnosis and mutational analysis is possible by direct sequencing of mRNA following RT-PCR [Tomatsu et al., 1991; Shipley et al., 1993; Vervoort et al., 1996]. Genomic sequencing is more challenging because of multiple unprocessed pseudogenes, but Shipley et al. [1993] described conditions of amplifying and characterizing genomic sequences of the true GUS gene, despite the background of related sequences.

The analysis of GUS mutations in MPS VII reveals considerable molecular heterogeneity, reflecting the diversity of clinical phenotypes. A total of 5 out of 49 unique mutations occurred over five times, accounting for 36.9% of all the analyzed mutant alleles. The most prevalent mutation, p.L176F, accounted for 20.4% of the analyzed mutant alleles. For diagnosis and prognosis in MPS VII, molecular testing should follow direct enzyme assay in leukocytes and cultured skin fibroblasts. The patients' clinical severity generally can be correlated with their genotype, the predicted effect of missense mutations on the tertiary structure of the enzyme, and the residual activity by in vitro expression study. Many of the reported patients had the severe form of MPS VII. These patients mainly demonstrate frameshifts or other mutations resulting in premature truncations, as well as deletions and splicing-site mutations. Other severe patients had missense mutations affecting conserved amino acid residues in the hydrophobic core or active site region for maintaining the tertiary structure of the protein.

Patients who are compound heterozygotes, having a combination of an attenuated and a severe mutation, manifest clinically milder symptoms than patients homozygous for a severe mutation. It appears that only a small percentage of normal GUS activity provided by one allele (2–3%) can protect against severe phenotypes. The protective effect provided by small amounts of enzyme activity from enzyme replacement and/or gene therapies augers well for effective treatments for MPS VII in the future.

MPS VII Models

Seven murine, one feline, and one canine model of MPS VII are now available to experimentally test pharmaceutical agents, bone marrow transplantation, ERT, and gene therapy (Table 5). These models result from missense mutations or deletions in the GUS gene, which are responsible for over 95% of mutant human alleles. These models should greatly contribute to evaluating the effectiveness of treatment.

The original natural MPS VII murine model, $gus^{mps/mps}$, showed a 1-bp deletion (c. 1470delC), which created a frameshift mutation in exon 10. This frameshift mutation introduces a premature stop codon at codon 497 in exon 10 (mP490RfsX8) and explains the molecular, biochemical, and pathological abnormalities associated with the $gus^{mps/mps}$ phenotype [Birkenmeier et al., 1989; Sands and Birkenmeier, 1993]. The second natural MPS VII mouse model, $gus^{mps2J/mps2J}$, is deficient in GUS because of insertion of an intracisternal A particle element into intron 8 of the gus structural gene. Mice with the $gus^{mps2J/mps2J}$ genotype had <1% of normal GUS activity and secondary elevations of other lysosomal enzymes. The phenotype includes shortened life-span, dysmorphic features, and skeletal dysplasia. Lysosomal storage of GAGs is widespread and affects the brain, skeleton, eye, ear, heart valves, aorta, and the fixed tissue macrophage system. Thus the phenotypic and pathologic alterations in $gus^{mps2J/mps2J}$ mice are similar to those in patients with MPS VII although milder than those in $gus^{mps/mps}$ mice [Gwynn et al., 1998; Vogler et al., 2001].

To enhance the value of the $gus^{mps/mps}$ model for enzyme and gene therapy using the human GUS gene product, we produced a transgenic mouse expressing the human GUS cDNA with an amino acid substitution at the active site nucleophile (p.E540A) and bred it onto the MPS VII ($gus^{mps/mps}$) background [Sly et al., 2001]. The mutant mice expressed the inactive human GUS from the mutant human transgene. We also used homologous recombination to simultaneously introduce a human cDNA transgene expressing inactive human GUS (p.E540A) into intron 9 of the murine Gus gene and a targeted active site mutation (mE536A) into the adjacent exon 10 [Tomatsu et al., 2003]. These two models retained the clinical, morphological, biochemical, and histopathological characteristics of the original MPS VII ($gus^{mps/mps}$) mouse. However, they were now tolerant to immune challenge with human GUS. These tolerant MPS VII mouse models became useful for preclinical trials evaluating the effectiveness of enzyme and/or gene therapy with the human gene products likely to be administered to human patients with MPS VII [Vogler et al., 2001].

To study missense mutant models of murine MPS VII with phenotypes of varying severity, we used targeted mutagenesis to produce mE536A and mE536Q, corresponding to active-site nucleophile replacements p.E540A and p.E540Q in human GUS, and also mL175F, corresponding to the most common human mutation, p.L176F. The mE536A mouse had no GUS activity in any tissue and displayed a severe phenotype like that of the originally described MPS VII mice carrying a deletion mutation ($gus^{mps/mps}$). The mE536Q and mL175F mice had low levels of residual activity and milder phenotypes [Tomatsu et al., 2002b].

In the MPS VII feline model, there was a G-to-A transition in the affected feline cDNA (c. 1074G>A) that predicted a fE351 K substitution, and eliminated GUS enzyme activity in expression studies. Multiple species comparisons with the crystal structure of human GUS indicated that E351 is a highly conserved residue most likely essential in maintenance of the enzyme's conformation [Fyfe et al., 1999]. An affected male cat 12–14 weeks old had walking difficulties and an enlarged abdomen. Other findings included facial dysmorphism, plump paws, corneal clouding, granulation of neutrophils, vacuolated lymphocytes, and a positive urine test for sulfated GAGs. Thus, the MPS VII cat had the phenotypic characteristics of human MPS VII patients [Gitzelmann et al., 1994].

In the MPS VII dog model, the G-to-A change at nucleotide position 559 in the affected canine cDNA sequence (c.559G>A) causes a cR166 H mutation. Introduction of the G-to-A substitution at position 559 into the normal canine GUS cDNA nearly eliminated the GUS enzyme activity expressed in mammalian cells [Haskins et al., 1984,1991;Ray et al., 1998]. The same cR166 H mutation was found in another German shepherd dog [Silverstein et al., 2004]. This 12-week-old male German shepherd dog was evaluated because of a 3-week

history of a progressive inability to ambulate. Clinical and laboratory findings included skeletal deformities, corneal cloudiness, cytoplasmic granules in the neutrophils and lymphocytes of blood and CSF, and GAGs in a urine sample.

Future Prospects

Improvements in diagnostic techniques should allow identification of uncharacterized mutations in MPS VII patients (8% of mutant alleles are undefined). To date, most investigations have been carried out by PCR-mediated strategies and subsequent analysis by direct sequencing. Large and complex rearrangements, deletions, inversions, or mutations in the intronic sequence of the GUS gene escape detection by the published PCR-based strategies. These mutations might be detected by array comparative genomic hybridization [Lu et al., 2007].

Defining the genotype/phenotype relationship remains one of the most challenging tasks for MPS VII professionals since the clinical manifestations of MPS VII patients are so variable. Still needed are long-term clinical observations as well as attempts to characterize the modifying factors that influence phenotype, posttranslational processing and stabilization of the mutant enzymes, and the efficient catabolism of DS, HS, and CS.

Longitudinal studies require a larger number of cases of the same age and genotype to clarify the relationship between genotype, the chemical phenotype in blood and urine DS, HS, and CS levels, and the clinical course. Additionally, investigations on the relationship between the GUS residual activity in MPS VII patients and accumulation of each GAG, particularly in bone and brain, will provide more precise information about the mechanisms causing systemic bone dysplasia and central nervous system (CNS) involvement in each mutant form, and may provide a rational basis for more efficient treatment.

Finally, recent development of identification of each GAG by tandem mass spectrometry will facilitate screening for MPS VII and monitoring therapy [Oguma et al., 2007]. These techniques will also enhance prospects for newborn screening for lysosomal storage disorders (LSDs), the importance of which is emphasized by the correlation between early treatment and favorable response to therapy.

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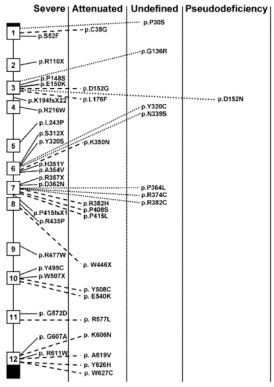

Figure 1.

Location of GUS gene mutations in MPS VII patients. The exons are presented by open boxes and the untranslated regions are filled boxes. Clinical phenotypes associated with missense or nonsense mutations are described.

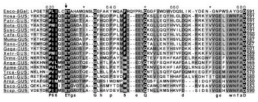

Figure 2.

Multiple amino acid alignment of GUS from human (Hosa-GUS), chimpanzee (Patr-GUS), cow (Bota-GUS), pig (Susc-GUS), dog (Cafa-GUS), mouse (Mumu-GUS), rat (Rano-GUS), chicken (Gaga-GUS), frog (Xetr-GUS), fruit fly (Drme-GUS), mosquito (Anga-GUS), honey bee (Apme-GUS), red flour beetle (Trca-GUS), nematode (Cael-GUS), gram-positive bacteria (Ars*p*.-GUS), enterobacteria (Esco-GUS), and fungi (Scs*p*.-GUS), along with bacterial β-galactosidase (Esco-β-Gal). The arrow indicates the active site (residue E540) of human GUS. GenBank reference sequences: *Homo sapiens*, NM_010368.1, NP_034498.1; Pan troglodytes, XP_001138789.1; Macaca mulata, XM_001087699; Mus musculus, NM_010368.1, NP_034498.1; *Rattus norvegicus*, NP_058711; *Felis catus*, NM_001009310.1, NP_001009310.1; *Canis familiaris*, NM_001003191.1, NP_001003191.1; *Bos taurus*, NM_001083436.1; *Sus scrofa*, AK232674.1; *Gallus gallus*, NP_001034405; *Xenopus tropicalis*, CT030620; *Danio renio*, XM_695030; *Drosophila melanogaster*, NP_001014535.1; *Anopheles gambiae*, XP_320660.2; *Apis mellifera*, XM_393305; *Tribolium castaneum*, XM_964260.1; *Caenorhabditis elegans*, NP_493548.1; *Arthrobacter sp*., RP10, AAV91790; *Scopulariopsis sp*., RP38.3, AAV91788; *Escherichia coli*, AAB30197.

Table 1

Mutations in the GUSB Gene Causing MPS VII*

Nucleotide change[a]	Effect on amino acid	Exon	Degree of conservation of aa[b]	Phenotype defined	Detected alleles (n)	Population[c]	References
c.f8C>T	p.P30S	1	2	Undefined	1	Un	Vervoort (unpublished results)
c.112T>G	p.C38G	1	4	Attenuated	2	It	Vervoort et al. [1998a]
c.155C>T	p.S52F	1	2	Severe	1	Cz	Vervoort et al. [1997]
c.328C>T	p.R110X	2	3	Severe	1	Be	Vervoort et al. [1997]
c.406G>A	p.G136R	3	2	Undefined	2	Bt	Vervoort et al. [1996]
c.442C>T	p.P148S	3	1	Severe	1	Cc	Yamada et al. [1995]
c.448G>A	p.E150K	3	1	Severe	1	Ch	Vervoort et al. [1996]
c.455A>G	p.D152G	3	3	Attenuated	1	Un	Sly (unpublished results)
c.526C>T	p.L176F	3	1	Attenuated	21	Sp, Br, Mn, Mx	Wu et al. [1994]; Vervoort et al. [1996]; Schwartz et al. [2003]
						Am, Fr-Ca, Bt, Tu	Sly (unpublished results); Young (unpublished results)
c.581+1G>A	p.K194fsX22	Intron 3		Severe	1	Pk	Young (unpublished results)
c.646C>T	p.R216W	4	1	Severe	4	Du, Be, Un	Vervoort et al. [1993,1996,1997]
c.728T>C	p.L243P	5	2	Severe	1	Bt, Tu	Young (unpublished results)
c.935C>G	p.S312X	6	4	Severe	1	Be	Vervoort et al. [1996]
c.959A>G	p.Y320C	6	1	Undefined	1	Ge	Vervoort et al. [1996]
c.959A>C	p.Y320S	6	1	Severe	1	Fi	Vervoort et al. [1996]
c.1016A>G	p.N339S	6	1	Undefined	1	Am	Sly (unpublished results)
c.1050G>C	p.K350N	6	2	Attenuated	1	Ge	Storch et al. [2003]
c.1051C>T	p.H351Y	6	1	Severe	1	Fi	Vervoort et al. [1996]
c.1061C>T	p.A354V	6	4	Severe	1	Cc	Wu and Sly [1993]
c.1069C>T	p.R357X	7	2	Severe	7	Be, Ch, Du, Am	Shipley et al. [1993]; Vervoort et al. [1996, 1997]; Sly (unpublished results)
c.1081_1107del27	p.F361-D369del	7		Severe	2	Du, Be	Vervoort et al. [1997]
c.1084G>A	p.D362N	7	1	Severe	2	Mx	Sly (unpublished results)
c.1091C>T	p.P364L	7	3	Undefined	1	Am	Sly (unpublished results)

Nucleotide change[a]	Effect on amino acid	Exon	Degree of conservation of aa[b]	Phenotype defined	Detected alleles (n)	Population[c]	References
c.1120C>T	p.R374C	7	2	Undefined	2	Cz, Ge	Vervoort et al. [1996,1997]
c.1144C>T	p.R382C	7	1	Undefined	4	Jp, Fr	Fukuda et al. [1991]; Tomatsu et al. [1991]; Vervoort et al. [1996]
c.1145G>A	p.R382H	7	1	Attenuated	1	Fr	Vervoort et al. [1996]
c.1222C>T	p.P408S	7	2	Attenuated	5	Mx	Islam et al. [1996]; Islam et al. [1998]; Sly (unpublished results)
c.1244C>T	p.P415L	7	4	Attenuated	5	Mx	Islam et al. [1996,1998]; Sly (unpublished results)
c.1244+1G>A	p.P415fsX1	Intron 7		Severe	2	Mx	Vervoort et al. [1997]
c.1304G>C	p.R435P	8	2	Severe	2	Tu	Vervoort et al. [1996]
c.1337G>A	p.W446X	8	1	Attenuated	1	Sw	Vervoort et al. [1998a]
c.1429C>T	p.R477W	9	1	Severe	4	Kw, Ma	Vervoort et al. [1996]
c.1454_1457del4	p.S485fsX13	9		Severe	1	Be	Vervoort et al. [1997]
c.1484A>G	p.Y495C	10	2	Severe	1	Cc	Yamada et al. [1995]
c.1520G>A	p.W507X	10	1	Severe	1	Fr	Vervoort et al. [1996]
c.1521G>A	p.W507X	10	1	Severe	2	Cc, Am	Yamada et al. [1995]; Sly (unpublished results)
c.1523A>G	p.Y508C	10	1	Attenuated	1	Fr	Vervoort et al. [1996]
c.1616_1653del38	p.S539RfsX7	10		Severe	1	Cc	Yamada et al. [1995]
c.1618G>A	p.E540K	10	1	Attenuated	1	Un	Sly (unpublished results)
c.1715G>A	p.G572D	11	4	Severe	2	In	Vervoort et al. [1996]
c.1730G>T	p.R577L	11	2	Attenuated	2	Ge, Mx	Storch et al. [2003]; Sly (unpublished results)
c.1775delT	p.F592SfsX2	11		Undefined	1	Fr	Vervoort et al. [1998b]
c.1818G>T	p.K606N	12	2	Attenuated	2	Al	Vervoort et al. [1996]
c.1820G>C	p.G607A	12	1	Severe	1	Un	Sly (unpublished results)
c.1831C>T	p.R611W	12	1	Severe	1	Cc	Wu and Sly [1993]
c.1856C>T	p.A619V	12	1	Attenuated	5	Jp	Tomatsu et al. [1990,1991]
c.1874_1875delGA	p.R625IfsX6	12		Severe	1	Be	Vervoort et al. [1996]
c.1876T>C	p.Y626H	12	1	Attenuated	1	It	Vervoort et al. [1998a]
c.1881G>T	p.W627C	12	3	Attenuated	2	Am	Shipley et al. [1993]; Vervoort et al. [1996]

* The numbers for the nucleotide changes are reported in accordance with GenBank entry NM_010368.1, NP_034498.1.

[a] The DNA mutation numbering is based on cDNA sequence. Nucleotides are numbered from the ATG initiation codon as suggested by den Dunnen and Antonarakis et al. [2001] and per HGVS guidelines (www.hgvs.org).

[b] 1, conserved among all species; 2, vertebrate specific; 3, mammal specific; 4, nonconserved.

[c] Al, Algerian; Am, American; Be, Belgian; Br, Brazilian; Bt, British; Cc, Caucasian; Ch, Chilean; Cz, Czechoslovakian; Du, Dutch; Fi, Finnish; Fr, French; Fr-Ca, French-Canadian; Ge, German; In, Indian; It, Italian; Jp, Japanese; Kw, Kuwaiti; Ma, Maghrebi; Mn, Mennonite; Mx, Mexican; Pk, Pakistani; Sp, Spanish; Sw, Swiss; Tu, Turkish; Un, unknown.

aa, amino acid(s).

Table 2

Nonpathogenic Variants of the GUSB Gene*

Base change[a]	Codon change	Effect	Region	Reference
c.454G>A	GAC>AAC	p.D152N	Exon 3	Vervoort et al. [1995]; Vervoort et al. [1998a]
c.1740T>C	TAT>TAC		Exon 11	Vervoort et al. [1998a]
c.1946C>T	CCG>CTG	p.P649L	Exon 12	Tomatsu et al. [1991]; Wu et al. [1994]; Vervoort et al. [1997]; Vervoort et al. [1998a]

*The numbers for the nucleotide changes are reported in accordance with GenBank entry NM_010368.1, NP_034498.1.

[a]The DNA mutation numbering is based on cDNA sequence. Nucleotides numbered from the ATG initiator codons as suggested by den Dunnen and Antonarakis et al. [2001] and per HGVS guidelines (www.hgvs.org).

Table 3

Combination of Alleles Producing Attenuated Phenotypes*

Base change[a] (first allele)	Amino acid change[b]	Factors defined as attenuated	Base change (second allele)	Amino acid change
c.112T>G	p.C38G	3	c.1876T>C	p.Y626H
c.455A>C	p.D152G	4	c.1618G>A	p.E540K
c.526C>T	p.L176F	1,2,3	c.526C>T	p.L176F
c.1050G>C	p.K350N	3	c.1730G>T	p.R577L
c.1061C>T	p.A354V	3	c.1831C>T	p.R611W
c.1144C>T	p.R382C	1,3	c.1144C>T	p.R382C
c.1145G>A	p.R382H	2,3	c.1523A>G	p.Y508C
c.1222C>T	p.P408S	1,3	c.1244C>T, c.1222C>T	p.P415L, p.P408S
c.1244C>T	p.P415L	1,3	c.1244C>T, c.1222C>T	p.P415L, p.P408S
IVS8+0.6kbdelTC	?	3	p.13376G>A	p.P446X
c.1523A>G	p.Y508C	3	c.1145G>A	p.R382H
c.1730G>T	p.R577L	3	c.1050G>C	p.K350N
c.1856C>T	p.A619V	1,3	c.1856C>T	p.A619V
c.1876T>C	p.Y626H	3	c.112T>G	p.C38G
c.1881G>T	p.W627C	3,4	c.1069C>T	p.R357X

*The numbers for the nucleotide changes are reported in accordance with GenBank entry NM_010368.1, NP_034498.1.

[a]The DNA mutation numbering is based on cDNA sequence. Nucleotides numbered from the ATG initiator codons as suggested by den Dunnen and Antonarakis et al. [2001] and per HGVS guidelines (www.hgvs.org).

[b]The correlation of a mutation with an attenuated phenotype is based on four factors: 1) homozygosity of the mutation in the patients with attenuated phenotypes; 2) prediction from the likely structural change in the protein; 3) prediction from in vitro expression studies; and, 4) mutant allele permitting residual enzyme activity in primary fibroblasts or leukocytes, which would be dominant over an allele permitting no activity.

Table 4

In Vitro Mutagenesis Data of *GUSB* Missense Mutations

Mutation	Phenotype	Percentage of normal cDNA	Location of the mutation	Conservativeness of amino acid changes	Evolutionary conservation[a]	References
p.C38G	Attenuated	7.0	Surface	Nonconservative	4	Vervoort et al. [1998a]
p.S52F	Severe	0.7	Surface	Nonconservative	2	Vervoort et al. [1997]
p.G136R	Undefined	0.6	Surface	Nonconservative	2	Vervoort et al. [1996]
p.P148S	Severe	1.2	Hydrophobic core	Semiconservative	1	Yamada et al. [1995]
p.E150K	Severe	4.0	Hydrophobic core	Conservative	1	Vervoort et al. [1996]
p.D152N	Pseudodeficiency	28	Hydrophobic core	Conservative	3	Vervoort et al. [1995]
p.L176F	Attenuated	84.1	Hydrophobic core	Conservative	1	Wu et al. [1994]
p.R216W	Severe	0.0	Hydrophobic core	Nonconservative	1	Vervoort et al. [1996]
p.Y320C	Undefined	1.4	Hydrophobic core	Nonconservative	1	Vervoort et al. [1996]
p.Y320S	Severe	1.0	Hydrophobic core	Nonconservative	1	Vervoort et al. [1996]
p.K350N	Attenuated	13	Hydrophobic core	Nonconservative	2	Storch et al. [2003]
p.H351Y	Severe	0.0	Hydrophobic core	Semiconservative	1	Vervoort et al. [1996]
p.A354V	Attenuated	37.7	Hydrophobic core	Semiconservative	4	Wu and Sly [1993]
p.R374C	Undefined	5.5	Surface	Nonconservative	2	Vervoort et al. [1996]
p.R382C	Attenuated	12.3	Salt bridge	Nonconservative	1	Tomatsu et al. [1991]
p.R382H	Attenuated	2.3	Salt bridge	Conservative	1	Vervoort et al. [1996]
p.P408S	Attenuated	61	Hydrophobic core	Semiconservative	2	Islam et al. [1996]
p.P415L	Attenuated	112	Surface	Nonconservative	4	Islam et al. [1996]
p.R435P	Severe	0.5	Hydrophobic core	Nonconservative	2	Vervoort et al. [1996]
p.R477W	Severe	0.7	Hydrophobic core	Nonconservative	1	Vervoort et al. [1996]
p.Y495C	Severe	0.8	Surface	Nonconservative	2	Yamada et al. [1995]
p.Y508C	Attenuated	19	Surface	Nonconservative	1	Vervoort et al. [1996]
p.G572D	Severe	2.1	Hydrophobic core	Nonconservative	4	Vervoort et al. [1996]
p.R577L	Attenuated	4.0	Surface	Nonconservative	2	Storch et al. [2003]
p.K606N	Severe	6.7	Hydrophobic core	Nonconservative	2	Vervoort et al. [1996]
p.R611W	Severe	0.0	Hydrophobic core	Nonconservative	1	Wu and Sly [1993]
p.A619V	Attenuated	9.1	Hydrophobic core	Semiconservative	1	Tomatsu et al. [1990,1991] Yamada et al. [1995]
p.Y626H	Attenuated	4.0	Hydrophobic core	Semiconservative	1	Vervoort et al. [1998a]

Mutation	Phenotype	Percentage of normal cDNA	Location of the mutation	Conservativeness of amino acid changes	Evolutionary conservation[a]	References
p.W627C	Attenuated	39	Surface	Nonconservative	3	Shipley et al. [1993]
p.P408S/	Attenuated	12.5				Islam et al. [1996]
p.P415L						
p.P649L	Polymorphism	88.3		Nonconservative	4	Tomatsu et al. [1991]

[a] 1, conserved among all species; 2, vertebrate species; 3, mammalian species; 4, nonconserved.

Table 5

Animal Models of MPS VII*

Animal	Description	Base changea	Amino acid change	Phenotype	Reference
Murine	*gus*^{mps}/*gus*^{mps}	c.1470delC	mP490RfsX8	Severe	Birkenmeier et al. [1989]; Sands and Birkenmeier [1993]
Murine	*gus*^{mps2J}/*gus*^{mps2J}	Intron 8 ins5.4-kb	NDb	Attenuated	Gwynn B et al. [1998]; Vogler et al. [2001]
Murine	MPSVII/E540ATg	c.1470delC + ins hGUSBcDNA with c.1618A>C	p.E540A · mP490RfsX8	Severe	Sly et al. [2001]
Murine	*Gus*$^{tm(hE540A \cdot mE536A)Sly}$	c.1606A>C + intron 9 ins hGUSBcDNA with c.1618A>C	p.E540A · mE536A	Severe	Tomatsu et al. [2003b]
Murine	*Gus*$^{tm(E536A)Sly}$	c.1606A>C	mE536A	Severe	Tomatsu et al. [2002b]
Murine	*Gus*$^{tm(E536Q)Sly}$	c.1607G>C	mE536Q	Attenuated	Tomatsu et al. [2002b]
Murine	*Gus*$^{tm(L175F)Sly}$	c.523C>T	mL175F	Attenuated	Tomatsu et al. [2002b]
Feline	NA	c.1074G>A	fE351K	Severe	Gitzelmann et al. [1994]; Fyfe et al. [1999]
Canine	NA	c.559G>A	cR166H	Severe	Haskins et al. [1984]; Haskins et al. [1991]; Ray et al. [1998]
Canine	NA	c.559G>A	cR166H	Severe	Silverstein et al. [2004]

*The numbers for the nucleotide changes are reported in accordance with GenBank entry human: NM_010368.1, NP_034498.1; murine: NM_010368.1, NP_034498.1; feline: NM_001003910.1, NP_001003910.1; canine: NM_001003191.1, NP_001003191.1.

[a]The DNA mutation numbering is based on cDNA sequence. Nucleotides are numbered from the ATG initiation codon as suggested by den Dunnen and Antonarakis et al. [2001] and per HGVS guidelines (www.hgvs.org).

NA, not available.