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Identification of nine new IDS alleles in mucopolysaccharidosis II. Quantitative evaluation by real-time RT-PCR of mRNAs sensitive to nonsense-mediated and nonstop decay mechanisms

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Abstract

The present study aimed to characterize mutant alleles in Mucopolysaccharidosis II and evaluate possible reduction of mRNA amount consequent to nonsense-mediated or nonstop mRNA decay pathways. A combination of different approaches, including real-time RT-PCR, were used to molecularly characterize seventeen patients. Fifteen alleles were identified and nine of them were new. The novel alleles consisted of three missense mutations (p.S71R, p.P197R, p.C432R), two nonsense (p.Q66X, p.L359X), two frameshifts (p.V136fs75X, p.C432fs8X), one allele carrying two in-cis mutations [p.D252N;p.S369X], and a large deletion (p.G394_X551). Analysing these results it emerged that most of the alterations resulted in mutants leading to mRNAs with premature termination codons, and therefore, potentially sensitive to mRNA surveillance pathway. By using real-time RT-PCR, the mRNAs resulting (i) from substitutions that changed one amino acid to a stop codon (L359X, and S369X), or caused the shifted reading frame with premature introduction of a stop codon (C432fs8X), (ii) from large deletion (p.G394_X551) that included the termination codon, seemed to be subject to degradation by nonsense-mediated (i) or nonstop decay (ii) mechanisms, as mRNA was strongly underexpressed. On the contrary, two mutations (Q66X and V136fs75X) produced transcripts evading mRNA surveillance pathway despite both of them fulfilled the known criteria. These results confirm the wide variability of the mRNA expression levels previously reported and represent a further exception to the rules governing susceptibility to nonsense-mediated decay. A close examination of the molecular basis of the disease is becoming increasingly important for optimising the choices of available or forthcoming therapies such as, enzyme replacement therapy or enzyme enhancement therapy.

Keywords: Hunter; Mucopolysaccharidosis; IDS; real-time RT-PCR; Genotype–phenotype correlation; mRNA decay

1. Introduction

Mucopolysaccharidosis type II (MPS II, Hunter syndrome; OMIM #309900) [1] is a rare X-linked recessive lysosomal storage disorder caused by the deficiency of the enzyme iduronate-2-sulfatase (*IDS*; EC 3.1.6.13) involved in the heparan sulfate and dermatan sulfate degradation. Two clinical entities, mild and severe, are conventionally recognised. The major clinical manifestations of the severe form are dysostosis multiplex, organomegaly, mental retardation and death before 15 years. Normal intelligence, short stature, survival into adulthood characterize the mild form [2].

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However, the two types represent two ends of a wide spectrum of clinical severity. In absence of a standardized index to score the severity, however, the classification of the clinical phenotypes is difficult and generally is based on the age at onset, degree of mental retardation and death age.

The human *IDS* gene spans approximately 24 kb in Xq28 and contains nine exons (Genbank-EMBL no. AF011889.1) [3]. Its 1653-bp open reading frame (Genbank-EMBL no. NM_000202) encodes a polypeptide of 550 amino acids (Genbank-EMBL no.NP_000193) [3]. About 20 kb distal to the functional *IDS* gene, a homologous pseudogene *IDS2* (*IDSP1*) facilitating genetic rearrangements, is located (Genbank-EMBL no. AF011889.1) [3]. To date, more than 300 different mutations have been reported in the *IDS* gene as the cause of a wide spectrum of clinical severity. The mutational profile consists of point mutations leading to missense (44%), nonsense (10%) or splice-site alterations (9%); the small insertions/deletions account for the 26% of the total mutations, the remaining 11% are gross deletions, insertions, duplications or complex rearrangements [4]. Amongst the different mutations, it emerges that more than half of the alterations (56%) results in mutant alleles that might lead to premature termination codons.

Our MPS II series consists at present of 55 patients. The molecular characterization of part of the group was reported in previous studies [5–10]. We now report nine additional novel mutant alleles, including missense, nonsense, frameshift mutations and a large deletion, encountered in MPS II patients with diverse phenotypes and ethnic backgrounds. In order to evaluate possible reduction of mRNA amount consequent to nonsense-mediated or nonstop mRNA decay pathways, we used the real-time RT-PCR for quantification of mRNAs with nonsense or frameshift mutations or lacking in-frame termination codons [11,12]. A close examination of the molecular basis of the disease is becoming increasingly important for optimising the choices of available and forthcoming therapies such as, enzyme replacement therapy (ERT) or enzyme enhancement therapy (EET) [13,14].

2. Materials and methods

2.1. Patients

Seventeen unrelated patients with diverse phenotypes and ethnic backgrounds were examined (Table 1). The diagnosis was based on clinical data as well as on demonstration of high excretion of dermatan sulfate and heparan sulfate in urine, and *IDS* deficiency in peripheral white blood cells or fibroblasts or lymphoblasts. Table 1 reports the patients' clinical phenotype that ranged from mild (no mental retardation, short stature), intermediate (mild retardation and moderate hyperkinetic behaviour) to severe (early mental retardation, pronounced behavioural disturbance, death before 15 years).

2.2. Controls

The control group consisted of 50 DNA samples from non-correlated female healthy subjects.

The samples were obtained from the "Cell Line and DNA Bank from Patients affected by Genetic Diseases" collection, situated at G. Gaslini Institute [15].

2.3. Ethical aspects

Following ethical guidelines, the patient and control samples were obtained for analysis and storage with written informed consent. The consent has been sought using a form approved by local Ethics Committee.

2.4. Enzymatic assay

Iduronate-2-sulfatase activity was determined in homogenates of leukocytes and/or cell lines using the fluorescent substrate, 4-methylumbelliferyl-alpha-iduronide-2-sulfate [16].

Table 1
IDS mutant alleles encountered in 17 MPS II patients

Patient	Age at diagnosis	Age at present	Age at death	Mutant alleles[a]	Clinical phenotype[b]	Ethnic background
H2	10 y		23 y	p.C432R	Intermediate	Italian
H4	3 y		15 y 6 m	p.Q66X	Intermediate/severe	Italian
H14	n.a.		n.a.	p.G394 _ X551	n.a.	Spanish
H25	5 y		10 y	p.L359X	Severe	Italian
H30	n.a.		n.a.	[p.C432fs8X;(p.T146T[c])]	n.a.	Portuguese
H118	3 y	10 y		p.P197R	Intermediate/severe	African
H145	3 y	9 y		p.E375_G394del	Mild/intermediate	Italian
H146	4 y	9 y		[p.D252N;p.S369X]	Mild	Italian
H147	8 y	10 y		unknown (p.T146T[c])	Mild	Ukrainian
H148	4 y	6 y		p.C422R	Mild/intermediate	Italian
H150	7 y	12 y		unknown (p.T146T[c])	Mild	Italian
H151	1 y	3 y		p.V136fs75X	Intermediate	Italian
H152	8 y	10 y		p.E375_ G394del	Mild/intermediate	Albanian
H153	3 y	4 y		Gene-Pseudogene Rec	Intermediate	Romanian
H155	5 m	6 m		p.P358R	‡	Italian
H156	15 m	16 m		p.S71R	‡	Italian
H157	1 y	7 y		p.S333L	Intermediate	Italian

y = years; m = months; n.a. = data not available; ‡ phenotypic classification is not applicable due to the patient's young age.
[a] New alleles are underlined.
[b] Classification based on degree of mental retardation and death age.
[c] Polymorphic variant.

2.5. IDS mutational analysis

Total DNA and RNA were extracted from cultured fibroblasts (patients H2, H4, H14, H25, H30, H118, H146, H150, H152) or lymphoblasts (patients H145, H147, H151, H153, H155) or peripheral lymphocytes (patients H148, H156, H157) using standard protocols or suitable kits (Qiagen).

IDS gene exons and exon–intron boundaries were amplified by using specific primers designed on the genomic sequence (GenBank-EMBL accession no. AF011889.1), as previously reported [5,7,17].

Putative mutations were confirmed by sequencing in both directions duplicate PCR products as well as by digesting PCR products with the specific restriction endonuclease whose recognition site was consequently altered. If the mutations neither created nor destroyed a restriction site, the amplification was carried out by PCR-mediated site direct mutagenesis that introduced a new cleavage site of restriction [18]. The hypothesis of new possible genetic polymorphisms was also excluded ascertaining that none of the 50 female control individuals had these alterations.

Reverse transcription-polymerase chain reaction (RT-PCR) conditions were previously reported [7].

The products from PCR and/or RT-PCR were purified and directly sequenced using an ABI 377 DNA automated sequencer with dye terminator cycle sequencing kits (Applied Biosystems).

Patients who resulted negative to conventional mutation screening of the *IDS* gene underwent a PCR-based methodology, previously developed to rapidly detect possible gene/pseudogene recombinations [10].

2.6. Real-time PCR on PE biosystems prism 7700

Primers and probe were designed (using the Primer Express 1.0 software program, Applied Biosystems) to yield a product of 151 bp, specific to exons 7 and 8. The probe contained 6FAM as the reporter fluorophore at its 5′-end and TAMRA as the quencher at its 3′-end. FPLC-purified primers and HPLC-purified probe were purchased from Roche Diagnostics. The following primers (HF and HR) and probe (HPB) were used for the analysis: HF: 5′-TGGACGATCTTCAGCTGGC-3′, HR: 5′-GCCGTCCTTCCAGGAACATA-3′, HPB: 5′-FAM-ATCATGGGTGGGCTCTAGGTGAACATGG-TAMRA-3′. GAPDH cDNA control was amplified using GAPDH Probe (JOE™ Probe), GAPDH Forward Primer, and GAPDH Reverse Primer supplied by Applied Biosystems (TaqMan® GAPDH Control Reagents-Human). ABI Prism 7700 sequence was used as detection system (Applied Biosystems). Reaction volumes of 25 µl were prepared in 96-well MicroAmp optical plates with MicroAmp optical caps (Applied Biosystems). GAPDH and IDS amplifications were run in separate wells. The reagents included the TaqMan 23 Universal Master Mix [1X TaqMan buffer A, 5 mM MgCl2 400 µM dUTP, 200 µM dATP, dCTP, dGTP (each), 8% glycerol, AmpliTaq Gold (0.025 U/µl), AmpErase UNG (0.01 U/µl)], 200 nM of each forward and reverse primer, 100 nM of each probe and cDNA. Standard wells contained 500, 200, 100, 50, 20, 10 pg of a normal male cDNA, test sample wells contained 100 pg of cDNA. No-template-control wells (without DNA) were included as negative controls. Each test sample and standard were run in triplicate. The same cDNA from a normal male served as standard in all the procedures. GAPDH and IDS samples were always run simultaneously. PCR conditions were 50 °C for 2 min, 95 °C for 10 min, then 50 cycles of 95 °C for 15 s and 60 °C for 1 min. The runs were monitored via the Sequence Detection Software 1.5. After each run, a semi-log amplification plot was shown, in which the progression of the reactions along the PCR cycles was indicated by the increase of the normalized reporter fluorescence emission (ΔR_n). A threshold in the linear region of the semi-log amplification plot was established at an arbitrary value of the ΔR_n normalized reporter fluorescence emission. For each reaction, a CT value, which is the number of cycles necessary to reach the threshold, was identified. Following the standard curve method two standard curves were generated, one for the GAPDH amplifications and one for the IDS amplifications, using the CT values obtained from the standard wells and their known cDNA amounts [19,20]. They were used to determine expression of the IDS and GAPDH genes in each triplicate. IDS gene expression was determined by the ratio between the mean value obtained for IDS and GAPDH.

2.7. Mutation nomenclature

All mutations are described according to mutation nomenclature [21].

3. Results and discussion

3.1. Mutation profile

Table 1 reports the mutational spectrum of *IDS* gene in the seventeen unrelated patients of the present study. Fifteen alleles were identified and nine of them were new (Table 2) and consisted of three missense mutations (p.S71R, p.P197R, p.C432R), two nonsense (p.Q66X, p.L359X), two frame shift changes (p.V136fs75X and p.C432fs8X), one complex allele carrying two in-cis mutations [p.D252N;p.S369X], and a large deletion (p.G394_X551). Table 2 summarized the localization of new alterations in relation to cDNA nucleotide numbering and the resulting changes at protein level. The missense S71R due to a C>G transversion at position c.213 differs from the already described S71R that resulted from the C>A transition in the same position [22]. The missense C432R mutation (Table 2) is novel but another missense mutation affecting the same codon (C432Y) was previously reported [23]. A third mutation affecting the same codon [a duplication of the nucleotide

Table 2
IDS new mutations

Exon	cDNA[a] mutation	Restriction enzyme site	Protein[b] mutation	Effect on coding
2	c.196C>T	PvuII (−)[c]	p.Q66X	Premature chain termination at codon 66
2	c.213C>G	NlaIV (+)	p.S71R	Polar uncharged Ser→polar positively charged Arg
3	c.408_411del CTTT	MaeIII (+)	p.V136fs75X	Frameshift and chain termination at codon 211
5	c.590C>G	MaeIII (+)	p.P197R	Polar uncharged Pro→polar positively charged Arg
6; 8	[c.754G>A;c.1106C>G]	MboI (−); Eco57I (−)[c]	[p.D252N;p.S369X]	Polar negatively charged Asp→polar uncharged Asn and chain termination at codon 369
8	c.1075delC	HphI (−)[c]	p.L359X	Premature chain termination at codon 359
9	c.1294T>C	BbvI (+)	p.C432R	Polar uncharged Cys→polar positively charged Arg
9	c.1295_96dupTG	–	p.C432fs8X	Frameshift and chain termination at codon 440
9	c.1191−?_1653+?del	–	p.G394_X551	?

[a] Genbank-EMBL: NM_000202. For cDNA numbering +1 corresponds to the A of the first ATG translation initiation codon.
[b] Genbank-EMBL: NP_000193; (+) and (−) symbols are for creation of a restriction site and loss of an existing one, respectively.
[c] New cleavage site of restriction introduced by PCR-mediated site direct mutagenesis [18].

stretches TG (c.1295_1296dupTG) resulting in a shift of reading frame (C432fs8X)] was also identified in our series (Tables 1 and 2). C432fs8X was associated with the polymorphism T146T [24].T146T was the only alteration identified in 2 mild MPSII patients of our series (Table 1).

3.2. Real-time RT-PCR of mRNAs with nonsense and frameshift mutations and a deletion

Recently, it has been reported that mutations introducing premature translation termination codons (PTCs) at a position that is more than 50–55 nucleotides upstream of a splicing-generated exon–exon junction trigger nonsense-mediated decay (NMD). This mechanism is aimed at eliminating mRNAs containing PTCs and thus helping limit the synthesis of abnormal proteins [12]. Moreover, distinct mRNA surveillance process, called nonstop decay, is responsible for depleting mRNAs lacking in frame termination codons [11]. Hence, the new substitutions we found to change one amino acid to a stop codon (Q66X, L359X, and S369X), or cause the shifted reading frame with premature introduction of a stop codon (V136fs75X and C432fs8X), could result in unstable mRNAs, subject to degradation by NMD [12]. Similarly, the nonstop decay mechanism might also be hypothesised for the large deletion that included exon 9 (G394_X551) with the start point at an unknown position in the intron 8 and an end at an unknown position downstream from polyA signal.

Possible mRNAs liable to be eliminated through NMD or nonstop decay pathways, were evaluated by real-time RT-PCR (Fig. 1). As expected, the transcripts that contained a nonsense codon more than 50–55 nucleotides upstream of the splicing-generated exon–exon junction (that are L359X, S369X) were most likely subjected to NMD, as a reduction of 30% in mRNA level was observed in comparison to the control samples. On the contrary, real-time RT-PCR showed an impressive increase of expression of the transcript with Q66X that we expected to escape NMD because of a nonsense codon <50 nucleotides upstream of the respective exon–exon junction. With regards to the two mRNAs with frameshift, the results obtained by real-time RT-PCR indicated that the mRNA with C432fs8X most likely elicited the NMD mechanism, since its expression was decreased, to 70% of the control samples. In contrast, the mRNA with V136fs75X evaded the RNA surveillance and, like the transcript mentioned before (Q66X), showed a notable increase of the expression. These results confirm the wide variability of the mRNA expression levels previously reported in several genetic diseases [25–32] and represent a further exception to the rules governing susceptibility to NMD pathway. Several genes are known to produce aberrant PTC-transcripts that fulfil all the known criteria but still evade NMD [25–31]. The proximity of non-sense codons to the native initiation codon has been reported as possible determinant of NMD evasion; this observation is supported by the knowledge that mRNAs with short open reading frames may have a general resistance to NMD [29,30]. NMD appears to be circumvented also by reinitiation of translation downstream of the premature termination codon [28,31]. It is clear, however, that the existence of additional, identified [27] and unidentified [26] determinants function to modulate the NMD sensitivity. More recently, several studies indicate that the posttranscriptional control of gene expression is much more complicated than expected with mRNA metabolism being subject to diverse regulatory mechanisms [33].

The other mechanism, nonstop decay, that functions to remove cellular mRNAs lacking in-frame termination codons, was hypothesised for the large deletion identified in the patient #H14 and confirmed by real-time RT-PCR showing that mRNA was strongly under expressed (to 9% of controls) (Fig. 1).

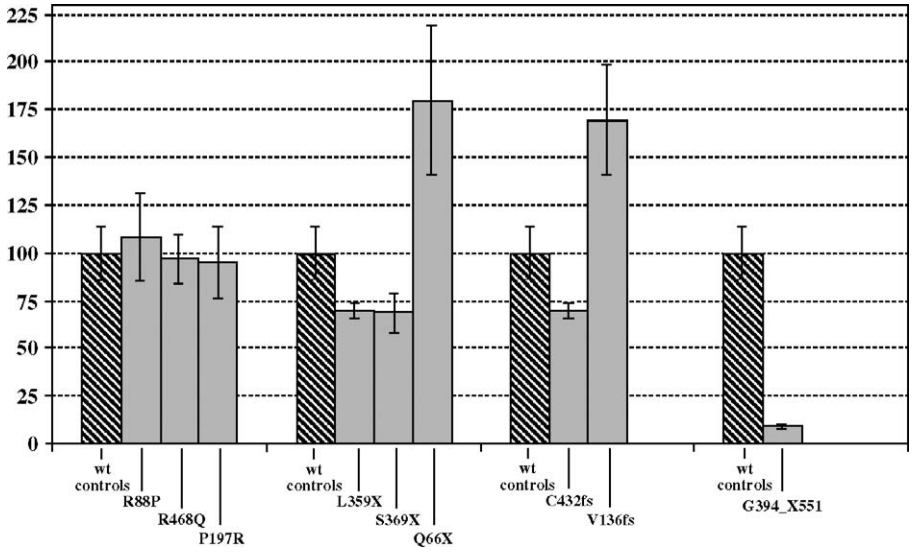

Fig. 1. Real-time RT-PCR. Real-time RT-PCR quantification of mRNAs with nonsense (Q66X, L359X, S369X), frameshift (C432fs, V136fs) mutations and a deletion (G394_X551). The wild-type (wt) controls (4 different normal males) are represented by stripped grey bars. mRNAs with missense mutations (R88P, R468Q, P197R) were used as counter-evidence. Note that the two missense R88P and R468Q were identified in a previous study [7]. Error bars represent standard deviations.

Four different male control individuals were included in the experiment and, as counter-evidence, 3 Hunter patients carrying missense mutations (R88P, R468Q, P197R). As expected, the expression of the mRNAs carrying missense mutations was in the range of the controls (Fig. 1).

3.3. Genotype–phenotype correlations

Genotype–phenotype correlation, for MPS II disease, was studied by means of expression of mutations in COS cells to evaluate enzyme activity, processing and intracellular localization [34] as well as by measurements of residual protein/activity in plasma samples of affected patients [35,36]. In the present study, the new 3 missense mutations (Table 2) seem to be functionally important since two of the substituted amino acids (S71 and P197) are evolutionarily conserved in human and mouse iduronate sulfatase [37]; moreover, all the amino acidic substitutions resulted in charge changes (S71R, P197R and C432R). The supposed deleterious nature of these mutations correlated with the phenotypes of the pt #H118 (P197R) and the pt #H2 (C432R), that ranged from intermediate to severe (Table 1). The rare mutations C422R and S333L were found associated with severe clinical manifestations in previous reports [38–40]. However, we detected these rare mutations in 2 patients (#H148 and #H157) who showed phenotypes mild and intermediate at the present age of 6 and 7 years old, respectively. Concerning the frequent E375_G394del (commonly named G374G) that activates a new cryptic splice site with consequent loss of 20 amino acids, previous studies demonstrated mostly association with mild subtype likely to be imputable to the presence of an additional normal transcript [40–45]. Others reported the same mutation associated with intermediate and severe phenotypes, although less frequently [23,46]. In our series, this panethnic mutation (E375_G394del) always seems to correlate with a phenotype ranging from mild to intermediate. To clarify characterization of this mutation, we analysed the available RNAs of our patients according to the methodological approach reported by Bunge et al. [44]. No additional normal transcript was found (data not shown), even though a possible failure due to technical conditions cannot be excluded. These findings support the hypothesis [41] that a deleted in-frame transcript lacking non-homologous region among human-derived sulfatase sequences [47] might produce a residual protein/activity consistent with the phenotypes observed by us.

With regard to the nonsense (Q66X, L359X) and frameshift mutation (V136fs) resulting in the production of markedly altered, generally truncated, IDS protein, the clinical phenotypes ranged from intermediate to severe (pts #H4, #H25, #151) (Table 1). The patient (#H146) carrying the mutant [D252N; S369X] in which the stop codon, S369X, coexisted with the missense D252N, which occurs on a completely-methylated CpG site of exon 6 [48], showed an unexpected mild phenotype. Currently, the patient, a 9-year-old boy, has mild coarsening of facial features, macrocephaly, moderate joint stiffness and normal development skills and intelligence.

3.4. Conclusion

In accordance with the results from a worldwide survey, in our series, in its entirety, gross rearrangements, frame shift changes and nonsense mutations, all leading to truncated IDS-peptides, account for 54% of the disease alleles. The remaining 46% of the alleles was due to missense mutations or small in frame deletions.

The findings of the study confirm the already known *IDS* mutational heterogeneity. The molecular characterization of index case is very valuable for identifying female carrier in at risk families. Also, the availability of molecular test corroborates the prenatal diagnosis procedures.

Finally, a close examination of the molecular basis of the disease, from transcription to protein function, is becoming increasingly important for optimising the choices of available and forthcoming therapies. Recently, enzyme replacement therapy (ERT) has been approved for MPS II and will soon be available for all patients. However, if the recombinant enzyme is an effective mean to reverse the substrate accumulation in peripheral organs, it is unable to cross the blood–brain barrier [49]. Another limitation is the problem of achieving enzyme uptake in connective tissue cells, particularly in bone and cartilagine tissue, known to be massively involved in MPS II. For patients carrying missense mutations outside the catalytic site, a possible treatment might rely on the development of other strategies such as enzyme enhancement therapy (EET) in which pharmacological chaperones are used to rescue misfolded or unstable proteins with the aim to increase protein function. The enormous advantage of these molecules is that they might cross the blood–brain barrier and diffuse through connective tissue matrices to reach target sites of pathology [13,14].

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References

[1] OMIM (on-line Mendelian inheritance in man), http://www.ncbi.nlm.nih.gov/Omim.
[2] E.F. Neufeld, J. Muenzer, The Mucopolysaccharidoses, in: C.R. Scriver, A.L. Beaudet, W.E. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, 8th ed., Mc Graw-Hill, New York, 2001, pp. 3421–3452.
[3] Genbank-EMBL, http://www.ncbi.nlm.nih.gov/Genbank/index.html.
[4] HGMD (Human Gene Mutation Database), http://archive.uwcm.ac.ukwcm/mg/hgmd0.htm.
[5] G. Bonuccelli, S. Regis, M. Filocamo, F. Corsolini, F. Caroli, R. Gatti, A deletion involving exons 2–4 in the iduronate-2-sulfatase gene of a patient with intermediate Hunter syndrome, Clin. Genet. 53 (1998) 474–477.
[6] G. Bonuccelli, M. Filocamo, S. Regis, F. Corsolini, R. Mazzotti, R. Gatti, A novel mutation, Y103X, and exon skipping in a patient with Hunter disease, Human Mutat. 15 (2000) 389.

[7] M. Filocamo, G. Bonuccelli, F. Corsolini, R. Mazzotti, R. Cusano, R. Gatti, Molecular analysis of 40 Italian patients with mucopolysaccharidosis type II: new mutations in the iduronate-2-sulfatase (IDS) gene, Human Mutat. 18 (2001) 164–165.
[8] V. Ricci, M. Filocamo, S. Regis, F. Corsolini, M. Stroppiano, M. Di Duca, R. Gatti, Expression studies of two novel in CIS-mutations identified in an intermediate case of Hunter syndrome, Am. J. Med. Genet., A 120 (2003) 84–87.
[9] V. Ricci, S. Regis, M. Di Duca, M. Filocamo, An Alu-mediated rearrangement as cause of exon skipping in Hunter disease, Hum. Genet. 112 (2003) 419–425.
[10] S. Lualdi, S. Regis, M. Di Rocco, F. Corsolini, M. Stroppiano, D. Antuzzi, M. Filocamo, Characterization of iduronate-2-sulfatase gene-pseudogene recombinations in 8 patients with Mucopolysaccharidosis type II revealed by rapid PCR-based method, Human Mutat. 25 (2005) 491–497.
[11] E. Wagner, J. Lykke-Andersen, mRNA surveillance: the perfect persist, J. Cell Sci., Suppl. 115 (2002) 3033–3038.
[12] L.E. Maquat, Nonsense-mediated mRNA decay in mammals, J. Cell Sci., Suppl. 118 (2005) 1773–1776.
[13] R.J. Desnick, E.H. Schuchman, Enzyme replacement and enhancement therapies: lessons from lysosomal disorders, Nat. Rev. Genet. 3 (2002) 954–966.
[14] R.J. Desnick, Enzyme replacement and enhancement therapies for lysosomal diseases, J. Inherit. Metab. Dis. 27 (2004) 385–410.
[15] "Cell Line and DNA Bank from Patients affected by Genetic Diseases" collection at http://www.gaslini.org/labdppm.htm.
[16] Y.V. Voznyi, J.L. Keulemans, O.P. van Diggelen, A fluorimetric enzyme assay for the diagnosis of MPS II (Hunter disease), J. Inherit. Metab. Dis. 24 (2001) 675–680.
[17] R.H. Flomen, E.P. Green, P.M. Green, D.R. Bentley, F. Giannelli, Determination of the organisation of coding sequences within the iduronate sulphate sulphatase (IDS) gene, Hum. Mol. Genet. 2 (1993) 5–10.
[18] E.I. Schwartz, S.P. Shevtsov, A.P. Kuchinski, P. Kovalev Yu, O.V. Plutalov, A. Berlin Yu, Approach to identification of a point mutation in apo B100 gene by means of a PCR-mediated site-directed mutagenesis, Nucleic Acids Res. 19 (1991) 3752.
[19] User Bulletin #2. Relative quantitation of gene expression. Standard curve method. ABI Prism 7700 Sequence Detection System (1997, updated 2001) Applied Biosystems. http://www.appliedbiosystems.com.
[20] S. Regis, S. Grossi, S. Lualdi, R. Biancheri, M. Filocamo, Diagnosis of Pelizaeus–Merzbacher disease: detection of proteolipid protein gene copy number by real-time PCR, Neurogenetics 6 (2005) 73–78.
[21] J.T. den Dunnen, Nomenclature for the description of sequence variations, http://www.hgvs.org/mutnomen/.
[22] P. Li, A.B. Bellows, J.N. Thompson, Molecular basis of iduronate-2-sulphatase gene mutations in patients with mucopolysaccharidosis type II (Hunter syndrome), J. Med. Genet. 36 (1999) 21–27.
[23] S. Karsten, E. Voskoboeva, S. Tishkanina, U. Pettersson, X. Krasnopolskaja, M.L. Bondeson, Mutational spectrum of the iduronate-2-sulfatase (IDS) gene in 36 unrelated Russian MPS II patients, Hum. Genet. 103 (1998) 732–735.
[24] P. Li, P. Huffman, J.N. Thompson, Mutations of the iduronate-2-sulfatase gene on a T146T background in three patients with Hunter syndrome, Human Mutat. 5 (1995) 272–274.
[25] R. Asselta, S. Duga, S. Spena, E. Santagostino, F. Peyvandi, G. Piseddu, R. Targhetta, M. Malcovati, P.M. Mannucci, M.L. Tenchini, Congenital afibrinogenemia: mutations leading to premature termination codons in fibrinogen A alpha-chain gene are not associated with the decay of the mutant mRNAs, Blood 98 (2001) 3685–3692.
[26] S. Danckwardt, G. Neu-Yilik, R. Thermann, U. Frede, M.W. Hentze, A.E. Kulozik, Abnormally spliced beta-globin mRNAs: a single point mutation generates transcripts sensitive and insensitive to nonsense-mediated mRNA decay, Blood 99 (2002) 1811–1816.
[27] J. Denecke, C. Kranz, D. Kemming, H.G. Koch, T. Marquardt, An activated 5′ cryptic splice site in the human ALG3 gene generates a premature termination codon insensitive to nonsense-mediated mRNA decay in a new case of congenital disorder of glycosylation type Id (CDG-Id), Human Mutat. 23 (2004) 477–486.
[28] L.W. Harries, C. Bingham, C. Bellanne-Chantelot, A.T. Hattersley, S. Ellard, The position of premature termination codons in the hepatocyte nuclear factor-1 beta gene determines susceptibility to nonsense-mediated decay, Hum. Genet. 25 (2005) 1–11.
[29] A. Inacio, A.L. Silva, J. Pinto, X. Ji, A. Morgado, F. Almeida, P. Faustino, J. Lavina, S.A. Liebhaber, L. Romao, Nonsense mutations in close proximity to the initiation codon fail to trigger full nonsense-mediated mRNA decay, J. Biol. Chem. 279 (2004) 32170–32180.
[30] L. Perrin-Vidoz, O.M. Sinilnikova, D. Stoppa-Lyonnet, G.M. Lenoir, S. Mazoyer, The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1mRNAs bearing premature termination codons, Hum. Mol. Genet. 11 (2002) 2805–2814.
[31] J. Zhang, L.E. Maquat, Evidence that translation reinitiation abrogates nonsense-mediated mRNA decay in mammalian cells, EMBO J. 16 (1997) 826–833.
[32] E. Pappalardo, L.C. Zingale, M. Cicardi, C1 inhibitor gene expression in patients with hereditary angioedema: quantitative evaluation by means of real-time RT-PCR, J. Allergy Clin. Immunol. 114 (2004) 638–644.
[33] M.J. Moore, From birth to death: the complex lives of eukaryotic mRNAs, Science 309 (2005) 1514–1518.
[34] G.R. Villani, A. Daniele, N. Balzano, P. Di Natale, Expression of five iduronate-2-sulfatase site-directed mutations, Biochim. Biophys. Acta 1501 (2000) 71–80.
[35] E.J. Parkinson, V. Muller, J.J. Hopwood, D.A. Brooks, Iduronate-2-sulphatase protein detection in plasma from mucopolysaccharidosis type II patients, Mol. Genet. Metab. 81 (2004) 58–64.
[36] E. Parkinson-Lawrence, C. Turner, J. Hopwood, D. Brooks, Analysis of normal and mutant iduronate-2-sulphatase conformation, Biochem. J. 386 (2005) 395–400.
[37] A. Daniele, C.J. Faust, G.E. Herman, P. Di Natale, A. Ballabio, Cloning and characterization of the cDNA for the murine iduronate sulfatase gene, Genomics 16 (1993) 755–757.
[38] T.C. Olsen, H.G. Eiken, P.M. Knappskog, B.F. Kase, J.E. Mansson, H. Boman, J. Apold, Mutations in the iduronate-2-sulfatase gene in five Norwegians with Hunter syndrome, Hum. Genet. 97 (1996) 198–203.
[39] E. Vafiadaki, A. Cooper, L.E. Heptinstall, C.E. Hatton, M. Thornley, J.E. Wraith, Mutation analysis in 57 unrelated patients with MPS II (Hunter's disease), Arch. Dis. Child. 79 (1998) 237–241.
[40] I. Moreira da Silva, R. Froissart, H. Marques dos Santos, C. Caseiro, I. Maire, D. Bozon, Molecular basis of mucopolysaccharidosis type II in Portugal: identification of four novel mutations, Clin. Genet. 60 (2001) 316–318.
[41] J.J. Hopwood, S. Bunge, C.P. Morris, P.J. Wilson, C. Steglich, M. Beck, E. Schwinger, A. Gal, Molecular basis of mucopolysaccharidosis type II: mutations in the iduronate-2-sulphatase gene, Human Mutat. 2 (1993) 435–442.
[42] E. Popowska, M. Rathmann, A. Tylki-Szymanska, S. Bunge, C. Steglich, E. Schwinger, A. Gal, Mutations of the iduronate-2-sulfatase gene in 12 Polish patients with mucopolysaccharidosis type II (Hunter syndrome), Human Mutat. 5 (1995) 97–100.
[43] M. Rathmann, S. Bunge, M. Beck, H. Kresse, A. Tylki-Szymanska, A. Gal, Mucopolysaccharidosis type II (Hunter syndrome): mutation "hot spots" in the iduronate-2-sulfatase gene, Am. J. Hum. Genet. 59 (1996) 1202–1209.
[44] S. Bunge, C. Steglich, C. Zuther, M. Beck, C.P. Morris, E. Schwinger, A. Schinzel, J.J. Hopwood, A. Gal, Iduronate-2-sulfatase gene mutations in 16 patients with mucopolysaccharidosis type II (Hunter syndrome), Hum. Mol. Genet. 2 (1993) 1871–1875.
[45] R. Froissart, I. Moreira da Silva, N. Guffon, D. Bozon, I. Maire, Mucopolysaccharidosis type II-genotype/phenotype aspects, Acta Paediatr. 91 (2002) 82–87.
[46] L. Gort, A. Chabas, M.J. Coll, Hunter disease in the Spanish population: molecular analysis in 31 families, J. Inherit. Metab. Dis. 21 (1998) 655–661.
[47] P.J. Wilson, C.P. Morris, D.S. Anson, T. Occhiodoro, J. Bielicki, P.R. Clements, J.J. Hopwood, Hunter syndrome: isolation of an iduronate-2-

sulfatase cDNA clone and analysis of patient DNA, Proc. Natl. Acad. Sci. 87 (1990) 8531–8535.

[48] S. Tomatsu, K.O. Orii, Y. Bi, M.A. Gutierrez, T. Nishioka, S. Yamaguchi, N. Kondo, T. Orii, A. Noguchi, W.S. Sly, General implications for CpG hot spot mutations: methylation patterns of the human iduronate-2-sulfatase gene locus, Human Mutat. 23 (2004) 590–598.

[49] N.J. Weinreb, J. Charrow, H.C. Andersson, P. Kaplan, E.H. Kolodny, P. Mistry, G. Pastores, B.E. Rosenbloom, C.R. Scott, R.S. Wappner, A. Zimran, Effectiveness of enzyme replacement therapy in 1028 patients with type 1 Gaucher disease after 2 to 5 years of treatment: a report from the Gaucher Registry, Am. J. Med. 113 (2002) 112–119.