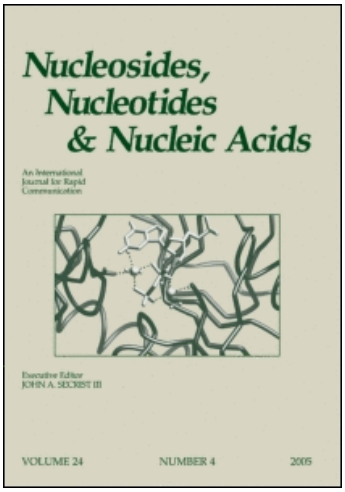

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Genetic Analysis of the First 4 Patients with β-Ureidopropionase Deficiency

A. B. P. van Kuilenburg[a]; R. Meinsma[a]; B. Assman[b]; G. F. Hoffman[c]; T. Voit[d]; A. Ribes[e]; I. Lorente[f]; R. Busch[g]; E. Mayatepek[b]; N. G. G. M. Abeling[a]; R. A. Wevers[h]; F. Rutsch[g]; A. H. van Gennip[i]
[a] Department of Clinical Chemistry, Academic Medical Center, Emma Children's Hospital, Amsterdam, the Netherlands [b] Department of General Pediatrics, University Children's Hospital, Düsseldorf, Germany [c] Department of Pediatrics, University Hospital Heidelberg, Heidelberg, Germany [d] Department of Pediatrics, University Hospital Essen, Essen, Germany [e] Institut de Bioquímica Clínica, CDB, Hospital Clínic, Barcelona, Spain [f] Hospital Parc Taulí, Sabadell, Barcelona, Spain [g] Klinik für Kinder- und Jugendmedizin, Klinikum Dortmund gGmbH, Dortmund, Germany [h] Institute of Neurology, University Medical Center Nijmegen, Nijmegen, the Netherlands [i] Department of Clinical Genetics and Clinical Chemistry, Academic Hospital Maastricht, Maastricht, the Netherlands

To cite this Article van Kuilenburg, A. B. P. , Meinsma, R. , Assman, B. , Hoffman, G. F. , Voit, T. , Ribes, A. , Lorente, I. , Busch, R. , Mayatepek, E. , Abeling, N. G. G. M. , Wevers, R. A. , Rutsch, F. and van Gennip, A. H.(2006) 'Genetic Analysis of the First 4 Patients with β-Ureidopropionase Deficiency', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 1093 — 1098
To link to this Article: DOI: 10.1080/15257770600956870
URL: http://dx.doi.org/10.1080/15257770600956870

Nucleosides, Nucleotides, and Nucleic Acids, 25:1093–1098, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600956870

Taylor & Francis
Taylor & Francis Group

GENETIC ANALYSIS OF THE FIRST FOUR PATIENTS WITH β-UREIDOPROPIONASE DEFICIENCY

A. B. P. van Kuilenburg and R. Meinsma □ *Department of Clinical Chemistry, Academic Medical Center, Emma Children's Hospital, Amsterdam, the Netherlands*

B. Assman □ *Department of General Pediatrics, University Children's Hospital, Düsseldorf, Germany*

G. F. Hoffman □ *Department of Pediatrics, University Hospital Heidelberg, Heidelberg, Germany*

T. Voit □ *Department of Pediatrics, University Hospital Essen, Essen, Germany*

A. Ribes □ *Institut de Bioquímica Clínica, CDB, Hospital Clínic, Barcelona, Spain*

I. Lorente □ *Hospital Parc Taulí, Sabadell, Barcelona, Spain*

R. Busch □ *Klinik für Kinder- und Jugendmedizin, Klinikum Dortmund gGmbH, Dortmund, Germany*

E. Mayatepek □ *Department of General Pediatrics, University Children's Hospital, Düsseldorf, Germany*

N. G. G. M. Abeling □ *Department of Clinical Chemistry, Academic Medical Center, Emma Children's Hospital, Amsterdam, the Netherlands*

R. A. Wevers □ *Institute of Neurology, University Medical Center Nijmegen, Nijmegen, the Netherlands*

F. Rutsch □ *Klinik für Kinder- und Jugendmedizin, Klinikum Dortmund gGmbH, Dortmund, Germany*

A. H. van Gennip □ *Department of Clinical Genetics and Clinical Chemistry, Academic Hospital Maastricht, Maastricht, the Netherlands*

Address correspondence to A.B.P. van Kuilenburg, Department of Clinical Chemistry, Academic Medical Center, Emma Children's Hospital, Amsterdam, the Netherlands. E-mail: a.b.vanKuilenburg@amc.uva.ni

□ *β-Ureidopropionase is the third enzyme of the pyrimidine degradation pathway and it catalyses the irreversible hydrolysis of N-carbamyl-β-aminoisobutyric acid or N-carbamyl-β-alanine to β-aminoisobutyric acid or β-alanine, ammonia, and CO_2. Analysis of the β-ureidopropionase gene (UPB1) of the first 4 patients presenting with a complete enzyme deficiency, revealed the presence of 2 splice-site mutations (IVS1-2A>G and IVS8-1G>A) and one missense mutation (A85E). RT-PCR analysis of the complete β-ureidopropionase cDNA suggested that both splice-site mutations lead to a variety of alternative splice variants, with deletions of a single or several exons. The alanine at position 85 was not conserved in other eukaryotic β-ureidopropionase protein sequences.*

Keywords Pyrimidines; β-Alanine synthase; β-Ureidopropionase; *UPB1*

INTRODUCTION

In man, the pyrimidine bases uracil and thymine are degraded via a three-step pathway. Dihydropyrimidine dehydrogenase is the initial and rate-limiting enzyme, catalysing the reduction of thymine and uracil to 5,6-dihydrothymine and 5,6-dihydrouracil, respectively. The second step consists of a hydrolytic ring opening which is catalysed by dihydropyrimidinase. Finally, the resulting N-carbamyl-ß-aminoisobutyric acid or N-carbamyl-ß-alanine are converted in the third step to ß-aminoisobutyric acid or ß-alanine, ammonia and CO_2 by β-ureidopropionase.

To date, approximately 50 patients have been described with a defect of dihydropyrimidine dehydrogenase and 9 patients with a deficiency of dihydropyrimidinase. The clinical phenotype of these patients was highly variable but centered around neurological problems. Recently, the first 4 patients with a defect of ß-ureidopropionase have been reported.[1] Analysis of the β-ureidopropionase gene (*UPB1*) of these patients revealed the presence of two splice-site mutations (IVS1-2 A>G and IVS8-1G>A) and one missense mutation (A85E).[1] In this paper, we analysed the effect of the 2 splice-site mutations and the A85E mutation at the cDNA and protein sequence level, respectively.

MATERIALS AND METHODS

The human β-ureidopropionase cDNA was amplified as follows. Reverse transcription was performed with 200 U of Moloney Murine Leukemia Virus reverse transcriptase (New England Biolabs Inc., Beverly, MA, USA) using 4 μg of total RNA isolated from peripheral blood mononuclear (PBM) cells and a $(dT)_{15}$-oligonucleotide and/or random hexamers as primer. The first amplification resulted in a fragment of 1,402 bp (see Table 1). A nested PCR reaction was subsequently carried out in order to amplify three overlapping fragments extending from –32 to +471, +371 to +903 and +803 tot +1339, respectively (Table 1). Forward primers contained an

TABLE 1 PCR Primers to Amplify the Human β-Ureidopropionase cDNA

cDNA[a] position	Forward primer (5′-> 3′)	Reverse primer (5′-> 3′)	Product (bp)
−44 to +1358	gcaggcagttcgtgcgc	gctaacccttttggggac	1402
−32 to +471	tgcgcggacacaagcact[b]	gttcttcgccagcttctg[b]	503
+371 to +903	cctttgccttctgtacgag[b]	tcccgaggtaaactcgttc[b]	533
+803 to +1339	cccatcgaggccagaaac[b]	cttatatcccccactccac[b]	537

[a]cDNA positions are according to the published cDNA sequence, GenBank accession nr.AF163312.

[b]Forward primers contained an 5′-TGTAAAACGACGGCCAGT-3′ extension whereas reverse primers contained an 5′-CAGGAAACAGCTATGACC-3′extension at their 5′ends.

5'-TGTAAAACGACGGCCAGT-3' extension at their 5'-ends, whereas reverse primers contained an 5'-CAGGAAACAGCTATGACC-3' extension at their 5' ends. These sequences are complementary to fluorescent labelled –21M13 and M13 reversed primers used in the dye-primer sequence reaction (Dye-primer cycle-sequence-ready reaction kit, Applied Biosystems (San Jose, CA, USA).

Amplification was carried out in 50 μl reaction mixtures containing 10 mM Tris/HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1 μM of each primer, 0.2 mM dNTPs. After initial denaturation for 5 minutes at 95°C, 2 U of Taq polymerase was added and amplification was carried out for 30 cycles (30 seconds 95°C, 1 minute 60°C, 2 minutes 72°C). In case of the nested PCR, amplification was carried out for 30 cycles (30 secconds 95°C, 1 minutes 60°C, 1 minute 72°C). PCR products were separated on 1% agarose gels, visualized with ethidium bromide and purified using a Qiaquick Gel Extraction kit or used for direct sequencing. Sequence analysis was performed on an Applied Biosystems 377 automated DNA sequencer.

RESULTS

The genomic organisation of the *UPB1* gene and the mutations identified in the 4 patients are shown in Figure 1A. Total RNA was isolated from PBM cells of one of the patients, who proved to be compound heterozygous for a splice acceptor site mutation IVS1-2 A>G in intron 1 and a splice acceptor site mutation IVS8-1G>A in intron 8, and his parents and subjected to RT-PCR in order to amplify three overlapping regions encompassing the complete β-ureidopropionase cDNA. Although this procedure worked well with a number of control samples, yielding single fragments of the expected size, amplification of the patient's sample resulted in a series of faint bands differing from the expected size for both the 5' fragment (-32 tot +471) and the middle fragment (+371 tot +903) (see Materials and Methods, Table 1), whereas the 3' fragment (+803 tot +1339) could hardly be amplified at all.

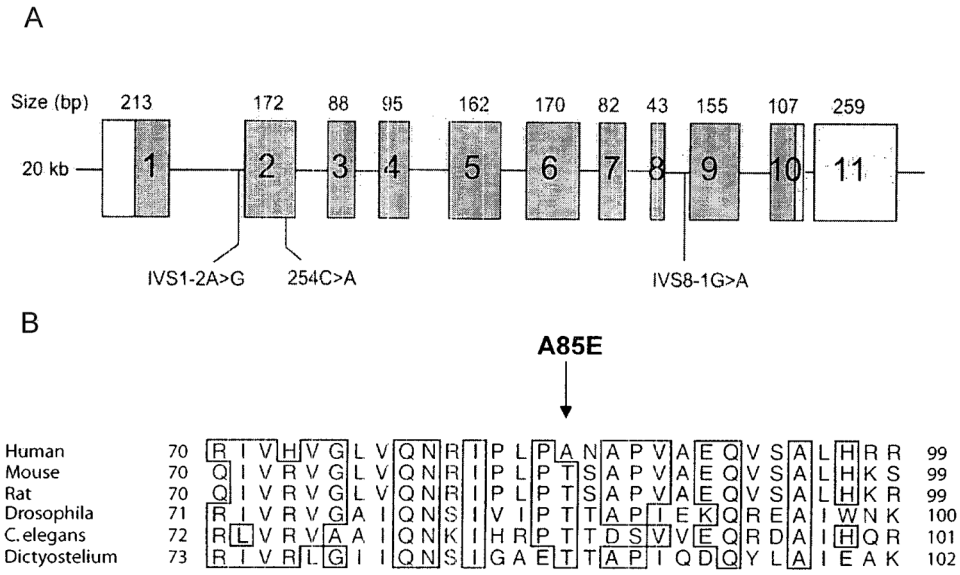

FIGURE 1 Genomic organization and mutation analyses of the *UPB1* gene. Panel A shows the *UPB1* gene which consists of 11 exons with an open reading frame of 1152 bp (depicted in gray). The different mutations identified in the three patients with a complete β-ureidopropionase deficiency are indicated. Panel B shows the alignment of the amino-acid sequences of β-ureidopropionase from human, mouse, rat, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Dictyostelium discoideum*. (GenBank accession numbers NP_057411, NP_598756, NP_446297, NP_649732, NP_495261, AF333186, respectively). Conserved amino-acid sequences are boxed and the arrow indicates the position of the mutation A85E in human β-ureidopropionase.

Apparently, both mutations lead to a variety of alternative splice variants with deletions of a single up to several exons. Sequence analysis of 2 abundant splice variants showed skipping of exon 2 (172 bp) in one product and deletion of exon 6 (170 bp) in another. Both of these deletions lead to a frameshift and probably a nonfunctional transcript. Since the second mutation is located in intron 8, one would also expect a transcript lacking only exon 9. This product could, however, not be detected, probably due to instability of this transcript. Analysis of the parents revealed that the father is heterozygous for the intron 1 mutation, and the mother is heterozygous for the intron 8 mutation. Gel electrophoresis showed that the splice variant lacking exon 2 could be detected in the paternal sample and was absent from the maternal sample, whereas the splice variant lacking exon 6 could be detected in the maternal sample, but not in the paternal sample.

Analysis of the genomic sequences of *UPB1* showed that one of the patients was homozygous for a missense mutation 254C>A in exon 2 of *UPB1,* leading to the amino acid substitution A85E (Figure 1). Alignment of various eukaryotic β-ureidopropionase protein sequences revealed that the Alanine at position 85 is replaced by a Threonine in β-ureidopropionase from mouse, rat, *Drosophila melanogaster*, *Caenorhabditis elegans*, and

Dictyostelium discoideum (Figure 1B). However, heterologous expression of the mutant enzyme in *Escherichia coli* showed that the A85E mutation resulted in a mutant β-ureidopropionase enzyme without residual activity.[1]

DISCUSSION

Recently, we have shown that the analysis of *UPB1* of 4 patients suffering from a complete β-ureidopropionase deficiency revealed the presence of two splice-site mutations (IVS1-2 A>G and IVS8-1G>A) and one missense mutation (A85E). Although the Alanine at position 85 was not conserved in the human sequence compared to other eukaryotic β-ureidopropionase protein sequences, heterologous expression of the mutant enzyme in *E. coli* showed that the mutant enzyme did not possess any residual activity.[1] The introduction of a negative charge at position 85 might result in mis-folding of the protein and/or inactivation of the enzyme. It should be noted that mammalian β-ureidopropionase appears to have been only relatively conserved throughout evolution, as a comparison of the deduced amino acid sequences of human β-ureidopropionase with that of rat, showed a homology of 84%. Nevertheless, the analysis of a variety of eukaryotic β-ureidopropionases showed that they are functionally related, despite a high degree of structural diversity.[2]

Systematic analysis of the sequences present in the vicinity of the splice junctions has led to consensus sequences for both the splice acceptor and splice donor sites.[3] A scoring system developed by Shapiro and Senapathy showed that most naturally occurring splice acceptor and splice donor sites yielded scores above 0.70. Analysis of the consensus sequence of the authentic splice acceptor site of exon 2 showed that the A→G point mutation reduces the consensus value from 0.80 (wild type) to 0.63 (mutant). Furthermore, the G→A mutation in the splice acceptor site of exon 9 reduces the consensus value from 0.81 (wild type) to 0.65 (mutant).

β-Ureidopropionase is expressed mainly in liver and kidney and only a very low activity can be detected in other tissues.[4] Nevertheless, with RT-PCR we were able to amplify the mRNA coding for human β-ureidopropionase in PBM cells. Apparently, both splice acceptor site mutations IVS1-2 A>G and IVS8-1G>A led to a variety of alternative splice variants with deletions of a single up to several exons. Mutations in splice acceptor sites often lead to the use of cryptic acceptor sites within the downstream exon or within the upstream intron, exon skipping or inclusion of the upstream intron.[5] The abundance and stability of mRNA generated by the use of these alternative sites depends on whether the products are in frame, whether they lead to premature termination codons within the coding sequences and whether the termination codon is succeeded by an intron.[5]

Pyrimidines play an important role in the regulation of the central nervous system and metabolic changes affecting the levels of pyrimidines may lead to abnormal neurological activity. Furthermore, defects in the first two enzymes of the pyrimidine degradation pathway have been associated with severe life-threatening toxicities when (partially) deficient individuals were treated with the widely used chemotherapeutic agent 5-fluorouracil.[6,7] Thus, it is conceivable that patients with a complete β-ureidopropionase deficiency are also at risk of developing severe 5-fluorouracil toxicity. The diagnosis and characterisation of patients with a deficiency in one of the enzymes of the pyrimidine degradation pathway is of paramount importance, not only to avoid severe toxicity during treatment of tumour patients with 5-fluorouracil but also to gain further insight in the relationship between the genetic and biochemical abnormalities and the clinical phenotype.

REFERENCES

1. van Kuilenburg, A.B.P.; Meinsma, R.; Beke, E.; Assmann, B.; Ribes, A.; Lorente, I.; Busch, R.; Mayatepek, E.; Abeling, N.G.G.M.; van Cruchten, A.; Stroomer, A.E.M.; van Lenthe, H.; Zoetekouw, L.; Kulik, W.; Hoffmann, G.F.; Voit, T.; Wevers, R.A.; Rutsch, F.; van Gennip, A.H. β-Ureidopropionase deficiency: an inborn error of pyrimidine degradation associated with neurological abnormalities. *Hum. Mol. Genet.* **2004**, 13, 2793–2801.
2. Gojković, Z.; Sandrini, M.P.B.; Piskur, J. Eukaryotic beta-alanine synthases are functionally related but have a high degree of structural diversity. *Genetics* **2001**, 158, 999–1011.
3. Shapiro, M.B.; Senapathy, P. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. *Nucleic. Acids. Res.* **1987**, 15, 7155–7174.
4. van Kuilenburg, A.B.P.; van Lenthe, H.; van Gennip, A.H. Activity of pyrimidine degradation enzymes in normal tissues. *Nucleos. Nucleot. Nucl.* **2006**, 25 (this issue).
5. Antonarakis, S.E.; Krawczak, M.; Cooper, D.N. The nature and mechanisms of human gene mutation. *In The Metabolic & Molecular Bases of Inherited Disease*,. Scriver, C.R.; Beaudet, A.L.; Sly, W.S.; Valle, D., Eds., McGraw-Hill: New York, 2001, 8th ed., 343–377.
6. van Kuilenburg, A.B.P. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur. J. Cancer* **2004**, 40, 939–950.
7. van Kuilenburg, A.B.P.; Meinsma, J.R.; Zonnenberg, B.A.; Zoetekouw, L.; Baas, F.; Matsuda, K.; Tamaki, N.; van Gennip, A.H. Dihydropyrimidinase deficiency and severe 5-Fluorouracil toxicity. *Clin. Cancer Res.* **2003**, 9, 4363–4367.