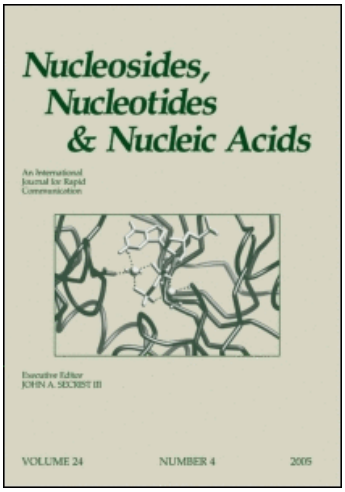

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Dihydropyrimidine Dehydrogenase Deficiency Caused by a Novel Genomic Deletion c.505_513del of *DPYD*

A. B. P. van Kuilenburg[a]; J. Meijer[a]; G. Gökcay[b]; T. Baykal[b]; M. E. Rubio-Gozalbo[c]; A. N. P. M. Mul[a]; C. E. M. de Die-Smulders[c]; P. Weber[d]; A. Capone Mori[e]; J. Bierau[c]; B. Fowler[d]; K. Macke[f]; J. O. Sass[g]; R. Meinsma[a]; J. B. Hennermann[h]; P. Miny[d]; L. Zoetekouw[a]; J. Roelofsen[a]; R. Vijzelaar[i]; J. Nicolai[c]; R. C. M. Hennekam[a]
[a] Academic Medical Center, Amsterdam, the Netherlands [b] Istanbul University, Istanbul, Turkey [c] University Hospital Maastricht, Maastricht, the Netherlands [d] University Children's Hospital Basel, Basel, Switzerland [e] Children's Hospital Aarau, Aarau, Switzerland [f] Rheinhessen-Fachklinik, Alzey, Germany [g] Universitätsklinikum Freiburg, Freiburg, Germany [h] Charité Universitaetsmedizin Berlin, Berlin, Germany [i] MRC-Holland b.v., Amsterdam, the Netherlands

Online publication date: 11 June 2010

To cite this Article van Kuilenburg, A. B. P. , Meijer, J. , Gökcay, G. , Baykal, T. , Rubio-Gozalbo, M. E. , Mul, A. N. P. M. , de Die-Smulders, C. E. M. , Weber, P. , Mori, A. Capone , Bierau, J. , Fowler, B. , Macke, K. , Sass, J. O. , Meinsma, R. , Hennermann, J. B. , Miny, P. , Zoetekouw, L. , Roelofsen, J. , Vijzelaar, R. , Nicolai, J. and Hennekam, R. C. M.(2010) 'Dihydropyrimidine Dehydrogenase Deficiency Caused by a Novel Genomic Deletion c.505_513del of *DPYD*', Nucleosides, Nucleotides and Nucleic Acids, 29: 4, 509 — 514
To link to this Article: DOI: 10.1080/15257771003730227
URL: http://dx.doi.org/10.1080/15257771003730227

Nucleosides, Nucleotides and Nucleic Acids, 29:509–514, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003730227

DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY CAUSED BY A NOVEL GENOMIC DELETION c.505_513del of *DPYD*

A. B. P. van Kuilenburg,[1] J. Meijer,[1] G. Gökcay,[2] T. Baykal,[2] M. E. Rubio-Gozalbo,[3] A. N. P. M. Mul,[1] C. E. M. de Die-Smulders,[3] P. Weber,[4] A. Capone Mori,[5] J. Bierau,[3] B. Fowler,[4] K. Macke,[6] J. O. Sass,[7] R. Meinsma,[1] J. B. Hennermann,[8] P. Miny,[4] L. Zoetekouw,[1] J. Roelofsen,[1] R. Vijzelaar,[9] J. Nicolai,[3] and R. C. M. Hennekam[1]

[1]*Academic Medical Center, Amsterdam, the Netherlands*
[2]*Istanbul University, Istanbul, Turkey*
[3]*University Hospital Maastricht, Maastricht, the Netherlands*
[4]*University Children's Hospital Basel, Basel, Switzerland*
[5]*Children's Hospital Aarau, Aarau, Switzerland*
[6]*Rheinhessen-Fachklinik, Alzey, Germany*
[7]*Universitätsklinikum Freiburg, Freiburg, Germany*
[8]*Charité Universitaetsmedizin Berlin, Berlin, Germany*
[9]*MRC-Holland b.v., Amsterdam, the Netherlands*

□ *Dihydropyrimidine dehydrogenase (DPD) deficiency is an autosomal recessive disorder of the pyrimidine degradation pathway. In a patient presenting with convulsions, psychomotor retardation and Reye like syndrome, strongly elevated levels of uracil and thymine were detected in urine. No DPD activity could be detected in peripheral blood mononuclear cells. Analysis of the gene encoding DPD (DPYD) showed that the patient was homozygous for a novel c.505_513del (p.169_171del) mutation in exon 6 of DPYD.*

Keywords Dihydropyrimidine dehydrogenase; *DPYD*; pyrimidine; deletions

INTRODUCTION

Dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2) is the initial and rate-limiting enzyme in the catabolism of the pyrimidine bases. It catalyzes the reduction of uracil and thymine to 5,6-dihydrouracil and 5,6-dihydrothymine, respectively. DPD deficiency is an autosomal recessive disorder characterized by thymine-uraciluria in patients with a complete

Address correspondence to A. B. P. van Kuilenburg, Academic Medical Center, Amsterdam, Laboratory Genetic Metabolism Diseases F0-224, Meibergrdreef 9, 1105 AZ Amsterdam, the Netherlands. E-mail: a.b.vanKuilenburg@amc.uva.nl

enzyme deficiency. In children, deficiency of DPD is often accompanied by a neurological disorder, but a considerable phenotypic variability has been reported among these patients.[1–4]

DPD is also responsible for the breakdown of the widely used antineoplastic agent 5-fluorouracil (5FU). In this light, a pharmacogenetic disorder has been described concerning oncologic patients with a complete or partial deficiency of DPD suffering from severe toxicity, including death, following the administration of 5FU.[5,6] A number of these patients proved to be heterozygous for a mutant allele of the gene encoding DPD (*DPYD*).[7] The identification of novel genetic events underlying a DPD deficiency is essential to gain an understanding of the phenotypic variability of the disease, as observed in patients with a complete deficiency, and to identify cancer patients at risk of fluoropyrimidine toxicity.

MATERIALS AND METHODS

Analysis of Pyrimidine Bases and DPD Activity

The pyrimidine bases uracil and thymine in urine and plasma were analyzed using HPLC electrospray tandem mass spectrometry, as described before.[8] The activity of DPD was determined in peripheral blood mononuclear (PBM) cells using radiolabeled thymine followed by separation of radiolabeled thymine from radiolabeled dihydrothymine using reversed-phase HPLC.[9]

Mutation Analysis of DPYD

DNA was isolated from EDTA blood using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany). PCR amplification of all 23 coding exons and flanking intronic regions was carried out using intronic primer sets, as described before.[7] Sequence analysis of genomic fragments amplified by PCR was carried out on an Applied Biosystems model 3730 automated DNA sequencer using the dye-terminator method.

RESULTS

The patient was a 16-month-old girl born by caesarean section because of foetal distress. Initial symptoms were poor sucking, respiratory distress, generalized convulsions and Reye like syndrome at the age of 10 days. The patient was treated with peritoneal dialysis and benzoate with good response. At the age of 40 days, the patient presented with mild hepatomegaly. Subsequently, the patient showed a transient failure to thrive, hypertonia, and developmental milestones were mildly delayed. Initial laboratory analysis showed hypoglycaemia, elevated blood lactate and ammonia levels.

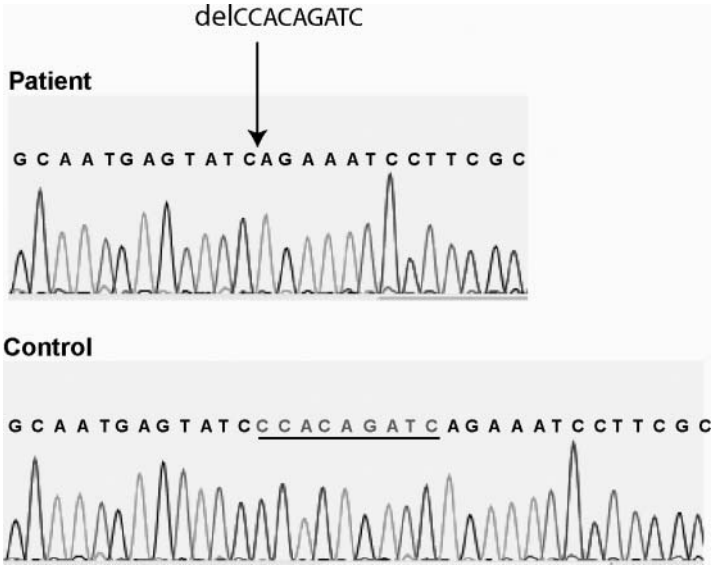

FIGURE 1 Sequence analysis of part of exon 6 of *DPYD*. Part of the coding sequence of exon 6 (c.493_526) is shown for the patient and a control. The deleted region c.505_513 is indicated by the arrow and underlined in the control.

Quantitative amino acid analysis in urine gathered in the period of acute illness showed a generalized hyperaminoaciduria, which was thought to be explained by the illness and renal tubular immaturity. The acylcarnitine profile was unremarkable. Analysis of a urine sample for purine and pyrimidine metabolism disturbances showed strongly elevated levels of uracil (766 μmol/mmol creatinine; controls 7 $\pm$ 6 μmol/mmol creatinine, $n = 112$) and thymine (637 μmol/mmol creatinine; controls 0.1 $\pm$ 0.3 μmol/mmol creatinine, $n = 112$). In addition, 5-hydroxymethyluracil was present (25 μmol/mmol creatinine) which is normally not detected in urine. Highly elevated concentrations of uracil (27 μM; controls <0.4 μM; $n = 40$) and thymine (41 μM; controls <0.1 μM; $n = 40$) were detected in plasma as well. The DPD activity in PBM cells was undetectable (<0.018 nmol/mg/h; controls: 9.9 $\pm$ 2.8 nmol/mg/h).

Analysis of *DPYD* for the presence of mutations showed that the patient was homozygous for a novel c.505_513del mutation in exon 6 of *DPYD* (Figure 1). As a result the mature mRNA lacks a 9 nucleotide segment encoding the amino acids 169–171. Carrier analysis showed that the parents of the patient were heterozygous for the c.505_513del (p.169_171del) mutation.

DISCUSSION

Dihydropyrimidine dehydrogenase (DPD) deficiency is an infrequently described autosomal recessive disorder of the pyrimidine degradation pathway. Various mutations and polymorphisms have been identified in the DPD

gene (*DPYD*) leading to a phenotype that is mainly characterized by mental and motor retardation and convulsions. To date, the pathological mechanism underlying the various clinical abnormalities is still not known. An altered homeostasis of the *β*-aminoisobutyric acid, a downstream product of thymine, might underlie some of the cerebral dysfunctions often seen in patients with a DPD deficiency.[10]

The analysis of *DPYD* in paediatric patients with a complete DPD deficiency has contributed significantly to the identification of disease-causing mutations.[1–3] *DPYD* is present as a single copy gene on chromosome 1p21.3 and consists of 23 exons.[11] A physical map indicates that *DPYD* is at least 950 kb in length with 3 kb of coding sequence and an average intron size of about 43 kb.[11] Recently, it has been shown that the common fragile site *FRA1E* extended over 370 kb within *DPYD* and the region with the highest fragility encompassed the central part of *DPYD* including exons 13–16.[12] Common fragile sites represent chromosomal structures that are particularly prone to breakage under replication stress and the genomic instability can give rise to deletions, translocations, and amplifications.[12]

To date, only four deletions have been described in patients with a complete DPD deficiency, resulting in a deletion of 1 to 4 nucleotides of the coding exons of *DPYD* (Figure 2). These include the mutations c.100delA,[13] c.299_302delTCAT,[14] c.1041_1041delTG,[3] and the c.1897delC.[15] In addition, large intragenic rearrangements of *DPYD* have been identified in severely affected patients with a DPD deficiency.[16] In three patients, including two siblings, a 13.8 kb deletion of exon 12 was found and in one patient a 122 kb deletion of exon 14–16 of *DPYD*. In the fifth patient, a c.299_302delTCAT mutation in exon 4 was found in addition to loss of heterozygosity of the entire DPD gene. Further analysis demonstrated a de novo deletion of approximately 14 Mb of chromosome 1p13.3–1p21.3, which included *DPYD*.[16] Thus, the finding of a novel c.505_513del mutation in exon 6 of *DPYD* in our patient indicates that screening for genomic deletions

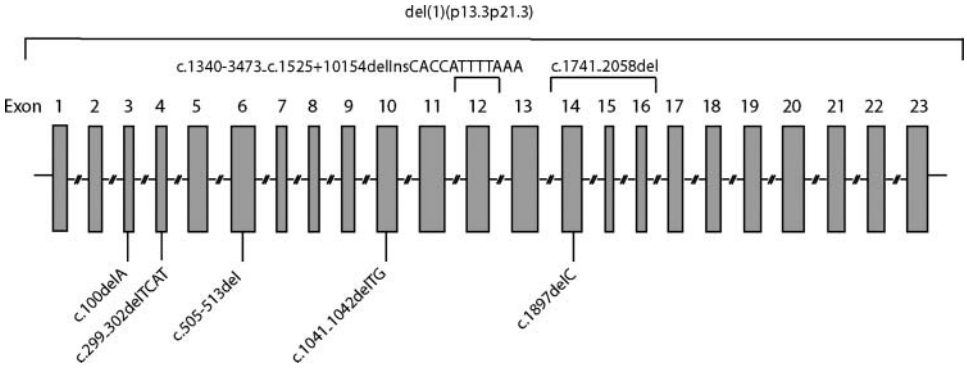

FIGURE 2 Organization of *DPYD*: *DPYD* consists of 23 exons with an open reading frame of 3075 bp. The different deletions identified in patients with a complete DPD deficiency are indicated.

should be considered using for example sequence analysis combined with multiplex ligation-dependent probe amplification and array-based comparative genomic hydridization for the relative quantification of genomic sequences.

REFERENCES

1. Fernandez-Salguero, P.M.; Sapone, A.; Wei, X.; Holt, J.R.; Jones, S.; Idle, J.R.; Gonzalez, F.J. Lack of correlation between phenotype and genotype for the polymorphically expressed dihydropyrimidine dehydrogenase in a family of Pakistani origin. *Pharmacogenetics* **1997**, 7, 161–163.
2. van Kuilenburg, A.B.P.; Vreken, P.; Abeling, N.G.G.M.; Bakker, H.D.; Meinsma, J.R.; van Lenthe, H.; De Abreu, R.A.; Smeitink, J.A.M.; Kayserili, H.; Apak, M.Y.; Christensen, E.; Holopainen, I.; Pulkki, K.; Riva, D.; Botteon, G.; Holme, E.; Tulinius, M.; Kleijer, W.J.; Beemer, F.A.; Duran, M.; Niezen-Koning, K.E.; Smit, G.P.A.; Jakobs, C.; Smit, L.M.E.; Moog, U.; Spaapen, L.J.M.; Van Gennip, A.H. Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Hum. Genet.* **1999**, 104, 1–9.
3. van Kuilenburg, A.B.P.; Dobritzsch, D.; Meinsma, J.R.; Haasjes, J.; Waterham, H.R.; Nowaczyk, M.J.M.; Maropoulos, G.D.; Hein, G.; Kalhoff, H.; Kirk, J.M.; Baaske, H.; Aukett, A.; Duley, J.A.; Ward, K.P.; Lindqvist, Y.; van Gennip, A.H. Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. *Biochem. J.* **2002**, 364, 157–163.
4. Au, K.M.; Lai, C.K.; Yuen, Y.P.; Shek, C.C.; Lam, C.W.; Chan, A.Y.W. Diagnosis of dihydropyrimidine dehydrogenase deficiency in a neonate with thymine-uraciluria. *Hong. Kong. Med. J.* **2003**, 9, 130–132.
5. van Kuilenburg, A.B.P. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur. J. Cancer* **2004**, 40, 939–950.
6. Diasio, R.B.; Beavers, T.L.; Carpenter, J.T. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. *J. Clin. Invest.* **1988**, 81, 47–51.
7. van Kuilenburg, A.B.P.; Haasjes, J.; Richel, D.J.; Zoetekouw, L.; van Lenthe, H.; De Abreu, R.A.; Maring, J.G.; Vreken, P.; van Gennip, A.H. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. *Clin. Cancer Res.* **2000**, 6, 4705–4712.
8. van Lenthe, H.; van Kuilenburg, A.B.P.; Ito, T.; Bootsma, A.H.; van Cruchten, A.G.; Wada, Y.; van Gennip, A.H. Defects in pyrimidine degradation identified by HPLC-electrospray tandem mass spectrometry of urine specimens or urine-soaked filter paper strips. *Clin. Chem.* **2000**, 46, 1916–1922.
9. van Kuilenburg, A.B.P.; van Lenthe, H.; Tromp, A.; Veltman, P.C.J.; van Gennip, A.H. Pitfalls in the diagnosis of patients with a partial dihydropyrimidine dehydrogenase deficiency. *Clin. Chem.* **2000**, 46, 9–17.
10. van Kuilenburg, A.B.P.; Stroomer, A.E.M.; van Lenthe, H.; Abeling, N.G.G.M.; van Gennip, A.H. New insights in dihydropyrimidine dehydrogenase deficiency: a pivotal role for *beta*-aminoisobutyric acid? *Biochem. J.* **2004**, 379, 119–124.
11. Wei, X.; Elizondo, G.; Sapone, A.; McLeod, H.L.; Raunio, H.; Gonzalez, F.J. Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics* **1998**, 51, 391–400.
12. Hormozian, F.; Schmitt, J.G.; Sagulenko, E.; Schwab, M.; Savelyeva, L. FRA1E common fragile site breaks map within a 370kilobase pair region and disrupt the dihydropyrimidine dehydrogenase gene (DPYD). *Cancer Lett.* **2007**, 246, 82–91.
13. van Kuilenburg, A.B.P.; Meinsma, J.R.; Poll-The, B.T.; Zoetekouw, L.; van Gennip, A.H. Identification of a novel disease-causing mutation (100delA) in the dihydropyrimidine dehydrogenase gene. *Int. Arch. Biocsi.* **2002**, 1, 1096–1100.
14. Vreken, P.; van Kuilenburg, A.B.P.; Meinsma, J.R.; van Gennip, A.H. Identification of a four-base deletion (delTCAT296–299) in the dihydropyrimidine dehydrogenase gene with variable clinical expression. *Hum. Genet.* **1997**, 100, 263–265.
15. Vreken, P.; van Kuilenburg, A.B.P.; Meinsma, J.R.; van Gennip, A.H. Dihydropyrimidine dehydrogenase (DPD) deficiency: identification and expression of missense mutations C29R, R886H and R235W. *Hum. Genet.* **1997**, 101, 333–338.

16. van Kuilenburg, A.B.P.; Meijer, J.; Mul, A.N.P.M.; Hennekam, R.C.M.; Hoovers, J.M.N.; de Die-Smulders, C.E.M.; Weber, P.; Mori, A.C.; Bierau, J.; Fowler, B.; Macke, K.; Sass, J.O.; Meinsma, R.; Hennerman, J.B.; Miny, P.; Zoetekouw, L.; Vijzelaar, R.; Nicolai, J.; Ylstra, B.; Rubio-Gozalbo, M.E. Analysis of severely affected patients with dihydropyrimidine dehydrogenase deficiency reveals large intragenic rearrangements of DPYD and a de novo interstitial deletion del(1)(p13.3p21.3). *Hum. Genet.* **2009**, 125, 581–590.