

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)

André B. P. van Kuilenburg · Judith Meijer · Adri N. P. M. Mul · Raoul C. M. Hennekam · Jan M. N. Hoovers · Christine E. M. de Die-Smulders · Peter Weber · Andrea Capone Mori · Jürgen Bierau · Brian Fowler · Klaus Macke · Jörn Oliver Sass · Rutger Meinsma · Julia B. Hennermann · Peter Miny · Lida Zoetekouw · Raymon Vijzelaar · Joost Nicolai · Bauke Ylstra · M. Estela Rubio-Gozalbo

Received: 27 January 2009 / Accepted: 5 March 2009 / Published online: 19 March 2009
© The Author(s) 2009. This article is published with open access at Springerlink.com

Abstract Dihydropyrimidine dehydrogenase (DPD) deficiency is an infrequently described autosomal recessive disorder of the pyrimidine degradation pathway and can lead to mental and motor retardation and convulsions. DPD deficiency is also known to cause a potentially lethal toxicity following administration of the antineoplastic agent 5-fluorouracil. In an ongoing study of 72 DPD deficient patients, we analysed the molecular background of 5 patients in more detail in whom initial sequence analysis did not reveal pathogenic mutations. In three patients, 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 and also 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 includes *DPYD*. Haploinsufficiency of *NTNG1*, *LPPR4*, *GPSM2*, *COL11A1* and *VAV3* might have contributed to the severe psychomotor retardation and unusual craniofacial features in this patient. Our study showed for the first time the presence of genomic deletions affecting *DPYD* in 7% (5/72) of all DPD deficient patients. Therefore, screening of DPD deficient patients for genomic deletions should be considered.

A. B. P. van Kuilenburg · J. Meijer · R. Meinsma · L. Zoetekouw
Department of Clinical Chemistry, Emma Children's Hospital,
Academic Medical Center, University of Amsterdam,
Amsterdam, The Netherlands

A. N. P. M. Mul · J. M. N. Hoovers
Department of Clinical Genetics, Academic Medical Center,
Amsterdam, The Netherlands

R. C. M. Hennekam
Department of Pediatrics, Emma Children's Hospital,
Academic Medical Center, University of Amsterdam,
Amsterdam, The Netherlands

C. E. M. de Die-Smulders
Department of Clinical Genetics, University Hospital Maastricht,
Maastricht, The Netherlands

P. Weber
Department of Neuropaediatrics,
University Children's Hospital Basel, Basel, Switzerland

A. C. Mori
Department of Neuropediatrics, Children's Hospital Aarau,
Aarau, Switzerland

J. Bierau · M. E. Rubio-Gozalbo
Department of Inherited Metabolic Diseases,
University Hospital Maastricht, Maastricht, The Netherlands

B. Fowler
Department of Metabolic Diseases,
University Children's Hospital Basel, Basel, Switzerland

K. Macke
Department of Child Neurology, Rheinhessen-Fachklinik,
Alzey, Germany

J. O. Sass
Zentrum für Kinder- und Jugendmedizin,
Labor für Klinische Biochemie und stoffwechsel,
Universitätsklinikum Freiburg, Freiburg, Germany

J. B. Hennermann
Department of Pediatrics, Metabolic Unit,
Charité Universitaetsmedizin Berlin, Berlin, Germany

P. Miny
Division of Medical Genetics, University Children's Hospital,
Basel, Switzerland

Introduction

Dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2) is the initial and rate-limiting enzyme in the catabolism of pyrimidine bases. It catalyses the reduction of uracil and thymine to 5,6-dihydrouracil and 5,6-dihydrothymine, respectively. Deficiency of DPD (MIM 274270) causes thymine-uraciluria and is typically accompanied by neurological symptoms such as mental and motor delay and convulsions (van Kuilenburg et al. 1999, 2002a). However, a consistent phenotype has not yet emerged (Fernandez-Salguero et al. 1997; van Kuilenburg et al. 1999, 2002a). In addition, DPD plays an important role in the breakdown of the antineoplastic agent 5-fluorouracil (5FU). Patients with a partial or complete enzyme deficiency can suffer from severe and potentially lethal toxicity following 5FU administration (van Kuilenburg 2004). Therefore, reliable identification of DPD deficiency is essential to identify cancer patients at risk.

DPD deficiency is an autosomal recessive disorder and *DPYD* is present as a single copy gene on chromosome 1p21.3 and consists of 23 exons (Wei et al. 1998). Physically, *DPYD* is at least 950 kb in length with 3 kb of coding sequence and an average intron size of 43 kb (Wei et al. 1998). Recently, it has been shown that the common fragile site *FRA1E* extends over 370 kb within *DPYD* and the region with the highest fragility encompasses exons 13–16 of *DPYD* (Hormozian et al. 2007). Common fragile sites represent chromosome structures that are particularly prone to breakage under replication stress and the

genomic instability can give rise to deletions, translocations and amplifications (Hormozian et al. 2007). However, no DPD deficient patients have been identified with chromosomal deletions of one or multiple exons of *DPYD* (van Kuilenburg et al. 1999, 2002a, 2004). The commonly used mutation detection methods (exon sequencing; DHPLC) do not detect deletions or other rearrangements and for this purpose Southern blotting or quantitative PCR are often used. Recently, multiplex ligation-dependent probe amplification (MLPA) and array-based comparative genomic hybridization (array CGH) have emerged as alternative techniques for the relative quantification of genomic sequences (Schouten et al. 2002; Ylstra et al. 2006).

In this paper, we present five clinically severely affected patients out of a series of 72 patients with complete DPD deficiency, in whom initial sequence analysis did not reveal pathogenic mutations, and demonstrate for the first time the presence of large deletions in *DPYD* and a de novo 14 Mb-interstitial deletion of chromosome 1p, including *DPYD*.

Materials and methods

Analysis of pyrimidines in body fluids and DPD activity

The concentrations of pyrimidine bases and their degradation products in urine and plasma were determined using reversed-phase HPLC combined with electrospray tandem mass spectrometry (van Kuilenburg et al. 2004; van Lenthe et al. 2000). 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 (van Kuilenburg et al. 2000b).

Sequence analysis of coding exons of *DPYD*

DNA was isolated from leukocytes using the Wizard Genomic DNA Purification Kit (Promega Benelux, Leiden, The Netherlands). PCR amplification of all 23 coding exons and flanking intronic regions of *DPYD* was carried out using intronic primer sets, essentially as described before (van Kuilenburg et al. 2000a). Sequence analysis of genomic fragments amplified by PCR was carried out on an Applied Biosystems model 3100 automated DNA sequencer using the dye-terminator method (Applied Biosystems, Nieuwekerk a/d IJssel, The Netherlands). The *DPYD* sequence of patients was compared to that observed in controls and the reference sequence of *DPYD* (Ref Seq NM_000110.3; Ensembl ENST00000370192).

P. Miny

Department of Biomedicine, University of Basel,
Basel, Switzerland

R. Vijzelaar

MRC-Holland bv, Amsterdam, The Netherlands

J. Nicolai

Department of Neurology, University Hospital Maastricht,
Maastricht, The Netherlands

B. Ylstra

Department of Pathology, VU University Medical Center,
Amsterdam, The Netherlands

M. E. Rubio-Gozalbo

Department of Pediatrics, University Hospital Maastricht,
Maastricht, The Netherlands

A. B. P. van Kuilenburg (✉)

Academic Medical Center, Laboratory Genetic Metabolic
Diseases F0-224, Meibergdreef 9, 1105, AZ Amsterdam,
The Netherlands

e-mail: a.b.vankuilenburg@amc.uva.nl

Deletion-specific PCR analysis

Exon 12 of *DPYD* was amplified using the forward primer *DPYD12_f* (5'-ttcctgtatgtgagtgta-3') and reversed primer *DPYD12_r* (5'-gaagcacttatccattgg-3'). The forward primer contained a 5'-TGTA AACGACGGCCAGT-3' extension whereas the reverse primers included a 5'-CAG-GAAACAGCTATGACC-3' extension at their 5' ends. The deleted genomic region was analysed using the forward primer *IVS11_f* (5'-tgcttctcactcctccaagc-3') and reverse primer *IVS12_r* (5'-gacttcaaatgctggctgct-3'). cDNA analysis of exon 14–16 was performed using the forward primer *F₁₃* (5'-TTGATCTGGTGGACATTAGTG-3') and reverse primer *R₁₉* (5'-GAAACTGAAGACCACTTTCAG-3').

Amplification was carried out in 25 µl reaction mixtures containing 20 mM Tris/HCl pH 8.4, 50 mM KCl, 1.5–2 mM MgCl₂, 0.4 µM of each primer, 0.2 mM dNTPs and 0.02 U of Platinum Taq polymerase. After initial denaturation for 5 min at 95°C, amplification was carried out for 30 cycles (30 s 95°C, 30 s 55°C, 1 min 72°C) with a final extension step of 10 min at 72°C. PCR products were separated on 1.5% agarose gels, visualised with ethidium bromide or used for direct sequencing.

MLPA analysis

The MLPA test for *DPYD* (MRC-Holland, Amsterdam, The Netherlands) contains 37 exon probes for *DPYD* and 9 control probes specific for DNA sequences outside the *DPYD* gene. MLPA was performed essentially as described before (Schouten et al. 2002). Data analysis was performed using Genescan and Genotyper software (Applied Biosystems). The relative peak area of each probe was divided by the average relative peak area of this probe in control samples. In normal individuals this calculation will result in a value of 1.0 representing two copies of the target sequence in the sample.

Cytogenetic analysis

FISH analysis was performed with BAC clones (Wellcome Trust Sanger Institute) for *DPYD* (Clones RP11-272L13 and RP11-526F14) and the flanking genes *PTBP2* (RP11-122C9) and *SNX7* (RP11-296E3) on metaphase chromosomes from lymphocytes of probands and parents.

Array-based comparative genomic hybridization was performed using a 28.830 oligo-array, as described before (van den IJssel et al. 2005). Microarray image analysis and quality control were performed using BlueFuse version 3.2 (BlueGnome). Regions with five or more consecutive deleted or duplicated oligonucleotides were further analysed provided they were not known variants as listed in the database of genomic variants (<http://projects.tcag.ca/variation/>).

Results

Clinical and biochemical phenotype of patients with a DPD deficiency

Patient 1 is a boy born to consanguineous Italian parents. At the age of 2 years, he presented with two short generalised febrile seizures. Because of slow psychomotor development, he attended a special school for children with learning disabilities at the age of 6 years. At the age of 8 years, he suffered from mild adipositas. In addition, he had started to show abnormal social behaviour with an impulsive, explosive and aggressive attitude towards his classmates, sister and parents. Treatment with methylphenidate (Concerta®) resulted in significant amelioration of explosive behaviour. Informed consent was obtained from the parents.

Patient 2 and 3 are cousins from a highly consanguineous Turkish family. Patient 2 is a girl of consanguine Turkish parents. At the age of 12 months, she suffered from her first febrile seizure. Subsequently, she showed febrile and non-febrile seizures, severe developmental delay including a severe language developmental disorder with absence of active speech, muscular hypotonia with an inability to walk freely, microcephaly, strabism and an autistic-like behaviour. Informed consent was obtained from the parents.

Patient 3 is a boy born to consanguineous Turkish parents who, at the age of 12 months, presented with febrile seizures, developmental delay and hypotonia. During the subsequent years, he was noted to suffer from recurrent epileptic seizures during febrile infections and focal motor seizures without fever. At present, the patient shows severe psychomotor retardation with no verbal communication and he is unable to walk independently. No further seizures have occurred on treatment with valproate. Informed consent was obtained from the parents.

Patient 4 is a girl born to consanguineous Turkish parents who presented shortly after birth with amniotic infection syndrome, respiratory insufficiency and pulmonary hypertonia, leading to intubation, application of surfactant factor, nitric oxide and high frequency oscillatory ventilation until the seventh day of life. Neurological examination showed muscular hypotonia and sucking weakness from the first day of life. Seizures were not reported, but EEG showed multifocal spike discharges. At the age of 5 months, the developmental status was 6 weeks. Facial and skeletal abnormalities included long eyelashes, thick eyelids, retrognathia, depressed nasal bridge, short neck, palmar crease and short extremities with radiological signs of dysostosis multiplex. Informed consent was obtained from the parents.

Patient 5 is a boy from non-consanguineous Dutch parents who showed irritability and hypertonia from birth. He

had transient respiratory problems and feeding difficulties. During subsequent months, hypertonia and hyperreflexia changed into severe hypotonia and areflexia. Growth followed the 50th centile for height and the 97th centile for head circumference. He was profoundly retarded. At 3 years he showed macrocephaly, prominent forehead, hypertelorism, downward slanted palpebral fissures, low nasal bridge, full nasal tip, anteverted nares, long and prominent philtrum, open mouth appearance, everted lower vermillion, a highly arched palate and large lobules (Fig. 1). Eruption of his dentition was delayed, nails were short and thin, and X-rays showed epiphyseal dysplasia of the femoral head. Ophthalmologic examination showed myopia, astigmatism and nystagmus. Informed consent was obtained from the parents.

Analysis of urine and plasma samples of the patients showed strongly elevated levels of uracil and thymine. The DPD activity in PBM cells or fibroblasts proved to be undetectably low in all patients indicating a complete deficiency of DPD. Analysis of the DPD activity in the parents of patient 5 showed that the DPD activity in PBM cells of the mother (3.9 nmol/mg/h) was decreased compared with controls (9.9 ± 2.8 nmol/mg/h) and comparable to that observed for other obligate heterozygotes (van Kuilenburg et al. 2002b). Surprisingly, a normal DPD activity (9.5 nmol/mg/h) was detected in PBM cells from the father.



Fig. 1 Patient 5 at age 3 years. Note the macrocephaly, prominent forehead, low nasal bridge, anteverted nares, open mouth appearance, full lower vermillion, and large lobules. Informed consent was obtained from the parents

Molecular studies

Analysis of the genomic sequences of all exons of *DPYD*, including their flanking sequences, revealed no pathogenic mutations in patients 1–4. However, exon 12 and its flanking sequences could not be amplified with PCR in patients 1, 2 and 3 whereas exons 14–16 could not be amplified in patient 4. In patient 5, apparent homozygosity for the c.299_302delTCAT (formerly known as the c.295_298delTCAT mutation) in exon 4 was observed. Analysis of *DPYD* from the mother demonstrated that she was heterozygous for the c.299_302delTCAT mutation whereas in the father the mutation could not be detected. Biological parenthood was confirmed using multiplex genotyping (data not shown).

To investigate the presence of a deletion of one or more exons of *DPYD*, MLPA was performed in all patients and four controls (Fig. 2). These results suggested a deletion of exon 12 in patients 1, 2 and 3, a deletion of exons 14–16 in patient 4, and loss of heterozygosity of the entire *DPYD* gene in patient 5. A normal MLPA pattern was observed in the parents of patient 5.

Sequence analysis of *DPYD* showed that the patients 1, 2 and 3 were homozygous for a 13.8 kb deletion ranging from c.1,340–3,473 to c.1,524 + 10,154 (c.1,340–3,473_c.1,525 + 10,154del13812) (Fig. 3). In addition, a short repeat sequence present in intron 12 was inserted between intron 11 and intron 12 (Fig. 3b). cDNA analysis showed that this large genomic deletion led to the synthesis of an aberrant transcript lacking exon 12 (c.1,340_1,524del).

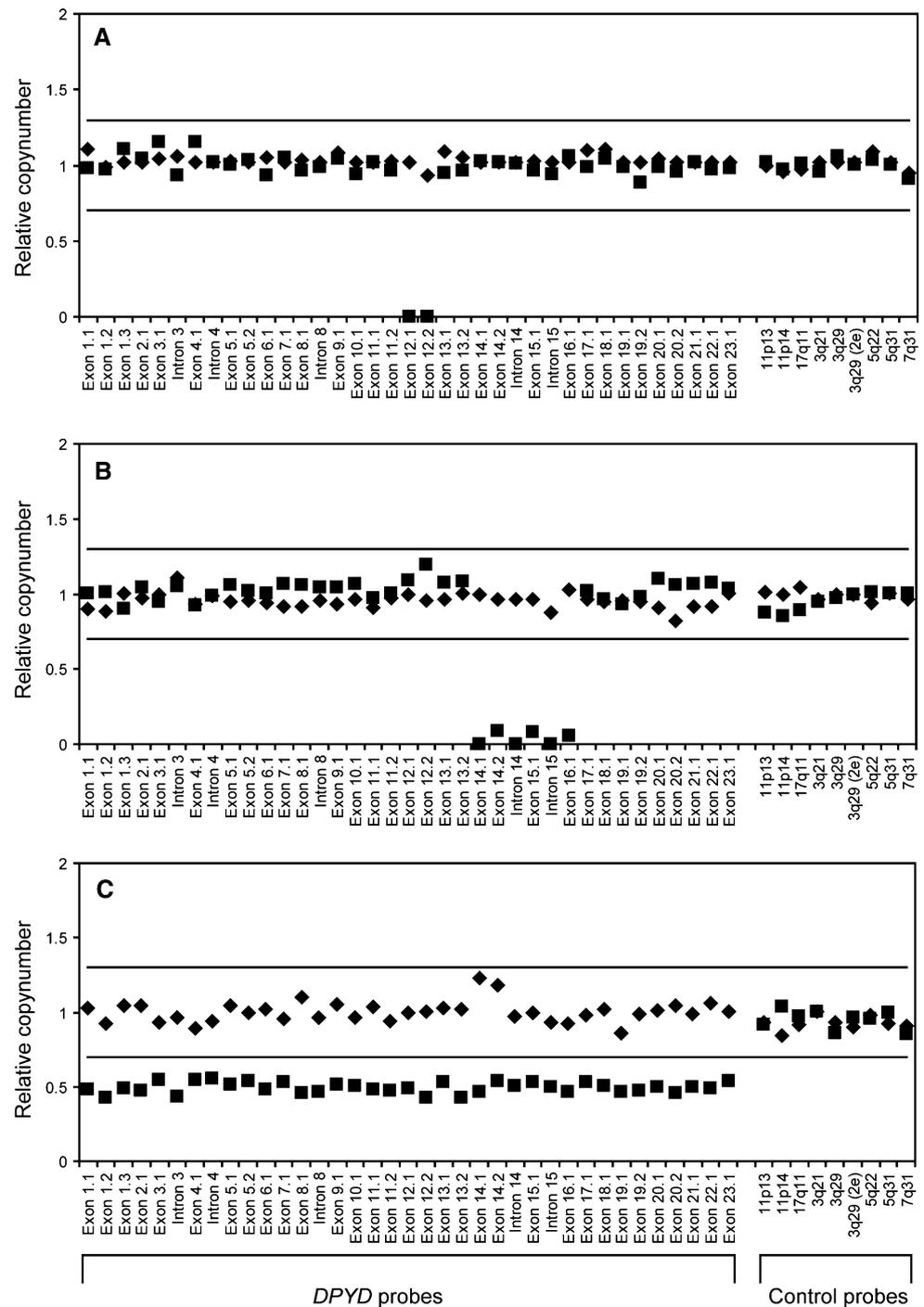
Analysis of the cDNA coding for DPD showed that patient 4 was homozygous for a deletion of exon 14–16 (c.1,741_2,058del) encoding the amino acids 581–686 (Fig. 4a). Analysis of *DPYD* showed that patient 4 had a deletion of approximately 122 kb ranging from 36 kb upstream exon 14 to 19.5 kb downstream of exon 16 (Fig. 4b).

Cytogenetic analyses

FISH analysis in patient 5 and his parents showed only one signal on chromosome 1p in the patient whereas both chromosomes were labelled in the parents (Fig. 5a), indicating a de novo deletion of *DPYD*. In addition to *DPYD*, also the flanking genes *PTBP2* and *SNX7* were deleted in the patient. Subsequent chromosome analysis with high resolution banding revealed a deletion of band p21 in the short arm of chromosome 1 (Fig. 5b).

Array-based CGH was performed to delineate the boundaries and size of the 1p21 deletion. Detailed analysis of the chromosome 1 region showed a deleted region of approximately 14 Mb situated between 1p13.3 and 1p21.3 (Fig. 5c). In this region, 57 different genes were localised

Fig. 2 Analysis of copy number changes in *DPYD* using MLPA. The results of the MLPA analysis are shown for patient 2 (a), patient 4 (b) and patient 5 (c). The quantitative analysis of the copy number of the 23 coding exons and 4 intronic sequences of *DPYD* and 9 control probes specific for DNA sequences outside *DPYD* was performed in the patient (square) and compared to that observed in a control (diamond). The solid lines represent the cut-off values indicative for amplification (relative copy number >1.3) or deletion (relative copy number <0.7) of that particular sequence



(UCSC Human Genome Browser Gateway <http://genome.ucsc.edu/>).

Discussion

Numerous mutations have been found in *DPYD* from DPD deficient patients but no patients have been described with genomic deletions of one or multiple exons (van Kuilen-

burg et al. 1999, 2002a, 2004). Here, we show the presence of large intragenic rearrangements of *DPYD* and a de novo interstitial deletion del(1)(p13.3p21.3) encompassing *DPYD*, in five patients.

Recently, it has been shown that a region of high genomic instability is located at chromosome 1p21-22 and the common fragile site *FRA1E* is located within *DPYD* (Hormozian et al. 2007). The entire genomic region of the *FRA1E* common fragile site extends from intron 8 to 18 of

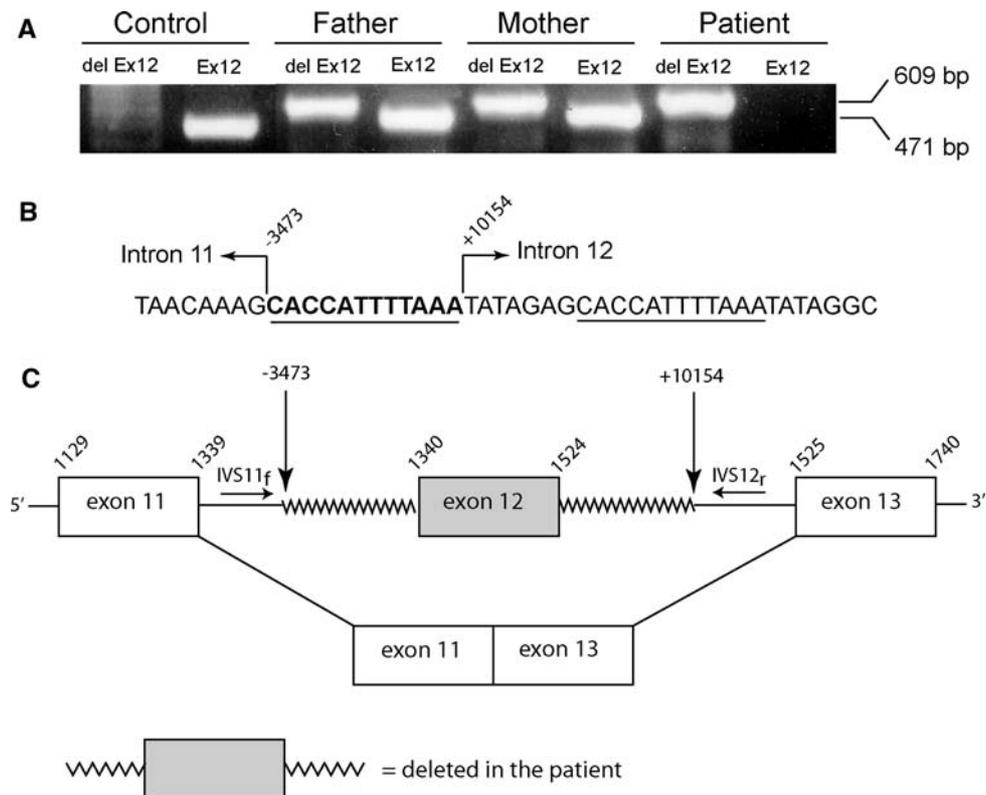


Fig. 3 PCR and sequence analysis of exon 12 and flanking regions of *DPYD*. **a** shows the PCR amplification of exon 12 (Ex12) and a genomic fragment (del Ex12) using PCR primers located 3.8 kb upstream and 10.4 kb downstream of exon 12. The deletion-specific genomic fragment could be detected in the patients 1, 2 and 3 and the obligate heterozygous parents whereas exon 12 was only detected in the control and parents of the patients. **b** shows the sequence of intron 11 joined to intron 12 via a repeat sequence of 12 bp (**bold and underlined**). The

repeat sequence in intron 12 is *underlined*. The *arrows* indicate the start of intron 11 and 12. **c** shows a schematic representation of the deleted region and its effect on the splicing of the DPD pre-mRNA and the generation of a mutant DPD mRNA. The locations of the forward primer IVS11_f (c.1,340–3,819) and reverse primer IVS12_r (c.1,524 + 10,398) used to amplify the region encompassing the deletion are indicated

DPYD with the region of the highest fragility encompassing the central part of *DPYD* including exons 13–16 (Hormozian et al. 2007). Thus, the presently described deletions involving exon 12 and 14–16 are located within *FRA1E*. A variation in the *DPYD* copy number was observed in a panel of human tumour xenografts (Kobunai et al. 2007). Copy-number variations (CNVs) are observed frequently in phenotypically normal individuals (Iafate et al. 2004; Redon et al. 2006). The most common CNV occurring in 49% of studied individuals encompassed the amylase alpha 1a/2a locus (*AMY1A–AMY2A*) at 1p13.3 and another CNV was identified at locus 1p21.3 (Iafate et al. 2004). Thus, the interstitial 1p13.3–21.3 deletion in the present patient 5 encompasses the entire region between these two CNVs. It remains uncertain whether the presence of the c.299_302delTCAT mutation on the other allele has had any influence in the origin of the interstitial deletion of chromosome 1p in patient 5. The occurrence of de novo point mutations or deletions combined with a mutation on the other allele has also been described for patients suffer-

ing from tyrosinase deficiency and Hutchinson-Gilford progeria syndrome (Coupry et al. 2001; Eriksson et al. 2003).

In patients with a complete DPD deficiency, considerable variation in the clinical presentation has been observed (Au et al. 2003; Fernandez-Salguero et al. 1997; van Kuilenburg et al. 1999, 2002a, 2005). Psychomotor retardation and convulsive disorders are relatively frequent manifestations whereas growth retardation, microcephaly, dysmorphia, autism, hypotonia and ocular abnormalities are less frequently observed (Au et al. 2003; van Kuilenburg et al. 1999, 2002a, 2005). The most conspicuous clinical abnormalities encountered in our patients were the severe psychomotor retardation, epilepsy, respiratory distress in the perinatal period, hypotonia, craniofacial dysmorphia and skeletal abnormalities. To date, no clear genotype-phenotype correlation has been established but it is noteworthy that our patients with gross deletions in *DPYD* presented with a severe phenotype when compared to that observed in other DPD patients (van Kuilenburg et al. 1999, 2002a).

Fig. 4 cDNA analysis of exon 14–16 and flanking regions of *DPYD* of patient 4. **a** shows the PCR amplification of a cDNA fragment using forward and reverse primers located in exon 13 and 19, respectively, in a control and the patient. **b** shows a schematic representation of the deleted region and its effect on the splicing of the DPD pre-mRNA and the generation of a mutant DPD mRNA

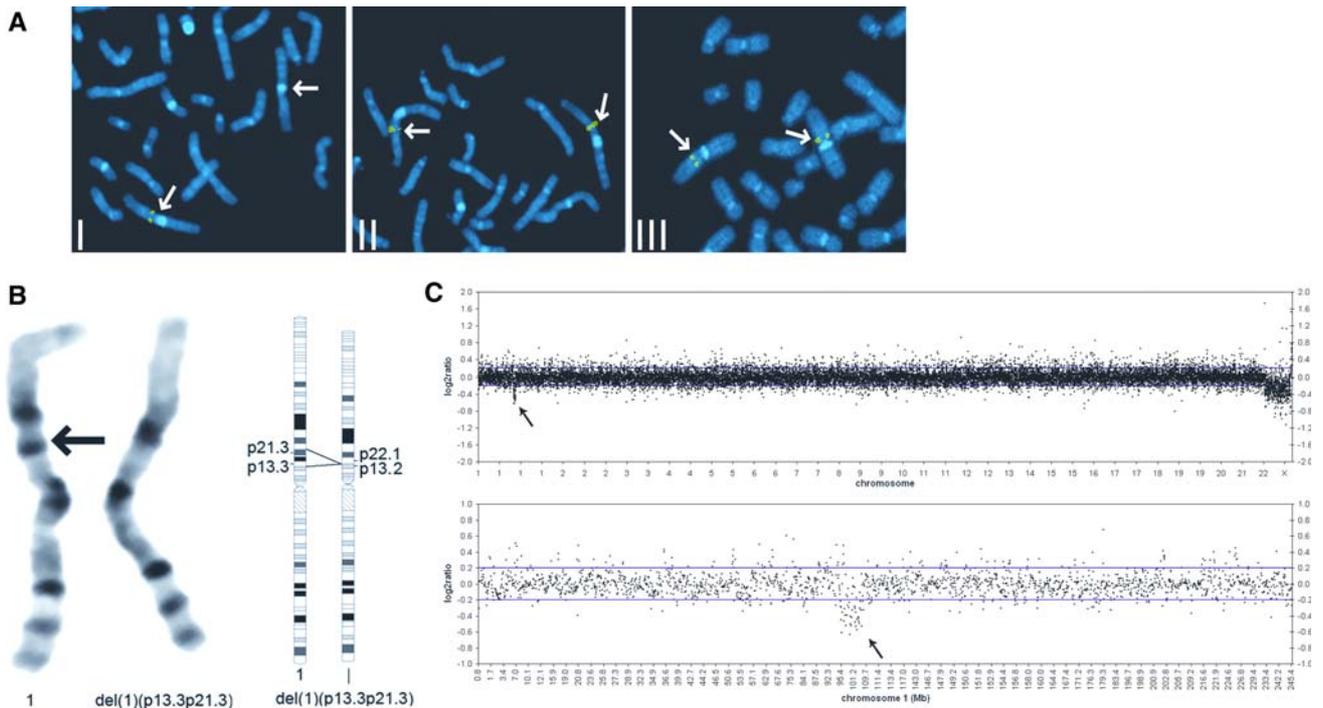
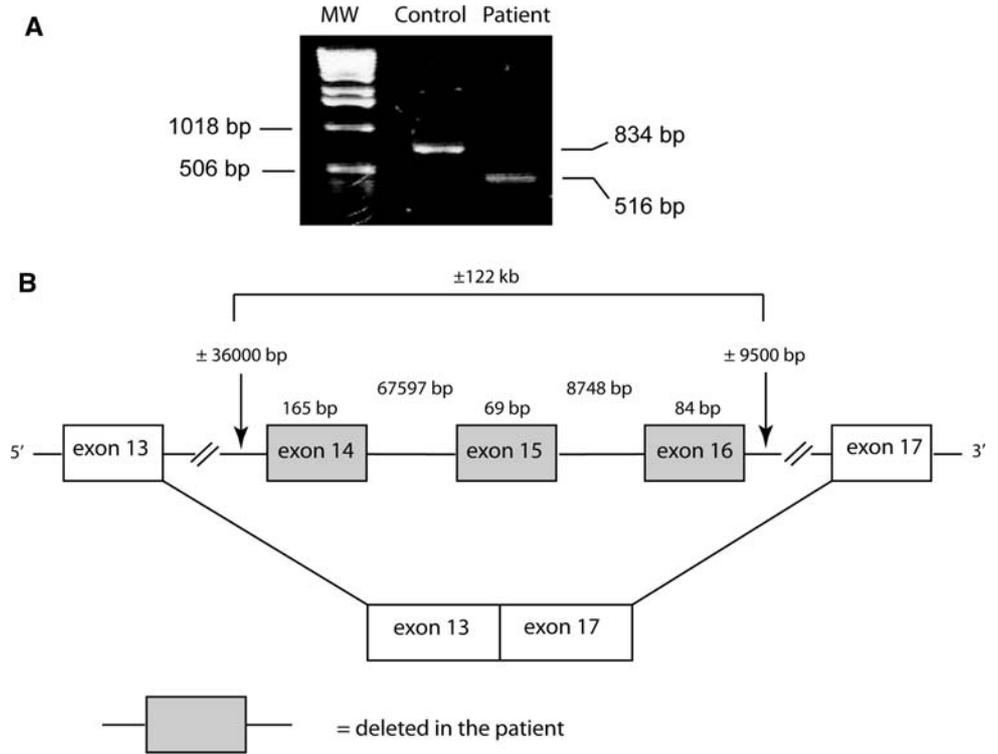


Fig. 5 Chromosomal analysis of *DPYD* and its flanking genes. **a** shows the FISH analysis of probe RP11-272L13 (*DPYD*) for patient 5 (I), his mother (II) and father (III). The arrow points at the short arm of chromosome 1 and the green fluorescent signal represents the probe RP11-272L13 (*DPYD*). **b** shows the ideogram and partial karyotype analysis of chromosome 1 of patient 5. The arrow indicates the interstitial deletion of (1)(p13.3p21.3). **c** shows the detection of copy number changes by oligo array-based genomic hybridization. The Log₂

ratio of oligos was plotted as a function of the whole genome (*upper panel*) and chromosome 1 in detail (*lower panel*). The Y-axis represents the Log₂ ratio of the intensities of test and reference DNA. On the X-axis oligos are ordered by chromosome or Mb position on the short arm of chromosome 1. The lines represent our selected criteria for considering gains (+0.2) and losses (−0.2). The arrows indicate the chromosomal region deleted in the patient which spans approximately 14 Mb

Table 1 Clinical presentation of patients with interstitial deletions of the short arm of chromosome 1

	Patient 5	Tabata et al. (1991)	Dockery and van der Westhuyzen (1991) (twins)	Mattia et al. (1992)	Bisgaard et al. (2007)
Deletion	1p13.3–1p21.3	1p13.3–1p22.3	1p13.3–1p22.3	1p13.3–1p22.3	1p13.3–1p21.1
Age at examination	3.5 years	2 months (died 7 months)	29 years	22 months	13 years
Birth weight	4,515 g (>P97)	3,000 g (term)	2,600 g (term)	4,000 g	3,400 g term
	42 weeks		2,380 g (term)	41.5 weeks	
Growth retardation/ short stature	96.5 cm (P10–P50)	nd	Final length 147 cm (<P5) Final length 146 cm (<P5)	P50	130 cm (<P5)
Head circumference	54 cm (P90–P97)	nd	51.5 cm (P3) 51 cm (P3)	nd	<3rd centile for age
Large anterior fontanel	+	+	nd	nd	nd
Frontal bossing	+	+	nd	nd	nd
Hypertelorism	+	nd	nd	nd	nd
Downslanting palpebral fissures	–	+	nd	–	nd
Epicanthal folds	+	+	nd	–	nd
Colobomata eye	–	nd	nd	nd	+
Long eyelashes	–	–	nd	+	nd
Low set ears	–	nd	nd	+	+
Malformed ears	–	–	+	+	+
Broad flat root of nose	+	+	nd	nd	+
Prominent nasal bridge and tip	–	–	nd	+	+
Anteverted nares	+	+	nd	+	+
Prominent philtrum	+	nd	nd	+	+
High arched palate	+	+	nd	nd	+
Microretrognathia	–	+	nd	+	nd
Low hair line	–	+	nd	nd	+
Short neck	–	+	nd	nd	nd
Widely spaced nipples	–	+	nd	nd	nd
Cyanotic heart diseases	–	+	–	–	–
Digitalised thumbs	–	–	+	+	nd
Toe anomalies	–	+	+	nd	+
Prominent calcanei	–	+	–	nd	nd
Equinovarus	–	+	+	nd	–
Psychomotor retardation	+ severe	+ severe	+ severe	+ moderate	+ severe
Seizures	+	+	nd	nd	+
Hypotonia	+	nd	nd	+	+

nd no data

Interstitial deletions of the short arm of chromosome 1 are rare (Bisgaard et al. 2007; Dockery and Van der Westhuyzen 1991; Mattia et al. 1992; Tabata et al. 1991). The phenotypic features in the few patients described with a comparable proximal interstitial deletion are summarised in Table 1 and are comparable to those of patient 5.

Psychomotor retardation and seizures were often noted in the patients. It is conceivable that defects in a number of genes located in the deleted region of the short arm of chromosome 1 underlie some of the clinical abnormalities

observed in patient 5. For example, the *NTNG1* gene encodes proteins providing axon guidance cues during vertebrate nervous system development and might play a role in late development stages of the central nervous system (Borg et al. 2005). In addition, G protein signalling modulator 2 (*GPSM2*) has been shown to regulate neuroblast self-renewal and the phospholipid phosphatase, PRG-1 (*LPPR4*), has been shown to be involved in axon growth and regenerative sprouting (Brauer et al. 2003; Lee et al. 2006). Thus, haploinsufficiency of *NTNG1*, *LPPR4*

and *GPSM2* might have contributed to the severe psychomotor retardation in the patient. In addition, mutations in *COL11A1* gene have been identified in patients suffering from Marshall syndrome and Stickler syndrome (Griffith et al. 1998; Majava et al. 2007). The Stickler syndrome is an autosomal dominant connective tissue disorder mainly characterised by ocular, orofacial and articular abnormalities (hereditary arthro-ophthalmopathy). The Marshall syndrome is a rare, autosomal dominant craniofacial disorder, which shows considerable phenotypic overlap with Stickler syndrome. The facial dysmorphism such as the hypertelorism, anteverted nares and a prominent philtrum, which are often observed in patients with Marshall syndrome were also present in the patient. Furthermore, nail dysplasia, delayed dentition, bony abnormalities (Meyer's dysplasia) and severe myopia are also found in patients heterozygous for mutations in the *COL11A1* gene. Interestingly, the *vav-3* gene (*VAV3*) coding for a Rho guanine nucleotide exchange factor, has been shown to regulate osteoclast function and bone mass (Faccio et al. 2005).

Within our group of 72 patients with DPD deficiency, we have been able to identify putative disease-causing mutations in 61 patients by sequence analysis (van Kuilenburg et al. unpublished). The large intragenic rearrangements and the de novo interstitial deletion of chromosome 1 were observed in 5 out of 11 DPD patients in whom sequence analysis did not provide pathogenic mutations. The fact that genomic deletions affecting *DPYD* were observed in 7% (5/72) of all DPD patients demonstrates that genomic *DPYD* deletions are not a rare event. Furthermore, the fact that the exon 12 deletion was observed in patients of different ethnic origins indicates that this mutation is not population specific and might be relatively frequent in the general population. It has been reported that in a significant number of tumour patients with a reduced DPD activity, no mutation could be identified in *DPYD* (Mattison et al. 2004; van Kuilenburg et al. 2000a). Although epigenetic regulation of the expression of *DPYD* through methylation of the promoter region may provide an explanation for some of these cases (Zhang and Diasio 2007), it is conceivable that genomic deletions encompassing part of or the entire *DPYD* gene can also provide a molecular explanation for patients with a phenotypically established DPD deficiency. Therefore, we suggest that patients with a DPD deficiency but without detectable mutation in *DPYD*, and especially in those with an unusual phenotype should be screened for such genomic *DPYD* deletions.

Acknowledgments This paper is dedicated to the memory of Dr. Albert H. van Gennip (1943–2007). This study was supported by the “Stichting Sacha Swarttouw-Hijmans”.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Au KM, Lai CK, Yuen YP, Shek CC, Lam CW, Chan AYW (2003) Diagnosis of dihydropyrimidine dehydrogenase deficiency in a neonate with thymine-uraciluria. *Hong Kong Med J* 9:130–132
- Bisgaard AM, Rasmussen LN, Moller HU, Kirchhoff M, Bryndorf T (2007) Interstitial deletion of the short arm of chromosome 1 (1p13.1p21.1) in a girl with mental retardation, short stature and colobomata. *Clin Dysmorphol* 16:109–112
- Borg I, Freude K, Kubart S, Hoffmann K, Menzel C, Laccione F, Firth H, Ferguson-Smith MA, Tommerup N, Ropers HH, Sargan D, Kalscheuer VM (2005) Disruption of Netrin G1 by a balanced chromosome translocation in a girl with Rett syndrome. *Eur J Hum Genet* 13:921–927
- Brauer AU, Savaskan NE, Kuhn H, Prehn S, Ninnemann O, Nitsch R (2003) A new phospholipid phosphatase, PRG-1, is involved in axon growth and regenerative sprouting. *Nat Neurosci* 6:572–578
- Coupry I, Taine L, Goizet C, Soriano C, Mortemousque B, Arveiler B, Lacombe D (2001) Leucodystrophy and oculocutaneous albinism in a child with an 11q14 deletion. *J Med Genet* 38:35–38
- Dockery H, Van der Westhuyzen J (1991) Monosomy of 1p13.3–22.3 in twins. *Clin Genet* 39:223–227
- Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS (2003) Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423:293–298
- Faccio R, Teitelbaum SL, Fujikawa K, Chappel J, Zallone A, Tybulewicz VL, Ross FP, Swat W (2005) Vav3 regulates osteoclast function and bone mass. *Nat Med* 11:284–290
- Fernandez-Salguero PM, Sapone A, Wei X, Holt JR, Jones S, Idle JR, Gonzalez FJ (1997) Lack of correlation between phenotype and genotype for the polymorphically expressed dihydropyrimidine dehydrogenase in a family of Pakistani origin. *Pharmacogenetics* 7:161–163
- Griffith AJ, Sprunger LK, Sirko-Osadsa DA, Tiller GE, Meisler MH, Warman ML (1998) Marshall syndrome associated with a splicing defect at the COL11A1 locus. *Am J Hum Genet* 62:816–823
- Hormozian F, Schmitt JG, Sagulenko E, Schwab M, Savelyeva L (2007) FRA1E common fragile site breaks map within a 370 kilobase pair region and disrupt the dihydropyrimidine dehydrogenase gene (*DPYD*). *Cancer Lett* 246:82–91
- Iafate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C (2004) Detection of large-scale variation in the human genome. *Nat Genet* 36:949–951
- Kobunai T, Ooyama A, Sasaki S, Wierzba K, Takechi T, Fukushima M, Watanabe T, Nagawa H (2007) Changes to the dihydropyrimidine dehydrogenase gene copy number influence the susceptibility of cancers to 5-FU-based drugs: data mining of the NCI-DTP data sets and validation with human tumour xenografts. *Eur J Cancer* 43:791–798
- Lee CY, Robinson KJ, Doe CQ (2006) Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. *Nature* 439:594–598
- Majava M, Hoornaert KP, Bartholdi D, Bouma MC, Bouman K, Carrera M, Devriendt K, Hurst J, Kitsos G, Niedrist D, Petersen MB, Shears D, Stolte-Dijkstra I, Van Hagen JM, la-Kokko L, Mannikko M, Mortier GR (2007) A report on 10 new patients

- with heterozygous mutations in the COL11A1 gene and a review of genotype-phenotype correlations in type XI collagenopathies. *Am J Med Genet A* 143:258–264
- Mattia FR, Wardinsky TD, Tuttle DJ, Grix A Jr, Smith KA, Walling P (1992) Interstitial deletion of the short arm of chromosome 1 (46XY, del(1)(p13p22.3)). *Am J Med Genet* 44:551–554
- Mattison LK, Ezzeldin H, Carpenter M, Modak A, Johnson MR, Diasio RB (2004) Rapid identification of dihydropyrimidine dehydrogenase deficiency by using a novel 2–13C-uracil breath test. *Clin Cancer Res* 10:2652–2658
- Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shaper MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME (2006) Global variation in copy number in the human genome. *Nature* 444:444–454
- Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 30:e57
- Tabata H, Sone K, Kobayashi T, Yanagisawa T, Tamura T, Shimizu N, Kanbe Y, Tashiro M, Ono S, Kuroume T (1991) Short arm deletion of chromosome 1: del(1)(p13.3 p22.3) in a female infant with an extreme tetralogy of Fallot. *Clin Genet* 39:132–135
- van den IJssel P, Tijssen M, Chin SF, Eijk P, Carvalho B, Hopmans E, Holstege H, Bangarusamy DK, Jonkers J, Meijer GA, Caldas C, Ylstra B (2005) Human and mouse oligonucleotide-based array CGH. *Nucleic Acids Res* 33:e192
- van Kuilenburg ABP (2004) Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer* 40:939–950
- van Kuilenburg ABP, Vreken P, Abeling NGGM, Bakker HD, Meinsma JR, van Lenthe H, De Abreu RA, Smeitink JAM, Kayserili H, Apak MY, Christensen E, Holopainen I, Pulkki K, Riva D, Botteon G, Holme E, Tulinius M, Kleijer WJ, Beemer FA, Duran M, Niezen-Koning KE, Smit GPA, Jakobs C, Smit LME, Spaapen LJM, van Gennip AH (1999) Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Hum Genet* 104:1–9
- van Kuilenburg ABP, Haasjes J, Richel DJ, Zoetekouw L, van Lenthe H, De Abreu RA, Maring JG, Vreken P, van Gennip AH (2000a) 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* 6:4705–4712
- van Kuilenburg ABP, van Lenthe H, Tromp A, Veltman PCJ, van Gennip AH (2000b) Pitfalls in the diagnosis of patients with a partial dihydropyrimidine dehydrogenase deficiency. *Clin Chem* 46:9–17
- van Kuilenburg ABP, Dobritzsch D, Meinsma JR, Haasjes J, Waterham HR, Nowaczyk MJM, Maropoulos GD, Hein G, Kalhoff H, Kirk JM, Baaske H, Aukett A, Duley JA, Ward KP, Lindqvist Y, van Gennip AH (2002a) Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. *Biochem J* 364:157–163
- van Kuilenburg ABP, Meinsma JR, Zoetekouw L, van Gennip AH (2002b) Increased risk of grade IV neutropenia after administration of 5-fluorouracil due to a dihydropyrimidine dehydrogenase deficiency: high prevalence of the IVS14 + 1 g > a mutation. *Int J Cancer* 101:253–258
- van Kuilenburg ABP, van Lenthe H, van Cruchten AG, Kulik W (2004) Quantification of 5, 6-dihydrouracil by HPLC-electrospray tandem mass spectrometry. *Clin Chem* 50:236–238
- van Kuilenburg ABP, Meinsma R, Beke E, Bobba B, Boffi P, Enns GM, Witt DR, Dobritzsch D (2005) Identification of three novel mutations in the dihydropyrimidine dehydrogenase gene associated with altered pre-mRNA splicing or protein function. *Biol Chem* 386:319–324
- van Lenthe H, van Kuilenburg ABP, Ito T, Bootsma AH, van Cruchten AG, Wada Y, van Gennip AH (2000) Defects in pyrimidine degradation identified by HPLC-electrospray tandem mass spectrometry of urine specimens or urine-soaked filter paper strips. *Clin Chem* 46:1916–1922
- Wei X, Elizondo G, Sapone A, McLeod HL, Raunio H, Gonzalez FJ (1998) Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics* 51:391–400
- Ylstra B, van den IJssel P, Carvalho B, Brakenhoff RH, Meijer GA (2006) BAC to the future! or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). *Nucleic Acids Res* 34:445–450
- Zhang X, Diasio RB (2007) Regulation of human dihydropyrimidine dehydrogenase: implications in the pharmacogenetics of 5-FU-based chemotherapy. *Pharmacogenomics* 8:257–265