Hum Genet (1999) 104:1–9

REVIEW ARTICLE

**A.B.P. Van Kuilenburg · P. Vreken · N.G.G.M. Abeling
H.D. Bakker · R. Meinsma · H. Van Lenthe
R.A. De Abreu · J.A.M. Smeitink · H. Kayserili
M.Y. Apak · E. Christensen · I. Holopainen · K. Pulkki
D. Riva · G. Botteon · E. Holme · M.Tulinius
W.J. Kleijer · F.A. Beemer · M. Duran · K.E. Niezen-Koning · G.P.A. Smit · C. Jakobs · L.M.E. Smit
U. Moog · L.J.M. Spaapen · A.H. Van Gennip**

Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency

Received: 25 August 1998 / Accepted: 24 November 1998

A.B.P. Van Kuilenburg (✉)[1] · P. Vreken · N.G.G.M. Abeling
H.D. Bakker · R. Meinsma · H. Van Lenthe · A.H. Van Gennip
Academic Medical Center, University of Amsterdam,
Emma Children's Hospital and Department of Clinical Chemistry,
PO Box 22700, 1100 DE Amsterdam, The Netherlands

R.A. De Abreu · J.A.M. Smeitink
University Hospital St. Radboud, Dept. of Pediatrics, Nijmegen,
The Netherlands

H. Kayserili · M.Y. Apak
University of Istanbul, Institute of Child Health, Div. Medical Genetics, Istanbul, Turkey

E. Christensen
Copenhagen University Hospital, Dept. of Pediatrics, Copenhagen, Denmark

I. Holopainen · K. Pulkki
Turku University Central Hospital, Dept. of Pediatric Neurology and Dept. of Clinical Chemistry, Turku, Finland

D. Riva · G. Botteon
Instituto Nazionale Neurologico Carlo Besta, Milan, Italy

E. Holme · M.Tulinius
Göteborg Universtity, Dept. of Clinical Chemistry & Transfusion Medicine and Dept. of Pediatrics, Göteborg, Sweden

W.J. Kleijer
Erasmus University Rotterdam, Dept. Clinical Genetics,
Rotterdam, The Netherlands

F.A. Beemer · M. Duran
University Children's Hospital, 'Het Wilhelmina Kinderziekenhuis' and Dept. of Clinical Genetics, Utrecht, The Netherlands

K.E. Niezen-Koning · G.P.A. Smit
University Groningen, Dept. Pediatrics, Groningen, The Netherlands

C. Jakobs · L.M.E. Smit
Free University Hospital, Dept. Clinical Chemistry and Dept. of Pediatrics, Amsterdam, The Netherlands

U. Moog · L.J.M. Spaapen
Maastricht University, Dept. Clinical Genetics, Maastricht, The Netherlands

[1]*Present address:*
Academic Medical Center, Laboratory Genetic Metabolic Diseases,
F0-224, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
Tel: +31 205665958, Fax: +31 206962596, e-mail: a.b.vanKuilenburg@amc.uva.nl

Abstract Dihydropyrimidine dehydrogenase (DPD) deficiency is an autosomal recessive disease characterised by thymine-uraciluria in homozygous deficient patients and has been associated with a variable clinical phenotype. In order to understand the genetic and phenotypic basis for DPD deficiency, we have reviewed 17 families presenting 22 patients with complete deficiency of DPD. In this group of patients, 7 different mutations have been identified, including 2 deletions [295–298delTCAT, 1897delC], 1 splice-site mutation [IVS14+1G>A)] and 4 missense mutations (85T>C, 703C>T, 2658G>A, 2983G>T). Analysis of the prevalence of the various mutations among DPD patients has shown that the G→A point mutation in the invariant splice donor site is by far the most common (52%), whereas the other six mutations are less frequently observed. A large phenotypic variability has been observed, with convulsive disorders, motor retardation and mental retardation being the most abundant manifestations. A clear correlation between the genotype and phenotype has not been established. An altered β-alanine, uracil and thymine homeostasis might underlie the various clinical abnormalities encountered in patients with DPD deficiency.

Introduction

In mammalian liver, the pathway for the catabolism of uracil and thymine consists of three consecutive steps (Fig. 1). 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. The second step consists of a hydrolytic ring-opening, which is catalyzed by dihydropyrimidinase (EC 3.5.2.2). Finally, β-ureidopropionic acid (N-carbamyl-β-alanine) or β-ureidoisobutyric acid (N-carbamyl-β-amin-

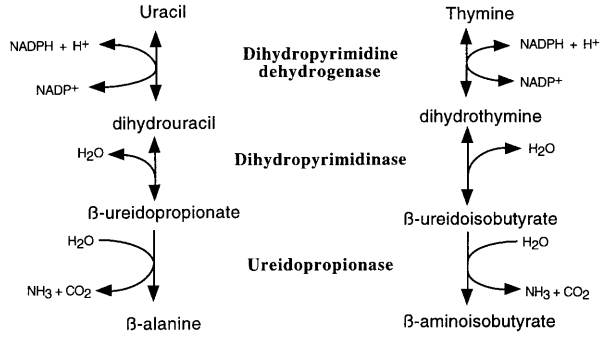

Fig. 1 Catabolic pathway of the pyrimidines uracil and thymine

oisobutyric acid) is converted to β-alanine or β-aminoisobutyric acid, ammonia and CO_2 by β-ureidopropionase (EC 3.5.1.6).

In children, a deficiency of DPD is often accompanied by a neurological disorder but a considerable variation in the clinical presentation among these patients has been reported (Van Gennip et al. 1994, 1997). In these patients, a large accumulation of uracil and thymine has been detected in urine, blood and cerebrospinal fluid, whereas no activity of DPD has been detected in fibroblasts and mononuclear cells (Bakkeren et al. 1984; Van Gennip et al. 1993, 1994, 1997).

DPD is also responsible for the breakdown of the widely used anti-neoplastic agent 5-fluorouracil (5FU). The catabolic route plays a significant role, since more than 80% of the administered 5-FU is catabolized by DPD (Heggie et al. 1987). In this light, a pharmacogenetic disorder has been described concerning cancer patients with a complete or partial deficiency of DPD and suffering from a severe or even life-threatening toxicity after the administration of 5FU. Recently, it has been shown that two such patients are genotypically heterozygous for a mutant allele of the gene encoding DPD (Wei et al. 1996; Van Kuilenburg et al. 1997a, 1998a, b). The detection of more than 30 patients of various nationalities with a (partial) DPD deficiency within 15 years in The Netherlands alone suggests that this type of inborn error is less rare than previously assumed (Van Gennip et al. 1997).

The recent cloning of the cDNA coding for human DPD and the sequence of the entire human DPD gene *(DPYD)* has allowed the detection of the defects at the molecular level. In this manuscript, we review the results of the analysis of the genotype and phenotype of 17 families presenting 22 patients who have complete deficiency of DPD (i.e. no detectable DPD activity in fibroblasts, <0.2% of controls) and who have been diagnosed in our laboratories. Clinical histories have been updated and three newly identified patients have been included. In addition, the possible pathological mechanisms underlying the various clinical abnormalities are discussed.

Structural and kinetic properties of dihydropyrimidine dehydrogenase

DPD catalyzes the NADPH-dependent reduction of uracil and thymine to 5,6-dihydrouracil and 5,6-dihydrothymine, respectively. The activity of DPD is exclusively present in the cytosol (Van Kuilenburg et al. 1997b) and can be detected in a variety of tissues with the highest activity being found in the liver (Naguib et al. 1985; Ho et al. 1986) and peripheral blood monocytes (Van Kuilenburg et al. 1997c, 1998c, d). The enzyme has been purified and extensively characterised from liver tissues of rat (Shiotani and Weber 1981), pig (Podschun et al. 1989), bovine (Porter et al. 1991) and man (Lu et al. 1992, 1993). These studies have demonstrated that the native mammalian enzyme has a molecular mass of approximately 210 kDa and is composed of two identical subunits. The C-terminal region of DPD is sensitive to proteolysis resulting in the cleavage of a 12-kDa peptide from the native enzyme. DPD contains various tightly associated prosthetic groups including two FMN, two FAD and at least two [4Fe–4 S] clusters (Podschun et al. 1989; Lu et al. 1992). With respect to the kinetic properties of the enzyme, a non-classical two-site ping-pong mechanism has been proposed for the pig liver enzyme, whereas a rapid equilibrium random kinetic mechanism has been put forward for the bovine liver enzyme (Podschun et al. 1990; Porter and Spector 1993). However, both kinetic mechanisms necessitate that two separate binding sites are available for uracil (or thymine) and NADPH, respectively.

Structural organisation of the human DPD gene

The cDNAs coding for human DPD, pig DPD and bovine DPD have been isolated and sequenced (Yokota et al. 1994; Albin et al. 1996). Mammalian DPD appears to be relatively conserved throughout evolution, since a comparison of the deduced amino acid sequences of bovine DPD with that of pig and human DPD has shown a homology of 93% and 92%, respectively. The human cDNA encodes a protein containing 1025 amino acids with a calculated molecular weight of 111 kDa (Fig. 2). The conserved domains corresponding to a possible NADPH-binding site and FAD-binding site have been found in the N-terminal and middle region of the enzyme. In the C-terminal region, typical motifs for [4Fe-4 S] clusters have been found between residues 953 and 964 and residues 986 and 997. On the basis of chemical modification studies, the putative uracil-binding site of DPD has been located between Gly-661 and Arg-678. Thus, the functional domains of DPD can be arranged, from the N-terminus, in the order of NADPH-FAD-uracil-[4Fe-4 S].

Recently, the entire human DPD gene has been isolated and shown to consist of 23 exons, with exon 15 (69 bp) being the smallest and exon 23 (961 bp) the largest (Johnson et al. 1997; Wei et al. 1998). A physical map indicates that the human DPD gene is a least 950 kb in length with 3 kb of

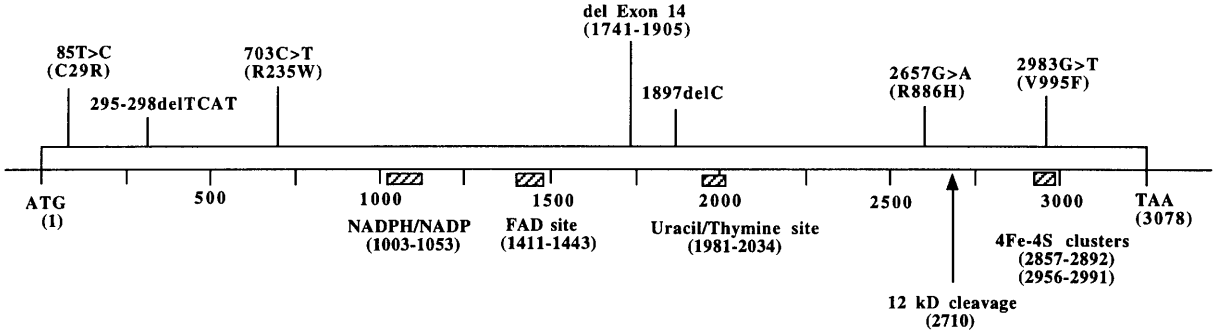

Fig. 2 Localisation of mutations in human DPD cDNA. The nucleotides involved in the three frameshift mutations are given. In addition, four missense mutations and their corresponding amino acid substitutions (given *in parenthesis*) are shown. The *hatched boxes* represent the localisation of conserved motifs corresponding to putative binding sites of the prosthetic groups. The nucleotide sequences involved are given *in parenthesis*. The proteolytic-sensitive site (*vertical arrow*) is close to the carboxy terminus and encompasses the iron-sulphur clusters

coding sequence and a minimal average intron size of about 43 kb (Wei et al. 1998). In addition, the human DPD gene has been mapped to chromosome 1p22 and is present as a single-copy gene (Takai et al. 1994; Wei et al. 1998).

DPD mutations in patients with DPD deficiency

In order to understand the genetic basis of DPD deficiency, we have analyzed 17 families presenting 22 patients with complete deficiency of DPD. In this large group of patients, we have identified 7 different mutations, including 1 splice-site mutation, 2 deletions and 4 missense mutations (Table 1). The G→A point mutation changes an invariant GT splice donor site to AT; this leads to the skipping of exon 14 immediately upstream of the mutated splice donor site, during the splicing of DPD pre-mRNA. As a consequence, a 165-bp fragment encoding amino acid residues 581–635 of the primary sequence of the DPD protein is lacking in the mature DPD mRNA (Meinsma et al. 1995; Vreken et al. 1996). Both the four-base deletion 295–298delTCAT and the 1897delC mutation cause a frameshift leading to a premature stop codon shortly thereafter (Vreken et al. 1997a, b). The TCAT(295–298) deletion is located in a TCAT tandem-repeat sequence and most probably results from slipped mispairing or unequal chromosome crossing-over. Expression of the missense mutations C29R, R235W, R886H and V995F in *Escherichia coli* has demonstrated that C29R, R235W and V995F result in mutant DPD proteins with no significant residual enzyme activity (Vreken et al. 1997c, 1998). However, the DPD protein with the R886H mutation still possesses a residual activity of 25% and it is therefore unlikely that this mutation is responsible for the observed complete deficiency in one patient who has proved to be homozygous for both the C29R and R886H mutations (Vreken et al. 1997c). The mutations identified so far are randomly distributed along the cDNA and there are no apparent hot spots present (Fig. 2). All mutations are located outside those regions known to be involved in the binding of the various substrates and prosthetic groups, with the exception of the V995F mutation, which is located in the C-terminal region of DPD, a region thought to be involved in the binding of a [4Fe-4 S] cluster.

The majority of the patients (68%) have proved to be homozygous for one of the identified mutations, whereas the remaining patients are compound heterozygotes. Analysis of family members of the index patients for the presence of mutant alleles has shown that the mutations segregate following a recessive pattern of inheritance (Meinsma et al. 1995; Vreken et al. 1997c), in accordance with the pattern

Table 1 Mutations in patients with DPD deficiency

Name[a]	Genotype[b]	Effect[c]	Location[d]	Allele frequency
Splicing				
*DPYD*2A*	IVS14+1G>A	Del (exon 14)	IVS14	23/44 (52%)
Frameshift				
*DPYD*7*	295–298delTCAT	Frameshift	EX4	7/44 (16%)
*DPYD*3*	1897delC	Frameshift	EX14	3/44 (7%)
Missense				
*DPYD*9A*	85T>C	C29R	EX2	7/44 (16%)
*DPYD*8*	703C>T	R235W	EX7	1/44 (2%)
	2657G>A	R886H	EX21	2/44 (4%)
*DPYD*10*	2983G>T	V995F	EX23	2/44 (4%)
	Unknown	-	-	1/44 (2%)

[a] Nomenclature according to McLeod et al. (1998)
[b] Nomenclature according to Antonarakis (1998)
[c] Effect of the mutation on DPD protein or mRNA
[d] According to Wei et al. (1998)

Table 2 Genotype and phenotype of patients with DPD deficiency at diagnosis (– none, + mild, ++ severe)

Patient[a]	Genotype	Convulsions	Motor retardation	Mental retardation	Growth retardation	Microcephaly	Dysmorphy	Autism	Ocular abnormalities	Others[b]
1 (NL)	ΔEX14/ΔEX14	–	+	++	–	–	–	–	+	1
2 (NL)	ΔEX14/ΔEX14	+	+	+	–	–	–	+	–	2
3 (DK)	ΔEX14/ΔEX14	+	+	+	–	–	–	–	–	3
4 (DK)	ΔEX14/ΔEX14	–	–	–	–	–	–	–	–	4
5 (S)	ΔEX14/ΔEX14	–	+	–	+	–	–	–	+	5
6 (DK)	ΔEX14/ΔEX14	++	++	++	–	–	–	–	–	
7 (NL)	ΔEX14/ΔEX14	–	–	–	–	–	–	–	–	6
8.1 (SF)	ΔEX14/ΔEX14	+	–	–	–	–	–	–	–	7
8.2 (SF)	ΔEX14/ΔEX14	–	–	–	–	–	–	–	–	8
9.1 (NL)	ΔTCAT/ΔTCAT	+	+	–	–	+	–	–	–	
9.2 (NL)	ΔTCAT/ΔTCAT	–	–	–	–	–	–	–	–	
9.3 (NL)	ΔTCAT/ΔTCAT	+	–	+	–	–	–	–	–	9
10 (IT)	C29R/C29R	–	+	++	–	–	+	++	–	10
11 (NL)	V995F/V995F	+	–	+	–	–	–	+	–	11
12.1 (TUR)	ΔC1897/ΔC1897	–	–	–	–	–	–	–	–	12
12.2 (TUR)	ΔC1897/R235 W	+	++	++	++	++	+	–	+	13
13 (TUR)	C29R, R886H/ C29R, R886H	–	–	–	+	–	–	–	–	14
14.1 (NL)	C29R/ΔEX14	+	–	–	–	–	–	–	–	15
14.2 (NL)	C29R/ΔEX14	–	–	–	–	–	–	–	–	
15 (NL)	ΔEX14/ΔTCAT	+	++	++	–	+	–	++	+	16
16 (NL)	ΔEX14/C29R	–	–	–	–	–	–	–	–	17
17 (NL)	ΔEX14/?	–	+	+	+	–	+	–	+	18
Total		10/22 (45%)	10/22 (45%)	10/22 (45%)	4/22 (18%)	3/22 (14%)	3/22 (14%)	4/22 (18%)	5/22 (23%)	

[a] The nationality of the patient is given in parenthesis

[b] (1) Ocular abnormalities (bilateral microphthalmia, iris and chorioidea coloboma) and nystagmus (Bakker et al. 1994). (2) CT scan showed strong contrast between white and grey matter. (3) Delayed development of speech (Christensen et al. 1998). (4) Lethargy (Christensen et al. 1998). (5) Bilateral ptosis, progressive external ophthalmoplegia, retinitis pigmentosa and muscle weakness caused by Kearns-Sayre syndrome with a verified mtDNA deletion and haemolytic anaemia resulting from hereditary spherocytosis. (6) Initially suffering from dizziness. (7) Status epilepticus, dizziness, minor difficulties in learning and in mathematics at school (Holopainen et al. 1997). (8) Minor difficulties in learning speech and language (Holopainen et al. 1997). (9) Generalised tonic clonic seizures; EGG showed generalised epileptic activity (Bakkeren et al. 1984; Braakhekke et al. 1987). (10) Slight white matter hyperintensity (Van Kuilenburg et al. 1999). (11) Delayed development of speech and hyperactivity (Van Gennip et al. 1981; Berger et al. 1984). (12) Suspected of having monoplegia. (13) Mild dysmorphic features (low set and posteriorly rotated ears, high arched palate) were noted. The child was in a state of unconsciousness with no response to verbal, sensory or physical stimuli; only massive motor reaction to pain was noted. There was no reaction to light with preserved corneal reflexes. Bilateral optic atrophy was present. Superficial abdominal and anal reflexes were absent. Tetraspasticity and flexion contractures were present. EEG revealed severe dysrhythmia and multifocal sharp/ spike waves. Generalised loss of white and grey matter and diffuse cerebral atrophy was observed on cranial MRI. Died at the age of 8 years. (14) Upper airway infection, Bartter's syndrome (hypokalaemia, 2.5 mM), enuresis nocturna. (15) Paroxysmal vertigo with attacks of 30 min to 2 h, hemiparaesthesia, diplopia, hemiparesis, headache. (16) Severe behavioural disorder and delayed development of speech, episodic tempers, chronic hypernatriemia, spastic diplegia, partial agenesis of corpus callosum, delayed myelination, hamartoid cerebral lesion, epileptic discharges, megalocorneae, hypopigmentation of the fundus and pallor of optic discs (Brockstedt et al. 1990). (17) ALTE with hypothermia (29°C) and shock. (18) Pseudostrabismus, ugly formed, coarse, notched and fawn-coloured teeth (Van Gennip et al. 1987)

observed for the DPD activity in mononuclear cells and fibroblasts (Meinsma et al. 1995; Van Gennip et al. 1995; Vreken et al. 1997b). Analysis of the prevalence of the various mutations among DPD patients has shown that the G→A point mutation in the invariant splice donor site leading to the skipping of exon 14 is by far the most common, whereas the other six mutations are less frequently observed (Table 1). In addition, there appears to be some homogeneity for the G→A point mutation in Northern Europe, since homozygosity for this mutation has been observed in 9 individuals from Denmark, Sweden, Finland and The Netherlands (Table 2). Furthermore, the majority of the DPD patients are of Dutch origin (55%); this probably reflects the fact that, in The Netherlands, screening for inborn errors of pyrimidine degradation is part of an intensive screening program for inborn errors in general.

So far, the frequency of these mutations in a normal population is not known. Based on the analysis of the DPD activity in various populations, it has been estimated that the frequency of heterozygotes might be as high as 3% (Gonzalez and Fernandez-Salguero 1995). Fortunately, the G→A point mutation destroys a unique *Mae*II restriction

Table 3 Onset phenotype of patients with DPD deficiency (*AS* asymptomatic, – not available for analysis)

Patient	Genotype	Age of onset of symptoms	Age at diagnosis	Epilepsy in family	Consanguinity	Treatment[a]
1	ΔEX14/ΔEX14	At birth	2 years, 1 months	No	No	
2	ΔEX14/ΔEX14	3 years	7 years	Yes	No	1
3	ΔEX14/ΔEX14	1–2 years	2 years	No	No	
4	ΔEX14/ΔEX14	At birth	At birth	–	No	
5	ΔEX14/ΔEX14	10 years 2 months	14 years, 6 months	No	No	
6	ΔEX14/ΔEX14	6 months	6 years, 2 months	No	No	2
7	ΔEX14/ΔEX14	Childhood	–	–	–	
8.1	ΔEX14/ΔEX14	7 years, 5 months	8 years, 1 month	Yes	No	3
8.2	ΔEX14/ΔEX14	Childhood	4 years, 5 months	Yes	No	
9.1	ΔTCAT/ΔTCAT	3 years	6 years	Yes	Yes	4
9.2	ΔTCAT/ΔTCAT	AS	4 years	Yes	Yes	
9.3	ΔTCAT/ΔTCAT	12 years	28 years	Yes	Yes	5
10	C29R/C29R	1 years	3 years	No	No	
11	V995F/V995F	1 years, 6 months	3 years, 1 months	No	No	
12.1	ΔC1897/ΔC1897	–	30 years	Yes	No	
12.2	ΔC1897/R235 W	4 months	6 years, 2 months	Yes	Yes	
13	C29R,R886H/ C29R, R886H	8 years	8 years	–	–	
14.1	C29R/ΔEX14	17 years, 6 months	18 years, 2 months	Yes	No	
14.2	C29R/ΔEX14	AS	26 years, 4 months	Yes	No	
15	ΔEX14/ΔTCAT	6 months	1 years, 3 months	No	No	6
16	ΔEX14/C29R	5 weeks	5 weeks	No	No	
17	ΔEX14/?	At birth	1 years, 10 months	–	No	

[a] (1) Responded to valproate. (2) No response to treatment with valproate. Good response to carbamazepine and lamotrigine. After interruption of medication, symptoms of complex partial epilepsy recurred. After reintroduction of medication, the patient is again seizure free (Christensen et al. 1998). (3) Good response to oxcarbazepine (Holopainen et al. 1997). (4) Phenobarbital and ethosuximide (Bakkeren et al. 1984; Braakhekke et al. 1987). (5) Phenytoin and phenobarbitone (Bakkeren et al. 1984). (6) Responded to pipamperon, clonazepam.

site present in an amplified genomic DNA fragment encompassing the skipped exon and its flanking sequences, allowing the rapid screening of this mutation in patients (Vreken et al. 1996; Wei et al. 1996). Screening for the presence of the G→A splice site mutation in a limited number of individuals of various nationalities has revealed heterozygosity for this mutation in 1% of the Finnish population (180 alleles analysed) and none in British (60 alleles), Japanese (100 alleles), African-American (210 alleles) or Dutch (100 alleles) populations (Wei et al. 1996, 1998; Vreken et al. 1996). Initially, an allele frequency of 5% was found for the G→A splice site mutation in Taiwanese subjects (72 alles analysed; Wei et al. 1996). However, in subsequent studies, the G→A splice site mutation has not been detected in a larger group of Taiwanese subjects (262 alleles analysed; Wei et al. 1998). In addition, neither the splice-site nor the 1897delC has been detected in 60 Caucasian subjects (Ridge et al. 1998).

Clinical phenotype of patients with DPD deficiency

A thorough investigation of clinical symptoms in the patients with complete DPD deficiency has shown a considerable variation in the clinical presentation among these patients (Table 2). Convulsive disorders (seizures and epileptic insults), motor retardation and mental retardation have been observed in approximately half of the patients, whereas growth retardation, microcephaly, autism and dysmorphy are less frequently observed. In this respect, it is worthwhile to note that, in 5 out of the 17 families, a history of convulsions is present (Table 3). A conspicuous finding has been the observation that five patients presented with ocular abnormalities. In one of the patients, the ocular symptoms are part of Kearns-Sayre syndrome with a verified mtDNA deletion but, even if this case is omitted, the four cases indicate a possible association with DPD deficiency. To our knowledge, a possible association of ocular abnormalities with DPD deficiency has not previously been recognised. Surprisingly, one patient suffers from a combined deficiency of DPD and the relatively rare syndrome of Kearns-Sayre, whereas one other patient suffers from a combined deficiency of DPD and Bartter's syndrome. The phenotypic variability of DPD deficiency is demonstrated by the finding that two asymptomatic patients have been identified and that seven other patients do not show the previously mentioned neurological and developmental abnormalities. However, 6 out of these 7 patients presented with other (neurological) abnormalities, such as lethargy, dizziness, monoplegia, an acute life-threatening event (ALTE) with hypothermia, and minor difficulties in learning speech and

language. This latter neurological abnormality has also recently been described in two other families with otherwise healthy DPD-deficient siblings (Henderson et al. 1995; Fernandez-Salguero et al. 1997). As discussed below, a number of the aforementioned symptoms might be explained by the altered β-alanine, uracil and thymine homeostasis in patients with DPD deficiency.

In all patients, the onset of the clinical phenotype occurred during childhood with the majority of the patients showing clinical abnormalities during the first years of life (Table 3). In general, a good response was noted when patients with convulsions/epileptic attacks were treated with anti-epileptic medication.

Phenotype-genotype relationship

An investigation into whether a correlation exists between the genotype and the observed clinical phenotype has revealed that all patients homozygous for the G→A splice site mutation presented with clinical abnormalities ranging from very mild (patients 5 and 8.2) to quite severe (Table 2). However, in a family presenting three subjects homozygous for the four-base deletion (295–298delTCAT), clinical abnormalities were clearly present in the index patient (no. 9.1) and his mother (no. 9.3), whereas the same genotype did not lead to a clinical phenotype in the brother of the patient (no. 9.2; Braakhekke et al. 1987; Vreken et al. 1997a). In addition, neurological abnormalities were seen in a patient who proved to be compound heterozygous for C29R/ΔEX14 (patient 14.1) but not in her sister (patient 14.2). Thus, the absence of a characteristic phenotype in some patients with complete DPD deficiency indicates that other factors play an important role in the clinical manifestation of this disorder. In this respect, it has been shown that the involvement of a second gene closely linked to the DPD gene on chromosome 1 in the expression of the clinical symptoms is not very likely (Fernandez-Salguero et al. 1997).

β-Alanine homeostasis

β-Alanine is a structural analogue of γ-aminobutyric acid (GABA) and glycine, which are the major inhibitory neurotransmitters in the central nervous system. It has been suggested that β-alanine itself is involved in synaptic transmission, since β-alanine is present in central nervous tissue (Martin Del Rio et al. 1977), is released upon depolarisation by high potassium (Sandberg and Jacobson 1981) or electrical stimulation (Kihara et al. 1989), inhibits neuronal excitability (Choquet and Korn 1988; Mathers et al. 1990) and is removed from the extracellular fluid by a high affinity uptake system shown to operate in brain slices (Kontro 1983), cerebellar granule cells (Saransaari and Oja 1993), synaptosomal preparations (Zafra et al. 1984) and glial cells (Schon and Kelly 1975; Holopainen and Kontro 1986; Mabjeesh et al. 1992). At least in chick spinal cord neurons and in mouse brain, it has been demonstrated that β-alanine activates both glycine and $GABA_A$ receptors with an efficacy similar to that for glycine and GABA, respectively (Choquet and Korn 1988; Horikoshi et al. 1988; Wu et al. 1993). Whether β-alanine is also able to bind to a unique receptor is still a matter of debate. Following synaptic release, GABA is transported into presynaptic endings and into glial cells where some of the neurotransmitter is metabolized by GABA transaminase. Most importantly, β-alanine has been shown to be a potent blocker of the uptake of GABA in glial cells (Mabjeesh et al. 1992). Reduced levels of β-alanine in patients with DPD deficiency might therefore have a profound effect on the degree of activation of the glycine and $GABA_A$ receptors and on GABA transport into glial cells. Since convulsions are often noted in patients with DPD deficiency, it is worthwhile to note that many anti-convulsant drugs act by potentiating the GABA-mediated inhibition in the nervous system and that GABA uptake blockers, such as β-alanine, possess profound anti-convulsant effects (Pfeiffer et al. 1996).

A conspicuous finding has been the presence of ocular abnormalities in 5 patients with a DPD deficiency. It has been suggested that, in addition to GABA, β-alanine is a neurotransmitter in the visual system (Sandberg and Jacobson 1981). Recently, a novel GABA receptor ($GABA_{\rho 1}$) has been detected in the retina; this receptor responds to glycine and β-alanine (Calvo and Miledi 1995). Therefore, an altered regulation of this $GABA_{\rho 1}$ receptor by decreased β-alanine concentrations may also play a role in the observed ocular abnormalities.

The regulation of body temperature takes place in the central nervous system in the hypothalamus and is affected by neurotransmitters such as serotonin and GABA (Dhumal et al. 1974). In addition, after the administration of β-alanine to mammals, both hypothermia and hyperthermia have been reported (Gomez et al. 1978; Peters et al. 1987). Whether the occurrence of severe hypothermia in one of the DPD deficient patients might have been related to reduced levels of β-alanine is not yet known.

Pyrimidine bases

There is an increasing awareness that pyrimidines play an important role in the regulation of the central nervous system and that metabolic changes affecting the levels of pyrimidines may lead to abnormal neurological activity (Connolly et al. 1996). Indeed, the anti-convulsant effects of uridine in animals with experimentally induced seizures indicate that pyrimidine compounds may play an important role in regulating the activity of the nervous system (Roberts 1973). Moreover, disturbances of pyrimidine metabolism by anti-metabolites in cancer treatment are thought to be responsible for neurotoxicity presenting as seizures (Wiley et al. 1982).

In patients with complete DPD deficiency, a large accumulation of uracil and thymine and a moderate amount of

5-hydroxymethyluracil is present in urine, blood and cerebrospinal fluid (Bakkeren et al. 1984; Van Gennip et al. 1993, 1994, 1997). Altered uracil and thymine homeostasis in addition to altered pools of their downstream products might conceivably underlie some of the clinical abnormalities of patients with DPD deficiency. Parenteral administration of low doses of uracil and thymine to mice has been shown to increase their spontaneous activity, whereas their activity is decreased at higher doses of these pyrimidine bases (Krooth et al. 1978). Very high concentrations of both uracil and thymine have even proved to be lethal. Most interestingly, the intraperitoneal administration of the degradation products has no effect on spontaneous activity, although the subcutaneous administration of high-doses of β-alanine leads to a moderate depression of activity (Krooth et al. 1978). The phenomena observed after the administration of pyrimidine bases to mice are in line with those observed in tumour patients treated with high doses of thymidine, which in vivo is rapidly catabolized into thymine. The central nervous system toxicities encountered in these patients include somnolence, headache, visual illusions and memory impairment (Chiuten et al. 1980). Thus, the elevated levels of pyrimidine bases in patients with complete DPD deficiency might underlie both the occurrence of lethargy and hyperactivity, as observed in two of the DPD-deficient patients.

Concluding remarks

In this paper, we have shown that a wide spectrum of clinical abnormalities, ranging from very mild to quite severe, are encountered in patients with complete DPD deficiency. The finding that some patients with complete deficiency of DPD do not present with any clinical abnormalities suggests that additional factors are involved determining the clinical outcome. Reasoning along these lines, we have speculated that an altered β-alanine homeostasis might be compatible with the various neurological symptoms seen in these patients. Since β-alanine can also be derived from dietary sources, such as carnosine, anserine and balanine (Van Gennip et al. 1993), the availability of these substances and of carnosinase activity in the relevant compartments may also affect the concentration of β-alanine in these compartments. In addition, we feel that not only a decreased level of β-alanine itself, but also the relative concentration of β-alanine compared with other neurotransmitters, such as GABA, might determine whether a clinical phenotype will emerge. Therefore, DPD deficiency is probably a necessary, but not a sole, prerequisite for the onset of a clinical phenotype. Nevertheless, the diagnosis of DPD deficiency is of paramount importance, not only in order to avoid severe toxicity during the 5FU treatment of tumour patients, but also in order to gain further insight into the relationship between the biochemical abnormalities and the onset of a clinical phenotype.

References

Albin N, Johnson MR, Diasio RB (1996) cDNA cloning of bovine liver dihydropyrimidine dehydrogenase. DNA Seq 6:243–250

Antonarakis SE (1998) Recommendations for a nomenclature system for human gene mutations. Hum Mutat 11:1–3

Bakker HD, Gonzalbo MER, Van Gennip AH (1994) Dihydropyrimidine dehydrogenase deficiency presenting with psychomotor retardation and ocular abnormalities. J Inher Metab Dis 17:640–641

Bakkeren JAJM, De Abreu RA, Sengers RCA, Gabreëls FJM, Maas JM, Renier WO (1984) Elevated urine, blood and cerebrospinal fluid levels of uracil and thymine in a child with dihydrothymine dehydrogenase deficiency. Clin Chim Acta 140:247–256

Berger R, Stoker-de Vries SA, Wadman SK, Duran M, Beemer FA, Bree PK de, Weits-Binnerts JJ, Penders TJ, Woude JK van der (1984) Dihydropyrimidine dehydrogenase deficiency leading to thymine-uraciluria. An inborn error of pyrimidine metabolism. Clin Chim Acta 141:227–234

Braakhekke JP, Renier WO, Gabreëls FJM, De Abreu RA, Bakkeren JAJM, Sengers RCA (1987) Dihydropyrimidine dehydrogenase deficiency. Neurological aspects. J Neurol Sci 78:71–77

Brockstedt M, Jakobs C, Smit LME, Van Gennip AH, Berger R (1990) A new case of dihydropyrimidine dehydrogenase deficiency. J Inher Metab Dis 13:121–124

Calvo DJ, Miledi R (1995) Activation of $GABA_{\rho 1}$ receptors by glycine and β-alanine. NeuroReport 6:1118–1120

Chiuten DF, Wiernik PH, Zaharko DS, Edwards L (1980) Clinical phase I-II and pharmacokinetic study of high-dose thymidine given by continuous intravenous infusion. Cancer Res 40:818–822

Choquet D, Korn H (1988) Does β-alanine activate more than one chloride channel associated receptor? Neurosci Lett 84:329–334

Christensen E, Cezanne I, Kjaergaard S, Hørlyk H, Faurholt-Pedersen V, Vreken P, Van Kuilenburg ABP, Van Gennip AH (1998) Clinical variability in three Danish patients with dihydropyrimidine dehydrogenase deficiency all homozygous for the same mutation. J Inher Metab Dis 21:272–275

Connolly GP, Simmonds HA, Duley JA (1996) Pyrimidines and CNS regulation. Trends Pharmacol Sci 17:106

Dhumal VR, Gulati OD, Raghunath PR, Sivaramakrishna N (1974) Analysis of the effects on body temperature of intracerebroventricular injection in anaesthetized dogs of gamma-aminobutyric acid. Br J Pharmacol 50:513–524

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

Gomez, MAM, Carlsson A, De Yebenes JG (1978) The effect of β-alanine on motor behaviour, body temperature and cerebral monoamine metabolism in rat. J Neural Transm 43:1–9

Gonzalez FJ, Fernandez-Salguero P (1995) Diagnostic analysis, clinical importance and molecular basis of dihydropyrimidine dehydrogenase deficiency. Trends Pharmacol Sci 16:325–327

Heggie GD, Sommadossi J-P, Cross DS, Huster WJ, Diasio RB (1987) Clinical pharmacokinetics of 5-fluorouracil and its metabolism in plasma, urine, and bile. Cancer Res 47:2203–2206

Henderson MJ, Jones S, Walker P, Duley J, Simmonds HA (1995) Heterogeneity of symptomatology in two male siblings with thymine uraciluria. J Inher Metab Dis 18:85–86

Ho DH, Townsend L, Luna MA, Bodey GP (1986) Distribution and inhibition of dihydrouracil dehydrogenase activities in human tissues using 5-fluorouracil as a substrate. Anticancer Res 6:781–784

Holopainen I, Kontro P (1986) High-affinity uptake of taurine and β-alanine in primary cultures of rat astrocytes. Neurochem Res 11:207–215

Holopainen I, Pulkki, K, Heinonen OJ, Näntö-Salonen K, Haataja L, Greter J, Holme E, Van Kuilenburg ABP, Vreken P, Van Gennip AH (1997) Partial epilepsy in a girl with a symptom-free sister: first two Finnish patients with dihydropyrimidine dehydrogenase deficiency. J Inher Metab Dis 20:719–720

Horikoshi T, Asanuma A, Yanagisawa K, Anzai K, Goto S (1988) Taurine and β-alanine act on both GABA and glycine receptors in *Xenopus* oocyte injected with mouse brain messenger RNA. Mol Brain Res 4:97–105

Johnson MR, Wang K, Tillmanns S, Albin N, Diasio RB (1997) Structural organization of the human dihydropyrimidine dehydrogenase gene. Cancer Res 57:1660–1663

Kihara M, Misu Y, Kubo T (1989) Release by electrical stimulation of endogenous glutamate, γ-aminobutyric acid, and other amino acids from slices of the rat medulla oblongata. J Neurochem 52:261–267

Kontro P (1983) β-alanine uptake by mouse brain slices. Neuroscience 8:153–159

Krooth, RS, Hsiao WL, Lam GFM (1978) Effects of natural pyrimidines and of certain related compound on the sponteneous activity of the mouse. J Pharmcol Exp Ther 207:504–514

Lu Z, Zhang R, Diasio RB (1992) Purification and characterization of dihydropyrimidine dehydrogenase from human liver. J Biol Chem 267:17102–17109

Lu Z, Zhang R, Diasio RB (1993) Comparison of dihydropyrimidine dehydrogenase from human, rat, pig and cow liver. Biochemical and immunological properties. Biochem Pharmacol 46:945–952

Mabjeesh NJ, Frese M, Rauen T, Jeserich G, Kanner BI (1992) Neuronal and glial γ–aminobutyric acid$^+$ transporters are distinct proteins. FEBS Lett 299:99–102

Martin del Rio R, Orensanz Muñoz LM, DeFeudis FV (1977) Contents of β-alanine and γ-aminobutyric acid in regions of rat CNS. Exp Brain Res 28:225–227

Mathers DA, Grewal A, Wang Y (1990) β-Alanine induced channels in the membrane of cultured spinal cord neurons. Neurosci Lett 108:127–131

McLeod, HL, Collie-Duguid ESR, Vreken P, Johnson MR, Wei X, Sapone A, Diasio RB, Fernandez-Salguero P, Van Kuilenburg ABP, Van Gennip AH, Gonzalez FJ (1998) Nomenclature for human DPYD alleles. Pharmacogenetics (in press)

Meinsma R, Fernandez-Salguero P, Van Kuilenburg ABP, Van Gennip AH, Gonzalez FJ (1995) Human polymorphism in drug metabolism: mutation in the dihydropyrimidine dehydrogenase gene results in exon skipping and thymine uraciluria. DNA Cell Biol 14:1–6

Naguib FNM, Kouni MH el, Cha S (1985) Enzymes of uracil catabolism in normal and neoplastic human tissues. Cancer Res 45:5405–5412

Peters GJ, Groeningen CJ van, Laurensse E, Kraal I, Leyva A, Lankelma J, Pinedo HM (1987) Effect of pyrimidine nucleosides on body temperatures of man and rabbit in relation to pharmacokinetic data. Pharm Res 4:113–119

Pfeiffer M, Draguhn A, Meierkord H, Heinemann U (1996) Effects of γ-aminobutyric acid (GABA) agonists and GABA uptake inhibitors on pharmacosensitive and pharmacoresistant epileptiform activity in vitro. Br J Pharmacol 119:569–577

Podschun B, Wahler G, Schnackerz KD (1989) Purification and characterization of dihydropyrimidine dehydrogenase from pig liver. Eur J Biochem 185:219–224

Podschun B, Cook PF, Schnackerz KD (1990) Kinetic mechanism of dihydropyrimidine dehydrogenase from pig liver. J Biol Chem 265:12966–12972

Porter DJT, Chestnut WG, Taylor LCE, Merrill BM, Spector T (1991) Inactivation of dihydropyrimidine dehydrogenase by 5-iodouracil. J Biol Chem 266:19988–19994

Porter DJT, Spector T (1993) Dihydropyrimidine dehydrogenase. Kinetic mechanism for reduction of uracil by NADPH. J Biol Chem 268:19321–19327

Ridge SA, Sludden J, Brown O, Robertson L, Wei X, Sapanone A, Fernandez-Salguero PM, Gonzalez FJ, Vreken P, Van Kuilenburg ABP, Van Gennip AH, McLeod HL (1998) Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. Br J Clin Pharmacol 46:151–156

Roberts CA (1973) Anticonvulsant effects of uridine: comparitive analysis of metrazol and penicillin induced foci. Brain Res 55:291–308

Sandberg M, Jacobson I (1981) β-Alanine, a possible neurotransmitter in the visual system? J Neurochem 37:1353–1356

Saransaari P, Oja SS (1993) Uptake and release of β-alanine in cerebellar granule cells in primary culture: regulation of release by glutamatergic and GABAergic receptors. Neurosci Lett 53:475–481

Schon F, Kelly JS (1975) Selective uptake of [^{3}H]β-alanine by glia: association with the glial uptake system for GABA. Brain Res 86:243–257

Shiotani T, Weber G (1981) Purification and properties of dihydothymine dehydrogenase from rat liver. J Biol Chem 256:219–224

Takai S, Fernandez-Salguero P, Kimura S, Gonzalez FJ, Yamada K (1994) Assignment of the human dihydropyrimidine dehydrogenase gene (DPYD) to chromosome region 1p22 by fluorescence in situ hybridization. Genomics 24:613–614

Van Gennip AH, Van Bree-Blom EJ, Wadman SK, Bree PK de, Duran M, Beemer FA (1981) Liquid chromatography of urinary pyrimidines for the evaluation of primary and secondary abnormalities of pyrimidine metabolism In: Hawk GL, Champlin PB, Hutton RF, Mol C (eds) Biological/biomedical applications of liquid chromatography III. Chromatographic science series, vol 18. Dekker, New York Basel, pp 285–296

Van Gennip AH, Bakker HD, Zoetekouw A, Abeling NGGM (1987) A new case of thymine uraciluria. Klin Wochenschr 65 (Suppl X):14

Van Gennip AH, Busch S, Elzinga L, Stroomer AEM, Van Cruchten A, Scholten EG, Abeling NGGM (1993) Application of simple chromatographic methods for the diagnosis of defects in pyrimidine degradation. Clin Chem 39:380–385

Van Gennip AH, Abeling NGGM, Stroomer AEM, Van Lenthe H, Bakker HD (1994) Clinical and biochemical findings in six patients with pyrimidine degradation defects. J Inher Metab Dis 17:130–132

Van Gennip AH, Van Lenthe H, Abeling NGGM, Bakker HD, Van Kuilenburg ABP (1995) Combined deficiencies of NADPH- and NADH-dependent dihydropyrimidine dehydrogenases, a new finding in a family with thymine-uraciluria. J Inher Metab Dis 18:185–188

Van Gennip AH, Abeling NGGM, Vreken P, Van Kuilenburg ABP (1997) Inborn errors of pyrimidine degradation: clinical, biochemical and molecular aspects. J Inher Metab Dis 20:203–213

Van Kuilenburg ABP, Vreken P, Beex LVAM, Meinsma R, Van Lenthe H, De Abreu RA, Van Gennip AH (1997a) Heterozygosity for a point mutation in an invariant splice donor site of dihydropyrimidine dehydrogenase and severe 5-fluorouracil related toxicity. Eur J Cancer 33:2258–2264

Van Kuilenburg ABP, Van Lenthe H, Wanders RJA, Van Gennip AH (1997b) Subcellular localization of dihydropyrimidine dehydrogenase. Biol Chem 378:1047–1053

Van Kuilenburg ABP, Blom MJ, Van Lenthe H, Mul E, Van Gennip AH (1997c) The activity of dihydropyrimidine dehydrogenase in human blood cells. J Inher Metab Dis 20: 331–334

Van Kuilenburg ABP, Vreken P, Beex LVAM, Meinsma R, Van Lenthe H, De Abreu RA, Van Gennip AH (1998a) Heterozygosity for a point mutation in an invariant splice donor site of dihydropyrimidine dehydrogenase and severe 5-fluorouracil related toxicity. Adv Exp Med Biol 431:293–298

Van Kuilenburg ABP, Vreken P, Beex LVAM, De Abreu RA, Van Gennip AH (1998b) Severe 5-fluorouracil toxicity caused by reduced dihydropyrimidine dehydrogenase activity due to heterozygosity for a G→A point mutation. J Inher Metab Dis 21:280–284

Van Kuilenburg ABP, Van Lenthe H, Blom MJ, Mul EPJ, Van Gennip AH (1998c) The activity of dihydropyrimidine dehydrogenase in human blood cells. Adv Exp Med Biol 431:823–826

Van Kuilenburg ABP, Van Lenthe H, Blom MJ, Mul EPJ, Van Gennip AH (1998d) Profound variation in dihydropyrimidine dehydrogenase activity in human blood cells. Major implications for the detection of partly deficient patients. Br J Cancer (in press)

Van Kuilenburg ABP, Vreken P, Riva D, Botteon G, Abeling NGGM, Bakker HD, Van Gennip AH (1999) Clinical and biochemical abnormalities in a patient with dihydropyrimidine dehydrogenase deficiency due to homozygosity for the C29R mutation. J Inher Metab Dis (in press)

Vreken P, Van Kuilenburg ABP, Meinsma R, Smit GPA, Bakker HD, De Abreu RA, Van Gennip AH (1996) A point mutation in an in-

variant splice donor site leads to exon skipping in two unrelated Dutch patients with dihydropyrimidine dehydrogenase deficiency. J Inher Metab Dis 19:645–654

Vreken P, Van Kuilenburg ABP, Meinsma R, De Abreu RA, Van Gennip AH (1997a) Identification of a four-base deletion (delTCAT296–299) in the dihydropyrimidine dehydrogenase gene with variable clinical expression. Hum Genet 100:263–265

Vreken P, Van Kuilenburg ABP, Meinsma R, Van Gennip AH (1997b) Identification of novel point mutations in the dihydropyrimidine dehydrogenase gene. J Inher Metab Dis 20:335–338

Vreken P, Van Kuilenburg ABP, Meinsma R, Van Gennip AH (1997c) Dihydropyrimidine dehydrogenase (DPD) deficiency: identification and expression of missense mutations C29R, R886H and R235W. Hum Genet 101:333–338

Vreken P, Van Kuilenburg ABP, Meinsma R, Beemer, FA, Duran M, Van Gennip AH (1998) Dihydropyrimidine dehydrogenase deficiency: a novel mutation and expression of missence mutations in *E. coli*. J Inher Metab Dis 21:276–279

Wei X, McLeod HL, McMurrough J, Gonzalez FJ, Fernandez-Salguero P (1996) Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. J Clin Invest 3:610–615

Wei X, Elizondo G, Sapone A, McLeod HL, Raunio H, Fernandez-Salguero P, Gonzalez FJ (1998) Characterization of the human dihydropyrimidine dehydrogenase gene. Genomics 51:391–400

Wiley RG, Gralla RJ, Casper ES, Kemeny N (1982) Neurotoxicity of the pyrimidine synthesis inhibitor N-phospho-acetyl-L-aspartate. Ann Neurol 12:175–183

Wu FS, Gibbs TT, Farb DH (1993) Dual activation of $GABA_A$ and glycine receptors by β-alanine: inverse modulation by progesterone and 5α-pregnan-3α-ol-20-one. Eur J Pharmacol 246:239–246

Yokota H, Fernandez-Salguero P, Furuya H, Lin K, McBride OW, Podschun B, Schnackerz KD, Gonzalez FJ (1994) cDNA cloning and chromosome mapping of human dihydropyrimidine dehydrogenase, an enzyme associated with 5-fluorouracil toxicity and congenital thymine uraciluria. J Biol Chem 269:23192–23196

Zafra F, Aragon MC, Valdivieso F, Gimenez C (1984) β-Alanine transport into plasma membrane vesicles derived from rat brain synaptosomes. Neurochem Res 9:695–707