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Inborn errors of pyrimidine degradation: Clinical, biochemical and molecular aspects

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Summary: The pyrimidines, uracil and thymine, are degraded in four steps. The first three steps of pyrimidine catabolism, controlled by enzymes shared by both pathways, result in the production of the neurotransmitter amino acid β-alanine from uracil and the nonfunctional (R)-(−)-β-aminoisobutyrate from thymine. The fourth step is controlled by several aminotransferases, which have different affinities for β-alanine, β-aminoisobutyrate and GABA. Defects concerning the first three steps all lead to a reduced production of β-alanine; defects of the transaminases involving the metabolism of β-alanine and GABA lead to accumulation of these neurotransmitter substances. In addition, other metabolites will accumulate or be reduced depending on the specific enzyme defect. Analysis of the abnormal concentrations of these metabolites in the body fluids is essential for the detection of patients with pyrimidine degradation defects. Clinically these disorders are often overlooked because symptomatology is highly aspecific. The growth in our knowledge concerning inborn errors of pyrimidine degradation has emphasized the importance of the clinical awareness of these defects as a possible cause of neurological disease and a contraindication for treatment of cancer patients with certain pyrimidine analogues. The various defects are discussed and attention is paid to clinical, genetic and diagnostic aspects.

In man, pyrimidines are degraded in four steps (Figure 1), catalysed by dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2), dihydropyrimidinase (DHP, EC 3.5.2.2), β-ureido-propionase (UP, EC 3.5.1.6) and three aminotransferases, (R)-(−)-β–aminoisobutyrate-pyruvate aminotransferase (BAIBPAT, EC 2.6.1.40), β-alanine-pyruvate aminotransferase (BAPAT, EC 2.6.1.18) and β-alanine-α-ketoglutarate aminotransferase (BAKAT, EC 2.6.1.19).

Inherited deficiency of BAIBPAT is known to be the defect in genetic high excretors of β-aminoisobutyric acid (Harris 1953; Kakimoto et al 1969); deficiency of BAKAT was hypothesized in the first reported patient with primary hyper-β-alaninaemia (Scriver et al 1966), and a partial deficiency has recently been established in fibroblasts of another case (Higgins et al 1994).

Two other defects of pyrimidine catabolism have been discovered more recently: inherited DPD deficiency was first proposed in 1981 in a patient with thymine-uraciluria (Van Gennip et al 1981a) and was demonstrated in 1984 (Bakkeren et al 1984); the first patient with presumed DHP deficiency as detected in 1990 (Duran et al 1990) and the enzyme defect was demonstrated in liver in 1996 (Van Gennip et al 1996a). Deficiencies of BAPAT and UP have not yet been discovered in man; UP deficiency has been shown in C57B1/6 mice (Dagg et al 1964).

Recent findings such as the detection of 33 patients of various nationalities with DPD deficiency within 15 years in The Netherlands alone suggest that inborn errors of pyrimidine degradation are less rare than has generally been assumed. With the exception of BAIPAT deficiency, the other enzyme defects of pyrimidine degradation can all present with severe neurological symptoms, although the clinical phenotype of patients suffering from these defects can be highly variable. In addition (partial) DPD deficiency can lead to severe neurotoxicity when affected individuals are treated with pyrimidine analogues such as 5-fluorouracil. For these reasons, intensification of the search for inherited disorders of pyrimidine degradation is highly recommended.

This review covers the clinical, biochemical and molecular aspects of the various disorders of pyrimidine degradation, with special attention given to the relatively new defects of DPD and DHP.

CLINICAL ASPECTS OF PYRIMIDINE DEGRADATION DEFECTS

Symptomatology

The predominant symptoms of patients with pyrimidine degradation defects involve neurological disorders. Epileptic or convulsive attacks were reported in more than half of the cases with a near complete deficiency of DPD (Van Gennip et al 1981a, 1987a, 1994, 1996b; Bakkeren et al 1984; Berger et al 1984; Wilcken et al 1985; Braakhekke et al 1987; Brockstedt et al 1990); in three out of the four patients with DHP deficiency (Duran et al 1990; Henderson et al 1993; Ohba et al 1994; Van Gennip et al 1997); in the one patient with presumed BAKAT deficiency (Scriver et al 1966); and in the patient with a proven partial deficiency (±30% of normal activity) of BAKAT in fibroblasts (Higgins et al 1994). Both of these last two patients also had a striking lethargy. Mental retardation was mentioned in about half of the patients with DPD and in two of the patients with DHP-deficiency. Growth retardation, dysmorphic features, microcephaly and motor retardation were less frequently found in DPD-deficient patients; it was seen in one of the DHP deficient patients and in the patient with partial BAKAT deficiency. The latter patient exhibited the characteristic facial appearance of Cohen syndrome. The severity of the manifestations has varied strongly between patients with DPD deficiency and even asymptomatic cases have been identified (Braakhekke et al 1987, a 4-year-old boy; Adolph et al 1991, a patient identified by newborn screening; Van Gennip et al 1994, an adult male found accidentally, patient T.S. in Table 1 of the paper). Hyper-β-aminoisobutyric aciduria due to BAIBPAT deficiency is thought to be a benign polymorphism.

Severe neurotoxicity due to 5-fluorouracil has been described in about 15 adult patients with profound (less than 10% of normal activity) DPD deficiency (Diasio et al 1988; Harris et al 1991) or partial (10–30% of normal activity) DPD deficiency (Lu et al 1994).
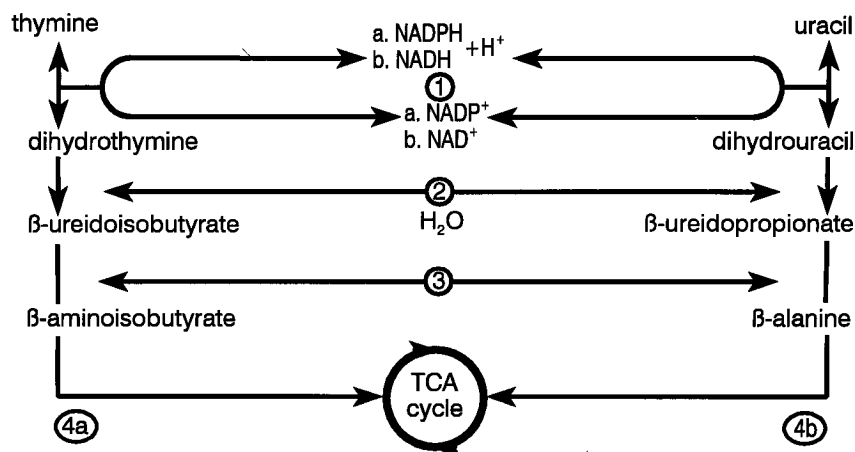
Figure 1 Scheme of pyrimidine degradation pathways showing the four steps and the enzymes involved in the catalysis of each step. (1) Dihydropyrimidine dehydrogenase (DPD): (a) NADPH dependent; (b) NADH dependent. (2) Dihydropyrimidinase (DHP). (3) Ureidopropionase (UP). (4) (a) (R)-(−)-β-aminoisobutyrate-pyruvate aminotransferase (BAIBPAT); (b) β-alanine-α-keto-glutarate aminotransferase (BAKAT)

5-Fluorouracil is a pyrimidine analogue widely used in the treatment of cancers of the gastrointestinal tract, ovary and breast. The drug is degraded by the same pathway as thymine and uracil, using the same enzymes (Figure 1). Although not yet reported, increased sensitivity for 5-fluorouracil neurotoxicity can also be expected in patients with (partial) dihydropyrimidinase deficiency, because of the reversibility of the first degradation step (Figure 1).

Mode of inheritance

DPD and DHP deficiency follow an autosomal pattern of inheritance. The conclusion is based on family studies in DPD deficiency and on the results of loading tests in a DHP family and the similarity of findings in unrelated DHP-deficient children from consanguineous kindreds. BAIBPAT deficiency seems to be determined by an incomplete recessive gene plus other familial factors (Scriver and Gibson 1995). BAKAT deficiency is so far very rare and therefore its mode of inheritance is not known.

Treatment

Patients with BAIBPAT deficiency need not be treated. Treatment of patients with DPD or DHP deficiency has not been described. Treatment for hyper-β-alaninaemia may be effective: in the first patient the metabolic, but not the clinical, phenotype improved with treatment with 10mg/day of pyridoxine (Scriver et al 1966); in the second patient both the metabolic as well as clinical phenotype improved dramatically on 100mg/day of pyridoxine (Higgins et al 1994).

BIOCHEMICAL ASPECTS

Implications of defects of pyrimidine degradation

The first three steps of the two pyrimidine degradation pathways for thymine and uracil, respectively, make use of the same three enzymes: DPD, DHP and UP (Figure 1). By these steps thymine and uracil are converted into β-aminoisobutyric acid and β-alanine, respectively. The fourth step of the two degradation pathways is catalysed by different enzymes: the conversion of (*R*)-β-aminoisobutyric acid into (*R*)-methylmalonic acid semi-aldehyde is catalysed by BAIBPAT; the conversion of β-alanine into malonic acid semi-aldehyde can be catalysed by BAPAT or BAKAT. β-Alanine can also be converted into carnosine, the reaction being catalysed by carnosine synthetase.

In patients with DPD deficiency, thymine and uracil accumulate and appear in elevated concentrations in the body fluids. In some cases 5-hydroxymethyluracil, which is a metabolite of thymine, can also be detected in body fluids. As expected, the concentrations of dihydropyrimidines, the *N*-carbamyl-β-amino acids (β-ureidoisobutyrate and β-ureidopropionate) and β amino acids are low or these metabolites may even be absent. In DHP-deficient patients dihydrothymine and dihydrouracil accumulate and because of the reversibility of the first step thymine and uracil also accumulate, and all these metabolites appear in elevated concentrations in the body fluids. The concentrations of the *N*-carbamyl-β-amino acids and β-amino acids are low or these metabolites are absent. Deficiency of UP (new name β-alanine synthase) has not yet been reported in man. In such patients high concentrations of *N*-carbamyl-β-amino acids and low concentrations of β-amino acids can be expected in the body fluids. Deficiency of BAIBPAT results in accumulation of (*R*)-β-aminoisobutyric acid, which is the enantiomer derived from thymine; (*S*)-β-aminoisobutyric acid originates from L-valine (Van Gennip et al 1981b). Deficiency of BAPAT or BAKAT will lead to accumulation of β-alanine.

As can be seen in Figure 1, all enzyme defects of pyrimidine degradation other than BAIBPAT deficiency will lead to altered β-alanine concentrations. In DPD or DHP deficiency the production of β-alanine will be reduced, but with BAKAT or BAPAT deficiency β-alanine will accumulate. The altered concentrations of the neurotransmitter β-alanine may be of relevance with respect to the cerebral dysfunction that occurs in patients with these defects. In DPD and DHP deficiency exposure of the nervous system to high concentrations of uracil and/or thymine also may be a contributing factor.

The severe neurotoxicity caused by 5-fluorouracil in patients with a (partial) deficiency of DPD may result from the exposure of the nervous system to relatively high concentrations of 5-fluorouracil, leading to increased incorporation of the drug into cellular RNA. However, shortage of β-alanine caused by complete DPD deficiency or by competition of 5-fluorouracil as substrate in partial DPD deficiency may also contribute to this condition. For similar reasons, although not yet reported, increased sensitivity for 5-fluorouracil neurotoxicity can also be expected in patients with (partial) DHP deficiency.

Detection and diagnosis

The preferential material for the screening of pyrimidine degradation disorders is urine, as all waste products accumulate in this body fluid. If urine is not available, blood and

cerebrospinal fluid (CSF) can be used for screening, but the accumulation of abnormal metabolites in these body fluids is much less prominent. The four known pyrimidine degradation defects can be detected by GC-MS analysis of urinary trimethylsilylated organic acid extracts (Wadman et al 1984) and by analysis of amino acids in urine before and after acid hydrolysis (Van Gennip et al 1993), procedures which are widely used for the screening of inborn errors of metabolism.

Strongly elevated amounts of the pyrimidine bases and dihydropyrimidines can be detected and identified by GC-MS, but quantification is not possible because of variable extraction yields. Specific methods such as two-dimensional TLC (Van Gennip et al 1990) or HPLC with or without prefractionation of urine (Van Gennip et al 1989) are more sensitive. Quantification requires sophisticated methods such as isotope dilution GC-MS (Jakobs et al 1991), HPLC with (diode-array) UV detection at various wavelengths in off-line fractions obtained by isolation and prefractionation of the bases and nucleosides, or on-line (dual-column methods) prepared fractions (Van Gennip et al 1987). Isotope dilution GC-MS has been used to measure uracil and thymine in amniotic fluid, after ethyl acetate extraction, as their trimethylsilyl ethers with $^{15}N_2$-labelled uracil as internal standard (Jakobs et al 1991). Figure 2 shows the GC profile (A), the TLC pattern (B) and the corresponding HPLC profile (C) of the urine from a patient with DPD deficiency. For comparison a normal TLC pattern is also presented (D). The elevated excretion of uracil and thymine was easily detected by all three methods, but 5-hydroxymethyluracil was not observed in the GC profile. Quantification by HPLC yielded excretion values of 280 and 37 mmol/mol creatinine for uracil and thymine, respectively, and a small amount of 5-hydroxymethyluracil. In the patients with DPD deficiency we found the following ranges for the excretion (mmol/mol creatinine) of the index compounds: uracil 56–683 (controls 3–33, $n = 100$); thymine 7–439 (controls 0–4, $n = 100$); and 5-hydroxymethyluracil 0–54 (controls not detectable, $n = 100$). In a patient with DHP deficiency we found for uracil 49 and for thymine 12 mmol/mol creatinine; 5-hydroxymethyluracil was below the detection limit. The dihydropyrimidines, which are almost undetectable in urine from DPD-deficient patients, are excreted in large amounts by patients with DHP deficiency and can easily be detected by GC-MS (for GC profile and relevant MS spectra, see Henderson et al 1993).

Screening for elevated urinary dihydropyrimidines and/or *N*-carbamyl-β-amino acids can be performed by amino acid analysis after conversion of these compounds into their corresponding β-amino acids by acid hydrolysis (Van Gennip et al 1993). Differential analysis of these compounds is possible using the same procedure after isolation of the dihydropyrimidines, *N*-carbamyl-β-amino acids and β-amino acids in separate fractions by cation- and anion-exchange chromatography (Van Gennip et al 1993). Application of this method to the urine of a DHP-deficient patient revealed the excretion of dihydrouracil to be 622 and of dihydrothymine to be 406 mmol/mol creatinine. Alternatively, the dihydro-pyrimidines in urine can also be measured by HPLC/FAB-MS with selected protonated molecular ion monitoring (Duran et al 1991).

The patient with BAKAT deficiency (Scriver et al 1966) and the patient with proven partial BAKAT deficiency (Higgins et al 1994) both presented with persistently elevated concentrations of β-alanine in plasma (patients, range 20–51 μmol/L; normal < 14), CSF (patient 1, 45 μmol/L; patient 2, elevated; normal < 0.06) and urine (patient 1, 100 times normal; patient 2, 28 μmol/24h; normal, trace). GABA was also reported to be elevated in
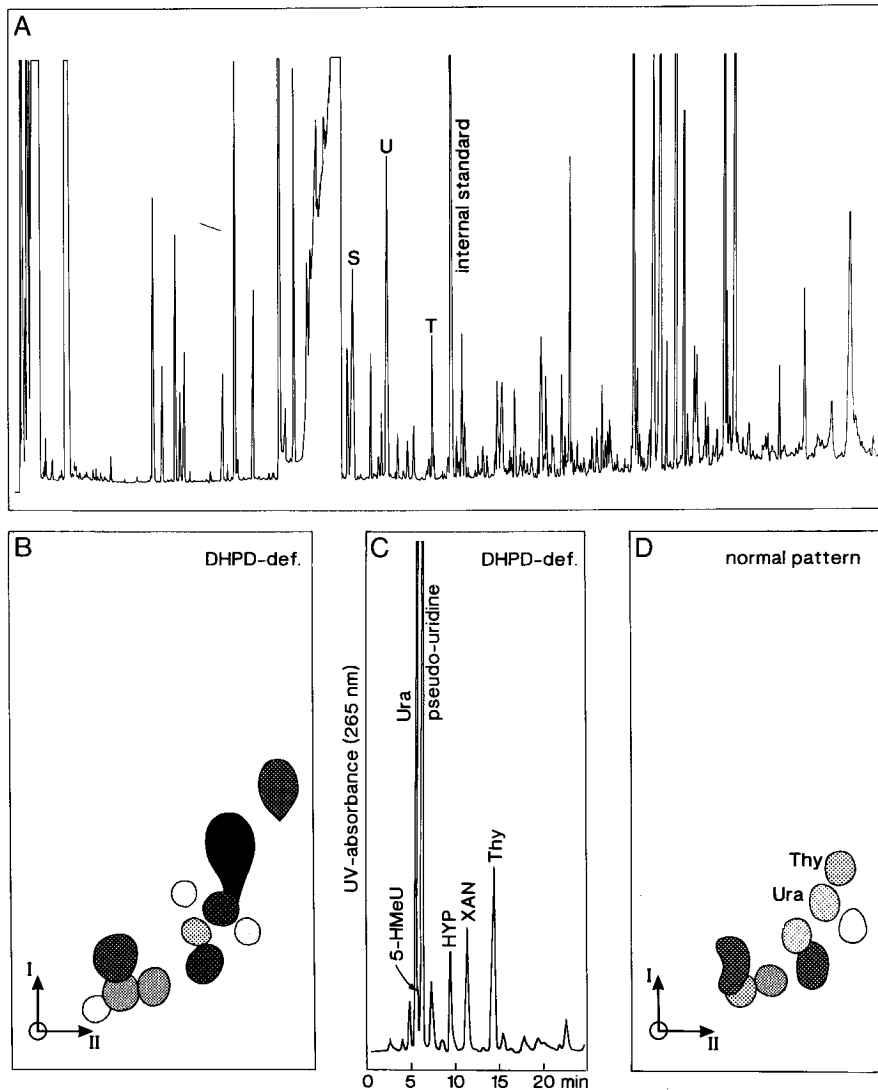
Figure 2 (A) GC profile of an organic acid extract from a urine sample of a patient with dihydropyrimidine dehydrogenase deficiency. Organic acids were analysed, after their extraction with ethyl acetate, as trimethylsilyl derivatives by capillary GC. (B) Two-dimensional TLC pattern of the same patient. (C) Corresponding HPLC profile. (D) Normal two-dimensional TLC pattern

the urine of both patients, and in plasma and CSF of patient 1. In the urine of patient 1 β-aminoisobutyric acid and taurine were also elevated. Individuals with BAIBPAT deficiency are only characterized by an elevated concentration of β-aminoisobutyric acid in urine. The patients with BAKAT deficiency or individuals with BAIBPAT deficiency can easily be detected by amino acid analysis of urine. Patients with UP deficiency or

BAPAT deficiency have not been reported yet, but in principle they can also be detected by the methods described above.

Pitfalls in the detection of pyrimidine degradation defects

Abnormal concentrations of pyrimidine catabolites simulating inborn errors of pyrimidine degradation can have various causes. Thymine-uraciluria suggesting DPD or DHP deficiency can also result from excessive tissue breakdown. In patients with this condition, urinary pseudouridine, β-aminoisobutyric acid and uric acid are also frequently elevated (Van Gennip et al 1993). Hyper-β-alaninuria combined with hyper-β-aminoisobutyric aciduria can simulate BAIBPAT deficiency. However, both conditions can also occur in cancer patients owing to an increased tissue degradation (Van Gennip et al 1987b). Hyper-β-alaninuria with hyper-β-amino isobutyric aciduria can also be caused by γ-vinyl-GABA, a drug that inhibits the relevant transaminase. Excessive urinary excretion of β-alanine and (*RS*)-β-aminoisobutyric acid in combination with β-hydroxypropionate, 3-hydroxyisobutyrate and (*S*)-2-(hydroxymethyl)butyrate has also been reported in a patient with reduced malonic semialdehyde dehydrogenase activity and deficient methylmalonic semialdehyde dehydrogenase (Pollitt et al 1985; Gray et al 1987). Bacterial metabolism can be the cause of *N*-carbamyl-β-amino aciduria instead of dihydropyrimidinuria in patients with DHP deficiency, thereby simulating UP deficiency (Van Gennip et al 1993). Uraciluria in combination with hypopseudouridinuria can also result from bacterial contamination of urine (Van Gennip et al 1993).

MOLECULAR ASPECTS

Confirmation of diagnosis by enzyme analysis

The diagnosis of DPD deficiency can be confirmed by analysis of DPD activity in liver tissue, fibroblasts and lymphocytes (Diasio et al 1994; Van Gennip et al 1995; Van Kuilenburg et al 1996a). Very recently, DPD activity was also demonstrated in human monocytes, gran-ulocytes and platelets, but not in erythrocytes (Van Kuilenburg 1996b). The DPD enzyme in human tissues is known to be dependent on NADPH as a cosubstrate. However, we discovered also substantial NADH-dependent DPD activity in human liver and fibroblasts, but only a very low activity in lymphocytes (Table 1). In our paediatric patients with DPD deficiency we could detect no activity with either cosubstrate in leukocytes and fibroblasts.

A prenatal diagnosis of DPD deficiency by analysing DPD activity in fetal liver has been reported, but in this case the activity of DPD in a control liver was also rather low. However, the diagnosis was confirmed by measurement of significantly elevated concentrations of thymine and uracil in amniotic fluid (Jakobs et al 1991).

In the adult patients with severe neurotoxicity due to 5-fluorouracil, activities of DPD have been reported varying from very low to about 30% of normal. In these cases only NADPH was used as cosubstrate (Diasio et al 1988; Harris et al 1991; Lu et al 1994). For comparison of the results of patients with controls and the interpretation of the results, one has to be aware of the fact that DPD activity shows a circadian rhythm, the difference between maximum and minimum activities being a factor 2 in humans.

Table 1   The activity of DPD in various tissue homogenates from different controls[a]

| Tissue | *Activity* (nmol/h per mg protein) | | Activity ratio NADPH/NADH |
	With NADPH	*With NADH*	
Lymphocytes	8.1±2.3 (12)	0.05; 0.03 (2)	124; 196 (2)
Fibroblasts	1.0±0.7 (21)	0.19±0.12 (19)	5.5±2.4 (13)
Liver	9.3±2.4 (8)	0.97±0.43 (8)	11.7±6.7 (8)

[a]Values are mean±SD. Number of controls is given in parentheses. For NADH-dependent activity in lymphocytes, only 2 values are available

Carrier detection of DPD deficiency by analysis of enzyme activity in lymphocytes or fibroblasts has been reported to be unreliable. However, it is our experience that carrier detection is possible if circadian rhythm is taken into consideration. Therefore, we recommend collection of samples from suspected carriers, patients and noncarriers at the same time of day.

DHP deficiency can only be demonstrated by analysis of the enzyme activity in liver, because the enzyme is not expressed in other more easily accessible tissues. In liver from a patient with DHP deficiency we could not detect DHP activity; in 8 control liver samples DHP activities ranged from 20 to 74 nmol/h per mg protein (Van Gennip et al 1996a).

Patients with UP (β-alanine synthase) deficiency have not been reported. The enzyme is only expressed in liver. We found that UP activity of human liver was inhibited by propionate at concentrations found in plasma of patients with propionic acidaemia (Van Gennip et al 1996b).

BAKAT and GABA-transaminase (4-aminobutyrate-α-ketoglutarate aminotransferase) are generally considered to be the same enzyme, although Mendelian phenotypes affecting β- and γ-amino acid metabolism imply that they are different enzymes (Scriver and Gibson 1995). Deficiency of GABA transaminase is documented in biopsied liver and in leukocytes, but the enzyme is not expressed in cultured skin fibroblasts (Jakobs et al 1993). In contrast, BAKAT activity is expressed in human skin fibroblasts and a (partial) deficiency of this enzyme in fibroblasts has been reported (Higgins et al 1994).

Measurement of the activity of BAIBPAT in patients with hyper-β-aminoisobutyric aciduria is not indicated, because this condition is thought to be a benign polymorphism. It has been reported that genetic high excretors have less than 10% normal transaminase activity in liver (Taniguchi et al 1972).

Determination of the gene defect

The final step and coping-stone in the diagnosis of an inborn error of metabolism is the determination of the underlying gene defect. Elucidation of the defect at the DNA and RNA levels will not only provide an important and reliable tool for categorizing patients, carrier detection and prenatal diagnosis, but will also be an essential step towards a better understanding of the pathogenesis of the disease.

The recent cloning of the DPD cDNA allows detection of the defect at the molecular level (Yokota et al 1994; Meinsma et al 1995). In 6 patients (5 paediatric, 1 adult) with complete DPD deficiency we could identify the molecular defects (Vreken et al 1996a,b). Three Dutch, one Danish and two Finnish patients were shown to have a 165 base pair

deletion in the mature DPD mRNA corresponding to an exon in the DPD gene. The exon skipping in these unrelated patients appeared to be caused by a single G→A point mutation in the invariant GT dinucleotide splice donor site downstream of the skipped exon. A Turkish patient was found to be heterozygous for a single base pair deletion at position 1897 (ΔC-1897). His father, who also had no residual activity, was homozygous for this mutation, which leads to a frameshift and premature termination of translation. Since the patient had no residual DPD activity, a second, yet unidentified, mutation is expected on the maternally inherited allele (Vreken et al 1996b). Another patient was shown to be homozygous for two point mutations (T85C and G2658A) resulting in amino acid substitutions which are probably responsible for the loss of enzyme activity (Vreken et al 1996b).

In patients with DHP deficiency, gene defects have not yet been reported. However, the cDNA sequence for human DHP has been submitted very recently (Hamajima et al 1996) and reports on the molecular defects underlying DHP deficiency can be expected fairly soon. As far as we know, no information has been published on the molecular defects in patients with BAKAT or BAIBPAT deficiency.

FINAL REMARKS

The growth in our knowledge of inborn errors of pyrimidine degradation over the past few years has been quite spectacular and has highlighted the importance of the clinical awareness of pyrimidine degradation defects as a possible cause of neurological disorders and a possible contraindication for treatment of cancer patients with certain pyrimidine analogues. Unfortunately, as in many inborn errors of metabolism, the symptomatology is very non-specific. However, the biochemical detection of pyrimidine degradation defects is not too difficult, as information on abnormal concentrations of the index metabolites in the body fluids can be obtained by procedures regularly used for the screening of inborn errors of metabolism. The causal relationship between the clinical symptoms and biochemical abnormalities is not yet clear, but is hypothesized to be at least partly related to the neurotransmitter function of β-alanine. Adequate treatment still has to be established. Therefore, a systematic collection of clinical data, the analysis of all the relevant metabolites in the body fluids, particularly cerebrospinal fluid, the measurement of residual enzyme activity, and the determination of the responsible gene defect are needed in addition to basic studies on the pathophysiological mechanisms.

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