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Asymptomatic and late-onset ornithine transcarbamylase (OTC) deficiency in males of a five-generation family, caused by an A208T mutation

In a large five-generation Polish family, late-onset ornithine transcarbamylase (OTC) deficiency in males segregated with the missense mutation Ala208Thr (A208T), and all heterozygous females were asymptomatic. No other mutations were found in the coding sequences and intron-exon boundaries of the OTC gene. Surprisingly, the mutation originated from the great-grandfather of the index patient who died at age 59 of liver carcinoma. He never had dietary restrictions or hyperammonemic spells throughout life and appears to be the oldest male reported with OTC deficiency. The index patient had a severe OTC deficiency (3% of normal). Eight males died suddenly at ages 4 months to 23 years (average 14 years) after a foudroyant episode triggered by a common infection. The patients remained undiagnosed for 28 years because a metabolic defect was not considered to be the cause of the acute episodes. Recognition of the familial pattern of inheritance was initially unnoticed since the patients were admitted to eight different hospitals. DNA analysis predicted that two ‘healthy’ boys also had OTC deficiency, which was confirmed by abnormal results of allopurinol challenge tests. Initial suspicion of OTC deficiency in such families is complicated, since symptoms can develop at any age, or even remain absent. This obscures the typical pattern of X-linked inheritance in small families.

Key words: asymptomatic – late onset – ornithine transcarbamylase deficiency – OTC deficiency

Ornithine transcarbamylase (OTC; E.C. 2.1.3.3) is one of the enzymes required for detoxification of waste nitrogen compounds, particularly ammonia. It catalyses the first step of the urea cycle: the synthesis of citrulline from carbamoyl phosphate and ornithine. Deficiency of OTC is an X-linked, partially dominant trait with milder expression in females than in males. Hemizygous males commonly present with acute neonatal hyperammonemia leading to coma and, in the absence of treatment, to early death. These patients have undetectable OTC activity in liver (for review see: Brusilow & Horwich 1995). An increasing number of males with OTC deficiency with late onset, up to early adulthood, has been reported (Krieger et al. 1979, DiMagno et al. 1986, Oizumi et al. 1984, Holmes et al. 1987, Cavard et al. 1988, Finkelstein et al. 1990, Drogari et al. 1988, Matsuda et al. 1991, Tuchman & Holzknecht 1991, Matsuura et al. 1993). The oldest clinically affected males with OTC deficiency were 46
and 58 years (Matsuda et al. 1991). In this paper we report a five-generation pedigree with late onset OTC deficiency caused by a new mutation which could be traced to the index patients’ great-grandfather, who had never had episodes characteristic of OTC deficiency and died at age 59.

Patients and methods

The pedigree of the family K is depicted in Fig. 1 and the clinical signs and symptoms of the male patients who died are summarised in Table 1. None of the females of the pedigree had clinical symptoms of OTC deficiency. Six males of generation III died after a remarkably similar course at ages between 6 and 23 year (average 15.4 years). The disease struck apparently normal males with good mental and physical capacities, even above average (III-19 was one of the best soccer players of his school and III-24 was junior tennis district champion). Four of them had no previous history of similar episodes, while two of them did (III-3 and III-24).

In generation IV, patient IV-7 had a similar clinical course to the patients of generation III, except that he was the only survivor of an acute episode of the disease. IV-16 died during infancy at 3 months and his clinical symptoms were reminiscent of females who are heterozygous for severe infantile OTC deficiency. These patients were the first in whom hyperammonemia was found.

The rapidly fatal course of the disease started with headache, nausea, abdominal pain, sometimes dizziness, and somnolence as the sequel to a viral or bacterial infection with fever (angina, severe pertussis, influenza, food poisoning or infectious diarrhea). Usually within 1 or 2 days (except case III-1 where this period lasted 5 weeks), mental disturbances appeared, ranging from somnolence to coma. Anxiety, irritability (sometimes with aggression) and euphoria were also noticed. At the same time characteristic cerebellar signs developed: uncertain gait and ataxia and seizures, as well as pyramidal signs with spasticity. Eventually, the progressive loss of consciousness led to irreversible coma and death. Lymphopenia was a frequent laboratory finding, probably caused by viral infections. During the acute phase which patient IV-7 survived, he developed a transient deficiency of T helper lymphocytes and an increase of T suppressor lymphocytes (index Th/Ts=0.2). In case III-19 the lymphopenia was associated with lack of rosette-forming ability. These findings corresponded to the post-mortem observations of lymph nodes (see below).

The six patients of generation III were seen in six different hospitals and a metabolic disease had not been considered because of the late onset and the apparent absence of previous disease-related episodes. In retrospect, a more detailed analysis of the histories revealed earlier milder episodes which
probably were caused by OTC deficiency (a total of seven episodes in three patients; see Table 1).

Post-mortem histological examination showed that the patients invariably had brain edema (Table 1). In the six available cases (III-1, 3, 19, 23 and 14; IV-16) spongiform changes were found, in II-24 as widespread status spongiosus of both grey and white matter. Cortical nerve cells and cerebellar Purkinje cells were lost, whereas the Bergman glia had proliferated. Severe and chronic acidophilic necrosis and ischemic changes of the nerve cells were present. In most cases intravascular clotting and numerous extravasations were seen.

Liver of four deceased patients was examined (III-3, 19, 23 and 24), generally showing necrotic areas, mainly central, and vacuolated hepatocytes. The portal area was often infiltrated with lymphoid cells and some hepatocytes were necrotic or vacuolated. In some sections central or diffuse fibrosis of hepatic globules was present. The histopathology was typical of chronic hepatitis.

Atrophy of lymph nodes with sparse lymph follicles or absence of germinal centers were observed in three cases (III-3, 19 and 24).

DNA analysis

Genomic DNA was extracted from peripheral leukocytes using routine procedures (Miller et al. 1988). SSCP analysis was done on the Phast System (Pharmacia) as described (Kneppers et al. 1995) with the PCR-amplified DNA fragment using the primers described by Matsuura et al. (1993), except for exon 1 where primers were chosen from the sequence data of Hata et al. (1988): forward primer upstream in exon 1 (TTA GTT TTT AGG TGG CCC C) and reverse primer in intron 1 (AAC CCA AGT CTC TGA CCA TCA). The presence of the A208T mutation was confirmed by digestion with restriction enzyme Hhal according to the manufacturer's instructions (New England Biolabs).

The intragenic MspI polymorphisms in the OTC gene were analyzed as described by Rozen et al. (1985). Exons were sequenced after PCR amplification using the intron primers described by Matsuura et al. (1993). Exons 4 and 7-8 were amplified in the presence of 10% DMSO, except for exon 1, where the intron primers of Hata et al. (1988) were used.

Enzyme analysis

OTC and carbamoylphosphate synthetase (CPS; reference enzyme) were determined as described by Nozum & Snodgrass (1976). OTC in control livers was 13-36 μmol/min/mg protein (n=7; mean=24). In the index patient IV-7, OTC activity was 0.8 μmol/min/mg, whereas CPS activity was within the normal range.

Table 1. Clinical signs & symptoms and post-mortem observation

<table>
<thead>
<tr>
<th>Acute episodes</th>
<th>III-1</th>
<th>III-3</th>
<th>III-4</th>
<th>III-19</th>
<th>III-23</th>
<th>III-24</th>
<th>IV-7</th>
<th>IV-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at fatal acute episode</td>
<td>23 y</td>
<td>23 y</td>
<td>14 y</td>
<td>14 y</td>
<td>6 y</td>
<td>19 y</td>
<td>14 y</td>
<td>3 mo</td>
</tr>
<tr>
<td>Duration of fatal episode</td>
<td>6 wk</td>
<td>10 d</td>
<td>5 d</td>
<td>6 d</td>
<td>8 h</td>
<td>7 d</td>
<td>2 d</td>
<td>10 d</td>
</tr>
<tr>
<td>Probable triggering event</td>
<td>angina</td>
<td>influenza</td>
<td>food poisoning</td>
<td>diarrrhoea</td>
<td>pertussis</td>
<td>angina</td>
<td>angina</td>
<td>fever</td>
</tr>
<tr>
<td>Earlier severe episodes</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>12 y</td>
</tr>
<tr>
<td>Possibly related milder episodes</td>
<td>none</td>
<td>7, 13, 23 y</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>12, 18, 18 y</td>
<td>13 y</td>
<td>none</td>
</tr>
</tbody>
</table>

Signs and symptoms

| Abdominal pain | + (+) | + (+) | + (+) | + (+) | + (+) | + (+) | + (+) | ? (+) |
| Headache (nausea) | + (+) | + (+) | + (+) | + (+) | + (+) | + (+) | + (+) | + (+) |
| Consciousness disturbances | anxiety | confusion | + | + | anxiety | + | somnolent | euphoric |
| Generalized seizures | + | + | + | + | + | + | - | + |
| Pyramidal tract anomalies, including decerebrate rigidity | + | + | + | + | + | + | - | + |
| Papilledema | - | + | + | + | + | + | - | + |
| Differential diagnosis at time of death | encephalitis | brain tumor | acute interm. | porphyria | brain tumor | adrenal failure | intoxication | brain tumor | Gilbert synd. | porphyria | cholelithiasis | OTCD | Reye synd. | OTCD |
| Post-mortem pathology | Brain edema, marked | + | + | + | +, hemiations | (transentorial, foraminal) | + | +, hemiations | (transentorial, foraminal) | + | + |

1) Retrospective data, not mentioned in anamnesis during fatal episodes.
Asymptomatic and late-onset OTCD

Fig. 2. Restriction enzyme analysis for A208T mutation in exon 6 of OTC gene. PCR-amplified exon 6 of the relevant members of family K. were digested with Hhal. The wild-type allele contains one Hhal site, leading to the formation of 102 bp and 58 bp fragments. The A208T mutation destroys this site, leaving the PCR product of exon 6 intact (160 bp). O: Females heterozygous for the A208T mutation. A black dot over the male symbols indicates the presence of the A208T mutation in males.

Results

The pedigree of family K. (Fig. 1) presents an X-linked recessive trait with eight deceased male patients and eight asymptomatic female carriers. The clinical phenotype was remarkably similar (see Patients section and Table 1). Without clear precedents, the mental and physical state of the patients deteriorated rapidly, resulting in coma, brain edema and death within 8 hours to 6 weeks. The first 7 patients all died undiagnosed at ages 6–23 years, except IV-16 who died at infancy. This patient, the second to last who died, was the first case in which metabolic screening was done and hyperammonemia was found. This had been attributed to Reye syndrome (frozen liver tissue from this patient and the previous ones was not available). Before OTC deficiency was demonstrated, a variety of diagnoses were considered (Table 1). Only when the last patient died (the index patient IV-7), was liver available for enzyme analysis and a severe OTC deficiency was demonstrated (3% of normal mean activity, see Methods section).

The search for the disease-causing mutation was started with SSCP analysis of the index patient’s DNA and the PCR product of exon 6 showed a bandshift (data not shown). Direct sequencing revealed a GCA→ACA mutation in codon 208, causing an Ala→Thr substitution (A208T) and destruction of an Hhal restriction site. PCR-amplified fragments of exon 6 from the available family members were then digested with this restriction enzyme and analyzed. Fig. 2 shows that the transmission of OTC deficiency in family K. is concordant with the segregation of the A208T mutation. Two males with the A208T mutation did not have clinical symptoms (IV-14 and V-4), but OTC deficiency was confirmed by abnormal orotic acid elevations of the allopurinol challenge tests (Table 2; liver biopsies were not allowed by the parents). The mother of V-4, an obligate heterozygote, had slightly abnormal orotic acid levels after allopurinol administration; her grandmother had completely normal results.

To exclude that the A208T substitution could be a polymorphism which segregated by chance with OTC deficiency, all exons of the OTC gene of the index patient IV-7 and the preclinical patient IV-14 were sequenced.

Table 2. Allopurinol challenge test of two males with the A208T mutation and the corresponding heterozygotes

<table>
<thead>
<tr>
<th>Pedigree #</th>
<th>Orotic acid in urine(^1), at various times after allopurinol administration(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>IV-14</td>
<td>1.2</td>
</tr>
<tr>
<td>V-4</td>
<td>3.9</td>
</tr>
<tr>
<td>Mother of V-4 (IV-6)</td>
<td>1.4</td>
</tr>
<tr>
<td>Great-grandmother of V-4 (II-4)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

\(^1\) μg/mmol creatinine; normal reference range: 1.2–3.2.
\(^2\) Dose of allopurinol was 300mg.
Table 3. Regions of the OTC gene sequenced: all 10 exons plus flanking intron boundaries

<table>
<thead>
<tr>
<th>Exon(^1)</th>
<th>Length of fragment sequenced (position towards exon)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1186 bp (-68 to +41)</td>
</tr>
<tr>
<td>2</td>
<td>202 bp (-30 to +33)</td>
</tr>
<tr>
<td>3</td>
<td>201 bp (-35 to +35)</td>
</tr>
<tr>
<td>4</td>
<td>111 bp (-8 to +15)</td>
</tr>
<tr>
<td>5</td>
<td>197 bp (-20 to +23)</td>
</tr>
<tr>
<td>6</td>
<td>164 bp (-20 to +30)</td>
</tr>
<tr>
<td>7+8</td>
<td>301 bp (-7 to +10)</td>
</tr>
<tr>
<td>9</td>
<td>224 bp (-7 to +10)</td>
</tr>
<tr>
<td>10</td>
<td>83 bp (-16 to +7)</td>
</tr>
</tbody>
</table>

\(^1\) Intron and exon sequences were compared to Matsuura et al. (1993) and Horwich et al. (1984).
\(^2\) Means upstream of exon and + means downstream.
\(^3\) Position upstream of start codon.

were sequenced, the flanking intron boundaries included (Table 3; for exon 1 only the coding sequences). No other base changes were detected, except for polymorphisms and discrepancies which had already been reported in the literature. Then, exon 6 from 60 Dutch and 60 Polish control alleles was investigated by digestion with HhaI and the A208T substitution was not found. These results indicate that A208T is the disease-causing mutation.

Since no material from generation I was available, we attempted to trace the origin of the mutation by determining the MspI haplotype in the offspring. The haplotypes of this intra-exonic polymorphism of the OTC gene are shown in the pedigree (Fig. 1). All obligate heterozygotes and the affected males have the G2 haplotype. Both normal males in generation II have the G3 allele, making this polymorphism uninformative. We then screened 12 polymorphisms at various distances from the OTC gene, looking for crossing-over events in 25 members of family K. The haplotypes of the 12 markers proved that the G2 OTC allele carrying the mutation must have been of paternal origin (I-9).

Discussion

In the last two decades it has become apparent that the clinical course of OTC deficiency in males is very heterogeneous. The classical form with neonatal onset and hyperammonemic coma is generally well recognised, but the late-onset forms can be missed (Matsuda et al. 1991). Recently, Tuchman et al. (1993) reported that only about 50% of their patients had onset in the neonatal period. Onset in the remaining half of their male patients was after the neonatal period, up to early adulthood. The two oldest reported patients with an acute episode were 46 and 58 years (Matsuda et al. 1991). The family K., described in Fig. 1, extends the heterogeneity of the disease in males because an asymptomatic male of 59 was found and all heterozygous females were asymptomatic. One of the obligate heterozygotes was 'normal' in the allopurinol challenge test. This confirms previous false negative results (Tsai et al. 1991), and once more illustrates the limitations of this test for detecting heterozygotes.

It took 28 years before the diagnosis was established in the family K. and it is of interest to analyze why it took so long. The first six patients who died at ages 6–23 years in a period of 15 years, were admitted to six different hospitals, which hampered comparison of the patients and recognition of the familial character of the disease. The X-linked inheritance of this disease was recognized by one of the authors (J. Z.) when the last patient of generation III died in a hospital with a clinical genetics department; the essential pedigree data were already available in a peripheral hospital 8 years earlier when patient III–4 died. All these six patients had died during the first acute episode of a disease which followed the course of increased intracranial pressure, coma and death. Initially, a metabolic coma was not suspected because the clinical features of the patients led to admission to neurology departments which were not familiar with late-onset acute metabolic diseases. Hence, urine analysis was not performed. Instead, in one case (III–4) was a brain tumor suspected and brain surgery performed (at that time CT scans and MRI were not available); two cases were suspected of encephalitis and one of acute intermittent porphyria (see Table 1). The familial and possibly metabolic character of the disease initiated a more detailed survey of the health of the patients prior to the fatal episode, and in only three out of eight patients had milder episodes, probably disease related, occurred (Table 1; overall seven episodes in three patients). When the 7th patient (IV–16) died, again in a different hospital, hyperammonemia was found and Reye syndrome was suspected. Liver tissue was not available for enzymatic analysis. By then, the striking clinical similarities of our male patients and the heterozygous female patients with OTC deficiency described by Rowe et al. (1986), were noticed (e.g. episodic irritability, ataxia, seizures in some cases, vomiting, lethargy and coma). Some of our patients also resembled a 29-year-old male with bizarre behavior reported by DiMagno et al. (1986). Interestingly, at the age of 16 years this case was suspected of Gilbert syndrome, like our case III-24.

Liver became available for OTC determination for the first time when the index patient IV–7 had his second acute and fatal episode of the disease. The
residual activity was only 3% under saturating conditions.

At present approximately 70 different mutations, including polymorphisms, are known leading to various degrees of OTC deficiency (Tuchman et al. 1995). The A208T mutation causing OTC deficiency in family K. has not been published earlier. It is remarkable that all females of generation II are heterozygote and both males are normal. The odds that this would occur through the usual maternal transmission are 1/32. Unfortunately the Mspl polymorphism was not informative, but extended haplotypeing showed that the male I-9 must have transmitted the OTC deficiency. This is also supported by four other males in generation I, who died of unknown causes (three in infancy and one at age 14 years; the fifth died of typhoid fever), which retrospectively are compatible with OTC deficiency.

The transmitting male I-9 died at age 59 years of liver carcinoma, without evidence of hyperammonemic episodes. It appears therefore that the disease remained latent for almost 60 years, which has not been reported before. Asymptomatic senior males with OTC deficiency were recently also found in a Dutch family carrying the same A208T mutation (Bakker et al. 1995, Ausems et al. 1996). The 97-year-old great-grandfather of a male patient who died of OTC deficiency at age 10 years (4% residual activity) has the mutation and has never had symptoms of the disease. Likewise, an uncle of the index patient, aged 41, has the A208T mutation and proven OTC deficiency, but is also asymptomatic. The independent occurrence of the same mutation in a Polish and a Dutch family with very similar conditions.

The independent occurrence of the same mutation in a Polish and a Dutch family with very similar clinical presentation suggests that the A208T mutation is a 'mild' mutation. An Ala to Thr substitution should have little effect on protein structure; however, the alanine 208 residue lies in a domain of OTC which has been conserved evolutionarily among man, mouse, rat, frog, yeast, fungus and various bacteria (Tuchman et al. 1995), suggesting that its substitution will have a major effect on the OTC.

We could not rule out that this exon mutation caused a splicing anomaly, since liver samples for RNA isolation were not available. This unusual condition in small families. This adds a new dimension to the existing heterogeneity of OTC deficiency.

Acknowledgements

We thank Prof. Hans Galjaard for his continuing support and Tom de Vries Lentsch for photography. The authors are grateful to Prof. Ewa Kostrzewska (Inst. Haematology, Warsaw) for drawing our attention to this interesting family and for excluding acute intermittent porphyria in case III-24, to Prof. Danuta Rozywnkowa (Dept. of Genetics, Medical School, Lublin) for doing immunological tests in case IV-7, to Dr. Janusz Zaremba for consultations about histopathological data and to all colleagues who made clinical and post-mortem data of the patients available to us. We thank Yvonne Zoet for technical assistance.

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