J. Inher. Metab. Dis. 21 (1998) 738–747

Homocystinuria due to cystathionine β-synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control

S. YAP and E. NAUGHTEN*
National Centre for Inherited Metabolic Disorders, The Children's Hospital, Temple Street, Dublin 1, Ireland

* *Correspondence*

MS received 27.06.97 Accepted 26.02.98

Summary: Homocystinuria (HCU) due to cystathionine β-synthase deficiency (Mudd et al 1964) was independently described by Gerritsen and colleagues (USA) and Carson and colleagues (Northern Ireland) in 1962. The worldwide frequency of HCU has been reported as 1 in 344 000, while that in Ireland is much higher at 1 in 65 000, based on newborn screening and cases detected clinically. The national newborn screening programme for HCU in Ireland was started in 1971 using the bacterial inhibition assay. A total of 1.58 million newborn infants have been screened over a 25-year period up to 1996. Twenty-five HCU cases were diagnosed, 21 of whom were identified on screening. The remaining four HCU cases were missed and presented clinically; three of these were breast-fed and one was pyridoxine responsive. Twenty-four HCU cases were pyridoxine nonresponsive. Once the status of pyridoxine responsiveness was identified, all pyridoxine nonresponsive cases, but one, were started on a low methionine, cystine-enhanced diet supplemented with pyridoxine, vitamin B_{12} and folate. Dietary treatment commenced within 6 weeks of birth (range 8–42 days) for those cases detected by screening, while for the late-detected cases treatment was started upon presentation and diagnosis. Biochemical control was monitored measuring deproteinized plasma methionine, free homocystine and cystine at least once a month. Review of the clinical outcome of the 25 HCU cases with 365.7 patient-years of treatment revealed no HCU-related complications in 18 screened, dietary-treated cases. Fifteen of these had lifetime medians of free homocystine $\leq 11\,\mu$mol/L (range 4–11). The remaining three cases with higher lifetime medians of free homocystine (18, 18 and 48 μmol/L) have developed increasing myopia recently. Among the three screened non-dietary-compliant cases, two have ectopia lentis, one has osteoporosis and two have mental handicap. Of the four cases missed on screening, three presented

with ectopia lentis after the age of 2 years. There were no thromboembolic events in any of the 25 HCU cases. The lifetime medians for methionine ranged from 47 to 134 μmol/L. The Irish HCU clinical outcome data suggest that newborn screening, early commencement of dietary treatment and a lifetime median of free homocystine of ≤ 11 μmol/L had significantly reduced the probability of developing complications when it was compared to the untreated HCU data (Mudd et al 1985).

Homocystinuria (HCU; McKusick 236200) due to cystathionine β-synthase (CBS; EC 4.2.1.22) deficiency is an autosomal recessive disorder with a frequency of 1 in 65 000 in Ireland, based on newborn screening and cases detected clinically, compared to a worldwide frequency of 1 in 344 000 (Mudd et al 1995). The national newborn screening programme was started in Ireland for HCU in 1971. Approximately one half of individuals with HCU worldwide are pyridoxine responsive, resulting in a decrease in plasma concentration of methionine and almost complete elimination of homocyst(e)ine in the blood and urine (Andria and Sebastio, 1996). Pyridoxine-nonresponsive cases have a more severe clinical presentation. The CBS gene has been mapped to the human chromosome 21q22.3 (Munke et al 1988). In Ireland, 70–71% of the defective CBS alleles are G307S, which heralds a more severe and earlier clinical presentation in association with pyridoxine nonresponsiveness (Gallagher et al 1995).

Individuals with HCU are clinically normal at birth. If untreated, they may present with varying degrees of the well-recognized clinical syndrome of ectopia lentis, dolichostenomelia, osteoporosis, thromboembolic events, and mental retardation. Of untreated patients who are pyridoxine nonresponsive, 82% will have ectopia lentis by the age of 10 years, 27% will have had a clinically detected thromboembolic event by the age of 15 years, 64% will have radiological evidence of spinal osteoporosis by the age of 15 years, and 23% will not survive to the age of 30 years (Mudd et al 1985). This paper presents the clinical outcome and biochemical control of 25 cases of HCU in Ireland detected in the 25-year period of national newborn screening with 365.7 patient-years of treatment. Four of these 25 cases were missed on screening.

PATIENTS AND METHODS

Subjects: This is a retrospective study of all 25 cases of HCU detected in Ireland between 1971 and 1996 either by the national newborn screening programme or by clinical presentation. The data were extracted from the clinical notes.

There are 12 females and 13 males from 19 families; one male died at the age of 8 years following a drowning accident. Twenty-four of the twenty-five patients are nonresponsive to pyridoxine. Twenty-one cases were identified by the national newborn screening programme. Of the remaining four cases missed on screening, three were breast-fed and one is pyridoxine responsive. All but one were started on treatment upon diagnosis.

Biochemical determinants: All 21 cases of HCU detected by newborn screening had high blood methionine concentration of more than 100 μmol/L, assayed by the bacterial inhibition assay (BIA) on the heel prick blood sample taken on day 3 to day 5 of life. A liquid blood sample, separated and deproteinized within 10 min of collection, was requested from these infants and assayed using an amino acid analyser for methionine, free homocystine and cystine. A high blood methionine and free homocystine with a low cystine confirmed the diagnosis for those detected on newborn screening and those that presented clinically. CBS enzyme activity in skin fibroblasts, assessed by Fowler according to his methods (Fowler et al 1978), was only available for 5 patients. Three patients showed no detectable CBS activity in both assays with or without added 1.0 mmol/L pyridoxal phosphate (normal CBS activity 4–25 nmol/h per mg). The remaining two patients exhibited very minimal CBS activity (0.05 and 0.037 increasing to 0.066 and 0.062 nmol/h per mg respectively with the addition of 1 mmol/L pyridoxal phosphate).

Clinical management and follow-up: All patients diagnosed as having HCU had their pyridoxine status determined clinically by commencement on oral pyridoxine 50 mg three times daily as inpatients. The deproteinized blood methionine, free homocystine and cystine were initially determined every 3 days while on daily oral pyridoxine. Pyridoxine responsiveness was indicated by a rapidly falling methionine level and clearing of free homocystine from the plasma, while pyridoxine nonresponsiveness was indicated by persistently high or rising plasma methionine and free homocystine.

In pyridoxine-nonresponsive patients, dietary management was commenced by restricting dietary methionine and using a methionine-free, cystine-supplemented synthetic mixture. Methionine was added as a proprietary food preparation or as breast milk and the amount was titrated against the plasma methionine and free homocystine concentration. Two-thirds of the total protein intake was derived from the synthetic methionine-free, cystine-supplemented mixture and the remaining third from the natural methionine-containing foods (P. Howard, personal communication, 1996). Plasma B_{12} and folate were assayed and, if they were found deficient, the patient was given supplements. Once stabilized, blood was drawn, deproteinized, and analysed for methionine, free homocystine and cystine at least once a month, or more frequently if clinically indicated, to monitor biochemical control. During all episodes of acute illness, prompt treatment of the illness with additional supportive care, including adequate hydration and aspirin or dipyridamole, was given to prevent blood stasis.

Lifetime medians and ranges of plasma methionine and free homocystine levels were calculated. Methionine and free homocystine levels during the initial periods of stabilization, pyridoxine challenge and subsequent periods of proven illness were excluded from the calculations for lifetime medians and ranges.

Upon discharge from hospital, patients initially attended the outpatient department fortnightly and thereafter at 4- to 6-weekly intervals. They were reviewed minimally four times per year. At every clinic visit, growth parameters were measured, a general physical examination was carried out by a doctor, and blood was drawn,

deproteinized and analysed for methionine, free homocystine and cystine to monitor biochemical control. Experienced dietitians provided dietary assessments and constant advice at each visit. Annual detailed ophthalmological examination was performed by a paediatric ophthalmologist experienced in HCU. Initially, skeletal radiology was performed yearly to detect evidence of osteoporosis. Up to 1990, annual clinical cardiovascular assessments including electrocardiographs and chest radiographs were performed by experienced paediatric cardiologists. Transthoracic echocardiograms were carried out when clinical abnormalities (e.g. murmurs, pulse differences) were detected. IQ assessments were performed at 2- to 6-yearly intervals. Psychological support was provided whenever necessary by the clinical psychologist.

RESULTS

Patient demographics: In 25 years of national newborn screening up to 1996, 21 cases of HCU were detected through the programme. All were pyridoxine non-responsive and five cases were breast-fed. Four other HCU cases were missed and presented clinically; three were breast-fed and one was pyridoxine responsive. Details of complications among the study group are categorized in Table 1.

Clinical and biochemical findings: The 25 cases of HCU are divided into three groups according to the mode of detection (screened and missed on screening) and the development of complications. Group 1 consisted of 18 HCU cases detected on screening, treated and remaining free from the recognized complications of untreated HCU (Table 2). Three patients who were detected by screening developed complications due to noncompliance with the prescribed diet, as indicated by dietary history and poor biochemical control, are in group 2 (Table 3). The four cases missed on screening presented with complications after the age of 2 years and are in group 3 (Table 4).

One of the four cases missed on screening and never treated was recently referred to our clinic aged 21 years with recognized complications of untreated HCU (case 4, Table 4). She had bilateral ectopia lentis and optic atrophy, mental retardation,

Table 1 Complications among the HCU patients

		Detected by screening		
	Total no.	*Without complications*	*With complications*	*Missed on screening*
Total no. detected	25	18[a]	3	4
Ectopia lentis	6	0	2	4
Osteoporosis (radiological)	2	0	1	1
Mental handicap	4	0	2	2
Thromboembolism	0	0	0	0

[a] One died at age 8 years due to a drowning accident.

Table 2 Summary of age data, lifetime medians and ranges of free homocystine (μmol/L) and methionine (μmol/L) for cases in group 1 (screened, on diet with no complications)

Case no.	Sex	Age (1996) (years)	Age therapy started (days)	Lifetime free homocystine			Lifetime methionine		
				No.	Median	Range	No.	Median	Range
1	M	2.5	15	24	4.0	0–17	31	56	5–651
2	M	4.3	15	29	6.0	0–19	26	56.5	35–76
3	F	5	8	32	5.5	0–19	33	48	5–80
4	F	6.5	10	51	6.0	0–19	51	47	6–110
5[a]	M	8	21	19	11	0–30	19	87.2	21–590
6[b]	F	12.7	28	64	5.0	0–47	62	61	11–173
7	M	12.8	22	68	11	0–34	70	64	7–232
8	M	13	7	41	8.0	0–22	41	64	19–132
9	M	13.9	13	66	10	0–25	67	57	13–187
10	F	14.6	15	85	6.0	0–36	84	67.4	21–300
11	M	14.6	21	52	5.5	0–21	52	63.5	18–314
12[b]	F	15.9	26	66	18	0–86	63	80	12–448
13	F	17.1	8	73	18	0–88	73	88	10–365
14	M	20.7	10	39	7.5	0–87	50	56.3	1–268
15[b]	M	20.9	10	60	8.5	0–55	8	97.5	24–642
16	M	21.8	36	65	9.0	0–70	73	134	23–762
17[b]	F	22.5	35	75	9.0	0–29	64	96	33–412
18[b]	M	23.4	42	97	48	0–172	98	99.5	27–523

[a] Case 5 died at the age of 8 years due to drowning accident
[b] Skin fibroblast assayed for CBS activity
M = Male, F = female

osteoporosis, dolichostenomelia (arm span 164 cm to height of 163 cm) but no documented clinical evidence of thromboembolic events.

The individual age at which diet was commenced, lifetime medians and ranges of plasma methionine and free homocystine levels are summarized in Tables 2, 3 and 4. Treatment was started before 6 weeks of age for patients in groups 1 and 2, while those in group 3 started upon presentation and diagnosis. The mean period of follow-up for the screened groups 1 and 2 was 14.3 years (range 2.5–23.4) and for the late-detected group 3 was 14.7 years (range 11.7–18.8).

Three patients (cases 12, 13 and 18) in group 1 (Table 2) developed increasing myopia in the last few years and all had higher lifetime median of plasma free homocystine (18, 18 and 48 μmol/L) compared to the remaining 15 cases, who all had lifetime medians of plasma free homocystine levels ≤ 11 μmol/L (Figure 1). No patient, whether detected by screening or clinically, developed any thromboembolic events, in contrast to the predicted outcome at their current age according to the time-to-event graphs by Mudd et al (1985; see Table 6). The lifetime median methionine levels ranged from 47 to 134 μmol/L.

Four patients had mental handicap: two noncompliant patients detected by screening (group 2: cases 1 and 2) and two who were missed on screening (group 3: cases 1 and 4). The remaining 21 patients have achieved age-appropriate education standards (Table 5).

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.