Neurological aspects of hyperinsulinism–hyperammonaemia syndrome

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Hyperinsulinism–hyperammonaemia syndrome (HHS) is a rare cause of congenital hyperinsulinism due to missense mutations in the GLUD1 gene, resulting in glutamate dehydrogenase (GDH) overactivity. The aim of this study was to document the spectrum of neurological disturbances associated with HHS and to identify possible phenotype–genotype correlations. We retrospectively analyzed the neurological outcomes of 22 consecutive patients (12 males, 10 females) aged from 18 months to 40 years and diagnosed with HHS. We analyzed demographic and clinical features and neuroradiological, biochemical, and genetic findings.

Fourteen patients had childhood-onset epilepsy. Learning disability* was found in 17 patients. Two patients had pyramidal involvement and one had generalized dystonia. Seizures were observed in 11 of 19 patients with documented GLUD1 mutations, and nine of these 11 patients had a mutation in the guanosine triphosphate (GTP) binding site. Our data demonstrate that neurological disorders in HHS are more frequent than previously thought and might suggest that mutations in the GTP binding site of GDH could be associated with more frequent epilepsy.

Hyperinsulinism–hyperammonaemia syndrome (HHS, OMIM No. 606762) is a clinically distinctive form of congenital hyperinsulinism caused by an inborn metabolic error. HHS is a rare cause of congenital hyperinsulinism1,2 and is identified by the accompanying hyperammonaemia.1 Patients with HHS have missense mutations in the GLUD1 gene (located in the 10q23.3 region), which encodes glutamate dehydrogenase (GDH). These mutations can occur de novo or be inherited in dominant mode.1 They are ‘activating’ mutations, that lead to a gain in enzyme function by reducing the sensitivity of GDH to allosteric inhibition by guanosine triphosphate (GTP) and adenosine triphosphate (ATP).1 The GLUD1 gene contains 13 exons encoding a 505-amino-acid mature enzyme.2 Mutations causing HHS have been identified in the antenna region of the enzyme encoded by exons 11 and 12 of the GLUD1 gene, as well as in the GTP binding site encoded by exons 6 and 7.1,4 However, up to now, the precise location of the mutations has never been shown to affect the phenotype.

The main clinical feature in children with HHS is recurrent episodes of symptomatic hypoglycaemia. These may occur during fasting or be provoked by high protein intake. The hypoglycaemia is usually not as severe as in infants with congenital hyperinsulinism due to defective ATP-sensitive potassium channels (mutations in SUR1 or Kir6.2).3 Children with HHS respond well to medical treatment with diazoxide and to protein restriction.1,2,9 HHS is not associated with large-for-dates birthweight and is often diagnosed several months after birth.5 The hyperammonaemia is typically mild to moderate (up to 3–5 times the upper limit of normal) and is resistant to detoxifying drugs and to protein restriction.1,6 In contrast to patients with hyperammonaemia due to urea cycle disorders, patients with HHS do not suffer from lethargy, headaches or acute hyperammonaemic crises.7 On the other hand, children with HHS may have neurological complications such as epilepsy and learning disability*.8,10–12 However, knowledge of the clinical features of HHS is largely based on small series (eight patients reported by Stanley et al.,1 14 patients reported by Santer et al.,8 five patients reported by Miki et al.,4 and 14 patients reported by Raizen et al.).10,11 but neurological and brain imaging findings have rarely been reported in depth.

Here we report a detailed study of neurological status in a large series of consecutive patients with HHS. We also analyze genetic, biochemical, and neuroimaging data, and correlations between the neurological phenotype and the location of GLUD1 mutations.

Method
We retrospectively enrolled 22 patients from 17 families with biochemically or genetically proven HHS, seen in various reference centres for metabolic diseases in France, Italy, and Belgium. Twelve of these patients (nos 1, 5, 6, and 8–16) have been described elsewhere2,12 but were included in the present series because more clinical and longer follow-up data were available.

All of the patients had recurrent hypoglycaemia with evidence of inappropriately elevated plasma insulin levels, low concentrations of plasma free fatty acids and ketones, a glycemic response to glucagon at times of hypoglycaemia, and

*North American usage: mental retardation.
plasma ammonia concentrations (>35μmol/L).\textsuperscript{1,2,11} The diagnosis of HHIS was confirmed by enzymatic or genetic investigations.

Biochemical investigations included measurements of GDH activity and GTP inhibition of GDH activity in lymphoblast homogenates, as previously described.\textsuperscript{1} Genetic testing was performed with the patients’ written informed consent. Screening for mutations in exons 5–13 of the GLUD1 gene was performed as previously described.\textsuperscript{9,13}

Clinical data (history and comprehensive neurological examination) were collected either by the principal investigators (NBB and ER; patients 1–4, 10, and 17–22) or from the patients’ medical charts.

In patients with epilepsy (nos 1–4, 10, and 17–22), we recorded the family history, age at seizure onset, type and severity of seizures, and ictal and interictal electroencephalogram (EEG) abnormalities. EEG focused on background activity during wakefulness, drowsiness, and sleep, and on interictal abnormalities and electrographic seizures. In addition, patients 1 to 4 had video-EEG monitoring.\textsuperscript{12}

All of the patients had magnetic resonance imaging (MRI) of the brain, and the findings were systematically re-evaluated by two investigators (NBB and NB). Because MRI studies were performed at different institutions over a 10-year period, the imaging sequences varied substantially, but all patients had sagittal, coronal and axial T1-weighted and T2-weighted studies. In addition, 10 patients had axial fluid-attenuated inversion recovery (FLAIR) studies.

Quantitative variables (means) were compared using the Wilcoxon signed-rank test, and qualitative variables (percentages) were compared using Fisher’s exact test.

The research was approved by the Necker Children’s Hospital ethics committee and informed consent was obtained from the participants or their parents.

### Results

Demographic data and individual neurological data are provided in Table I. The patients consisted of 12 males and 10 females, aged from 1 year 6 months to 40 years (mean 12y 11mo, [SD 10y 8mo], median 9y 10mo) at the time of evaluation. All of the patients were born at term, following uneventful delivery. Only four patients had large-for-gestational-age birthweights (mean 3135g, SD 435g).

Clinical signs of hypoglycaemia were noted within the first 3 days of life in four patients and later in infancy or childhood in the other patients. Median age at recognition of hypoglycaemia was 5 months (range 3d–18mo). Seventeen patients had hypoglycaemic seizures. The seizures were generalized tonic–clonic, except for two patients (nos 6 and 11) who had prolonged hemiclonic seizures. Four of the five patients who did not have seizures had repeated episodes of symptomatic hypoglycaemia (i.e. generalized hypotonia, drowsiness, head tremor, eye rolling), and the remaining patient was diagnosed through familial genetic screening. Nineteen patients were successfully treated with diazoxide, starting at a median age of 12 months (range 1–180mo), although a leucine-restricted diet or cornstarch was also effective. Two patients were treated with dietary measures alone, and one patient (no. 1) had persistent hypoglycaemia that required partial pancreatectomy (tail and half the body). The median interval between the onset of hypoglycaemia and the onset of effective treatment was 3.95 months (range 0.5–172mo).

Plasma ammonia concentrations were persistently elevated (mean 123.5μmol/L, SD 35.6). No episode of acute hyperammonaemia was reported. Hyperammonaemia was always stable, independent of protein intake, and insensitive to sodium benzoate and carbamyl glutamate administration (data not shown).\textsuperscript{1,2} Blood and cerebrospinal fluid (CSF) amino-acid levels (glutamine) and CSF neurotransmitter levels (catecholamine and gamma-aminobutyric acid [GABA]) were measured in four patients (nos 1, 2, 21, and 22) and were normal. The activity and allosteric response of GDH in lymphoblasts was evaluated in 14 patients, all of whom had normal basal GDH activity. The GTP inhibition of GDH activity was 70% in controls.

At the time of the evaluation, 14 patients had epilepsy and 17 had features of learning disability. Among the 14 patients with epilepsy, non-hypoglycaemic seizures started at a median age of 6 years (range 2–12y). Four of these patients had never had hypoglycaemic seizures. One patient (no. 6) initially had hypoglycaemic partial status epilepticus before developing generalized seizures with atypical absences and myoclonia. Seizure types included atypical absences lasting more than 30 seconds (nine patients) with eyelid myoclonia (five patients) and myoclonic jerks involving the proximal limbs (three patients), or with atactic seizures and falls (two patients). Other reported types were focal motor seizures in three patients, and generalized tonic–clonic seizures in another two patients. In 11 of 14 patients, seizures were controlled by a single antiepileptic drug (sodium valproate, phenobarbitone, or carbamazepine), with a seizure frequency below one per year. Three patients (nos 1, 2, and 7) had severe epilepsy with refractory atypical absences and myoclonic seizures. Two of these three patients were sisters (patients 1 and 2, family A), who had photosensitive atypical absences with myoclonic seizures. Treatment with clobazam reduced the frequency of myoclonic absences but did not abolish them. Carbamazepine transiently worsened the seizures, except in two patients with focal seizures. Interictal EEG was abnormal in all but two patients, showing generalized spike-and-wave discharges (eight patients) and sleep-activated photosensitive spike–wave paroxysms (two patients). In three patients (patients 1, 2 and 4, family A), polygraphic ictal EEG allowed us to record seizures. Absence seizures with myoclonia were associated with high-amplitude irregular generalized spike–wave discharges, that occurred both spontaneously and with photic stimulation. In the group of patients without epilepsy (n=8), background EEG activity was always normal and expected developmental features were present. Neither photosensitivity nor focal or generalized slowing was observed.

Head circumference was normal in all patients. Developmental milestones were delayed in 17 of the 22 patients. Cognitive impairment was identified in 17 patients, at a median age of 5 years (range 3–12y). On neuropsychological assessment, learning disability was graded as borderline (IQ 70–75) in five patients, mild (IQ 50–70) in nine patients, and moderate (IQ 35–50) in three patients. Mild behavioural problems were reported in four patients, with social withdrawal and attention difficulties possibly related to cognitive impairment. Eleven patients required specialized education and the four oldest patients were in protected employment. Learning disability was more likely to occur in patients with epilepsy, as 13 of the 17 patients with cognitive impairment...
<table>
<thead>
<tr>
<th>Patient no., sex, age y (median 9.7y)</th>
<th>GLUD1 mutation</th>
<th>Clinical features</th>
<th>Disease course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family A</td>
<td></td>
<td>Birth weight g</td>
<td></td>
</tr>
<tr>
<td>1. F, 8</td>
<td>p.Arg221Cys c.835C&gt;T 2800</td>
<td>7 GTC seizure</td>
<td>15 Diazoxide + pancreatectomy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treatment</td>
<td></td>
</tr>
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<td>2. F, 5</td>
<td>p.Arg221Cys c.835C&gt;T 3000</td>
<td>23 GTC seizure</td>
<td>24 Diazoxide</td>
</tr>
<tr>
<td>3. F, 40</td>
<td>p.Arg221Cys c.835C&gt;T 3000</td>
<td>12 GTC seizure</td>
<td>24 Diazoxide</td>
</tr>
<tr>
<td>5. M, 11</td>
<td>p.Arg265 Thr c.966G&gt;C 3900</td>
<td>&lt;72h GTC seizure</td>
<td>36 Diazoxide</td>
</tr>
<tr>
<td>6. F, 18</td>
<td>None           4000</td>
<td>&lt;72h Hemiclonic SE 6</td>
<td>Diazoxide</td>
</tr>
<tr>
<td>7. M, 39</td>
<td>None           2800</td>
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<td>12 Diazoxide</td>
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<td>8. F, 7</td>
<td>p.Tyr266Cys c.969A&gt;G 2500</td>
<td>3 Hypotonia</td>
<td>5 Diazoxide 6</td>
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<tr>
<td>9. M, 18</td>
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<td>8 GTC seizure</td>
<td>180 Diazoxide</td>
</tr>
<tr>
<td>11. M, 9</td>
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<td>&lt;72h Hemiclonic SE</td>
<td>Diazoxide</td>
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<td>12. M, 8</td>
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<td>2.5 Diazoxide</td>
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<td>1 Diazoxide</td>
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<td>5 GTC seizure</td>
<td>5.5 Diazoxide</td>
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<td>17. F, 5.5</td>
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<td>Family B</td>
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<td>Diazoxide</td>
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<td>12 Hypotonia</td>
<td>Diet</td>
</tr>
<tr>
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<tr>
<td>Family C</td>
<td></td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>22. Male, 18</td>
<td>p.Arg269His c.978G&gt;A Not known</td>
<td>6 GTC repeated seizure</td>
<td>Diazoxide</td>
</tr>
</tbody>
</table>

F, female; M, Male; GTC, generalized tonic–clonic; LD, learning disability; SE, status epilepticus.
also had epilepsy, whereas only one of the patients without epilepsy (patient no. 11) had learning disability.

Except for cognitive impairment, neurological findings were normal (including no upper motor neuron signs) in all but two patients (nos 21 and 22, family C). Patient no. 21 was diagnosed at age 25 years, with spastic gait, brisk tendon reflexes, and bilateral Babinski’s sign but no motor deficit. His brother, patient no. 22, also had pyramidal signs with no motor deficit and had progressive generalized dystonia from age 10 years, with early and prominent cervicofacial involvement. Four of the five patients with no apparent learning difficulties were either younger than 3 years (n = 5) or had a follow-up of less than 1 year (n = 1). The remaining patient (no. 9) had no learning disability at the last evaluation, at age 12 years, but had epilepsy.

Brain MRI was normal in all but one patient (no. 11), who had mild frontal atrophy, presumably secondary to hypoglycaemic status epilepticus.

Mean ammonia levels were not significantly different between patients with and without epilepsy (128.1 μmol/L [SD 38.4] vs 117.4 μmol/L [SD 29.0] respectively; p = 0.51) or between patients with and without intellectual impairment (125.2 μmol/L [SD 35.5] vs 120.8 μmol/L [SD 37.1], respectively; p = 0.80). We were unable to compare the GTP inhibition of GDH activity between patients with and without epilepsy or between patients with and without learning disability because too few values were available.

Among the 14 children who were followed until at least the age of 5 years, eight had epilepsy at age 6 years. One of these eight patients had a mutation in exons 11 and 12, which encode the hinge and antenna regions of GDH, while the other seven had mutations in exons 6 and 7, which encode the GTP binding site (p = 0.09). Among the 15 patients who were followed until at least the age of 3 years, 13 had learning disability; they comprised five patients with mutations in exons 11 and 12 and eight patients with mutations in exons 6 and 7 (p = 1).

Discussion

We describe the neurological outcome in 22 patients with HHS aged from 1 to 40 years. In keeping with previous reports, we found a high frequency of learning disability and epilepsy. This, together with pyramidal signs and dystonia in some patients, points to diffuse chronic brain insult in HHS. We also found putative genotype–phenotype associations, between childhood-onset epilepsy and mutations in exons 6 and 7 of the \textit{GLUD1} gene encoding the GTP-binding site.

More than 77% of our patients with HHS had borderline to moderate learning disability, which was frequently associated with epilepsy. This prevalence of learning disability is higher than the 51% found in the 57 previously reported patients in which relevant data were available,\(^1\),\(^4\),\(^8\),\(^10\),\(^11\),\(^13\) possibly owing to the longer follow-up in our series. Epilepsy was also more frequent in our series (64%) than previously reported (46%, 12/26)\(^8\),\(^10\) possibly owing to its relatively late onset in HHS. It is also noteworthy that in our study, neurological development and status were ascertained systematically, whereas previous reports focused on biochemical and metabolic features.\(^3\),\(^4\),\(^8\),\(^10\),\(^11\),\(^13\) Thus, even when glycaemic control is satisfactory, long-term follow-up of patients with HHS is needed to determine the neurological consequences of the enzyme deficiency. Indeed, careful and repeated neurological evaluations are needed to ensure timely symptomatic treatment, educational assistance, and social support.

We observed a distinctive electroclinical phenotype of epilepsy, consisting of atypical absences combined with myoclonia. These seizure types were seen in most patients in the present study, in one family previously reported, and also in three patients in Raizen’s series.\(^10\) A similar seizure type was reported in patients with chromosome abnormalities and succinic semialdehyde dehydrogenase deficiency.\(^14\),\(^16\) As in those patients, this form of myoclonic absence-like epilepsy in patients with HHS tends to be refractory to antiepileptic drugs.\(^14\),\(^15\)

To our knowledge our results are the first to suggest putative phenotype–genotype associations in HHS, according to the location of mutations in the \textit{GLUD1} gene sequence. Indeed, mutations in exons 6 and 7 (encoding the major GTP binding site, previously named GTP1 site; 9/11 patients; 81%) tended to be more frequently associated with epilepsy than those in exons 11 and 12 (encoding the hinge and antenna region; 2/8 patients; 25%). This tendency of an increased proportion of epilepsy in patients with mutations in exons 6 and 7 was not observed in the literature (with respectively 7/17 [41%] and 5/9 [56%] epileptic patients). In contrast, the location of mutations did not seem to influence the frequency of other neurological disturbances, in particular, the rate of learning disability.\(^1\),\(^4\),\(^8\),\(^10\),\(^11\),\(^13\)

Why mutations in exons 6 and 7 should be associated with more frequent epilepsy than mutations in exons 11 and 12 is unclear. Whatever their location, these mutations reduce the sensitivity of GDH activity to GTP inhibition.\(^2\),\(^9\),\(^17\) Correlations have been observed between serum ammonia concentrations and sensitivity to GTP inhibition, leading to the suggestion that altered GDH activity is responsible for the abnormal ammonia concentration.\(^9\) However, in our study, ammonia levels did not correlate with intellectual impairment or epilepsy. In addition, no such correlation has been found with hypoglycaemia.\(^9\)

The pathophysiology of brain damage associated with GDH overactivity is complex and probably multifactorial. Our results, together with previous reports, argue against direct hypoglycaemic insult through recurrent hypoglycaemia or prior hypoglycaemic injury.\(^1\),\(^12\) Indeed, in contrast to patients with hypoglycaemic brain insults, patients with HHS do not usually have focal deficits.\(^18\) In addition, the epilepsy observed in a subset of patients with HHS was generalized and not focal or multifocal as in patients with epilepsy secondary to hypoglycaemic insults.\(^19\) Imaging findings were normal in all but one of our patients, with none of the structural abnormalities previously observed in patients with HHS (i.e. left frontal encephalomalacia, cerebral atrophy, left frontal porencephalic cyst),\(^10\) and no evidence of hypoglycaemic brain injury.\(^18\),\(^20\) As in other series, the hypoglycaemia in our patients was less severe and more easily controlled than in patients with congenital hyperinsulinism without HHS, who do not usually have neurological disorders.\(^1\),\(^13\)

Although patients with HHS do not show the classical signs of hyperammonaemia\(^3\) (i.e. lethargy, headaches, vomiting) usually observed in patients with urea cycle disorders,\(^7\) chronic brain damage is not excluded. Even if it is hypothesized that the protective effects of GDH from neurotoxicity of hyperammonaemia is related to depletion of glutamate pools,\(^5\) other indirect consequences of chronic hyperammonaemia are not excluded, in particular in the developing
brain. In this view, attempts to lower ammonia levels with sodium benzoate or N-carbamoyl glutamate have not produced any apparent effect on levels of blood ammonia. Further studies are needed to demonstrate that such efforts might influence neurological outcome in patients with HHS.

The overactivity of GDH in the brain leads to chronic depletion of brain pools of glutamate and other amino acids that feed into the glutamate pool, especially glutamine. This depletion of glutamate pools could lead to disequilibrium between glutamate and GABA, with increased GABA levels. Although CSF measurements in four of our patients revealed normal levels of GABA, it may not exactly reflect the physiological balance between the two neurotransmitters (glutamate and GABA), which are known to play major roles in the developing brain. All together, this disequilibrium could contribute to the occurrence of neurological disorders, which could result in epilepsy or developmental delay.

Conclusion
Neurological disorders are underdiagnosed in patients with HHS and consist of cognitive impairments and various forms of epilepsy. Seizures may be related to hypoglycaemia in the early phase of the disease or may occur later in childhood as the result of the chronic metabolic defect. Although further studies are needed to identify the pathophysiology of such neurological impairment, we suggest that the underlying mechanisms may include hyperammonaemia.

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