The difficult management of persistent, non-focal congenital hyperinsulinism

A retrospective review from a single, tertiary center

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Christesen Henrik Thybo (Orcid ID: 0000-0001-5330-6853)

Title

The difficult management of persistent, non-focal congenital hyperinsulinism: A retrospective review from a single, tertiary center

Running title

Forty patients with persistent, non-focal CHI

Authors

Amalie Greve Rasmussen[1,2], Maria Melikian[3], Evgenia Globa[4], Sönke Detlefsen[2,5,6], Lars Rasmussen[5,7], Henrik Petersen[8], Klaus Brusgaard[9], Annett Helleskov Rasmussen[1], Michael Bau Mortensen[5,7], Henrik Thybo Christesen[1,2,5]

[1] Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark

[2] Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

[3] Endrocrine Research Center, Moscow, Russia, Department of Pediatric Endocrinology

[4] Ukrainian Research Center of Endocrine Surgery, Department of Children Endocrinology, Kyiv, Ukraine

[5] OPAC, Odense Pancreas Center, Odense University Hospital, Odense, Denmark

[6] Department of Pathology, Odense University Hospital, Odense, Denmark

[7] Department of Surgery, Odense University Hospital, Odense, Denmark

[8]Department of Nuclear Medicine, Odense University Hospital, Odense, Denmark

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[9]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark

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Abstract

Background. Congenital hyperinsulinism (CHI) is a rare, heterogeneous disease with transient or persistent hypoglycemia. Histologically, focal, diffuse, and atypical forms of CHI exist, and at least 11 disease-causing genes have been identified.

Methods. We retrospectively evaluated the treatment and outcome of a cohort of 40 patients with non-focal, persistent CHI admitted to the International Hyperinsulinism Center, Denmark, from January 2000 to May 2017.

Results. Twenty-two patients (55%) could not be managed with medical monotherapy (diazoxide or octreotide) and six (15%) patients developed severe potential side effects to medication. Surgery was performed in 17 (43%) patients with resection of 66-98% of the pancreas. Surgically treated

patients had more frequently K_{ATP}-channel gene mutations (surgical treatment 12/17 *vs*. conservative treatment 6/23, p=0.013), highly severe disease (15/17 *vs*. 13/23, p = 0.025) and clinical onset <30 days of age (15/17 *vs*. 10/23, p = 0.004).

At last follow-up at median 5.3 (range: 0.3-31.3) years of age, 31/40 (78%) patients still received medical treatment, including 12/17 (71%) after surgery. One patient developed diabetes after an 98% pancreatic resection. Problematic treatment status was seen in 7/40 (18%). Only 8 (20%) had clinical remission (three spontaneous, five after pancreatic surgery). Neurodevelopmental impairment (n=12, 30%) was marginally associated with disease severity (p=0.059).

Conclusion. Persistent, non-focal CHI remains difficult to manage. Neurological impairment in 30% suggests a frequent failure of prompt and adequate treatment. A high rate of problematic treatment status at follow-up demonstrates an urgent need for new medical treatment modalities.

Key words: Congenital Hyperinsulinism, Treatment, Surgery, Genetic mutations, Hypoglycemia.

Main text

Introduction

Congenital hyperinsulinism (CHI) is a rare, heterogeneous disease in terms of age at clinical onset, severity and prognosis. CHI is characterized by hypersecretion of insulin from pancreatic ß-cells, which lowers blood glucose and inhibits glycolysis, gluconeogenesis, lipolysis and ketone body synthesis. As a result, the brain is deprived of both its primary and secondary sources of energy, which leads to cerebral damage if not promptly and adequately treated[1-3].

CHI is divided into three histological types, a focal, a diffuse and atypical forms[1]. To date, at least 11 genes have been discovered to be involved in CHI: *ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *UCP2*, *SCL16A1*, *HNF4A*, *HNF1A*, *HK1*, and *PGM1*. Mutations are most frequently found in the ß-cell K_{ATP}-channel genes *ABCC8* and *KCNJ11*, which express the sulphonyl urea receptor 1 (SUR1) and the potassium inward rectifier 6.2 (Kir6.2), respectively[2].

The diffuse type can be inherited as a recessive or dominant trait, most commonly caused by homozygous, compound heterozygous or heterozygous *ABBC8/KCNJ11* mutations. The dominant

K_{ATP}-channel mutations usually result in milder disease in contrast to homozygous or compound heterozygous mutations. Focal CHI is usually severe and characterized by a unique two-hit etiology with a heterozygous paternal mutation in *ABCC8* or *KCNJ11* plus a focal, somatic loss of maternal chromosome 11p15 leading to loss of heterozygosity in the focal pancreatic lesion[2,4].

Hence, a non-dominant, heterozygous paternal K_{ATP}-channel mutation in blood analysis is indicative of focal CHI, whereas homozygous, compound heterozygous, or heterozygous maternal *ABBC8/KCNJ11* mutations indicate diffuse CHI[4,5]. In focal CHI, the focal lesion can be localized by ^{18}FDOPA PET/CT scan aiding surgeons to perform curative limited pancreatic resection, in addition to per-operative frozen sections and ultrasound[6-10].

Diffuse CHI may be caused by mutations not only in *ABCC8* or *KCNJ11*, but in any of the known genes related to CHI and has variable severity; the most severe being difficult to treat medically[1,11].

Atypical CHI may be caused by somatic events, such as mosaic paternal uniparental disomy, with or without germline gene mutations known to cause CHI[12,13]. Atypical CHI may be seen in Beckwith-Wiedemann Syndrome and other syndromes.

The conservative treatment constitutes primarily of diazoxide, or octreotide, as first choice[3,14-16], but a significant number of the patients are unresponsive or only partially responsive to medical treatment[4,15,17]. Alternatively, continuous glucagon, sirolimus or nifedipine among others have been used, however with variable success[1,18-21]. If responsive to octreotide, long acting somatostatin analogues may be preferred[22-25].

In the case of poor medical response or unacceptable side effects, near-total (95-98%) pancreatectomy was previously a routine procedure[26]. However, long-term follow-up studies have identified a very high risk of diabetes and malabsorption after near-total pancreatectomy, which has

led to almost abandoning this surgical procedure even in severe, diffuse CHI at many specialist centers[1,3,7,18,27]. To avoid these long term complications, partial pancreatectomy has been used, however with lesser success in obtaining clinical remission[1,7,28].

We aimed to review the medical and surgical treatment of non-focal CHI in a consecutive cohort of patients at one international CHI center over a 16.5-year period, to evaluate the need for improved treatment options.

Methods

Study design and setting

This study was performed as a retrospective cohort-study analyzing patients with diagnosed non-focal CHI admitted at The International Hyperinsulinism Centre, Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark, in the period between January 1, 2000 and May 1, 2017. Data were collected in the period September 1st 2016 to June 1, 2017.

Participants and study size

In a larger cohort of patients admitted to Hans Christian Andersen Children's Hospital with hypoglycemia and suspected hyperinsulinism (n=162), 40 pediatric patients with non-focal, persistent CHI were identified (Fig. 1 - Flowchart of patient selection). Hyperinsulinism was defined as plasma insulin (p-insulin) within or above the normal range during hypoketotic hypoglycemia. Of note, p-insulin was measured by a highly sensitive immunoassay (Cobas e 411

immunoassay analyzer, Roche Diagnostics GmbH, Mannheim, Germany) with ability to detect p-insulin down to 2 pmol/L (0.1 mU/L), far below the lower border of the normal range, 18 pmol/L (1 mU/L). Exclusion criteria were: lack of documented hyperinsulinemic hypoglycemia, hyperinsulinism due to insulinoma, transient hyperinsulinism with remission before six months and focal CHI. Focal CHI was defined by histology with focal adenomatous ß-cell hyperplasia with preserved normal lobular structure of the pancreas according to Rahier *et al*.[29]. All patients with a heterozygous paternal *ABCC8/KCNJ11* mutation underwent 18F-DOPA PET/CT scan and all patients with a focal lesion by 18F-DOPA PET/CT had focal CHI confirmed by surgery.

Diffuse CHI was defined histologically by well-described criteria[29] in patients who had pancreatic surgery, or by diffuse labeling on ^{18}F-DOPA-PET/CT scan and/or genetic results corresponding with previous literature of known diffuse type mutations in non-surgical cases. Atypical CHI was defined as absence of diffuse and focal histological criteria after surgery, with or without other characteristic histological features. The finally included patients were referred from Denmark, Norway, Sweden, Latvia, Russia, Ukraine, Kazakhstan, Belarus and Greenland.

Variables and data sources

Clinical and biochemical information was collected from patient's hospital records. The following variables were studied: Country of origin, gender, age at clinical presentation (early onset defined <30 days of age), age at last follow-up, laboratory values, genetic results, ^{18}F-DOPA-PET/CT scan results, maximal i.v. glucose need, medical treatments (drug doses, medical response and side effects), surgical treatment (age at surgery, type of surgery, percentage removed of the pancreas as recorded by the surgeons, effect of and complications to surgery based on the Clavien-Dindo

classification[30], nutrition and data on medical treatment and neurological impairment at last follow-up.

Patients were retrospectively described as having mild to moderate CHI if lowest registered blood glucose was 1.5 to 3.1 mmol/L and severe CHI if lowest registered blood glucose was <1.5 mmol/L and/or maximum registered i.v. glucose need was ≥15 mg/kg/min. Maximum medical treatment was registered as the maximum number of different drugs and the highest dose registered for the relevant drug. For the surgically treated patients, this was registered for the medical treatment received before surgery. Potential medical side effects were registered, including cerebral, cardio-pulmonal, gastro-intestinal, urogenital and dermatological symptoms and abnormal blood-values.

The patients were categorized as diazoxide-responsive if they received diazoxide as monotherapy; had no hypoglycemic episodes (blood glucose < 3.5 mmol/L); and had a normal diet and eating pattern for their age and no surgical treatment. The surgically treated patients were categorized as postsurgical diazoxide-responsive if they after surgery met the same criteria as the conservatively treated diazoxide-responsive patients.

Hypoglycemia control at last follow-up was categorized as good (clinical remission or dietary and/or medical treatment with no registered episodes of hypoglycemia), intermediate (1-9 registered episodes of hypoglycemia), or poor (≥ 10 registered episodes of hypoglycemia) for the given medication. Nutritional therapy was categorized as: none (normal diet and feeding), no-tube (need of extra carbohydrates or frequent feeding), or with tube (nasogastric or percutaneous gastric, intermittent or continuous tube feeding).

The patient's treatment status at last follow-up was defined as acceptable, if the patient was categorized as having "good" hypoglycemia control with "none" or "no-tube" nutritional therapy

and no severe medical side effects. The treatment status was defined as problematic if the patient was categorized as having "poor" hypoglycemia control, had severe medical side effects, was managed with tube feeding, or had developed diabetes as a complication to surgery. Neurological impairment was defined as the presence of psychomotor retardation, epilepsy, cerebral palsy, or blindness at latest follow-up. Data used for follow-up was the latest entry in the patient's hospital record regarding the abovementioned variables.

Genetic analysis

Before 2012, denaturing high-performance liquid chromatography (dHPLC, Wave, Transgenomics Ltd. Crewe, UK) with subsequent Sanger sequencing was performed in analysis of two (*ABCC8* and *KCNJ11*) to nine genes (*ABCC8*, *KCNJ11*, *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4A*, *SLC16A1 and UCP2*). From 2012, *INS* and *INSR* were added in a targeted Next-Generation Sequencing (NGS) CHI-panel using the Agilent targeted sequence capture method (SureSelectXT Reagent Kit, Agilant Technologies, DenmarkAps, Glostrup) was performed on the Illumina HiSeq1500 or NextSeq NGS platform (Illumina Inc, San Diego, CA) with validation of positive findings by bi-directional Sanger sequencing using BigDye Terminater v.3.1 Cycle sequencing on an ABI3730XL sequencer (ThermoFischer, Denmark, Roskilde). Splice site or missense mutations were described as pathogenic if they were previously described in our laboratory, or in the literature and/or HGMD[31]. If missense variants mutations were considered novel or only described once, Polyphen-2 and SIFT prediction software were consulted to assess the effect of the amino acid substitution alongside presence in dbSNP, ExAC, GenomAD, or EVS[32-34].

If splice site mutations were not previously described, the pathogenicity was estimated based on five splice site prediction programs (SpliceSiteFinder-like, MaxEntScan, GeneSplicer, NNSplice and Human Splicing Finder)[35-39] using Alamut version 2.1 (Interactive Biosoftware, Rouen, France). Uniparental disomy was detected by use of routine microsatelliteanalysis of for chromosome 11p. Mutation nomenclature was used according to HGVS[40]. Genebank references were as follows *ABCC8*; NM_001351295.1, *KCNJ11*; NM_000525.3, *GLUD1*; NM_005271.2, *HNF4A*; NM_000457.3.

Histological analysis

Pancreatic tissue histology was assessed by intraoperative frozen section analysis of all the surgically treated patients using hematoxylin-eosin staining with the addition of tolouidine blue staining and/or immunohistochemistry for synaptophysin in difficult cases. After surgery, the tissue specimens were analysed by microscopy of hematoxylin-eosin stained, formalin fixed, paraffin embedded 4 µm thick sections. If necessary, immunohistochemical staining for markers such as synaptophysin, insulin, glucagon, somatostatin and p57 was performed using the BenchMark Ultra immunostainer (Ventana Medical Systems, Tucson, AZ) with OptiView-DAB detection kit (Ventana Medical Systems, Tucson, AZ). Nuclear counterstaining was performed with a BenchMark Ultra instrument using Hematoxylin II (Ventana Medical Systems, Tucson, AZ) and coverslipping using a Tissue-Tek Film coverslipper (Sakura, Alphen aan den Rijn, The Netherlands).

Statistical analysis

Statistical analysis was performed with STATA software (V. 14.2 Stata Corporation LP, Texas, USA). The Shapiro-Wilk test was used to test for normal distribution of data. The Wilcoxon rank-sum test and Fisher's exact test were used where appropriate to test the equality among unmatched data. The immediate form of two-sample test was used to test for equality among proportions.

A p-value of <0.05 was considered statistically significant, p-values 0.05-0.1 were considered trends.

Ethics approval and consent to participate

Permission to collect data in patient records was acquired from the Research Ethics Committee (reference no. 54947). The project has been approved by the Danish Data Protection agency (journal no. 16/28242). The reporting of this study conformed to the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) statement.

Results

Patient characteristics

Of 162 patients with known or suspected hyperinsulinism, 68 patients had persistent CHI, of whom 40 (59%) had non-focal CHI, Figure 1. The patient characteristics are summarized in Table 1. Disease-causing mutations were found in 53% (n=21). Among non-Scandinavians, 94% (n=16) had severe disease *vs.* 52% (n=12) of Scandinavians (p=0.012). Severe CHI was associated with early clinical onset (p=0.009). As a probable chance finding, females more frequently exhibit severe CHI compared to males (p=0.012) combined with an earlier clinical onset (p=0.035). Four patients had

atypical CHI. We found no correlation between the type of CHI (diffuse or atypical) and disease severity, country of origin, or age at clinical onset. Pancreatic surgery was performed in 43% (n=17) of the patients. All patients were alive upon follow-up.

Genetic findings

The 21 patients with identified genetic alterations are presented in Table 2. We identified 14 known, and 10 novel mutations in our cohort. The novel mutations were, *ABCC8* c.149-54_290+1448del; p.?, p.(Val58Glu), p.(Leu225_Ser226insThr Lys*), p.(Leu366Phe), c.3466-?_3623+?del); p.?, p.(Gln1134Arg), p.(Tyr1287*), p.(Gly1401Trp), *KNCJ11* p.(Arg206His), and *HNF4A* p.(Gly82Val).

K_{ATP}-channel mutations were seen in 18 (86%). Significantly more females 13/18 (72%) had a CHI mutation compared to males 8/22 (36%), p=0.026. No difference regarding mutation status was detected between Scandinavians and non-Scandinavians (p=0.216).

Significantly more of the patients with detected mutations had severe CHI (p=0.002) and early clinical onset (p=0.000). All patients with heterozygous or compound heterozygous K_{ATP}-channel mutations had severe CHI. Of the patients with diffuse CHI, 18/35 (51%) had a K_{ATP}-channel mutation compared to none among atypical CHI.

Eight patients had only one, heterozygous K_{ATP}-channel mutation, of which four were paternal, one was maternal, and three were *de novo*. Two patients had the same, previously described dominant mutation, *ABCC8* p.(Glu1507Lys). Of the seven different heterozygous K_{ATP}-channel mutations found, four (*ABCC8* p.(Gln1459His), p.(Gly1479Arg), p.(Glu1507Lys) and p.(Ile1512Thr)) have previously been reported as dominant; one (*ABCC8* p.(Asp1472Asn)) as recessive; and two

(*ABCC8* p.(Leu366Phe) and *KCNJ11* p.(Arg206His)) were novel. While none of the five identified heterozygous parents had hypoglycemia or diabetes, Patient 20 with the novel, paternal *ABCC8* mutation, p.(Leu366Phe), had a younger sister with mild CHI and an elder sister with non-autoimmune diabetes. For the whole cohort with persistent CHI, a heterozygous, paternal K_{ATP}-channel mutation predicted non-focal disease in 13% (4/32), while 88% (28/32) had focal CHI.

Maximal medical treatment and diazoxide responsiveness

As maximal medical treatment, 45% (n= 18) of the patients received monotherapy with diazoxide, median (range) dose 10 (3.0-15) mg/kg/d, or octreotide 12.1 (4.4-18) ug/kg/d, Supplementary Table 1. Of the 55% (n= 22) patients treated with combined medical therapy, the combinations constituted primarily of diazoxide and octreotide with or without i.v. glucose (n= 12). Five patients received three or more different type of drugs (with or without i.v. glucose). The remaining five patients received different combinations of double therapy with diazoxide, i.v. glucose, lanreotide, sandostatin long acting release, sirolimus and hydrocortisone.

The number of drugs as maximal therapy was highest in those having pancreatic surgery (p=0.002). There was no significant difference between the doses received between conservative and surgically treated patients.

Diazoxide non-responsiveness was seen in 28/36 (78%) of the patients. Four patients were not categorized because of missing data. Mutations were equally frequent in diazoxide-unresponsive and diazoxide-responsive patients (p=0.43). K_{ATP}-channel mutations were seen in 50% of the diazoxide-unresponsive patients and 88% of those with K_{ATP}-channel mutations were diazoxide non-responders. None of the atypical patients responded to diazoxide.

No patients with compound heterozygous or homozygous K_{ATP}-channel mutations were diazoxide responsive. Of the eight patients with a heterozygous K_{ATP}-channel mutation, five were diazoxide unresponsive (*ABCC8* p.(Gln1459His) (need of surgery); p.(Gly1479Arg) (need of additional cornstarch); p.(Asp1472Asn) (need of surgery); p.(Glu1507Lys) (hypoglycemia attacks) and *KCNJ11* p.(Arg206His). The patient with the novel, heterozygous *KCNJ11* mutation p.Arg206His had the most severe disease among the heterozygous patients with need of monthly long-acting octreotide injections and dietary treatment even after an 80% pancreatic resection. Of note, both repeat 18F-DOPA PET/CT and an 68Ga-DOTATATE PET/CT showed diffuse disease, confirmed by histology after surgery.

Of the four mutation-positive patients with diazoxide responsiveness, three had a heterozygous *ABCC8* mutation (p.(Leu366Phe); p.(Glu1507Lys); p.(Ile1512Thr)) and one had a heterozygous *HNF4A* mutation. Significantly more of the conservatively treated patients were diazoxide responsive (p=0.005).

Side effects

Potential medical side effects of medication were registered in 37/40 (93%) of the patients. For patients treated with diazoxide (n=36), 24 (67%) had recorded hypertrichosis on a diazoxide median (range) dose of 10.2 (3.1-22.5) mg/kg/day; 17 (27%) had fluid retention on a dose of 10.0 (5.0-22.5) mg/kg/day; and two had intermittent ketosis (13.6 mg/kg/d and N/A).

Severe potential side effects were seen in six patients. Of these, four had severe cardiomyopathy and signs of heart failure during treatment with diazoxide (n=1, dose N/A); octreotide (n=1, 18 μg/kg/day); octreotide and diazoxide in combination (n=1, 22.5 mg/kg/day; 13 μg/kg/day); or

diazoxide, octreotide and glucagon in combination (n=1, 20.0 mg/kg/day; 20 μg/kg/day; 3 μg/kg/h). Two patients had severe thrombocytopenia (thrombocytes <20x10^9/l) during treatment of diazoxide (7 mg/kg/day), and diazoxide and octreotide in combination (doses N/A).

Surgery

Table 3 describes the 17 patients with non-focal CHI who underwent surgery. Compared to conservative treatment, surgically treated patients more often had a severe type of CHI (p=0.025) and an earlier clinical onset (p=0.004). Surgically treated patients more frequently had genetic mutations compared to conservatively treated (p=0.013). Of those with compound heterozygous or homozygous K_{ATP}-channel mutations, 9/10 (90%) had pancreatic surgery. The last patient had a dose of diazoxide of 11 mg/kg/d and a problematic treatment regimen at last follow-up (see below). Of note, three patients with severe CHI, who underwent pancreatic surgery, had diffuse CHI by 18F-DPA PET/CT and histology, but a heterozygous K_{ATP}-channel mutation only, of which two were paternal and one *de novo*. Four patients had no detected mutations by NGS panel.

Of the four patients with atypical CHI by histology after surgery, three had no mutations found with the CHI NGS panel and one had genetically verified Beckwith-Wiedemann Syndrome with paternal uniparental disomy.

Before surgery, two patients received only one type of medication (octreotide), nine patients had two different types and six patients had more than two different types of medications. The recorded indications for choosing surgical treatment were; severe side effects (two patients), no effect of the treatment (eight patients), or a combination of the two (two patients). For five patients, the surgical indication could not be detected retrospectively.

Of the 17 surgically treated patients, 12 had a single surgical resection; three had one additional resection and one had two additional resections in attempt to obtain normoglycemia. The median percentage of pancreas resected after the last surgery was 90% (range: 66-98%). Early complications to surgery according to the Clavien-Dindo classification were registered in 53% of the patients (n=9). Of these, six had mild complications (grade I-II, infection, parenteral nutrition, blood transfusion). Two patients had grade IIIb complications with need of re-operation due to adherences and ileus, one (Patient 35) had grade IVb complications with septicemia and multi-organ failure. This three-year-old patient had highly severe, very insufficiently treated CHI and a body mass index of >30 kg/m^2 (+6 SD), complicating the postoperative course. The patient needed intensive care for 45 days, but ultimately recovered.

Follow-up

Follow-up data are presented in Table 4. The median (range) age at last follow-up was 5.3 (0.3-31.3) years; surgery treated patients 4.1 (0.4-16.5) years, conservatively treated patients 6.3 (0.3-31.3) years. The median time from last surgery to last follow-up was 2.46 years (range 0.06-14.8 years). Seventy-seven percent (n=31) still received medical treatment for hypoglycemia. Conservatively treated patients with ongoing medication at follow-up more frequently had monotherapy with diazoxide compared to surgically treated patients (p=0.001). Eight patients were managed without medical treatment at follow-up, of which seven had clinical remission. Clinical remission at follow-up was recorded in 24% (4/17) of those with surgery and 13% (3/23) of the conservatively treated (p =0.432). The median (range) age at clinical remission was 7.4 (1.7-16.5) years after surgery and 14.2 (11.4-16.6) years after conservative treatment. All three with

spontaneous remission had late clinical onset, diffuse CHI and no detectable mutations. One of the patients had severe initial disease, the two others non-severe CHI.

Overall, hypoglycemia control was good in 26 patients (65%), intermediate in zero, poor in 7 (18%) and missing in 7 patients. Nutritional therapy with tube feeding was needed in 3 (8%). Treatment status (as defined by hypoglycemia control, nutritional therapy, medical side effects and eventual diabetes) was categorized as acceptable in 24 (60%), problematic in 7 (18%) and missing in 9 (23%). Excluding tube feeding from the definition of problematic treatment status did not change this result.

Of the seven children with problematic treatment status at follow-up, six had severe CHI (one had missing data), five had undergone pancreatic surgery (30% of the surgical patients) and five had K_{ATP}-channel mutations. Three of the patients were treated with diazoxide in a median dose of 5.8 mg/kg/d, two patients were treated with long acting somatostatin analogue, and one patient was treated with insulin. One patient had no medication at follow-up, but extra carbohydrates before sports. We found no statistical associations between the treatment status at last follow-up and country of origin, genetics, age at clinical onset, disease severity, number of drugs, or surgery.

Neurological impairment was found in 15 (38%) of the patients. This included psychomotor retardation (n=15), epilepsy (n=2), cerebral palsy (n=5) and cerebral blindness (n=1). Neurological impairment was not significantly associated with country of origin, genetics, age at clinical onset, number of drugs, or surgery, but marginally associated with disease severity (trend p=0.059).

Discussion

Non-focal, persistent CHI remains difficult to manage. In our cohort, the majority did not respond to medical monotherapy (diazoxide or octreotide) and surgery was performed in almost half of the patients. Surgically treated patients more frequently had highly severe disease, early onset and K_{ATP}-channel mutations, and almost three-fourths were still in need of medical treatment after surgery. At follow-up, only one fifth were in clinical remission without medical treatment, with or without pancreatic surgery.

Patient characteristics and genetics

Genetic mutations were found in 53% of our patients, consistent with some[41,42], but lower compared to other referral centers[43], possibly reflecting variations in referral patterns and inclusion/exclusion criteria, and advances in gene analysis methods. The importance of referral patterns was also seen by the fact that our non-Scandinavian patients more often had highly severe disease compared to Scandinavians. The correlation we found between early onset and K_{ATP}-channel mutations also has been found in other series[44].

Severe disease, genetic mutations, and early onset were more frequently seen in females than males, and surgery was more often performed in females in this cohort. We found no literature supporting a sexual dimorphism, therefore suggesting a chance finding[3,7,43,44].

Four of the patients with diffuse CHI had a paternal heterozygous K_{ATP}-channel mutation and a healthy father. While this usually is predictive of focal CHI[45], others have found similar exceptions[41,46,47]. In addition, we found one patient with a maternal *ABCC8* mutation and a healthy mother. Likely explanations could be an undiscovered additional mutation on the other allele, variable penetrance for a dominant mutation, under-diagnosis of hypoglycemia in the parent, or an

additional somatic, diffuse pancreatic mutation. We routinely recorded fasting p-glucose, insulin and HbA1c in our parents without detecting anyone affected (data not shown), but previous unrecognized CHI with seemingly remission in adulthood could not be ruled out.

Only two patients with diffuse CHI by 18F-DOPA PET/CT had other than K_{ATP}-channel mutations detected (after the scan was performed), one in *GLUD1* and one in *HNF4A.* The rareness of these "non-K_{ATP}-channelopathies" or "metabolopathies" is consistent with the literature[41,48].

Atypical CHI was found in 8% of our patients, comparable with other series based on histological criteria only[43,49]. One of our atypical patients had BWS, with a highly severe disease. The disease severity spectrum varies considerably in BWS from none, over mild transient, to severe persistent CHI[50]. None of the non-syndromic atypical patients had a positive gene mutation analysis, in keeping with other reports[51]. Somatic mosaic mutations in the pancreas may be responsible for CHI in such patients[52].

Medication

We only found mutations in 57% of the diazoxide-unresponsive non-focal patients, in contrast to the 91% found by Snider *et al.*[51]. However, large differences in the definition of diazoxide responsiveness between reports hamper comparisons. We were not able do formal fasting tests on diazoxide due to the retrospective nature of our study. Of our eight patients with a heterozygous K_{ATP}-channel mutation, five were diazoxide unresponsive. Others have reported severe CHI in dominant, non-focal CHI[41,53-55].

Twenty-three patients received short-acting octreotide either as monotherapy or as part of combination therapy. While octreotide is not labeled in many countries for children, and necrotizing

enterocolitis has been reported as a potential side effect, the use of octreotide in short-acting og long-acting release forms have become widespread in European countries[14,25,56].

Regarding side effects, severe hypertrophic cardiomyopathy and thrombocytopenia were considered to be severe potential medical side effects in six patients treated with diazoxide, octreotide, or both. However, hypertrophic cardiomyopathy may be the result of the anabolic effects of hyperinsulinism itself[57,58] and octreotide has been used to reduce hypertrophic cardiomyopathy in other patients[59]. Thrombocytopenia as well as neutropenia are rare, but recognized as side effects to treatment with diazoxide[44,60]. None of our non-focal patients died, developed necrotizing enterocolitis, glucagon-induced migratory necrolytic erythema, or had other adverse events described elsewhere in the treatment of CHI[20,21,61-64].

Among mild side effects, hypertrichosis was almost universal after diazoxide treatment. Two patients developed ketosis during diazoxide treatment; which reversed after cessation of the drug, as shown by others[65]. Among the patients treated with sirolimus (n=3), only mild potential side-effects were registered. The effect of sirolimus therapy in CHI has been discouraged because of the potential for severe side-effects[20,21,66].

Surgery

Nine out of ten patients with compound heterozygous or homozygous K_{ATP}-channel mutations had pancreatic surgery, and the tenth patient had problematic treatment status on diazoxide at follow-up, indicating this group of patients as especially difficult to treat. Non-responsiveness to medication was the major indication for surgery. The indications for pancreatic resections in non-focal CHI varied over the years, but always included a judgment of the chance of successful conservative

treatment for the individual *vs.* the chance of improved outcome by surgery, including the risk of complications. In our cohort with patients of many nationalities, both the medical treatment and decision of surgery were individualized and included parental preferences, the patient's family background, country of origin and possibilities of financial support for the specific family.

More than half of our surgically treated patients were not cured by the surgery, consistent with the results of the largest surgical cohort study reported by Adzick *et al.*[64]. We performed near-total pancreatectomy (95-98%) in four patients over one or more surgical procedures. In recent years, near-total pancreatectomy was only performed at our institution if all medical possibilities including long-acting release octreotide had been evaluated or considered. Near-total pancreatectomy led to euglycemia in two of the patients. Other studies have reported a relatively low cure rate even after near-total pancreatectomy[7,67].

At follow-up, one patient had diabetes 5½ years after a 98% pancreatic resection and one patient had exocrine insufficiency. Our follow-up time from last surgery to last follow-up was relatively short with a maximum of 14.8 years and a median of 2.5 years. In the Lord *et al.* cohort[68], 36% developed diabetes at a median of 7.7 years after near-total pancreatectomy. In a long-term follow-up study, the cumulative risk of insulin-dependent diabetes was 91% in CHI patients 14 years after near-total pancreatectomy[7]. Another study found peak onset of diabetes in the first year or between 12-16 years after pancreatectomy[3]. Based on this, near-total pancreatectomy in diffuse CHI is currently avoided in some centers[7], but still employed in others[68]. In the decision process of choosing near-total pancreatic surgery, the risk of further brain damage with poor possibilities of successful conservative treatment including the familial burden should be considered for each individual patient.

In our cohort, more limited pancreatic resections (66-90%) did not cure hypoglycemia in nine out of 12 patients (75%). The benefit of partial pancreatic resections in diffuse CHI is still a matter of debate. We were not able to discern a special genotype or phenotype for those with clinical remission after smaller pancreatic resections.

Reports of early complications to pancreatic surgery in CHI range from none, over mild and short-lasting to a single report of death[69,70]. Only one of our patients had severe postoperative complications with septicemia and long-standing, but transient multi-organ failure related to this patient's extreme obesity. Extreme obesity is a well-known cause of surgery complications, although rarely reported in children[71]. In general, the rate and severity of complications depend on both the reporting criteria used, patient co-morbidity, type of CHI and the complexity of the surgical procedure. We believe that the Clavien-Dindo classification is an appropriate reporting tool for surgical complications as used once before in CHI[10].

Follow-up

The median (range) age at follow-up for conservatively treated patients was 6.3 (0.3-31.3) years, of whom three (13%) had clinical remission. In a study from Manchester, UK[47], 71% of conservatively treated patients with K_{ATP}-channel mutations (focal or diffuse CHI) had resolution of CHI at a median (range) age of 3.1 (0.2-13) years.

The lower spontaneous remission rate in our cohort probably relates to our exclusion of those with transient CHI before six months' age, compared to inclusion of patients with spontaneous resolution down to two months' age in the UK study[47]. With prolonged follow-up, an increasing part of conservatively treated patients may enter clinical remission as shown by others[70].

The three conservatively treated patients with clinical remission in our study had some similarities. All patients had a late clinical onset, diffuse CHI and no detectable mutations. Currently, trio exome scans and tissue investigations are carried out to identify a genetic cause and possibly a genotype-phenotype correlation in these and other genetically unexplained patients.

Seven of our patients (17.5%) were defined as having problematic treatment status at follow-up. This percentage may furthermore increase with prolonged follow-up, as diabetes may be expected in some or all, with near-total pancreatectomy[3,68].

Neurological impairment

Neurological impairment was seen in 30% of our patients. Other studies[3,47,72,73] have shown mild or severe neurodevelopmental impairment in 31-45% in non-focal CHI. Regardless of limitations in follow-up time and cohort size variations, this high prevalence indicates an urgent need for optimizing the treatment of CHI. We found no correlation between age at disease onset, genetics, number of drugs, surgery or country of origin and neurological impairment. However, a trend towards neurological impairment was found for higher disease severity ($p=0.059$). In our recent larger study on neurodevelopmental impairment, including patients treated in Moscow, Russia, blood glucose recordings <1 mmol/L and more than five days' delay before treatment at an expert center were identified as risk factors associated with neurodevelopmental impairment[72]. This further emphasizes the need of improvements in early recognition and prompt, efficient treatment of severe hypoglycemia at all wards handling newborns and infants. This difficult task may be solved by national and international guidelines with screening for hypoglycemia in at-risk neonates[72,74]. However, not all patients with CHI may be recognized at-risk, and hypoglycemia may be difficult

to detect from clinical symptoms in neonates. Implementation of screening guidelines and spread of knowledge remain important tasks for national pediatric societies. Furthermore, hypoglycemia may potentially become part of the dry-spot screening program for all neonates, enabling identification of hypoglycemia after the first days of life.

Strength and limitations

Our study had the strength of presenting a mixed, international cohort with patients from Eastern Europe, Russia and Scandinavia. Our detailed information on treatment regimens, surgery complication rates and follow-up data provided a basis for in-depth judgement of the pros and cons for surgery in diffuse CHI.

Limitations included the relative small cohort size; a short median follow-up time, presumed selection bias by international referrals, and the retrospective sampling of hospital data from several countries.

Conclusion

Persistent, non-focal CHI remains difficult to manage. In our cohort, 30% had neurological impairment upon follow-up to a median of 5.3 years' age, suggesting lack of prompt and adequate treatment. Moreover, 18% had a problematic treatment status upon follow-up, including one patient with diabetes after near-total pancreatectomy. Improvements in early diagnosis and treatment and new medical treatment modalities are urgently warranted in CHI.

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Tables

Table 1. Patient characteristics in 40 patients with non-focal CHI.

	All, n = 40	Conservatively treated, n = 23	Surgically treated, n = 17	p-value (conservatively *vs.* surgically treated)
Gender				**0.033**
Female, n (%)	18 (45%)	7 (30.4%)	11 (64.7%)	
Male, n (%)	22 (55%)	16 (69.6%)	6 (35.3%)	
Country of origin				0.257
Scandinavia incl. Greenland[†], n (%)	23 (57.5%)	15 (65.2%)	8 (47%)	
Referred non-Scandinavians[‡], n (%)	17 (42.5%)	8 (34.8%)	9 (53%)	
Genetics				
K_{ATP}-channel mutations, n (%)	18 (45%)	6 (26.1%)	12 (70.6%)	**0.013**
Other[§], n (%)	3 (7.5%)	2 (8.7%)	1 (5.9%)	
No mutations found, n (%)	19 (47.5%)	15 (65.2%)	4 (23.5%)	
Clinical onset				
Median (range), days	3 (1-270)	49 (1-270)	1 (1-120)	**0.019**
Early (<30 days), n (%)	25 (62.5%)	10 (43.5%)	15 (88.2%)	**0.004**
Late (≥30 days), n (%)	12 (30%)	11 (47.8%)	1 (5.9%)	
Missing, n (%)	3 (7.5%)	2 (8.7%)	1 (5.9%)	
Severity[¶]				**0.025**
Non-severe, n (%)	10 (25%)	9 (39.1%)	1 (5.9%)	
Severe, n (%)	28 (70%)	13 (56.6%)	15 (88.2%)	
Missing, n (%)	2 (5%)	1 (4.3%)	1 (5.9%)	

Histological type				**0.029**
Diffuse, n (%)	35 (87.5%)	22 (95.7%)	13 (76.5%)	
Atypical, n (%)	4 (10%)	0 (0%)	4 (17.5%)	
Missing, n (%)	1 (2.5%)	1 (4.3%)	0 (0%)	
Age at last follow-up				
Median (range), years	4.8 (0.3-31-3)	6.3 (0.3-31.3)	3.2 (0.4-16.5)	0.374

†) Denmark; n=12, Norway; n=1, Sweden; n=9, Greenland; n=1, ‡) Russia; n=9, Ukraine; n=3, Kazakhstan; n=2, Belarus; n=1, Latvia; n=1 , Syria; n=1, §)11p15 paternal uniparental disomy (Beckwith-Wiedemann Syndrome), *GLUD1* and *HNF4A (*n=1 each), ¶) Less severe: lowest registered blood glucose 1.5 – 3.5 mmol/L, highly severe: lowest registered blood glucose < 1.5 and/or maximum registered i.v. glucose need ≥ 15 mg/kg/mi

Table 2. Genetic findings in 21 patients with non-focal CHI.

Gene/syndrome Patient no.	Age at presentation, days	Nucleoid sequence change /somatic event	Protein change	Transmission	Heredity Phase	Inheritance pattern	Previously reported	Type	Surgery (yes/no)	Diazoxide responsive (yes/no)
ABCC8										
4	150	c.4519G>A	p.(Glu1507Lys)	Heterozygous	de *novo*	Dominant	Yes	Diffuse	No	Yes
5	1	c.4377G>C	p.(Gln1459His)	Heterozygous	de *novo*	Dominant	Yes	Diffuse	Yes	Post
13	1	c.742C>T	p.(Arg248*)	Compound	Paternal	Recessive	Yes	Diffuse	Yes	No
		c.560T>A	p.(Val187Asp)	heterozygous	Maternal		Yes			
14	1	c.3643C>T	p.(Arg1215Trp)	Compound	Paternal	Recessive	Yes	Diffuse	Yes	No
		c.2117-1G>A	p.?	heterozygous	Maternal		Yes			
15	1	c.4631T>C	p.(Leu1544Pro)	Compound	Paternal	Recessive	Yes	Diffuse	Yes	No
		c.1630+1G>T	p.?	heterozygous	Maternal		Yes			
18	3	c.149-54_290+1448del	p.?	Homozygous		Recessive	Novel	Diffuse	Yes	No
20	n/a	c.1096C>T	p.(Leu366Phe)	Heterozygous	Paternal	Dominant†	Novel	Diffuse	No	No
22	1	c.173T>A	p.(Val58Glu)	Compound	Paternal	Recessive	Novel	Diffuse	No	No
		c.3751C>T	p.(Arg1251*)	heterozygous	Maternal		Yes			
25	2	c.4435G>A	p.(Gly1479Arg)	Heterozygous	Maternal	Dominant‡	Yes	Diffuse	No	No

26	1	c.3751C>T	p.(Arg1251*)	Compound	Paternal	Recessive	Yes	Diffuse	Yes	No
		c.1332G>T	p.(Gln444His)	heterozygous	Maternal		Yes			
28	1	c.4535T>C	p.(Ile1512Thr)	Heterozygous	de *novo*	Dominant	Yes	Diffuse	No	Yes
30	1	c.4519G>A	p.(Glu1507Lys)	Heterozygous	Paternal	Dominant‡	Yes	Diffuse	No	n/a
31	2	c.4201G>T	p.(Gly1401Trp)	Compound	Paternal	Recessive	Novel	Diffuse	Yes	No
		c.3466-?_3623+?del	p.?	heterozygous	Maternal		Novel			
33	1	c.3861C>A;	p.(Tyr1287*)	Compound	Paternal	Recessive	Novel	Diffuse	Yes	No
		c.3751C>T;	p.(Arg1251*)	heterozygous	Maternal		Yes			
35	3	c.3401A>G	p.(Gln1134Arg)	Compound	Paternal	Recessive	Novel	Diffuse	Yes	n/a
		c.4163_4165delTCT	p.(Phe1388del)	heterozygous	Maternal		Yes			
36	1	c.4414G>A	p.(Asp1472Asn)	Heterozygous	Paternal	Dominant‡	Yes	Diffuse	Yes	No
38	1	c.4014G>A	p.(Trp1338*)	Compound	Paternal	Recessive	Yes	Diffuse	Yes	No
		c.674_675insCACGAAGTAGCA	p.(Leu225_Ser226insThr Lys*)	heterozygous	Maternal		Novel			
KCNJ11										
6	4	c.617G>A	p.(Arg206His)	Heterozygous	Paternal	Dominant‡	Novel	Diffuse	Yes	No
GLUD1										
32	5	c.1495G>C	p.Gly499Arg	Heterozygous	de *novo*	Dominant	Yes	Diffuse	No	No
HNF4alfa										
7	1	c.245G>T	p.(Gly82Val)	Heterozygous	n/a	Dominant	Novel	Diffuse	No	Yes

Beckwith-Wiedemann Syndrome						
21	1	11p15 paternal uniparental disomy	Yes	Atypical	Yes	No

†) Phenotype of family members with the mutation: father healthy, one sisters with hypoglycemia, one with non-autoimmune diabetes.

‡) Carrier parent healthy suggesting variable penetrance, unidentified additional mutation, or additional somatic, diffuse pancreatic mutation

Table 3. Description of 17 surgically treated patients with non-focal CHI.

Pt no	Severity	Genetics	Treatment before first surgery	Indication for first surgery	Age at first surgery, years	Number of surgical procedures	Percentage of pancreas removed after last surgery	Histological type	Time from last surgery to follow-up, years	Complications after surgery Clavien-Dindo classification[†]	Treatment at last follow-up
5	Severe	*ABCC8*, heterozygous, *de novo*	Diazoxide, octreotide	Severe side effects	0.31	1	90	Diffuse	5.8	n/a	Diazoxide
6	Severe	*KCNJ11*, heterozygous, paternal	Diazoxide, octreotide, glucagon, sirolimus, S.LAR[‡]	No effect of medicine	0.47	1	80	Diffuse	0.43	n/a	S.LAR
10	Severe	Negative NGS panel	Hydrocortisone, diazoxide, octreotide, nifedipine	No effect of medicine	0.14	1	95	Diffuse	14.83	IIIb	None
12	Non-severe	Negative NGS panel	Octreotide	n/a	15.03	2	66	Atypical	0.28	I	None
13	Severe	*ABCC8*, compound heterozygous	Diazoxide, glucagon, octreotide	n/a	0.17	1	75	Diffuse	7.46	I	None
14	Severe	*ABCC8*, compound heterozygous	Diazoxide, octreotide	No effect of medicine	1.02	2	90	Diffuse	0.7	0	Octreotide, S.LAR

15	n/a	*ABCC8*, compound heterozygous	Diazoxide, octreotide	No effect of medicine	0.99	3	98	Diffuse	5.62	II	Insulin
18	Severe	*ABCC8*, homozygous	Hydrocortisone, octreotide, glucagon	n/a	0.98	1	n/a*	Diffuse	3.1	n/a	S.LAR
21	Severe	Paternal uniparental disomy	Diazoxide, octreotide, glucagon	No effect of medicine	0.06	1	90	Atypical	6.27	II	Diazoxide, S.LAR
26	Severe	*ABCC8*, compound heterozygous	Diazoxide, octreotide	No effect of medicine	0.54	1	85	Diffuse	0.08	IIIb	Diazoxide
31	Severe	*ABCC8*, compound heterozygous	Diazoxide, octreotide	n/a	0.35	1	85	Diffuse	1.02	n/a	S.LAR
33	Severe	*ABCC8*, compound heterozygous	Octreotide	Severe side effects	0.4	1	90	Diffuse	2.16	n/a	Octreotide
34	Severe	Negative NGS panel	Diazoxide, octreotide, lanreotide	No effect of medicine; side effects	2.22	1	80	Atypical	2.46	n/a	S.LAR
35	Severe	*ABCC8*, compound heterozygous	Octreotide, diazoxide	No effect of medicine	2.99	1	95	Diffuse	0.17	IVb	Diazoxide & kreon
36	Severe	*ABCC8*,	Diazoxide,	No effect	1.34	1	95	Diffuse	5.85	n/a	None

		heterozygous, paternal	S.LAR	of medicine; severe side effects							
38	Severe	*ABCC8*, compound heterozygous	Octreotide, diazoxide	No effect of medicine	0.38	2	80	Diffuse	0.06	I	S.LAR
39	Severe	Negative NGS panel	Octreotide, diazoxide	n/a	1.16	1	66	Atypical	0.08	I	None

†) Clavien-Dindo postoperative complications: Grade I; antibiotics for (suspected) infection, Grade II; blood-transfusion, enzymes and/or insulin, Grade IIIb; reoperation due to adherences, Grade IVb: multi-organ failure complicating septicemia.
‡) Abbreviation: S.LAR; Long-acting release somatostatin analogue

Table 4. Follow-up of 40 patients with non-focal CHI.

	All n=40	Conservatively treated n=23	Surgically treated n=17	p-value (conservative *vs.* surgical)
Age, median (range), years	5.33(0.3-31-3)	6.3(0.3-31.3)	4.1(0.4-16.5)	0.374
No treatment, n (%)	8 (20%)	3 (13%)	5 (29.4%)	0.199
Treatment for hypoglycemia				
Monotherapy, n (%)	28 (70%)	19 (82.6%)	9 (53%)	0.140
Diazoxide, n (%)	19 (47.5%)	16 (69.6%)	3 (17.6%)	**0.001**
mg/kg/d, median (range)	5.4 (0.9-11)	5.6 (0.9-11))	3.8 (2.5.4.4)	0.218
Octreotide, n (%)	2 (5%)	1 (4.3%)	1 (5.9%)	0.816
ug/kg/d, median	n/a	n/a	n/a	-
LAR-SA†, n (%)	6 (15%)	2 (8.7%)	4 (23.5%)	0.195
mg/month, median (range)	10 (3.3-40)	25 (10-40)	8.6 (3.3-20)	0.240
Lanreotide, n (%)	1 (2.5%)	0 (0%)	1 (5.9%)	-
mg/month, median	45		45	-
Combination therapy, n (%)	3 (7.5%)	1 (4.3%)	2 (11.8%)	0.373
Diazoxide + LAR-SA, n (%)	2 (5%)	1 (4.3%)	1 (5.9%)	0.818
Diazoxide, mg/kg/d, median (range)	9.4 (6.7-12.1)	12.1	6.7	0.317
LAR-SA, mg/month, median	10	10	10	-
Octreotide + LAR-SA, n (%)	1 (2.5%)	0 (0%)	1 (5.9%)	-
Octreotide, ug/kg/d	9.7		9.7	-

LAR-SA, mg/month	10		10	-
Insulin, n (%)	1 (2.5%)	0 (0%)	1 (5.9%)	-
Kreon for malabsorption, n (%)	1 (2.5%)	0 (0%)	1 (5.9%)	-
Treatment status‡				0.198(fishers)
Acceptable	24 (60%)	15 (65.2%)	9 (53%)	
Problematic	7 (17.5%)	2 (8.7%)	5 (29.4%)	
Missing	9 (22.5%)	6 (26.1%)	3 (17.6%)	
Neurological impairment§				0.107(fishers)
No, n (%)	25 (62.5%)	17(74%)	8 (47%)	
Yes, n (%)	15 (37.5%)	6(26%)	9(53%)	

†) Abbreviation: LAR-SA; Long-acting release somatostatin analogue, ‡) The patient's treatment status at last follow-up was defined as acceptable, if the patient was categorized as having good hypoglycemia control with no or no-tube nutritional therapy and no severe medical side effects. The treatment status was defined as problematic if the patient was categorized as having poor hypoglycemia control, had severe medical side effects, was managed with tube feeding, or had developed diabetes as a complication to surgery, §) Neurological impairment was defined as the presence of psychomotor retardation, epilepsy, cerebral palsy, or blindness at latest follow-up.

Figure legends

Figure 1. Flow chart of patient inclusion.

Figure 1. Patient inclusion flowchart.