

Clinical and Genetic Evaluation of Patients with K_{ATP} Channel Mutations from the German Registry for Congenital Hyperinsulinism

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Key Words

Congenital hyperinsulinism · Hypoglycemia · K_{ATP} channel · *ABCC8* · *KCNJ11*

Abstract

Congenital hyperinsulinism (CHI) causes hypoglycemia due to irregular insulin secretion. In infants, a rapid diagnosis and appropriate management to avoid severe hypoglycemia is mandatory. CHI is a heterogeneous condition at the clinical and genetic level, and disease-causing genes have been identified in about half of the patients. The majority of mutations have been identified in the *ABCC8* and *KCNJ11* genes encoding subunits of the K_{ATP} channel responsible for two distinct histological forms. The diffuse form is caused by autosomal recessive or dominant inherited mutations, whereas the focal form is caused by a paternally transmitted recessive mutation and a second somatic event. We report on an unselected cohort of 136 unrelated patients from the German CHI registry. Mutations in either the *ABCC8* or *KCNJ11* gene were identified in 61 of these patients (45%). In total, 64 different mutations including 38 novel ones were detect-

ed in this cohort. We observed biparental (recessive) inheritance in 34% of mutation-positive patients, dominant inheritance in 11% and paternal transmission of a mutation associated with a focal CHI type in 38%. In addition, we observed inheritance patterns that do not exactly follow the classical recessive or dominant mode, further adding to the genetic complexity of this disease.

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Introduction

Congenital hyperinsulinism (CHI) is a disorder of dysregulated insulin secretion resulting in persistent hypoglycemia with potentially harmful consequences. It manifests in nearly 50% of patients during the first hours or days of life [1]. The prevalence in the EU population (calculated from 8 EU countries) is 1.85 per 10,000, and the incidence has been reported as 1:40,000 [2]. CHI is characterized by

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clinical, histological and genetic heterogeneity (MIM 600509, 600937, 138130, 138079, 256450), but common to all types are inappropriately elevated levels of insulin in relation to low plasma glucose concentrations along with low levels of fatty acids and ketones [3, 4]. Diazoxide (DZX) is used as first-line medication, while in DZX-resistant cases of CHI, off-label therapy with octreotide analogues or glucagon has been used [5, 6]. The most severe forms of CHI are caused by loss of function of the *ABCC8* and *KCNJ11* genes encoding subunits of the ATP-sensitive K_{ATP} channel. This subgroup has been given the name channelopathies. They are usually associated with unresponsiveness of pancreatic β -cells to medical treatment. Recently, it has been suggested to implement rapid genetic analysis of *ABCC8* and *KCNJ11* in newborns with DZX-unresponsive CHI to improve patient management [7, 8]. Gene defects in medically responsive or partially responsive CHI include mutations in *GLUD1*, *GCK*, *HADH*, *SLC16A1*, *UCP2*, *HNF4A* and *HNF1A* [9]. This subgroup is due to dysregulation of the glucose-mediated signaling pathway promoting insulin release and is summarized as metabolopathies. Still, for more than 65% of children affected by CHI the genetic basis is unknown and the mechanisms of disease remain to be determined [10].

Four *ABCC8* (sulfonylurea receptor 1) and 4 *KCNJ11* (Kir6.2) protein subunits form the K_{ATP} channel as a hetero-octameric complex [11]. The majority of CHI-associated mutations (e.g. nonsense, frameshift, splice site) are predicted to cause a loss of protein expression, while missense mutations may act through perturbed trafficking of the subunits to the plasma membrane or interference with the formation or the gating function of the channel [12–15]. Electrophysiological evidence of K_{ATP} channel dysfunction has been identified in children with CHI mutations due to either *ABCC8* or *KCNJ11* [16]. Recessive inheritance of 2 inactivating mutations leads to functionally impaired K_{ATP} channels or their complete absence [17]. In healthy heterozygote carrier parents, the wild-type allele is sufficient to ensure the formation of intact channels. In contrast, dominant mutations are specific missense mutations in the *ABCC8* and *KCNJ11* genes which are presumed to be expressed as abnormal protein components and to integrate into the hetero-octameric complex, where they interfere with the proper assembly or function of the channel (dominant negative effect) [18]. In line with the proposed molecular mechanisms, biallelic inactivating mutations of the *ABCC8* or *KCNJ11* gene are usually associated with a severe phenotype and resistance to treatment with DZX, a component that is able to bind to and modify the function of K_{ATP} channels. In contrast,

dominant-acting mutations are often associated with a less severe clinical expression and DZX sensitivity [19].

Three histological CHI subtypes have been classified, which are commonly described as diffuse, focal and atypical forms [20]. The diffuse form affects all pancreatic β -cells and is most frequently associated with biallelic inactivating mutations in the *ABCC8* and *KCNJ11* genes and recessive inheritance of the disease. Occasionally, the diffuse form of CHI is caused by dominant-acting mutations [18, 19, 21].

In contrast, patients carrying only a paternally inherited heterozygous loss-of-function mutation of *ABCC8* or *KCNJ11* are suspected to have a focal subtype of CHI, where only a circumscribed lesion of the pancreas is affected. It has been proposed that in such a focus of dysregulated β -cells only the paternally transmitted mutant allele of *ABCC8* or *KCNJ11* is expressed while the maternal allele is absent due to a second somatic mutation event [22–26]. The circumscribed hypersecretion of insulin can be visualized by positron emission tomography/CT with increased [18 F]fluoro-L-3,4-dihydroxyphenylalanine uptake compared to the unaffected pancreas [27]. For these infants, limited pancreatectomy has been shown to be curative [28, 29].

Herein, we report the clinical and mutational spectrum in a large patient cohort from the German CHI registry and analyze the data for genotype-phenotype correlations.

Materials and Methods

Patients

A total of 146 patients with the clinical diagnosis of CHI were referred between January 2005 and November 2012 for further differential diagnosis and included in the registry. Patients belonged to 136 unrelated families originating from Central Europe ($n = 133$), Asia ($n = 1$) and South America ($n = 2$). The cohort included 125 sporadic cases and 21 kin with familial occurrence of CHI. CHI clinical diagnosis was based on recurrent hypoglycemia (<2.5 mmol/l plasma glucose) with low plasma levels of ketone bodies and free fatty acid together with measurable insulin levels at the time of hypoglycemia. DZX treatment was started at 5 mg/kg/day by oral application of 3 separate doses per day. Doses were increased up to 15 mg/kg/day if unresponsiveness was observed, i.e. glucose levels below 3.5 mmol/l. Written informed consent was obtained from the parents of the patients according to the approval by the local ethics committee and in agreement with the German law on genetic testing.

Sequence Analysis

The coding regions of all *ABCC8* exons and adjacent regions and the single exon of *KCNJ11* were analyzed by conventional Sanger sequencing. Oligonucleotide primer sequences and proto-

cols are available on request. Sequence variants were compared with the Single Nucleotide Polymorphism Database and the sequence changes reported in the Human Gene Mutation Database (Cardiff, UK) and by Bellanné-Chantelot et al. [30], Flanagan et al. [31], Kapoor et al. [32] and Snider et al. [33]. If applicable, sequence changes observed in the index patients were further evaluated in parents and further affected family members to confirm segregation with the phenotype. Novel missense mutations were scrutinized with the help of the in silico prediction programs PolyPhen-2 [34] and MutationTaster2 [35], and novel frameshift, nonsense and splice site mutations were analyzed with MutationTaster2.

Multiplex Ligation-Dependent Probe Amplification Analysis

The *ABCC8* gene was further analyzed by multiplex ligation-dependent probe amplification (MLPA) when sequencing revealed a heterozygous or no causative mutation in *ABCC8* or *KCNJ11*. The *ABCC8* MLPA kit (Kit 117, MRC Holland, Amsterdam, Netherlands) was applied as recommended by the manufacturer. MLPA products were separated on a capillary sequencer and analyzed using Sequence Pilot software (JSI medical systems GmbH, Kippenheim, Germany). Deletions detected by MLPA were verified by analyzing the parental DNAs and by exon-spanning PCR and agarose gel electrophoresis to detect a fragment of reduced size.

Results

In this patient cohort primarily composed of CHI patients from Germany and Central Europe, 53 different mutations were detected in the *ABCC8* gene (fig. 1a) and 11 different mutations were identified in the *KCNJ11* gene (fig. 1b). In total, causative mutations in one of the genes encoding components of the K_{ATP} channel were identified in 61 out of 136 unrelated families (45%).

*Spectrum of Mutations Detected in the *ABCC8* and *KCNJ11* Genes*

In the *ABCC8* gene, nonsense, frameshift and splicing mutations (presumable 'null' mutations) accounted for about half of the mutations (24 out of 53), and they were dispersed throughout the gene. A highly recurrent mutation suggesting a founder effect was not observed. In contrast, missense mutations ($n = 24$) were located more frequently within exons 1–10 and 29–38, with half of them occurring in exons 34–38, which encode the second nucleotide-binding domain of the sulfonylurea receptor 1 subunit (fig. 1a). This distribution pattern is quite similar to previously described patient cohorts [30–33, 36, 37]. Three different in-frame deletions and 2 different intragenic deletions encompassing exons 32 and 33 and exons 34–37, respectively, were detected in

unrelated patients. Most mutations were 'private', i.e. they were detected only in a single family. The most recurrent mutation, the previously described missense change p.Q444H, was observed in 5 unrelated families of our cohort. Nine of the 11 mutations detected in *KCNJ11* were missense mutations, including 8 novel changes, 1 mutation was a novel in-frame deletion of a single amino acid and 1 was a novel complex frameshift mutation at the 3' end predicting a Kir6.2 protein with an addition of 104 amino acids at the C-terminal end (fig. 1b; table 1).

The majority of mutations detected in *ABCC8* and *KCNJ11* in this study (38/64) have not previously been described. These novel mutations are summarized in table 1. To validate a pathogenic effect of novel mutations we performed in silico predictions using proven software programs (table 1). As expected, a clearly deleterious effect was predicted for all frameshift, nonsense and splicing mutations. The 'silent' mutation c.1176G>A p.(0=) of *ABCC8* in fact was predicted to affect splicing of exon 7, since c.1176G is the last nucleotide of exon 7 and within the consensus sequence at the splice donor site. Consistent prediction of a high probability of being pathogenic was obtained from PolyPhen-2 and MutationTaster2 for 10 of 14 and 4 of 6 novel missense mutations in *ABCC8* and *KCNJ11*, respectively (table 1). For 4 missense mutations (*ABCC8*: p.I1404M, p.V1430A; *KCNJ11*: p.A96T, p.P340H), the scores calculated by PolyPhen-2 were somewhat weaker than those from MutationTaster2, while discrepant prediction results were only received for 2 novel variants in the *ABCC8* gene, namely p.V17A and p.I148T. The former was identified in a patient with a diffuse CHI type as proved by histology, who carried a splice site mutation on the other *ABCC8* allele, thus supporting the pathogenic relevance of this change. The latter variant (*ABCC8*: p.I148T) was found as a heterozygous change only in an infant who is currently being treated with a somatostatin analogue and who responded well, and further imaging diagnosis was not performed. The variant was transmitted from the healthy father, thus allowing no definite classification. All other novel sequence changes listed in table 1 (including the latter 2 with the ambiguous prediction results) showed a segregation pattern within the respective families that corresponded with the clinically diagnosed CHI type (biparental transmission of mutations in diffuse CHI and recessive inheritance; heterozygous paternally inherited mutation in focal CHI), thus supporting their pathogenic significance (table 2).

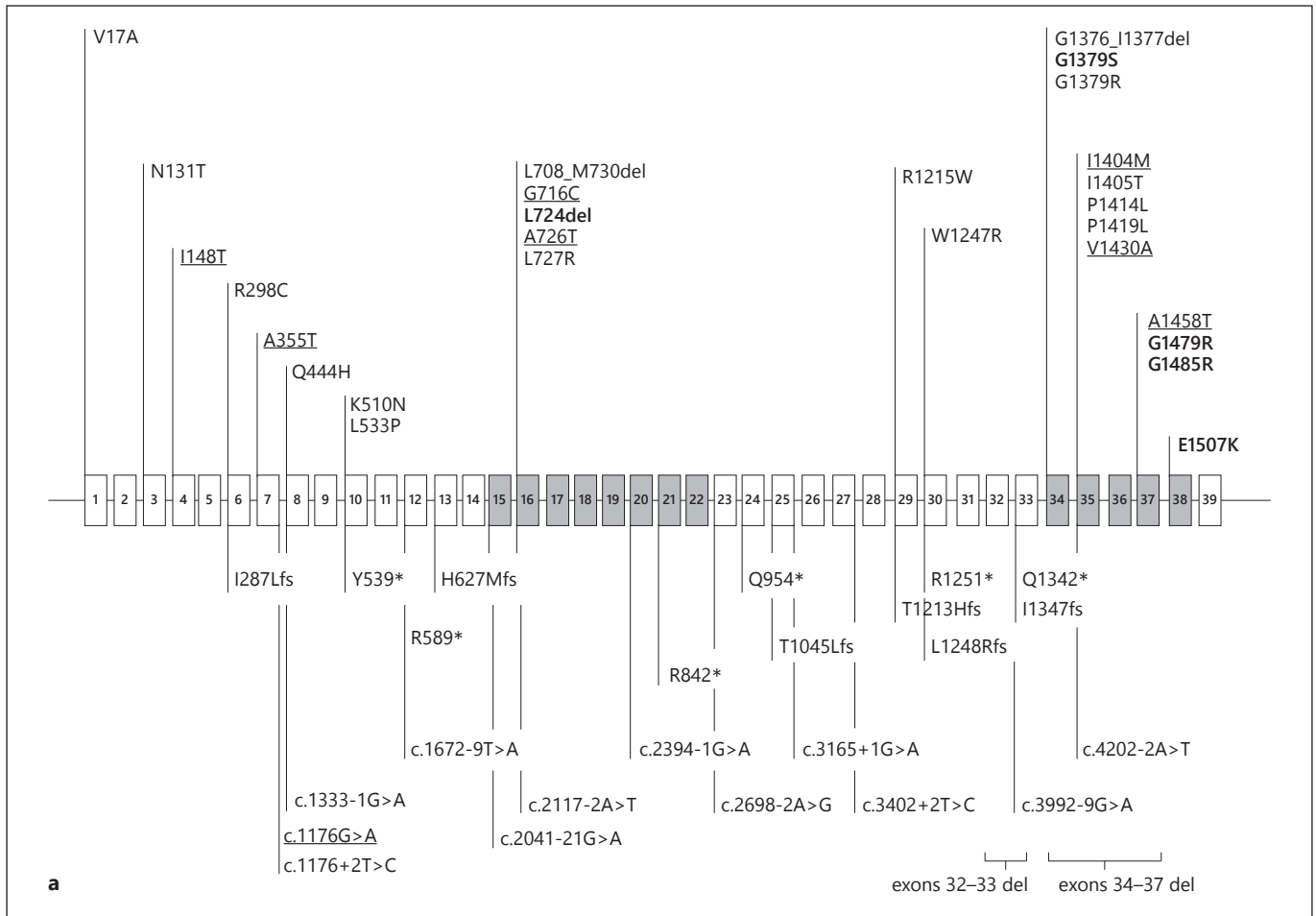
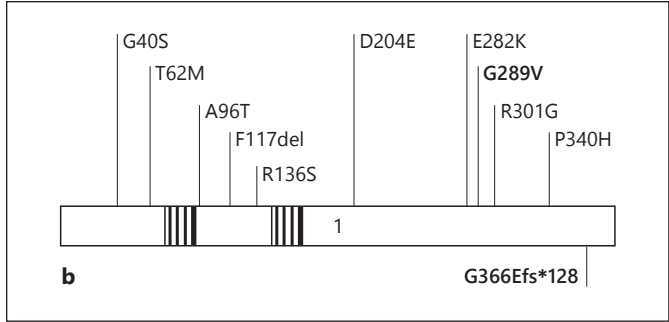


Fig. 1. Mutation spectrum of *ABCC8* and *KCNJ11* in patients from the German CHI registry. Schematic outlines of the *ABCC8* gene (a) and the single exon of *KCNJ11* (b) were adapted from Snider et al. [33]. Missense mutations are shown above the exons, while nonsense and frameshift mutations, splice mutations and intra-genic deletions are shown below the exons. *ABCC8* exons encoding the 2 nucleotide-binding domains are shaded (a), and the transmembrane-spanning domains encoded by the single exon of *KCNJ11* are indicated by black bars (b). Dominant mutations are indicated in bold letters, and unclassified mutations (recessive or dominant) are underlined.



Clinical Data
 Comprehensive clinical data, including birth delivery, birth weight related to gestational age, time of manifestation, DZX responsiveness and type of CHI determined by localization diagnostics and histology, were available from 47 affected individuals from the 61 families in whom K_{ATP} channel mutations could be identified. For the remaining 14 patients, only partial information was available. In 37% of patients (18/49), birth

weight was large for gestational age, indicating fetal onset of CHI. Initial hypoglycemia was detected within the first hours of life in 95% of patients (58/61) and within 10 days to 6 months in 5% (3/61; table 3). A trial with DZX was performed in 53 patients, and only 12 were reported to be (partially) responsive. In 49 patients (80%), conclusive results from imaging and/or histology were available. Twenty-five patients were diagnosed as having a diffuse form of CHI, while 21 exhibited a

Table 1. Novel *ABCC8* and *KCNJ11* mutations detected in the cohort

Gene	cDNA	Predicted protein	Mutation type	In silico prediction ²		Genetic status	Frequency ³
				Poly Phen-2	Mutation Taster		
<i>ABCC8</i> ¹							
Exon 1	c.50T>C	p.(V17A)	missense	0.194	0.9957	c-htz	1
Exon 3	c.392A>C	p.(N131T)	missense	0.990	0.9999	c-htz	1
Exon 4	c.443T>C	p.(I148T)	missense	0.211	0.9998	htz pat	1
Exon 6	c.835_851dup	p.(I287fs*78)	frameshift		1	htz pat	1
Exon 7	c.1176G>A	p.(0=) ⁴	splice site		1	htz pat	1
Intron 8	c.1333-1G>A	p.0? ⁴	splice site		1	htz pat	1
Exon 10	c.1530G>T	p.(K510N)	missense	1	0.9999	htz pat	1
Exon 10	c.1598T>C	p.(L533P)	missense	0.997	0.9999	hmz	1
Intron 11	c.1672-9T>A	p.0?	splicing		0.9999	c-htz	1
Intron 15	c.2117-2A>T	p.0?	splice site		1	c-htz	2
Exon 16	c.2123_2191del	p.(L708_M730del)	in-frame deletion		n.a.	hmz	1
Exon 16	c.2169_2171del	p.(L724del)	in-frame deletion		0.9999	htz mat; dom	1
Exon 16	c.2180T>G	p.(L727R)	missense	1	0.9999	c-htz	1
Intron 19	c.2394-1G>A	p.0?	splice site		1	c-htz; htz pat	3
Intron 22	c.2698-2A>G	p.0?	splice site		1	c-htz	1
Intron 27	c.3402+2T>C	p.0?	splice site		1	htz pat	1
Exon 29	c.3636_3637dup	p.(T1213Hfs*8)	frameshift		1	hmz	1
Exon 30	c.3739T>A	p.(W1247R)	missense	1	0.9999	c-htz	1
Exon 30	c.3743del	p.(L1248Rfs*18)	frameshift		1	c-htz	1
Exon 32	c.3871-31_3994del	p.0?	exon deletion		n.a.	c-htz	1
Exon 33	c.4038del	p.(I1347Sfs*4)	frameshift		1	htz pat	1
Exon 34	c.4123-?_4548+?del	p.0?	exon deletion		n.a.	c-htz	1
Exon 34	c.4126_4131del	p.(G1376_I1377del)	in-frame deletion		n.a.	htz pat	1
Exon 34	c.4135G>A	p.(G1379S)	missense	1	0.9999	htz mat	1
Intron 34	c.4202-2A>T	p.0?	splice site		1	c-htz	1
Exon 35	c.4212C>G	p.(I1404M)	missense	0.436	0.9994	htz pat; dom	1
Exon 35	c.4214T>C	p.(I1405T)	missense	0.999	0.9999	c-htz	1
Exon 35	c.4256G>T	p.(R1419L)	missense	1	0.9999	c-htz	1
Exon 35	c.4289T>C	p.(V1430A)	missense	0.610	0.9942	htz pat; dom (?)	1
Exon 37	c.4453G>A	p.(G1485R)	missense	1	0.9999	htz	1
<i>KCNJ11</i> ¹							
Exon 1	c.118G>A	p.(G40S)	missense	1	0.9999	htz pat	1
Exon 1	c.286G>A	p.(A96T)	missense	0.687	0.9999	htz pat	1
Exon 1	c.350_352del	p.(F117del)	in-frame deletion		0.9999	c-htz	1
Exon 1	c.406C>A	p.(R136S)	missense	1	0.9999	c-htz	1
Exon 1	c.612C>A	p.(D204E)	missense	0.999	0.9999	htz pat	1
Exon 1	c.866G>T	p.(G289V)	missense	1	0.9999	de novo	1
Exon 1	c.1019C>A	p.(P340H)	missense	0.795	0.9879	c-htz	1
Exon 1	c.1097_1109delinsAG	p.(G366Efs*128)	frameshift		n.a.	htz mat; dom	1

n.a. = Not applicable; c-htz = compound heterozygous; htz = heterozygous; hmz = homozygous; dom = dominant inheritance; de novo = de novo occurrence (not detected in parents); pat = paternal inheritance; mat = maternal inheritance.

¹ *ABCC8* reference transcript ENST00000302539 including the alternative codon in exon 17; *KCNJ11* reference transcript ENST00000339994.

² Scores refer to the probability of a damaging effect using prediction programmes. For PolyPhen-2, HumanVar scores are

shown; scores are evaluated as 0.000 (most probably benign) to 0.999 (most probably damaging). For MutationTaster, scores describe the probability of the prediction, i.e. a value close to 1 indicates a high 'security' of the prediction.

³ Frequency of occurrence in unrelated patients.

⁴ According to Human Genome Variation Society nomenclature: p.(0=) = probably no protein is produced or synonymous substitution; p.0? = effect on protein unknown.

Table 2. Summary of clinical and genetic results of *ABCC8* and *KCNJ11* mutation-positive patients

Patient No.	Gene	cDNA	Predicted protein	Genetic status	Parental origin	Birth delivery	Birth weight	Manifestation ¹	Maximum glucose uptake, mg/kg/min	DZX ²	Sur-gery	Histology
33	<i>ABCC8</i> exon 3 exon 21	c.392A>C c.2524C>T	p.(N131T) p.(R842*)	c-htz c-htz	pat mat	preterm	LGA	neonatal	20	++	no	n.d.
46	exon 7 exon 36	c.1063G>A c.4372G>A	p.(A355T) p.(A1458T)	c-htz c-htz	n.d. n.d.	preterm	LGA	neonatal	n.a.	++	no	n.d.
41	exon 35 intron 32	c.4214T>C c.3992-9G>A	p.(I1405T) p.0? ³	c-htz c-htz	pat mat	preterm	LGA	neonatal	n.a.	++	no	diffuse ⁴
37	exon 16 exon 32	c.2180T>G c.3871-31_3994del	p.(L727R) p.0?	c-htz c-htz	pat mat	term	LGA	infantile	>10	+	yes	diffuse
34	exon 30 intron 32	c.3743del c.3992-9G>A	p.(L1248Rfs*18) p.(?)	c-htz c-htz	pat mat	preterm	AGA	neonatal	>10	+	no	diffuse ⁴
7	exon 1 intron 19	c.50T>C c.2394-1G>A	p.(V17A) p.0?	c-htz c-htz	pat mat	term	LGA	neonatal	>20	-	yes	diffuse
40	exon 6 exon 37	c.892C>T c.4435G>A	p.(R298C) p.(G1479R)	c-htz c-htz	pat mat	preterm	LGA	neonatal	n.a.	-	yes	segmental mosaic
11	exon 8 intron 34	c.1332G>T c.4202-2A>T	p.(Q444H) p.0?	c-htz c-htz	n.d. n.d.	term	LGA	neonatal	10	-	yes	diffuse
3	exon 8 exon 13	c.1332G>T c.1879del	p.(Q444H) p.(H627Mfs*20)	c-htz c-htz	n.d. n.d.	term	LGA	neonatal	<10	-	yes	n.d.
26	exon 12 intron 15	c.1792C>T c.2117-2A>T	p.(R598*) p.0?	c-htz c-htz	pat mat	preterm	LGA	neonatal	n.a.	-	no	diffuse ⁴
24	intron 19 exon 30	c.2394-1G>A c.3751C>T	p.0? p.(R1251*)	c-htz c-htz	pat mat	preterm	LGA	neonatal	>10	-	no	diffuse ⁴
19	intron 22 intron 11	c.2698-2A>G c.1672-9T>A	p.0? p.0?	c-htz c-htz	pat mat	term	AGA	neonatal	>10	-	yes	diffuse
42	exon 35 exon 34	c.4256G>T c.4123-?_4548+?	p.(R1419L) p.0?	c-htz c-htz	pat mat	term	n.a.	neonatal	n.a.	-	yes	diffuse
2	exon 10 exon 30	c.1617T>A c.3739T>A	p.(Y539*) p.(W1247R)	c-htz c-htz	n.d. n.d.	n.a.	n.a.	neonatal	n.a.	n.a.	yes	diffuse
5	exon 10	c.1598T>C	p.(L533P)	hmz	bipar	term	LGA	neonatal	n.a.	n.a.	no	diffuse ⁴
13	exon 16	c.2123_2191del	p.(L708_M730del<9)	hmz	bipar	term	n.a.	neonatal	n.a.	n.a.	no	n.d.
22	exon 29	c.3636_3637dup	p.(T1213Hfs*8)	hmz	bipar	preterm	AGA	neonatal	>10	-	no	diffuse ⁴
50	exon 29	c.3643C>T	p.(R1215W)	hmz	n.d.	term	AGA	neonatal	>10	+	no	diffuse ⁴
21	exon 6	c.835_851dup	p.(I287Lfs*78)	htz	pat	preterm	LGA	neonatal	20	-	yes	focal
31	intron 7	c.1176+2T>C	p.0?	htz	pat	term	n.a.	neonatal	n.a.	n.a.	yes	focal
10	exon 8	c.1332G>T	p.(Q444H)	htz	pat	term	AGA	neonatal	>20	-	yes	focal
18	exon 8	c.1332G>T	p.(Q444H)	htz	pat	term	LGA	neonatal	>10	-	yes	focal
29	intron 8	c.1333-1G>A	p.0?	htz	pat	term	AGA	neonatal	n.a.	-	yes	focal
38	exon 10	c.1530G>T	p.(K510N)	htz	pat	term	AGA	infantile	n.a.	-	yes	focal
39	exon 12	c.1792C>T	p.(R598*)	htz	pat	term	AGA	neonatal	n.a.	-	yes	focal
43	intron 14	c.2041-21G>A	p.0?	htz	pat	term	n.a.	neonatal	n.a.	-	yes	focal
45	intron 15	c.2117-2A>T	p.0?	htz	pat	term	AGA	neonatal	n.a.	-	yes	focal
25	intron 19	c.2394-1G>A	p.0?	htz	pat	term	LGA	neonatal	n.a.	n.a.	yes	focal
32	exon 24	c.2860C>T	p.(Q954*)	htz	pat	term	AGA	neonatal	n.a.	++	yes	focal

Table 2. (continued)

Patient No.	Gene	cDNA	Predicted protein	Genetic status	Parental origin	Birth delivery	Birth weight	Manifestation ¹	Maximum glucose uptake, mg/kg/min	DZX ²	Surgery	Histology
16	exon 33	c.4024C>T	p.(Q1342*)	htz	pat	term	AGA	neonatal	>10	-	yes	focal
27	exon 33	c.4038del	p.(I1347Sfs*4)	htz	pat	term	AGA	neonatal	n.a.	-	yes	focal
20	exon 34	c.4126_4131del	p.(G1376_I1377del)	htz	pat	term	AGA	neonatal	>10	-	yes	focal
47	exon 34	c.4135G>C	p.(G1379R)	htz	pat	term	AGA	neonatal	n.a.	-	yes	focal
49	exon 35	c.4241C>T	p.(P1414L)	htz	pat	term	AGA	neonatal	>10	-	yes	focal
15	exon 7	c.1176G>A	p.(0=) ³	htz	pat	preterm	AGA	neonatal	>10	-	no	diffuse ⁴
30	exon 25	c.(3133_3152del)	p.(T1045Lfs*63)	htz	pat	term	AGA	neonatal	>10	-	no	diffuse ⁴
23	exon 35	c.4289T>C	p.(V1430A)	htz	pat	preterm	AGA	neonatal	n.a.	-	no	nonfocal ⁵
35	exon 4	c.443T>C	p.(I148T)	htz	pat	term	AGA	infantile	<10	-	no	n.d.
9	intron 25	c.3165+1G>A	p.0?	htz	pat	term	AGA	neonatal	n.a.	n.a.	no	n.d.
4	intron 27	c.3402+2T>C	p.0?	htz	pat	term	AGA	neonatal	>10	-	no	n.d.
1	exon 35	c.4212C>G	p.(I1404M)	htz	pat	term	AGA	neonatal	n.a.	n.d.	no	n.d.
17	exon 37	c.4519G>A	p.(E1507K)	htz dom	pat	term	AGA	neonatal	n.a.	++	no	diffuse ⁴
14	exon 34	c.4135G>A	p.(G1379S)	htz dom	mat	preterm	LGA	neonatal	20	++	no	diffuse ⁴
6	exon 16	c.2169_2171del	p.(L724del)	htz dom	mat	preterm	n.a.	neonatal	n.a.	-	yes	diffuse
36	exon 37	c.4519G>A	p.(E1507K)	htz	de novo	term	AGA	neonatal	n.a.	++	yes	diffuse
48	exon 37	c.4519G>A	p.(E1507K)	htz	de novo	term	AGA	neonatal	n.a.	-	yes	diffuse
8	exon 8	c.1332G>T	p.(Q444H)	htz	n.d.	term	AGA	neonatal	n.a.	-	yes	focal
44	exon 16	c.2146G>T	p.(G716C)	htz	n.d.	term	n.a.	neonatal	n.a.	-	yes	diffuse
28	exon 16	c.2176G>A	p.(A726T)	htz	n.d.	preterm	AGA	neonatal	n.a.	-	no	diffuse ⁴
12	exon 37	c.4453G>A	p.(G1485R)	htz	n.d.	term	AGA	neonatal	>10	-	no	diffuse ⁴
<i>KCNJ11</i>												
57	exon 1 exon 1	c.350_352del c.1019C>A	p.(F117del) p.(P340H)	c-htz c-htz	bipar bipar	term	n.a.	neonatal	n.a.	-	yes	n.a.
52	exon 1	c.185C>T	p.(T62M)	hmz	bipar	term	LGA	neonatal	n.a.	-	yes	diffuse
54	exon 1	c.185C>T	p.(T62M)	hmz	bipar	term	LGA	neonatal	n.a.	-	no	diffuse ⁴
51	exon 1	c.118G>A	p.(G40S)	htz	pat	term	LGA	neonatal	n.a.	-	yes	focal
55	exon 1	c.286G>A	p.(A96T)	htz	pat	term	AGA	neonatal	n.a.	-	yes	focal
56	exon 1	c.612C>A	p.(D204E)	htz	pat	preterm	AGA	neonatal	n.a.	-	yes	focal
59	exon 1	c.844G>A	p.(E282K)	htz	pat	term	n.a.	neonatal	n.a.	-	yes	focal
53	exon 1	c.901C>G	p.(R301G)	htz	pat	preterm	AGA	neonatal	n.a.	-	yes	focal
60	exon 1	c.1097_1109delinsAG	p.(G366Efs*128)	htz dom	mat	n.a.	n.a.	neonatal	n.a.	++	no	n.d.
61	exon 1	c.866G>T	p.(G289V)	de novo	de novo	n.a.	n.a.	neonatal	n.a.	++	no	n.d.
58	exon 1	c.406C>A	p.(R136S)	htz	n.d.	n.a.	n.a.	neonatal	n.a.	n.a.	no	n.d.

c-htz = Compound heterozygous; htz = heterozygous; hmz = homozygous; dom = dominant inheritance; de novo = de novo occurrence (not detected in parents); mat = maternal inheritance; pat = paternal inheritance; bipar = biparental; AGA = appropriate for gestational age; LGA = large for gestational age; n.a. = not available; n.d. = not done.

¹ Neonatal: 0–30 days of life; infantile: 1–12 months.

² + = Partially responsive to DZX; ++ = responsive to DZX; - = resistant to DZX.

³ According to Human Genome Variation Society nomenclature: p.(0=) = probably no protein is produced or synonymous substitution; p.0? = effect on protein unknown.

⁴ Only imaging was performed.

⁵ Positron emission tomography/CT showed an irregular pattern without further histology.

Table 3. Clinical presentation of patients with mutations in *ABCC8* or *KCNJ11*

Genotype	Number	Birth weight	First onset of hypoglycemia ¹	DZX response	Familial cases	
Heterozygote with paternal inheritance	<i>ABCC8</i> 23	<i>ABCC8</i>	<i>ABCC8</i>	<i>ABCC8</i>	<i>ABCC8</i>	0/23
		LGA	3/23	neonatal	21/23	
		AGA	18/23	infantile	2/23	
	<i>KCNJ11</i> 5	n.a.	2/23			
		<i>KCNJ11</i>	<i>KCNJ11</i>	<i>KCNJ11</i>	<i>KCNJ11</i>	0/5
		LGA	1/5	neonatal	5/5	
AGA	3/5					
n.a.	1/5					
Recessive/biparental inheritance	<i>ABCC8</i> 24	<i>ABCC8</i>	<i>ABCC8</i>	<i>ABCC8</i>	<i>ABCC8</i>	0/24
		LGA	11/24	neonatal	23/24	
		AGA	9/24	infantile	1/24	
	<i>KCNJ11</i> 5	n.a.	4/24			
		<i>KCNJ11</i>	<i>KCNJ11</i>	<i>KCNJ11</i>	<i>KCNJ11</i>	0/5
		LGA	2/5	neonatal	5/5	
AGA	0/5					
n.a.	3/5					
Dominant inheritance	<i>ABCC8</i> 3	<i>ABCC8</i>	<i>ABCC8</i>	<i>ABCC8</i>	<i>ABCC8</i>	3/3
		LGA	1/3	neonatal	3/3	
		AGA	1/3			
	<i>KCNJ11</i> 1	n.a.	1/3			
		<i>KCNJ11</i>	<i>KCNJ11</i>	<i>KCNJ11</i>	<i>KCNJ11</i>	1/1
		n.a.	1/1	neonatal	1/1	

AGA = Appropriate for gestational age; LGA = large for gestational age; n.a. = not available.

¹ Neonatal: 0–30 days of life; infantile: 1–12 months.

focal type. In 1 patient with compound heterozygosity of missense mutations in *ABCC8*, an unusual histology distributed over the pancreas was found. Thirty-five affected individuals underwent surgery, which was subtotal pancreatectomy in 7 and partial pancreatectomy or excision of a focus in 28 patients (table 2).

Response to Medical Treatment

DZX sensitivity was reported in 12 infants. In 5 infants, a known or presumed dominant mutation within *ABCC8* or *KCNJ11* was detected (*ABCC8*: 2× p.E1507K, p.G1379S; *KCNJ11*: p.G289V, p.G366Efs*128). Four patients had compound heterozygous mutations of *ABCC8*. In 3 of them, one allele carried a missense mutation, and in 2 of them, one allele was the Ashkenazi founder mutation c.3992-9G>A, which is known to be associated with a more variable phenotype [38]. Two of these individuals received octreotide in addition to DZX to achieve sufficient metabolic control, thus indicating that there was only partial DZX sensitivity. One patient who was reported as DZX responsive before surgery had a pater-

nally inherited *ABCC8* nonsense mutation (c.2860C>T, p.Q954*) with a proven focal type and relatively mild manifestation.

Genotype-Phenotype Correlations

Fifteen patients from unrelated families in this cohort harbored compound heterozygous mutations, including 8 known and 17 novel mutations, and 6 patients were homozygous with clinically unaffected carrier parents with known or suspected consanguinity in all of them. This genetic constellation leads to a clear diagnosis of autosomal recessive inheritance of the disease in 34% of the families. Such a genotype is suggestive of a diffuse form of CHI, which was further supported in all of the patients who underwent imaging or surgery (n = 16).

A single heterozygous mutation was recognized in 40 unrelated patients (66%), including 11 known and 25 novel mutations. In 23 cases, the mutation was inherited from a clinically unaffected father, a constellation that is generally suggestive of a focal form of CHI. Concomitantly, the observed genetic changes included 13 putative

'null' mutations, 9 missense mutations and 1 in-frame deletion; of the latter two groups, 6 are known as recessive mutations while 4 were novel ones. All of these patients who had a focal type of CHI inherited a monoallelic mutation from their healthy fathers, and the diagnosis was confirmed by imaging and/or pancreatic histology highly suggestive of recessive mutation types that are associated with a focal form of CHI.

The *ABCC8* missense mutations p.I1404M and p.V1430A, which were observed as paternally inherited heterozygous changes in 2 unrelated patients, were called unclassified (recessive or dominant) because of insufficient clinical data from the patients and their fathers. The individual who carried the paternally inherited novel *ABCC8* change p.I1404M had severe hypoglycemic episodes during infancy and childhood that resolved spontaneously during adolescence. The other novel *ABCC8* mutation, c.1176G>A (with probable splicing effect) was observed as a paternally inherited heterozygous change in a patient with a diffuse CHI type by imaging diagnostics. Inheritance of the *ABCC8* mutation p.G716C in 2 further siblings was shown to be nonmaternal, suggesting paternal transmission. However, clinical data and DNA from the father were not available; therefore, this mutation was also called unclassified (recessive or dominant). In these cases, we cannot exclude the possibility that the mutation acts in a dominant fashion with incomplete penetrance or that a second maternal mutation was missed.

Maternal inheritance was shown for the novel *ABCC8* mutation p.G1379S, which was classified as dominant because the mother was pancreatectomized during infancy and a diffuse form of CHI was confirmed by imaging in the patient. Two novel heterozygous mutations (*ABCC8*: p.L724del; *KCNJ11*: p.G366Efs*128) showed dominant maternal inheritance, because the mothers and/or their progeny were affected, and the missense mutation p.G289V in *KCNJ11* was observed as a heterozygous de novo alteration, which is rather indicative of a dominant mutation type. CHI was diagnosed in the patient at the age of 6 months, when the patient presented with hypoglycemia and seizures. The patient responded to medical treatment with DZX. Finally, the known dominant mutation p.E1507K was detected 3 times in this cohort including 2 de novo genetic events. Diffuse type was confirmed by imaging and histology.

In 3 cases, the parent of origin of a heterozygous mutation could not be determined. These mutations included 3 missense changes in the *ABCC8* gene. The first was p.Q444H, a known and recurrent mutation considered to act as a recessive allele. In the patient, a focal lesion was detected by imag-

ing and confirmed by histology, suggesting paternal transmission of the mutation. The following 2 missense mutations were classified tentatively as dominant mutations: p.A726T, which was previously described as a variant of uncertain clinical significance [8], and the novel change p.G1485R, which is suggested to be a dominant mutation because of its localization within a cluster of dominant mutations in conjunction with a diffuse type of CHI in the patient.

Discussion

CHI represents a group of clinically, genetically and morphologically heterogeneous entities [39]. To date, defects in K_{ATP} channel function have accounted for the majority of cases in which a genetic diagnosis can be established, and they are often associated with a severe expression of the disease. Since these patients are at risk of hypoglycemic brain damage and subsequent significant neurological sequelae, rapid diagnosis and disease management are mandatory [4, 40–42]. In our cohort, 61 out of 136 investigated unrelated patients (45%) carried mutations in either *ABCC8* or *KCNJ11*. This proportion corresponds quite well with previously published cohorts [2, 32, 36]. It has been shown that among patients selected for early onset of the disease and DZX resistance, the proportion of cases related to K_{ATP} channel mutations may be up to 91% [30, 33].

Genetic testing may be a useful tool not only for confirming the diagnosis of CHI but also for distinguishing between the different genetic and biological mechanisms underlying CHI, thus having an impact on clinical management and genetic counseling of affected families [9, 33, 37, 43, 44]. In the cohort presented here, biparental inheritance of *ABCC8/KCNJ11* gene mutations was found in 21 of 61 K_{ATP} CHI patients (34%), with two thirds of them carrying a compound heterozygous genotype. The majority of patients with proven recessive *ABCC8/KCNJ11*-related disease showed severe phenotypes, including unresponsiveness to medical treatment and diffuse pancreatic involvement. A paternally inherited heterozygous recessive mutation suggestive of a focal form of CHI was detected in 23 of 61 patients (38%). Currently, these patients benefit most from genetic testing because after visualization of the focus by modern imaging methods, localized pancreatic surgery is able to cure them [28, 45]. In this cohort, 21 out of 23 patients with a heterozygous mutation inherited from the unaffected father were confirmed to have focal CHI, and all proven focal cases had the typical genetic constellation,

thus confirming a high specificity and sensitivity, respectively, in terms of the correct classification of the CHI type on the basis of genetic test results. In 5 of 61 cases a dominant mutational effect was evident, either because of a known dominant mutation or dominant segregation of the phenotype with the mutation within a family. Two additional mutations can be tentatively assigned as dominant ones, i.e. the *ABCC8* mutation p.G1485R, which was observed in a sporadic case with insufficient clinical data about the parents, and the *KCNJ11* mutation p.G289V, which was observed as a de novo event in a sporadic case. In 7 patients, the classification of the identified mutations was less obvious because of conflicting results from the prediction programs, a phenotype that was inconsistent with the genetic results or unavailability of parental genotypes or phenotypes. Particularly for the novel variant p.I148T and the previously described p.A726T, it also has to be considered that the identified variants might be nonpathogenic. This emphasizes the need to integrate results from the in silico prediction with segregation analysis and clinical phenotype in order to obtain a reliable classification of mutations that may be used in future for genetically based prognosis and decision-making. Currently, it is not possible to unequivocally predict the functional impact of the large number of private missense mutations. Whereas the majority of nonsense, frameshift and splice site mutations, as well as exon deletions, can be presumed to represent functional null alleles, suggesting that they act in a recessive fashion, the interpretation of novel missense mutations or small in-frame deletions/insertions may be challenging. This illustrates the need for large patient registries that collect and integrate clinical and genetic data of many CHI patients in order to establish genotype-phenotype correlations that can be incorporated in future individualized treatment decisions. There is also a possibility that mutations in the investigated genes (e.g. intronic or promoter changes) can be missed by the employed mutation screening methods, thus explaining apparent inconsistencies between genotype and phenotype. Indeed, it has recently been reported, for example, that a deep intronic mutation detected by next-generation sequencing is able to create a cryptic splice site and an out-of-frame pseudoexon in the *ABCC8* transcript [46].

In the course of this study, we observed inheritance patterns that do not exactly follow the classical recessive or dominant mode, thus further expanding the genetic complexity of CHI. In 1 patient, compound heterozygosity was detected for the *ABCC8* missense mutations p.R298C and p.G1479R. The former was inherited from

an unaffected father and has been recently classified as a recessive mutation [33]. The latter mutation, p.G1479R, inherited from the mother with lack of clinical data, has been previously identified in independent families with dominant inheritance, albeit with incomplete penetrance, and it was reported to be associated with a milder, DZX-responsive CHI phenotype [19, 36, 47]. Compound heterozygosity for a recessive and a mild dominant *ABCC8* missense mutation was associated with a relatively severe disease manifestation in our patient. A similar genetic situation, e.g. coinheritance of a dominant and a predicted recessive missense mutation in *ABCC8*, has previously been reported in another patient with a much more severe phenotype compared to individuals with the sole presence of the dominant mutation [48]. In addition, we detected homozygosity for the *KCNJ11* missense mutation p.T62M in 2 patients from independent consanguineous families of Turkish descent. This mutation has been recently proposed as a dominant one associated with a DZX-responsive phenotype [33]. The parents of our patients had no obvious phenotype, but unfortunately, no detailed clinical data were available from them to exclude a minor phenotypic effect of the mutation in the heterozygous state. In the homozygous state, as observed in our patients, the phenotype was severe and indistinguishable from recessive cases with other genotypes. This suggests that the *KCNJ11* mutation p.T62M represents an intermediate mutation type that may occur as a recessive allele and as reflected by semi-dominant inheritance as a dominant incompletely penetrant mutation, depending on the genetic context [49]. Finally, the novel heterozygous missense mutation p.G716C in *ABCC8* was detected in 2 siblings affected with a diffuse form of CHI. This mutation was not transmitted from the mother, as determined by routine genetic testing. Although the father was not available for testing, paternal inheritance has been presumed because both children are affected. Different missense mutations at codon position 716 in *ABCC8* have been classified as recessive or dominant [17, 30], which makes prediction of the mutation type for p.G716C difficult, in particular, because we cannot exclude an as yet undetected maternally derived second mutation.

In contrast to small sequence changes, intragenic single or multiple exon deletions in the *ABCC8* gene are not very common. They have been previously observed in independent cohorts at low frequencies in 2 of 89 patients (2.2%) with *ABCC8/KCNJ11* defects [30] and in 4 of 125 DZX-unresponsive patients (3.2%) [50]. We observed a similar frequency of 2 of 61 patients (3.3%) with *ABCC8*/

KCNJ11 mutations in our cohort. Both patients were compound heterozygous for the deletion and a missense mutation in the *ABCC8* gene. In 2 other large cohorts, one multiple exon deletion was identified in 109 patients (0.9%) [32] and a single duplication of 4 exons of the *ABCC8* gene was identified in 1 of 265 patients (0.4%) with *ABCC8/KCNJ11* mutations [33].

DZX is regarded as first-line medication in CHI. Most cases with this diagnosis are therefore at least trialled on this drug to determine DZX responsiveness. Since the pharmacological effect of DZX is based upon binding of the compound to the extracellular domain of the K_{ATP} channel and modification of its conductivity, it is obvious that the complete or near-complete lack of one of the channel's components is generally associated with resistance to this drug. In this cohort, DZX sensitivity was reported in only 12 patients, 5 of whom had a known or presumed dominant mutation. This is consistent with previous experience and with the proposed biological mechanism of dominant mutations which are assumed to lead to functional impairment but not complete absence of K_{ATP} channels. Four patients reported to be (partially) DZX responsive instead were recessive cases with compound heterozygosity for mutations in *ABCC8*. Notably, in all of them there was at least one allele that might be compatible with the expression of a protein that exhibits residual function, namely different missense mutations and in 2 cases the known Ashkenazi founder mutation

c.3992-9G>A, which is known to be associated with a variable phenotype. In the case of a patient with focal CHI and a paternally inherited *ABCC8* nonsense mutation, we have no definite explanation for the observed DZX response.

In conclusion, genetic analysis of the K_{ATP} channel genes *ABCC8* and *KCNJ11* revealed mutations in 45% of patients from an unselected CHI patient cohort. No major recurrent mutation indicative of a founder effect was detected in this cohort, which was mainly composed of patients from Central Europe. Rapid genetic testing in infants with DZX-resistant CHI appears to be a useful tool for determining the best individual clinical management. In particular, the presence of a heterozygous paternally transmitted recessive mutation has a very good sensitivity and specificity in predicting the focal form of CHI, which can be cured by lesional resection. Concerted efforts to integrate clinical and genetic results in large registries and databases are necessary to further improve the classification of rare mutations and their impact on individualized treatment and genetic counseling of families.

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