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Monoallelic *ABCC8* mutations are a common cause of diazoxide-unresponsive diffuse form of congenital hyperinsulinism

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Abstract

ABCC8 encodes a subunit of the β-cell potassium channel (K_{ATP}) whose loss of function is responsible for congenital hyperinsulinism (CHI). Patients with two recessive mutations of *ABCC8* typically have severe diffuse forms of CHI unresponsive to diazoxide. Some dominant *ABCC8* mutations are responsible for a subset of diffuse diazoxide-unresponsive forms of CHI. We report the analysis of 21 different *ABCC8* mutations identified in 25 probands with diazoxide-unresponsive diffuse CHI and carrying a single mutation in *ABCC8*. Nine missense *ABCC8* mutations were subjected to *in vitro* expression studies testing traffic efficiency and responses of mutant channels to activation by MgADP and diazoxide. Eight of the 9 missense mutations exhibited normal trafficking. Seven of the 8 mutants reaching the plasma membrane had dramatically reduced response to MgADP or to diazoxide (<10% of wild type response). In our cohort, dominant K_{ATP} mutations account for 22% of the children with diffuse unresponsive-diazoxide CHI. Their clinical phenotype being indistinguishable from that of children with focal CHI and diffuse CHI forms due to two recessive K_{ATP} mutations, we show that functional testing is essential to make the most reliable diagnosis and offer appropriate genetic counseling.

Keywords

ABCC8; congenital hyperinsulinism; In vitro expression studies; molecular genetics; potassium channel; SUR1

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Conflict of interest

The authors declare that they have no conflict of interest.

Introduction

Congenital hyperinsulinism (CHI; MIM #256450) characterized by an inappropriate oversecretion of insulin from pancreatic β-cells is the most frequent cause of persistent hypoglycemia. It is mostly associated with molecular defects of the β-cell ATP-sensitive potassium (K_{ATP}) channel genes, *KCNJ11* and *ABCC8*, respectively encoding the channel subunits Kir6.2 and SUR1 (1). Patients with recessive mutations of these genes typically have complete loss of K_{ATP} channel function resulting in severe forms of diazoxide-unresponsive CHI. They are related to two distinct molecular mechanisms, each one characterized by a histopathological presentation of pancreatic β-cells. The histological "focal" form is characterized by the combination of two events: (i) a paternally inherited K_{ATP} channel gene mutation and (ii) the paternal isodisomy and loss of the corresponding maternal allele in a circumscribed group of pancreatic β-cells. Conversely, when both recessively inherited K_{ATP} channel alleles are invalidated in all β-cells, the patient has a form histologically described as "diffuse". However, we and others found that not all of diazoxide-unresponsive patients with diffuse CHI had two recessive *ABCC8* or *KCNJ11* mutations (1–3). Interestingly, it has been reported that some dominant *ABCC8* mutations, a type of mutation that had primarily been associated with mild CHI responsive to diazoxide (4), were responsible for a subset of diffuse diazoxide-unresponsive forms of CHI (5, 6). The *in vitro* expression studies showed severely impaired responses to diazoxide and MgADP for these *ABCC8* diazoxide-unresponsive dominant mutations while recessive mutations result in channel subunits that do not reach the plasma membrane (5).

We recently reported the molecular spectrum of a series of 64 children with diffuse CHI unresponsive to diazoxide. In one third of patients, only one K_{ATP} channel heterozygous mutation was identified (2). Here, we reviewed the molecular etiology of 25 children with diazoxide-unresponsive CHI and carrying a monoallelic *ABCC8* mutation. It is critical, both in terms of clinical management and genetic counseling, to determine whether these patients indeed represent dominant diffuse cases or misdiagnosed focal cases, associated with recessive mutations. We therefore characterized missense mutations using *in vitro* expression studies to help determine whether they act in a recessive or dominant fashion.

Materials and Methods

Patients

Twenty-five probands with a diagnosis of diffuse CHI were investigated, including 18 patients from a previous report (2) and 7 novel patients (#7–9, 15, 17, 19, 23) carrying monoallelic *ABCC8* mutations. We obtained written informed consent in agreement with the local Ethics Committee. Clinical diagnosis of CHI was made as previously described (2). The diagnosis of diffuse form was based either on the pancreatic histopathological analysis (7) or on positron emission tomography (PET) (8).

Genetic analysis

Sequencing and search for genomic rearrangements of *ABCC8* (NM_000352.3) were performed as previously described (2). *De novo* mutations were assessed by genotyping 10 short tandem repeats (Kit AmpflSTR SGM Plus, Life Technology).

Bioinformatics analyses were performed for missense variants using Polyphen, Sift, Mutation Taster and GVGD.

Functional Analysis of Mutant Channels

Functional analyses of K_{ATP} channels, including $^{86}Rb^{+}$ efflux assays, immunoblotting, immunostaining and inside-out patch-clamp recordings were conducted in COSm6 cells transiently expressing wild-type (WT) or mutant K_{ATP} channels as described previously (5, 9). Detailed protocols are provided in the online supporting information.

Results

Systematic screening of the K_{ATP} channel genes unexpectedly revealed that one third of all patients diagnosed with a diffuse CHI unresponsive to diazoxide only carried a monoallelic *ABCC8* mutation (2). Twenty-five children (11 boys, 14 girls) were gathered and clinical characteristics at diagnosis and after a mean follow-up duration of 6.8 years were analyzed (Table 1). All were severe CHI cases, mainly diagnosed as neonates (n= 22/25, 88%) and managed with octreotide +/− glucose-enriched feedings.

When both parents were available (n=18), the mutation was mostly inherited from the father (n=11) or *de novo* (n=6). Only one child inherited her mutation from her mother. In most transmitting parents, there was no reported history of glycemic disorder, only 2 had history of mild hypoglycemia in infancy (Table 1, #3 and 20).

Twenty-one distinct mutations were identified in these 25 patients: 14 missense mutations (67%), 4 truncating mutations (19%) and 3 splice mutations (14%) (Table 1). Two novel mutations (G1379S and I1512S) were added to the cases reported in (2). Missense mutations were mostly located in the first and second nucleotide binding domains (93%, 13/14, Fig. S1A). They were all considered as “Disease causing” with p>0.97 according to Mutation Taster. At least, one other algorithm (Sift, GVGD and/or PolyPhen2) also predicted a pathogenic effect for all mutations except for I1512S (Table S1). All were absent from public databases with the exceptions of S1387F (dbSNP130) and R1353P (dbSNP133) with no recorded information on allele frequency.

Functional analyses were available in the literature for 5 missense mutations (Table 1) (5, 9, 10). We therefore analyzed the 9 remaining missense mutations.

When co-transfected in COSm6 cells with hKir6.2, all mutated hSUR1 proteins except for G1379S exhibited a similar profile as the WT hSUR1 on Western Blot (Fig. S1B). The two-band profile of mutant proteins indicated they were correctly glycosylated, whereas the single lower band seen in G1379S corresponding to the core glycosylated form indicated misprocessing. Surface immunostaining confirmed that all mutants retained cell membrane

expression except for G1379S (Fig. S1C). Inside-out patch-clamp recordings were consistent with the expression data and showed that all mutants produced a current amplitude not statistically different from WT channels (ranging ~70–150% of WT), with the exception of G1379S (~20%, significantly lower than WT, $p<0.01$, Student's t-test) (Table 2). Taken together, the results indicated only G1379S corresponded to a mistrafficking mutation.

$^{86}Rb^{+}$ efflux was subsequently examined to determine whether the mutant channels at the plasma membrane were functional (Table 2). All mutants except K890T exhibited greatly reduced efflux compared to WT (>85%). K890T showed a 50% decrease of its function compared to the WT.

The different pattern of K890T was confirmed with channel responses to MgADP and diazoxide (Table 2). The K890T channel showed ~50% and ~70% of the WT response to MgADP and diazoxide, respectively, consistent with the reduced but significant activity in Rb efflux assays. All the other mutants had dramatically reduced response to MgADP or to diazoxide (<10% of WT).

Discussion

Even with recent advances in histology and genetic methods, molecular diagnosis of CHI in some patients remains challenging. We and others have reported patients diagnosed with a diffuse form of CHI unresponsive to diazoxide and carrying a monoallelic *ABCC8* mutation (1–3, 5, 6, 11). However, whether all these mutations are indeed dominant mutations causing diffuse CHI is unknown since monoallelic *ABCC8* mutations can also act in a recessive manner in focal CHI which may be missed in the initial imaging diagnosis. MacMullen *et al*. have shown that dominant diffuse CHI mutations are strongly correlated with severe defects in channel response to MgADP and diazoxide without compromising channel trafficking to the cell surface (5). In our series, we analyzed 1/ the expression of the mutated channel at the plasma membrane which determined whether the mutation was dominant or recessive, and 2/ for the channels that reached the membrane, their electrophysiological properties and thereby the extent of their pathogenicity.

We analyzed 21 distinct monoallelic *ABCC8* mutations identified in 25 children with a diagnosis of diffuse diazoxide-unresponsive CHI. Exonic deletions of *ABCC8* as well as mutations in *KCNJ11* and *GCK* were excluded.

Two-thirds of the patients (17/25) in this series had a heterozygous *ABCC8* missense mutation. None were previously reported in diazoxide-responsive forms of CHI associated with dominant *ABCC8* mutations. All missense mutations but one (L511M) were located in one of the two NBDs. Flanagan *et al* noticed that most of the mutations producing channels that got to the membrane but were not functional were located in the NBDs (13). And indeed, out of the 14 missense mutations in our study, all exhibited normal or slightly reduced surface expression with the exceptions of R1353P and G1379S ((5, 9, 10) and Fig. S1C). This suggested that R1353P and G1379S were probably recessive mutations and that patients #7 and #8 who had a paternal inheritance were misdiagnosed focal forms.

Furthermore, R1353P had previously been identified both in a focal form (14) and in a recessive diffuse form (1).

For 11 out of 12 mutated SUR1 channels reaching the plasma membrane, response to MgADP and diazoxide was practically abolished (Table 2, (5, 9)), consistent with the lack of response to metabolic inhibition in Rb efflux assays. The K890T mutation was the sole missense mutation that retained a partial channel function. Further examination of the clinical data of patient #5 showed she had actually displayed partial response to diazoxide between the ages of 2 and 4 months, then had required glucose-enriched feeding and ultimately, a total pancreatectomy. The K890T mutation had also been previously reported twice. One patient had a diffuse form unresponsive to medication and only one heterozygous mutation ((15) and personal communication); the other one was a diffuse case unresponsive to diazoxide with a homozygous K890T (16).

Bioinformatical analyses have predicted K890T to be pathogenic (Table S1). However, functional studies subsequently showed it had limited effect on channel activity. Conversely, *in silico* analysis was not in favor of a pathogenic effect for the I1512S mutation while functional study showed severely impaired responses to MgADP or diazoxide. These findings highlight the limitation of bioinformatics tools and emphasize that functional analyses are helpful to classify novel missense mutations.

One third of the monoallelic *ABCC8* mutations were truncating mutations. When their inheritance could be determined (6/8), it was always paternal. Five of these truncating mutations were previously reported, either in a focal form or in a biallelic diffuse form (1). This strongly suggests that these patients (#18–25) were either focal cases in which the PET-scan analysis failed to detect a focal lesion or biallelic diffuse cases for which a second event was missed, such as a germline deep intronic mutation or a post-zygotic event (12).

Snider *et al* outlined the risk of misdiagnosed focal cases (1). In their large series, 25 diffuse unresponsive-diazoxide cases only had one mutation, 16 were dominant and 9 recessive mutations. Re-examination of these cases had suggested that the latter may be misdiagnosed focal forms. Similarly in the 18 monoallelic diffuse cases from (2), we concluded here that 12 were probably dominant mutations whereas 6 were recessive mutations. Dominant *ABCC8* cases therefore accounted for 22% of the patients with a diffuse form of diazoxide-unresponsive CHI in this series, and 14% in the series reported by Snider *et al* (1).

Clinical discrimination of patients with focal forms *versus* diffuse forms with one or two K_{ATP} recessive mutations is challenging. We confirmed the subtle differences reported between focal and diffuse cases (17, data not shown). Elsewhere, MacMullen *et al* (5) found 76% of neonates in dominant cases *vs.* 100% in our series. In contrast, the proportion of neonates in recessive forms was the same between the two series (100% *vs.* 97%). The LGA frequency in dominant cases was similar in both series (65% (5) and 60%) whereas contradictory results were observed for recessive cases (87% (5) and 55.5% in this series).

Accurate classification of CHI mutations between dominant and recessive is of utmost importance for appropriate clinical management and genetic counseling of the disease. First, the care of these patients is based on a multidisciplinary approach where the genetic

diagnosis is complementary to the PET analysis, particularly for the decisive exclusion of a focal form. Second, the characterization of the mutation is critical to the assessment of the recurrence risk that varies between ½ for dominant forms, ¼ for recessive diffuse forms and 1/1200 for focal forms (18). It is therefore critical to include functional testing to the genetic diagnosis to make the most reliable diagnosis for this condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Clinical characteristics of 25 probands diagnosed with diazoxide-unresponsive diffuse CHI and carrying a monoallelic *ABCC8* mutation. Patients 1 to 17 have missense mutations for which the functional properties of the mutants were investigated. Patients 18 to 25 carry truncating mutations, considered as recessive mutations.

#	Nucleotide sequence change	Protein effect		mutation type	Inheritance	Family history	Sex	Birthweight	Gestation (weeks)	LGA	age at presentation	Histolopathological/radiological diagnosis	Surgery	Treatment	Follow-up duration (years)	Ethnicity	Functional conclusion
1	c.1531C>A	p.Leu511Met	L511M	missense	De novo	none	F	3560	40	No	Neonate	Histology	Yes	Octreotide + glucose-enriched feeding*	3	Caucasian	Dominant
2	c.1531C>A	p.Leu511Met	L511M	missense	Not maternal	none	F	2461	31	Yes	Neonate	Histology	Yes	glucose-enriched feeding*	7,17	Caucasian	Dominant
3	c.2147G>A	p.Gly716Asp	G716D	missense	Maternal	hypoglycemic mother	F	4010	37	Yes	Neonate	PET	No	Octreotide + glucose-enriched feeding* + adalate	0,67	Caucasian	Dominant (5)
4	c.2473G>A	p.Glu825Lys	E825K	missense	Paternal	Twin sister affected. Two brothers and the father are asymptomatic carriers	M	2730	33	Yes	Neonate	PET	No	Octreotide	9	Caucasian	Dominant
5	c.2669A>C	p.Lys890Thr	K890T	missense	Paternal	none	F	3220	40	No	Neonate	Histology	Yes	Diazoxide + glucose-enriched feeding*	5,17	Caucasian	Dominant**
6	c.2672T>C	p.Leu891Pro	L891P	missense	De novo	none	F	3720	37	Yes	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding* + adalate	6	Caucasian	Dominant
7	c.4058G>C	p.Arg1353Pro	R1353P	missense	Paternal	none	F	2970	39	No	Neonate	PET	No	Diazoxide+ octreotide + frequent feeding	0,5	Caucasian	Recessive (10)
8	c.4135G>A	p.Gly1379Ser	G1379S	missense	Paternal	none	M	4040	38	Yes	Neonate	PET	No	Diazoxide + octreotide	4,5	Caucasian	Recessive
9	c.4160C>T	p.Ser1387Phe	S1387F	missense	NA	none	F	3900	35	Yes	Neonate	Histology	Yes	Diazoxide + octreotide + glucose-enriched feeding*	11	Caucasian	Dominant (5,9)
10	c.4160C>T	p.Ser1387Phe	S1387F	missense	NA	none	F	4250	40	Yes	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding* + glucose infusion	0	Sub-Saharan Africa	Dominant (5,9)
11	c.4166C>A	p.Ser1389Tyr	S1389Y	missense	Not maternal	none	M	4100	39	Yes	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	9	Caucasian	Dominant (5)
12	c.4169T>C	p.Leu1390Pro	L1390P	missense	De novo	none	F	3940	39	No	Neonate	Histology	Yes	Diazoxide + glucose-enriched feeding*	12	North Africa	Dominant (9)
13	c.4373C>T	p.Ala1458Val	A1458V	missense	Paternal	none	M	4310	38	Yes	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	7,25	Caucasian	Dominant
14	c.4442A>T	p.Asn1481Ile	N1481I	missense	De novo	none	F	3530	39	No	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	0,25	Caucasian	Dominant
15	c.4518C>G	p.Asp1506Glu	D1506E	missense	De novo	none	M	4050	38	Yes	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	2	Caucasian	Dominant
16	c.4518C>G	p.Asp1506Glu	D1506E	missense	NA	hypoglycemic paternal uncle	M	2960	36	No	Neonate	Histology	Yes	glucose-enriched feeding*	NA	Caucasian	Dominant
17	c.4535T>G	p.Ile1512Ser	I1512S	missense	De novo	none	F	2930	37	No	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	2,5	Sub-Saharan Africa	Dominant

#	Nucleotide sequence change	Protein effect		mutation type	Inheritance	Family history	Sex	Birthweight	Gestation (weeks)	LGA	age at presentation	Histolopathological/radiological diagnosis	Surgery	Treatment	Follow-up duration (years)	Ethnicity	Functional conclusion
18	c.655C>T	p.Gln219*	Q219*	nonsense	Paternal	none	F	3150	39	No	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	10	Caucasian	ND
19	c.655C>T	p.Gln219*	Q219*	nonsense	Not maternal	none	F	3180	41	No	4 months	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	0,5	Caucasian	ND
20	c.1176G>A	p.?		Splice defect	Paternal	Mild hypoglycemia in infancy in father and paternal grandmother	F	3000	36	No	3 months	NA	NA	Octreotide	NA	Caucasian	ND
21	c.1630+1G>T	p.?		Splice defect	Paternal	none	M	5000	NA	Yes	Neonate	PET	No	Diazoxide + octreotide + glucose-enriched feeding*	3,7	Caucasian	ND
22	c.2153delG	p.Gly718fs	G718fs	frameshift	Paternal	none	M	3640	40	No	Neonate	Histology	Yes	Diazoxide + glucose-enriched feeding*	27	Caucasian	ND
23	c.2800C>T	p.Arg934*	R934*	nonsense	NA	NA	M	NA	NA	NA	3 months	Histology	Yes	NA	32	Caucasian	ND
24	c.2924-9G>A	p.?		Splice defect	Paternal	none	M	2650	32	Yes	Neonate	PET	No	Octreotide + glucose-enriched feeding*	2,58	Lebanon	ND
25	c.4390delG	p.Val1464*	V1464*	nonsense	Paternal	none	M	3800	38	No	Neonate	PET	No	Octreotide	0,75	Caucasian	ND

LGA: Large for gestational age. NA: not available. ND: not determined.

*frequent or continuous feeding with a glucose enriched preparation, to maintain glycemia >55mg/dl.

**partial alteration of the channel functions.

Table 2

Functional data of mutant channels expressed in COSm6 cells[1]

	% Rb efflux[2] (n = 2)	% Average patch current amplitude[3] (n = 60 for WT; n = 5–15 for mutants)	ATP sensitivity % current in 0.1 mMATP[4] (n = 24 for WT; n = 3–9 for mutants)	MgADP stimulation % current in 0.1 mMATP/0.5 mM ADP[4] (n = 24 for WT; n = 3–8 for mutants)	diazoxide stimulation % current in 0.1 mM ATP/0.2 mM diazoxide[4] (n = 22 for WT; n = 3–5 for mutants)
WT	**100.0 ± 0.0**	**100.0 ± 12.8**	**7.5 ± 0.8**	**77.2 ± 5.4**	**91.9 ± 7.9**
SUR1 mutation					
L511M	−6.6 ± 3.8	46.2 ± 14.2	1.2 ± 1.1	0.5 ± 0.1*	0.9 ± 0.1*
E825K	14.0 ± 7.5	37.5 ± 5.6	3.0 ± 1.1	3.3 ± 1.2*	3.8 ± 1.3*
K890T	47.3 ± 11.2	48.6 ± 12.2	4.9 ± 1.1	50.4 ± 5.2*	66.4 ± 5.2*
L891P	−4.9 ± 2.9	54.5 ± 17.7	2.3 ± 1.1	1.4 ± 0.2*	0.7 ± 0.4*
G1379S	−4.0 ± 4.5	9.0 ± 2.8*	5.8 ± 2.4	3.6 ± 3.0*	0.9 ± 1.3*
A1458V	4.2 ± 3.5	46.2 ± 10.1	1.8 ± 0.3	1.9 ± 0.4*	1.3 ± 0.3*
N1481I	−1.8 ± 2.9	65.6 ± 17.0	4.2 ± 2.1	3.1 ± 1.7*	0.9 ± 0.2*
D1506E	−1.9 ± 2.7	55.6 ± 22.6	2.7 ± 0.6	1.6 ± 0.7*	1.8 ± 0.2*
I1512S	−4.0 ± 4.2	29.9 ± 5.6	1.1 ± 0.4	1.4 ± 0.7*	0.5 ± 0.3*

[1] WT or mutant human SUR1 and WT human Kir6.2 cDNAs were transfected into COSm6 cells and cells used for functional analysis 48–72 hours post-transfection.

[2] Net efflux during a 40-min period after substracing background efflux in untransfected cells is shown as percentage of efflux observed in WT channels. The value is the mean ± difference of two independent experiments.

[3] Current amplitude measured by inside-out patch-clamp recording and expressed as percent that of WT channels. The value is the mean ± standard error of the mean.

[4] ATP, MgADP or diazoxide response assessed by inside-out patch-clamp recording. Currents observed in test solutions were normalized to those seen in K-INT and expressed as % currents. The value is the mean ± standard error of the mean.

* $p < 0.01$, comparison between mutant and WT by unpaired Student's *t* -test.