

*MUTATION IN BRIEF***Mutation Spectra of *ABCC8* Gene in Spanish Patients with Hyperinsulinism of Infancy (HI)**

Ana Fernández–Marmiesse<sup>1,2\*</sup>, Antonio Salas<sup>2,3</sup>, Ana Vega<sup>1,3</sup>, José Ramón Fernández–Lorenzo<sup>4</sup>, Jesús Barreiro<sup>4</sup>, and Ángel Carracedo<sup>1,2,3</sup>

<sup>1</sup>Unidad de Medicina Molecular, Fundación Pública Galega de Medicina Xenómica, SERGAS, Hospital Clínico Universitario de Santiago, Galicia, Spain; <sup>2</sup>Unidad de Genética, Instituto de Medicina Legal, Facultad de Medicina, Universidad de Santiago de Compostela, Galicia, Spain; <sup>3</sup>Centro Nacional de Genotipado (CeGen), Hospital Clínico Universitario, 15706; Galicia, Spain; <sup>4</sup>Servicio de Pediatría, Hospital Clínico Universitario de Santiago, Galicia, Spain

\*Correspondence to: Ana Fernández-Marmiesse, Unidad de Medicina Molecular, Hospital Clínico Universitario de Santiago, Galicia, Spain; Tel.: +34 981 251490; E-mail: ana.fernandez.marmiesse@sergas.es

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**Hyperinsulinism of Infancy (HI) is a clinical disorder characterized by deregulation of insulin secretion that leads to profound hypoglycemia. Mutations in genes encoding the ATP-regulated potassium channels of the pancreatic  $\beta$ -cell, namely *ABCC8* (*SUR1*) and *KCNJ11* (*Kir6.2*), are the major genetic known cause of the disease. To elucidate the genetic etiology of HI in the uncharacterized Spanish population, we conducted extensive sequencing analysis of the *ABCC8* (83.5Kb) and *KCNJ11* (1.7Kb) genes in 34 Spanish HI patients. Mutations in *ABCC8* were detected for both alleles in 13 patients, while ten patients carried only one mutation in one of the *ABCC8* alleles. We have detected 22 novel and seven previously described mutations in *ABCC8*, ~60% of them lead to a premature termination signal, which would result in truncated *SUR1* proteins. No mutations were found in the *KCNJ11* gene. In addition, we report for the first time a 3914bp macrodeletion associated with the HI disorder. The potential pathogenicity of several additional variants is discussed. The spatial pattern of three pathological mutations suggests possible geographical founder effects. This work reveals for first time the involvement of KATP channels in the pathogenesis of an important proportion (~68%) of Spanish HI patients. The spectrum of mutations in Spanish HI patients provides an important tool for diagnosis and prognosis of HI patients in the Spanish population, as well as for genetic counseling of HI families. © 2006 Wiley-Liss, Inc.**

KEY WORDS: hyperinsulinism; *ABCC8*; *KCNJ11*; mutation spectra; Spanish

**INTRODUCTION**

ATP-sensitive potassium channels (KATP) are major regulators of glucose-induced insulin secretion in pancreatic  $\beta$  cells (Aguilar-Bryan et al. 1996). Mutations in *ABCC8* (MIM# 600509) and *KCNJ11* (MIM# 600937) genes have been shown to result in defects in the KATP channel function leading to Hyperinsulinism of Infancy HI, a disease characterized by deregulated insulin secretion that leads to severe and recurrent hypoglycaemias

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(Dunne et al. 2004; references therein). Estimates for the incidence of this disorder vary from 1/40.000 live births in Northern Europe (Bruining et al. 1990) to 1/2675 live births in Saudi Arabia (Mathew et al. 1988). The severity of the disease varies from a mild form, which responds to medical treatment, to severe drug-resistant form, which may need resection of the pancreas. Early diagnosis is important to avoid irreversible brain damage due to profound hypoglycaemia. Clinical manifestations of HI, which occurs predominantly in neonates and infants, include large birth weight for gestational age, seizures, cyanosis and coma (Hussain et al. 2000).

Although both autosomal dominant and recessive inheritance of HI have been described, this disorder is inherited as an autosomal recessive trait in the majority of familial cases. Most of HI cases (>95%) are sporadic, and two histopathologic lesions can be distinguished, a diffuse and a focal form, each with a prevalence of ~50% (Rahier et al. 2000). It has been demonstrated that focal cases show a specific loss of maternal alleles of the imprinted chromosome region 11p15 in cells from focal lesion but not in the adjacent normal pancreatic  $\beta$  cells. This somatic event appears in association with a germ line ABCC8 mutation on the paternal allele. The reduction to hemizygoty of the ABCC8 mutation is proposed as the cause of focal HI (De Lonlay et al. 1997; Ryan et al. 1998; Vekarre et al. 1998; Glaser et al. 1999; Fournet et al. 2001). The distinction between these two forms should be of paramount importance, because infants suffering from the focal form may be cured by a partial pancreatectomy, nevertheless the reference method used up to now (selective catheterization) is not readily available in Spanish hospitals. A new promising method (PET with 18F-fluoro-L-DOPA) has been proposed by Ribeiro et al. (2005) as an accurate non-invasive technique to distinguish between focal and diffuse forms of HI.

The first mutations associated with HI were identified in the second nucleotide binding fold (NBF2) of SUR1 (Thomas et al. 1995). Subsequently, mutations along the entire gene were described (Thomas et al. 1996; Nestorowicz et al. 1996, 1998). Kane et al. (1996) and Dunne et al. (1997) demonstrated that  $\beta$ -cells from patients with HI and ABCC8 mutations lack functioning KATP channels confirming the importance of these channels in the pathogenesis of the disease. Mutations of KCNJ11 have also been found to cause HI, but to date only five point mutations have been identified (Thomas et al. 1996; Nestorowicz et al. 1997; Tornovsky et al. 2004). Huopio et al. (2000) reported a missense mutation in ABCC8 gene associated with a mild, diazoxide-responsive form of HI and, for the first time, showing a dominant mode of inheritance. Glaser et al. (2003) showed that mutation E1507K in the ABCC8 gene caused loss of insulin secretory capacity in early adulthood, and diabetes in middle-age. Subsequently, other mutations in ABCC8 have been described which show a dominant mode of inheritance (Huopio et al. 2003; Thornton et al. 2003) also associated with a mild, diazoxide-responsive form of HI.

Up to now, HI Spanish patients have been treated generally with the removal of 95% of pancreas when hypoglycaemias cannot be managed with medical treatment, with no distinction between focal and diffuse forms. The genetic causes of HI in Spanish population have not been studied before and the molecular etiology of the condition remained unknown. In this work we have analyzed the entire coding region of the ABCC8 and KCNJ11 genes and exon-intron boundaries in each one of the 34 HI patients collected from different Spanish hospitals. We aim to identify mutations involved in HI disease in Spanish patients, which would lead to improve strategies for genetic screening and prenatal diagnosis. Genetic analysis can also help to distinguish the two histopathological forms of HI, which would lead to a clear improvement in the treatment of HI and in genetic counseling for the condition.

## MATERIALS AND METHODS

### Patients

Thirty four HI probands were collected from different Spanish Hospitals. Only one family possessed two affected siblings. HI was diagnosed using generally accepted criteria (Hussain et al. 2000). Peripheral blood samples were collected from each patient and first-degree relatives. Written informed consent was obtained from each patient.

### Mutational analysis of ABCC8 and KCNJ11 genes

Leukocyte DNA was extracted from peripheral blood samples of the affected individuals using a standard proteinase K-phenol/chloroform procedure. The ABCC8 mRNA (human ABCC8 cDNA sequence accession numbers L78207 and U63421) contains a single open reading frame that encodes for 1581 amino acids. Each of the 39 exons of ABCC8 gene, including flanking genomic splice-sites sequences was amplified by polymerase chain reaction (PCR). Some primers used to amplify ABCC8 fragments were taken from the literature

(Nestorowicz et al. 1996), but some were new designs (including primers designed to delimit a macrodeletion found in patient P19) using the software Primer3 ([http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)). Primer sequences will be provided under request. The entire coding region of the KCNJ11 gene was amplified in three overlapping fragments from genomic DNA following Someya et al. (2000). Each amplicon was checked in a T9/C5 polyacrylamide gel and purified using MICROSPIN HR-S300 columns (Applied Biosystems, Foster City, California) before cycle sequencing. Sequence reactions were carried out using the ABI PRISM® dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (ABI). The sequencing profile for 36 cycles was 96° C for 15 s, 50° C for 10 s and 60° C for 2 min, followed by an extension cycle at 60° C for 10 min. Sequence products were denatured with desionized formamide and run in the ABI Prism® 3100 Genetic Analyser.

### Population studies of putative polymorphisms

To check the incidence in the Spanish population of some polymorphisms found in HI Spanish patients, we interrogated 50 DNA control samples for the presence of such substitutions. Exons of interest were amplified from genomic DNA and screened for the presence of mutations using SSCP analysis with the PhastSystem separation and control unit (Pharmacia Biotech) using Phast Gel™ Homogeneous 20 and Native buffer strips.

### Haplotype analysis

Haploview 3.0 (Barrett et al. 2005) was used to infer the haplotype block structure of ABCC8/KCNJ11 gene region based on a high density set of SNPs genotyped by the HapMap project (thirty U.S. trios from the Centre d'Etude du Polymorphisme Humain (CEPH) panel; The International HapMap Consortium, 2003) that covers the ABCC8 and KCNJ11 region analyzed in the present paper. In order to define blocks, we followed Gabriel et al. (2002); however, consistent results were obtained when using other methods implemented in Haploview. In addition, the information provided by Applied Biosystems SNPbrowser™ Version 1.0.19 (Applied Biosystems, Database Loaded: NCBI 33) was used to obtain an independent view of haplotype blocks in the same genomic region. This resource is based on the genotyping of 180 'Caucasian' unrelated individuals. The information obtained from these resources is summarized in Figure 1c.

### Paternity testing

A paternity test was carried out with Proband P21 following the protocols of the Instituto de Medicina Legal of the Universidad de Santiago de Compostela (Galicia, Spain).

### Evaluation of the functional impact of the novel missense mutations and silent SNPs

PolyPhen (Polymorphism Phenotyping; <http://www.bork.embl-heidelberg.de/PolyPhen/>) is a web tool for the prediction of potential impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations (Sunyaev et al. 2000, 2001; Ramensky et al. 2002). BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) was used to perform comparison of inter and intra-specific amino acidic SUR1 sequences. Changes affecting highly conserved positions are generally interpreted as having damaging effects. The web tool ESEfinder (<http://rulai.cshl.edu/tools/ESE/>) was used to detect alterations in exonic splicing enhancers (ESE) due to nucleotide changes. Only ESE sites with scores over the threshold (as indicated in Cartegni et al. 2002, 2003) are taken into account in the analysis (Table 3).

## RESULTS AND DISCUSSION

### Clinical Findings

Table 1 shows the clinical characteristics of HI Spanish patients carrying at least one mutation. Only 45% of patients were large for gestational age (>p90) and all but one (P17) had neonatal onset (<3 days). Patients P9 and P27 underwent two consecutive surgeries because the first intervention failed to control the hypoglycaemias. Ten patients were successfully treated medically. No significant response to diazoxide was detected in any of the patients studied with the exception of P7 and P16, both without mutations in ABCC8/KCNJ11. Three patients (P12, P14 and P28) had familial histories of neonatal deaths and three patients (P25, P30, P34) died.

### Identification of mutations in the ABCC8 gene

The present study aimed to identify the genetic causes of HI in a sample of Spanish patients. To date, about 100 ABCC8 mutations have been reported in HI patients (most of them reported in HGMD: Gene ID ABCC8; <http://uwcmml1s.uwcm.ac.uk/uwcm/mg/search/>; see references therein); however, it is important to emphasize that genetic screening has failed to define the genetic basis of disease in >50% of diffuse HI and ~30% of focal HI patients (Dunne et al. 2004).

In the present work, a total of 48 nucleotide sequence variants within the ABCC8 gene were identified and are summarized in Figure 1a and Table 2. Twenty eight of these sequence changes were classified as potential disease-causing mutations and involve defective KATP channels in ~68% of HI Spanish cases. An important proportion (60%) of the mutations found in Spanish probands is predicted to introduce premature termination signals for translation of SUR1 mRNA and this predicts the synthesis of a truncated SUR1 molecule. Since the C-terminus of SUR1 has an anterograde signal which is required for KATP channels to exit the ER/cis-Golgi compartments and transit to the cell surface, a deletion of as small portion as the last seven amino acids from SUR1 markedly reduces surface expression of KATP channels (Sharma et al. 1999).

Mutations in ABCC8 were detected for both alleles in 13 patients, while ten patients carried only one mutation in one of the ABCC8 alleles (Table 1). Interestingly, among these nine patients, seven inherited the mutation from the father; five of them were pancreatectomized but none of them were classified as Fo-HI. Although a focal form scenario is more probably due to the genetic context, we are aware of the fact that histological classification of pancreatic samples from HI patients is still highly controversial (Suchi et al. 2003). This fact could suggest deficiencies in histopathological determination of HI since recent estimates from France, Israel, and the US now suggest that 40-65% of all patients with HI have the focal form of HI (De Lonlay et al. 1997; Glaser et al. 1999; Stanley et al. 2002). The failure to detect ABCC8/KCNJ11 mutations in eleven patients may be due to several reasons: 1) mutations may be present in intronic regions not analyzed in the present report, 2) macrosomic deletions or insertions in the heterozygous state, 3) intronic or silent mutations assumed as benign may have functional effects (e.g. those that alter ESE elements), 4) some cases of HI may be attributable to mechanisms affecting gene expression, and 5) locus heterogeneity of the disease could occur. Further studies are needed to elucidate the molecular causes of HI in these patients.

Our results also seem to suggest the presence of possible founder mutations in particular Spanish regions. Patients P3 and P4 carry the same mutation in homozygous state (c.584\_585insA) and both came from the same geographical area of Galicia (NW Spain). Due to the particular features showed by this relatively isolated population (Salas et al. 1998; 2000), we suggest that this could be the result of a founder effect. Mutation c.1732\_1746dup has also been found in two unrelated patients from Catalonia and the macrodeletion found in homozygous state is also suggestive of a founder effect. The detection and localization of founder mutations have important advantages in diagnosis and prognosis of the disease.

Five mutations that could result in aberrant splicing (AS) were identified. Two of them are novel and affects position -2 of the 3' splice-site of introns 19 and 38. These mutations are considered to be pathogenic because they occur at the essentially invariant AG dinucleotide located at the end of introns (splice acceptor). It was not possible to carry out cDNA studies to confirm this point because pancreatic tissue from patients was not available. However, this kind of splice-site mutation usually results in skipping of the next exon or more frequently in the utilization of closely situated cryptic splice-sites due to the inactivation of the normal splice-site (Krawczak et al. 1992). Mutation identified in patient P21 (c.2394-2 A>G) was not present in either of the probands parents or in the unaffected sibling, so it was carried out paternity test to discard possible mistakes with parent's samples. The probability of paternity was 99.99998% (using an a priori of 0.5). This result confirms that the G to A substitution occurred de novo in proband P21. In addition, two previously well characterized splice site mutations (c.3992-9G>A and c.4310G>A) were also detected. Both mutations have been shown previously to activate cryptic splice sites in vitro, and are predicted to result in frame shifts and premature termination of translation of SUR1 mRNA (Thomas et al. 1995).

The present study also describes a homozygous macrodeletion of 3914 bp (c.1332+4438\_1631-9207del) which encompasses exons 9 and 10 of ABCC8 gene. The design of primers inside introns 8 and 11 allow us to delimit such deletion. To our knowledge, this mutation represents the first instance of a macrodeletion reported for HI patients. Note that this macrodeletion would pass unnoticed in heterozygous state which suggest the need for systematic screening of macrodeletions in those HI patients with only one, or indeed any, detected mutation in

ABCC8 gene. The presence of this macrodeletion in homozygosis could be indicative of a founder effect in the island of Lanzarote (Canary Islands).

Eleven missense mutations, eight novel and three previously identified mutations were detected. Mutations c.220C>T (p.R74W), c.331G>C (p.G111R), and c.563A>G (p.N188S) are situated in the TMD0 domain of SUR1 which is implicated in the strong association between SUR1 and Kir6.2 and modulate trafficking and gating of the channel (Chan et al. 2003). Although R74W was found for first time in our population, Nestorowicz et al. (1998) described a different amino acid change at the same codon (R74Q). It was recently demonstrated that the second substitution G111R constitutes a pathological change (Tornovsky et al 2004). Finally, mutation N188S detected in homozygosis in patient P25 had been previously reported by Nestorowicz et al. (1998) associated with severe clinical disease, although it was reported by Shyng et al. (1998), that this mutation alters channel function only minimally *in vitro*. Indeed, we found this patient carried the pathogenic splicing mutation c.4123-19C>T in homozygous state. This substitution appears as a pathogenic mutation in the Human Gene Mutation Database which seems to support that N188S produces a non pathogenic change.

The second sets of missense mutations (c.698T>G (p.M233R) and c.928G>A (p.D310N)) are placed in the CL3 domain of SUR1, also implicated in modulate gating of the channel (Babenko et al 2003). These changes affect highly conserved residues.

The amino acid change c.2156A>C (p.K719T) is positioned in the cytoplasmatic domain CL6 (NBF1) of SUR1 and constitutes a highly conserved residue. It was previously described a mutation which affected the same codon and promoted the change K719P (Gribble et al. 1997) and functional experiments showed that it provoked a reduced response to ADP-Mg. The mutation c.4352T>C (p.L1451P) affects the NBF2 domain of SUR1, changing a highly conserved amino acid. It has been previously reported (Shyng et al. 1998; Tanizawa et al. 2000; Tornovsky et al. 2004; Nichols et al. 1996; Taschenberger et al. 2002) that missense mutations placed in the NBF2 of SUR1 are shown to provoke a diminished KATP sensitivity to ADP-Mg or are predicted to prevent normal trafficking of KATP channels.

Although evidences abound about the pathogenic characteristics of most of the missense mutations found in the Spanish cohort, further research is needed in order to address their functional significance upon SUR1 regulation of KATP channel activity.

In addition to the 28 ABCC8 mutations described above, another 20 variants in the ABCC8 gene and five in KCNJ11 gene were identified. The intronic c.2256-44T>G mutation in the ABCC8 gene has been classified as unclassified variant, but the possibility that this variant is a causal mutation cannot be ruled out at present. This sequence change was detected in only one patient and was not found in 50 control samples. The variant V1573I was also classified as non-pathogenic because previous papers considered it (Nestorowicz et al. 1996) but we suggest it could play a role in the pathogenesis of the disease.

Although synonymous mutations are typically assumed to have no effects, recent studies have demonstrated that certain nucleotide substitutions may affect mRNA splicing sites, by inactivating an exonic splicing enhancer (ESE) (Cartegni et al. 2003; Liu et al. 2001; Fairbrother et al. 2002). We have used the ESEfinder web-based resource for the identification of putative ESEs. Several of the nucleotide changes detected in our patients lead to the lost and/or gain of ESE elements (Table 3) but direct experimental evidence needs to be in place before safely concluding that a particular sequence is acting as an ESE and that any of these nucleotide changes have implications for HI syndrome when disrupting certain ESEs.

Finally, we have observed that both genes (ABCC8 and KCNJ11) have a genomic structure comprising blocks of strong linkage disequilibrium (LD), as inferred from independent sets of available SNP genotypes; although, at least in the case of ABCC8, these LD-blocks are interrupted by hotspots of recombination (Fig. 1c). There are SNPs that are in perfect LD within blocks which suggest that some of these variants could also be in LD with certain, as yet undetected, HI causal mutations.

To sum up, the present study has important implications for the early diagnosis and genetic counselling of affected children and their families in the uncharacterized Spanish population, especially if we take into account that we have detected mutations in 68% of the HI patients studied, 57% of them characterized by two mutations in both alleles of ABCC8. The remaining 43% of patients were found to carry only one mutation, 75% of which were found in the paternal allele, but none of them were classified as Focal HI. This strongly suggests that some of these cases could be missed focal forms of HI and point out to an important underestimation of this histopathological HI type in our country. This deficiency would have devastating consequences in treatment and prognosis of the patients. We hope that the implantation of sequence analyses of ABCC8/KCNJ11 genes, in

addition to the new imaging method proposed by Ribeiro et al. (2005) will contribute to a better diagnosis and prognosis of HI Spanish patients.

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**Table 1.** Clinical characteristics of HI Spanish patients that carry at least one mutation in ABCC8

P <sup>a</sup>	O <sup>b</sup>	Sex	M <sup>c</sup>	T <sup>d</sup>	PC <sup>e</sup>	PT <sup>f</sup>	Mutations <sup>g</sup>	
							Pchr <sup>h</sup>	Mchr <sup>h</sup>
1a	Gal	M	>p90	DZ	5 (>90%)	OT, NF, GC	p.R248X	c.3576delG
1b	Gal	F	>p50	DZ, OT, NF	–	OT, NF, NGT	p.R248X	c.3576delG
3	Gal	F	>p90	DZ	2 (95%)	–	c.584 585insA	c.584 585insA
4	Gal	M	>p75	DZ	4 (95%)	DZ, NGT	c.584 585insA	c.584 585insA
5	Gal	M	>p50	OT, DZ	16 (90%)	–	c.1347 1348delGA	–
8	Cast	M	>p75	DZ, OT, GC	–	–	p.M233R	–
9	Cast	F	>p75	DZ	0.5 (85%)	DZ, OT, PC (99%)	p.G111R	–
12	And	M	–	–	–	–	c.4612 –2 A>T	p.D310N
14	Cat	M	>p75	DZ	–	–	p.R934X	c.3992-9 G>A
17	Cat	F	>p90	DZ, OT	–	–	c.3133 3152del	c.4619 4620insT
18	Cat	M	<p50	DZ, CNF	0.5 (95%)	DZ	c.1732 1746dup	–
19	Can	M	<p50	DZ, NF, OT	2 (99%)	–	c.1332+4438 1631-9207del	c.1332+4438 1631-9207del
20	Cat	M	–	DZ, NF, GC	–	–	c.2142delG	p.T1131P
21	Cat	F	>p50	DZ, NF	–	–	– i	– i
23	Bal	M	>p90	CNF	–	–	c.4310 G>A	c.1732 1746dup
25	Mor	M	–	DZ, OT	yes	(EXITUS)	p.N188S, c.4123-19 C>T	p.N188S, c.4123-19 C>T
27	Cast	F	>p75	DZ, CNF	24 (75%)	PC (99%)	p.R598X	p.L1451P
28	Cat	M	>p90	DZ, CNF	–	–	p.R1251X	p.L1148R
30	Cast	M	>p90	DZ, OT	5 (95%)	DZ, OT (EXITUS)	p.R74W	–
31	Gal	F	>p90	DZ	0.5 (95%)	DZ	–	p.K719T
32	Cat	F	>p90	DZ	–	–	–	p.N1296K
33	Cast	F	>p75	DZ, OT	1 (95%)	DZ, OT	c.3291 3292delGC	–
34	Val	F	>p90	DZ, NF	–	(EXITUS)	p.P551R	–

<sup>a</sup> P = patient.

<sup>b</sup> O = geographical origin: Cat = Catalanian (northeast Spain); Gal = Galician (northwest Spain); Cast = Castellan (central Spain); And = Andalusian (south Spain); Bal = Balearic Island; Can = Canary Island; Mor = Moroccan; Val = Valencian.

<sup>c</sup> M = macrosomia; “p” refers to percentile.

<sup>d</sup> T = treatment before pancreatectomy: DZ = diazoxide; OT = octreotide; NF = nifedipine; CNF = continuous night feeding.

<sup>e</sup> PC = pancreatectomy (age of surgery in months; extension of the pancreatectomy).

<sup>f</sup> TP = treatment after pancreatectomy.

<sup>g</sup> Nucleotide and codon position numbering are according to the full-length human ABCC8 cDNA sequence (accession numbers L78207 and U63421).

<sup>h</sup> Pchr = paternal chromosome; Mchr = maternal chromosome.

<sup>i</sup> Patient 21 carries mutation 2394-2A>G which has not been detected in their parents.

**Table 2.** Genetic variants found in ABCC8 gene from HI Spanish cohort

Mutations considered pathogenic									
nt change <sup>a</sup>	aa change <sup>a</sup>	Type	E/I <sup>c</sup>	Domain <sup>d</sup>	Patient	Ref <sup>e</sup>	PSIC <sup>f</sup>	Polyphen <sup>g</sup>	C <sup>h</sup>
c.220C>T	p.R74W	MIS	E2	CL1	P30	NR	2.257	PrD	Highly
c.331G>C	p.G111R	MIS	E3	TM3	P9	[1]	1.672	PsD	Moderately
c.563A>G	p.N188S	MIS	E4	TM5	P25	[2]	1.494	Benign	Highly
c.698T>G	p.M233R	MIS	E5	CL3	P8	NR	2.428	PrD	Highly
c.584_585insA	p.Y195X	FS	E5	—	P3, P4	NR	—	—	—
c.742C>T	p.R248X	NON	E5	—	P1a, P1b	[3]	—	—	—
c.928G>A	p.D310N	MIS	E6	CL3	P12	NR	1.614	PsD	Highly
c.1347_1348delGA	p.V449VfsX493	FS	E9	—	P5	NR	—	—	—
c.1332+4438_1631-9207del	p.I445FfsX447	FS	—	—	P19	NR	—	—	—
c.1652C>G	p.P551R	MIS	E11	TM10	P34	NR	2.1	PsD	Highly
c.1732_1746dup	p.A578_L582dup	IFins	E12	—	P18, P23	NR	—	—	—
c.1792C>T	p.R598X	NON	E12	—	P27	NR	—	—	—
c.2156 A>C	p.K719T	MIS	E16	CL6	P31	NR	1.927	PsD	Highly
c.2142delG	p.Q714QfsX724	FS	E16	—	P20	NR	—	—	—
c.2394-2A>G	—	AS	I19	—	P21	NR	—	—	—
c.2800C>T	p.R934X	NON	E23	—	P14	NR	—	—	—
c.3133_3152del	p.L1045LfsX1107	FS	E25	—	P17	[6]	—	—	—
c.3291_3292delGC	p.L1097LfsX1113	FS	E26	—	P33	NR	—	—	—
c.3391A>C	p.T1131P	MIS	E27	CL7	P20	NR	1.777	PsD	Moderately
c.3443T>G	p.L1148R	MIS	E28	TM14	P28	NR	1.722	PsD	Highly
c.3576delG	p.L1191LfsX1207	FS	E29	—	P1a, P1b,	NR	—	—	—
c.3751C>T	p.R1251X	NON	E30	—	P28	NR	—	—	—
c.3888C>G	p.N1296K	MIS	E32	TM17	P32	NR	1.924	PsD	Highly
c.3992-9G>A	—	AS	I32	—	P14	[4]	—	—	—
c.4123-19C>T	—	AS	I33	—	P25	[5]	—	—	—
c.4310G>A	—	AS	E35	—	P23	[4]	—	—	—
c.4352T>C	p.L1451P	MIS	E36	CL9	P27	NR	1.797	PsD	Highly
c.4612-2 A>T	—	AS	I38	—	P12	NR	—	—	—
c.4619_4620insT	p.H1540AfsX1559	FS	E39	—	P17	NR	—	—	—
Polimorphisms and unclassified variants									
nt change <sup>a</sup>	aa change <sup>a</sup>	Type	E/I <sup>c</sup>	SNPid	Patients <sup>i</sup>	Controls	NCBI <sup>j</sup>	Exclusion	
c. 207T>C	p.P69P	SYN	E2	rs1048099	28/46	—	0.50	S	
c. 330C>T	p.A110A	SYN	E3	rs8192695	2/48	—	0.04	S	
c. 423G>A	p.V141V	SYN	E4	—	1/48	1/112	—	S	
579+14T>C	none	INT	I 4	rs2301703	23/46	—	—	I	
c. 1686C>T	p.H562H	SYN	E12	rs1799857	21/44	—	0.42	S	
c. 1707C>T	p.A569A	SYN	E12	—	1/48	0/96	—	S	
c. 1947G>A	p.K649K	SYN	E14	rs1799858	5/48	—	0.14	S	
2117-3C>T	none	INT	I 15	rs1799854	22/42	—	0.42	I	
2256-65C>T	none	INT	I 17	rs12293228	8/48	7/96	—	I	
2256-50C>T	none	INT	I 17	rs4148626	6/48	16/96	—	I	

2256-44T>G	none	INT	I 17	—	1/48	0/96	—	I
c. 2280C>T	p.T760T	SYN	E18	rs1801261	2/48	3/96	0.05	S
2294-34T>C	none	INT	I 18	rs4148629	3/48	—	—	I
2294-36C>T	none	INT	I 18	rs4148628	3/48	—	0.15	I
c. 2488C>T	p.L830L	SYN	E21	rs4148633	8/48	—	—	S
c. 3822G>A	p.R1274R	SYN	E31	rs4148643	11/48	—	0.38	S
c. 4108T>G	p.S1370A	MIS	E33	rs757110	17/44	—	0.37	NC
4123-27T>C	none	INT	I 33	rs739689	16/46	—	0.25	I
4612-40A>G	none	INT	I 38	rs1109591	3/7	—	—	I
c. 4717G>A	p.V1573I	MIS	E39	rs8192690	3/48	—	0.07	C
c. 67G>A	p.E23K	MIS	KCNJ11	rs5219	18/48	—	0.34	NC
c. 570C>T	p.A190A	SYN	KCNJ11	rs5218	12/48	—	0.29	S
c. 801C>G	p.L267L	SYN	KCNJ11	rs5216	2/48	—	0.04	S
c. 1009A>G	p.I337V	MIS	KCNJ11	rs5215	18/48	—	0.39	NC
c. 1143G>A	p.K381K	SYN	KCNJ11	rs8175351	1/48	—	0.02	S

a Nucleotide and codon position numbering are according to the full-length human ABCC8 cDNA sequence (accession numbers L78207 and U63421).

b MIS = missense; FS = frame shift; NON = nonsense; AS = aberrant splicing; IFins = in-frame insertion; SYN = synonymous; INT = intronic.

c E = exon; I = intron; KCNJ11 = the unique exon of KCNJ11.

d Domain of SUR1 in which is situated the mutation (indicated for missense mutations only); CL = cytoplasmatic domain; TM = trans-membrane domain.

e Ref = references: NR = not reported; [1] = Tornovsky et al. (2004); [2] = Nestorowicz et al. (1998); [3] = Aguilar-Bryan et al. (1999); [4] = Thomas et al. (1995); [5] = INSERM U383 (2001) ABCC8 Locus-specific database; [6] = Fournet et al. (2001).

f PSIC score: Polyphen value which correlates with the probability of one missense mutation to be pathogenic.

g Polyphen prediction: PsD = possible damaging; PrD = probably damaging.

h C = conservation of amino-acidic SUR1 sequences across 27 different sequences from six different species using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>): *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Tetraodon nigroviridis*, *Oryctolagus cuniculus*, Golden hamster.

i Frequency of SNP in cases and controls (number of chromosomes).

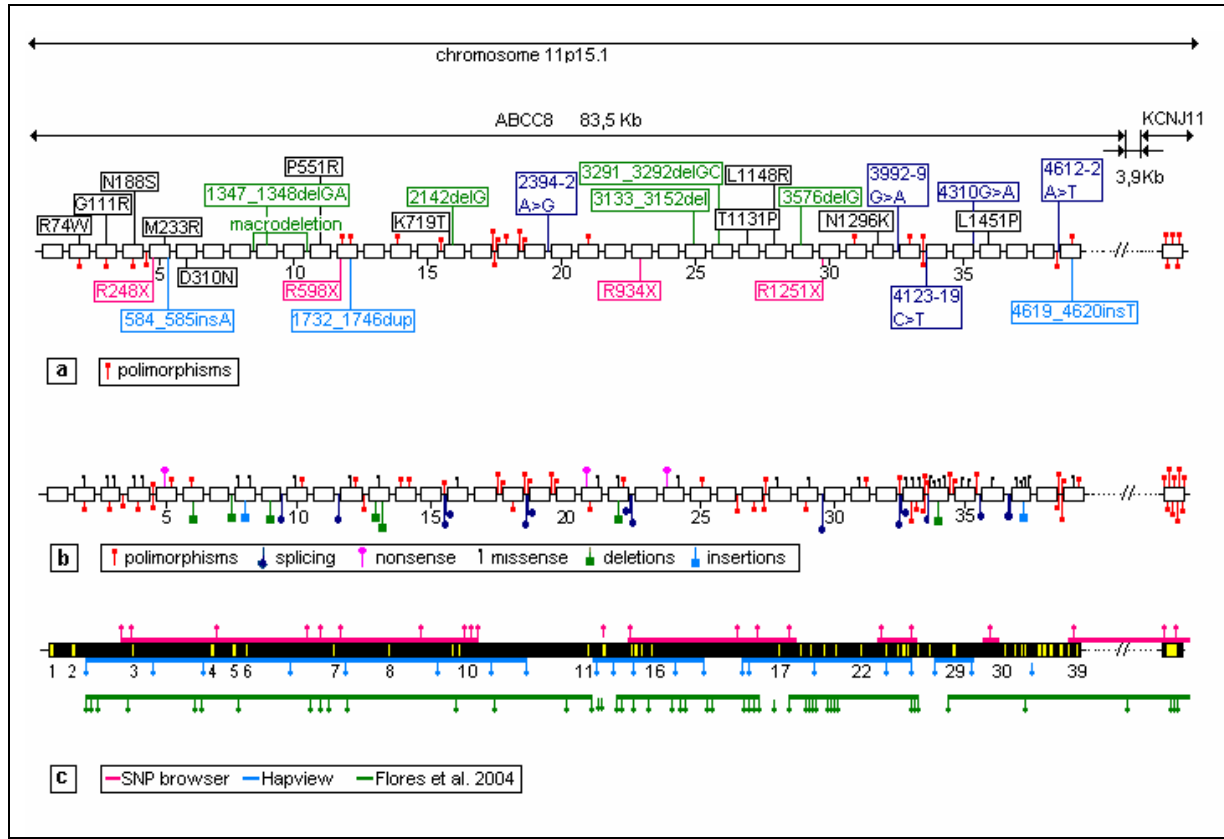
j Frequencies taken from NCBI SNPs database for those SNPs typed in population of European ancestry.

k Reason for being classified as non-pathogenic: S = silent; I = intronic; C = conservative; NC = found in normal chromosomes.

**Table 3.** ESEfinder score change of some variants of ABCC8 gene

nt change	aa change	ESE changes <sup>a</sup>			
		SF2-ASF	SC35	SRp40	SRp55
Mutations					
c.220C>T	R74W	Lost 2.9	Gain 0.64	Gain 1.49	Lost 1.55
c.331G>C	G111R	M 2.58	-	-	-
c.563A>G	N188S	-	M 0.36	-	Gain 0.61
c.2156 A>C	K719T	-	-	-	Gain 2.14
c.3391A>C	T1131P			Lost 1.74	
c.3443T>G	L1148R	M +2.12		Gain 2.39	
c.3888C>G	N1296K	—	—	Lost 1.73	—
c.4352T>C	L1451P	M -0.42	Lost 0.65	Gain 1.54	—
Polymorphisms and unclassified variants					
c. 207T>C	P69P	Gain 1.458	M -0.645	—	—
c. 330C>T	A110A	M -2.524	—	—	—
c. 1686C>T	H562H	—	Lost-0.645	M -0.356	M +1.551
c. 1707C>T*	A569A	Lost 2.397	—	—	—
c. 1947G>A	K649K	—	—	Lost 0.846	M -0.609
c. 2280C>T	T760T	Lost 2.524	—	M +0.356	—
c. 3822G>A	R1274R	M -2.824		Lost 2.382	—
c. 4108T>G	S1370A	—	M +0.648	—	—
c. 4717G>A	V1573I	—		—	M -0.609

<sup>a</sup>Lost (or gain) of an ESE below (above) the threshold values (SF2-ASF = 1.956; SC35 = 2.383; SRp40 = 2.67; SRp55 = 2.676; Cartegni et al. 2003) are indicated. In brackets we show the difference in score. If an ESE is maintained but the score has changed is indicated (M).



**Figure 1. a:** Schematic diagram showing the locations of 29 mutations and 20 putative polymorphisms identified in the *ABCC8* gene in HI Spanish patients. All 39 exons of the *ABCC8* gene are depicted as closed boxes and are not drawn to scale. Different types of mutations (splice site, insertions, deletions, missense, and nonsense) are indicated in different colours. The two nucleotide binding domains are also indicated (NBF1/2). **b:** Locations of all the mutations identified in the *ABCC8* and *KCNJ11* genes in different populations as recorded in the Human Gene Mutation Database. **c:** Colour fringes indicate haplotype blocks as inferred using data from SNPbrowser (pink; upper) and Haploview (blue; lower) (see material and methods). Here, exon numbering is partially omitted by convenience and polymorphisms are located as in SNPbrowser and Haploview. We add for comparison the haplotype structure and polymorphisms (green) reported by Florez et al. (2004).

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