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ORIGINAL ARTICLE

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Sudden infant death syndrome and long QT syndrome: an epidemiological and genetic study

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Abstract Sudden infant death syndrome (SIDS) is a frequent cause of death among infants. The etiology of SIDS is unknown and several theories, including fatal ventricular arrhythmias, have been suggested. We performed an epidemiological and genetic investigation of SIDS victims to estimate the presence of inherited long QT syndrome (LQTS) as a contributor for SIDS. Forty-one consecutively collected and unrelated SIDS cases were characterized by clinical and epidemiological criteria. We performed a comprehensive gene mutation screening with single-strand conformation polymorphism analysis and sequencing techniques of the most relevant LQTS genes to assess mutation frequencies. In vitro characterization of identified mutants was subsequently performed by heterologous expression experiments in Chinese hamster ovary cells and in *Xenopus laevis* oocytes. A positive family history for LQTS was suspected by mild prolonged Q-T interval in family members in 2 of the 41 SIDS cases (5%). In neither case, a family history of sudden cardiac death was present nor a mutation could be identified after thorough investigation. In another SIDS case, a heterozygous missense mutation (H105L) was identified in the N-terminal region of the *KCNQ1* (LQTS 1) gene. Despite absence of this mutation in the general population and a high conservational degree of the residue H105 during evolution, electrophysiological investigations failed to show a significant difference between wild-type and $KCNQ1_{H105L}$/minK-mediated I_{Ks} currents. Our data suggest that a molecular diagnosis of SIDS related to LQTS genes is rare and that, even when an ion channel mutation is identified, this should be regarded with caution unless a pathophysiological relationship between SIDS and the electrophysiological characterization of the mutated ion channel has been demonstrated.

Keywords Sudden infant death syndrome · Long QT syndrome · Arrhythmia · Genetics

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Introduction

Despite considerable decline in incidence, sudden infant death syndrome (SIDS) is still the leading cause of death among infants between 8 and 365 days of age [1]. In

Germany, the incidence of SIDS decreased from 602 cases reported in 1998 to 359 in 2002, which is a relative decline of 0.77 to 0.5 per 1,000 live births [2]. Similar statistics have been reported by most industrialized countries [3].

Several theories on the causes of SIDS have been proposed in the past, but only a few have clearly been proven mostly in observational cases. These theories also include cardiovascular causes (e.g. viral myocarditis, abnormal reflexes and abnormalities of the cardiac conduction system) [4, 5], but the extent of cardiac and non-cardiac causes for SIDS remains unclear. In addition, ventricular tachycardia or fibrillation, often found in the absence of structural heart disease (so-called primary electrical disorders), has been associated with sudden cardiac death (SCD) in neonates and raised the suspicion about a potential role in SIDS [6, 7]. One such disorder is the long QT syndrome (LQTS), which is characterized by increased vulnerability of the myocardium with an increased propensity to develop ventricular fibrillation. It had already been proposed as a cause for SIDS in 1976 [8]. Since that time, several studies have investigated a potential association of prolonged Q-T interval in the surface electrocardiogram (ECG) and SIDS, but the results were not concordant [9–14]. So far, only one study used a genetic approach in SIDS by systematically screening cardiac ion channel genes that are known to cause congenital LQTS [15, 16]. In addition, case reports of post-mortem analysis in LQTS genes that show a relationship with SIDS exist [7, 17].

The aim of the present study was to investigate a sample of consecutive SIDS cases for epidemiological and genetic post-mortem evaluation by mutation analysis of major LQTS genes.

Methods

Clinical and epidemiological investigations

In a total of 41 SIDS cases of sudden and unexpected infant death, which occurred in 1991/1992 and 1995/1996 in the northwestern area of Germany, a post-mortem examination was performed. The investigation included a standardized autopsy protocol similar to the International Standardized Autopsy Protocol [18], with x-ray investigation, extensive histology, neuropathology, toxicology, microbiology, virology and clinical chemistry [19]. Death scene examination was performed by police officers and, in some cases, by specialists in legal medicine. Finally, an interdisciplinary case conference with a paediatrician, a forensic pathologist, an epidemiologist and a microbiologist took place. The internationally accepted definition of SIDS, first introduced in 1969 [20] and modified in 1989 by the National Institute of Child Health and Human Development [21], was applied. Cases were classified into three categories [22]: (1) SIDS cases (inconspicuous in their previous history, no pathological findings); (2) SIDS cases with minor pathological changes, not being causative for death and (3) cases with pathomorphologically determined cause of death (non-SIDS). Cases belonging to category 3 were not included in this study.

Genetic analysis of LQTS genes

For DNA isolation, specimens were taken from the liver, the spleen and the thymus, which have been frozen in liquid nitrogen and stored at −80°C before. Extraction was done using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany). The purified DNA was eluted according to the manufacturer's instruction with 60 µl elution buffer and stored at −20°C [23].

Screening for mutations of *KCNQ1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* was performed with the polymerase chain reaction (PCR), followed by single-strand conformation polymorphism (SSCP) analysis (80% of amplicons) and direct sequencing (20% of amplicons) using standard procedures [24–27]. Fluorescence-labeled specific PCR primers reported by Wang et al. [28], Splawski et al. [29] and Abbott et al. [30] were used to amplify all coding exons and exon/intron boundaries of LQTS 1–3 and 5–6. Due to the size of some exons, we divided some of them with overlapping primers (e.g. *HERG* exon 6-1 to 6-2). Each exon was analysed at two specific temperatures (12 and 18°C), and fluorograms were compared to a known wild type. SSCP analysis was performed on an ABI Prism 3700 Genetic Analyzer (Applied Biosystems, Boston, MA, USA). Aberrant conformers were detected by differences in separation patterns compared to the wild type and were subsequently analysed by DNA sequencing (ABI Prism 3700 Genetic Analyzer, Applied Biosystems) on the same devices.

Restriction enzyme analysis was used for independent mutation detection of particular mutations. Ten microliter of the PCR product was incubated with the restriction endonuclease according to the manufacturer's conditions (New England Biolabs, Inc., Beverly, MA, USA). After the reaction was completed, the DNA digests and the control samples were loaded on an agarose gel for further electrophoresis.

Heterologous expression of mutant LQT 1 ion channel protein

Chinese hamster ovary (CHO) cells were grown in MEM Eagle's Alpha medium (Life Technologies, Paisley, Scotland) containing 10% fetal calf serum (Biochrom, Berlin, Germany), penicillin (100 U/ml) and streptomycin (100 mg/ml) (Life Technologies) in 50-ml flasks (NUNC, Roskilde, Denmark) at 37°C in a humidified atmosphere (5% CO_2). Cells were subcultured in monodishes (NUNC) before electrophysiological experiments. Transfection was performed using 3.5 µl of the lipofectamine reagent (Gibco BRL, Rockville, USA) and 0.5 µg of KCNQ1, 0.5 µg of KCNE1 and 0.5 µg EGFP plasmid DNA.

Oocytes from South African clawed frogs (*Xenopus laevis*) were removed surgically under anaesthesia with ethyl 3-aminobenzoate (Sigma, Munich, Germany). Two nanograms of cRNA of KCNQ1 or 0.05 ng of KCNQ1 together with 0.05 ng of KCNE1 cRNA in distilled water (50 nl) was injected, and oocytes were maintained under culture conditions at 18°C until used for experiments (50 μg/ml gentamicin).

CHO currents were measured using an EPC-9 amplifier and Pulse software 8.11 (HEKA Elektronik, Lambrecht, Germany). Oocytes were voltage-clamped with an Oocyte Clamp OC-725 amplifier (Warner Instruments, Hamden, CT, USA). Recording electrodes were pulled from borosilicate glass capillary tubes (World Precision Instruments, Saratoga, USA) and filled with (mM) 160 KCl, 0.5 MgCl, 10 HEPES, 2 Na-ATP; pH 7.2 (adjusted with KOH). The external solution contained (CHO/oocytes, mM) 135/79.5 NaCl, 5/2 KCl, 2/2 $CaCl_2$, 2/1 $MgCl_2$, 5/5 HEPES, 10 sucrose, 0.1 mg/ml phenoled (all from Sigma, Deissenhofen, Germany); pH 7.4/7.5 (adjusted with NaOH). The holding potential was −80 mV (CHO) and −100 mV (oocytes), and the depolarizing test potentials (5 s) ranged from −90 to +80 mV in 10-mV steps. Tail currents (500 ms) were recorded at −40 mV (CHO) and −30mV (oocytes).

The mutant KCNQ1 H105L was generated by site-directed mutagenesis. The triplet CAC coding for histidine was changed to TTA coding for leucine. The PCR fragment was cloned into the pGEM KCNQ1 wild-type vector by digestion with PauI and Bsp1407. The cRNA was synthesized with the T7 mMesssage mMachine-Kit (Ambion, Austin, TX, USA). For expression in mammalian cells, the mutation was subcloned into the pcDNA3 KCNQ1 vector by digestion with Eco47III and Bsp1407. The primer was (sense) TGGCGCGCACCTTAGTCCAGGGC, (antisense) GGAGATGGCAAAGACAGAGAAGCAG.

Results

Epidemiology of 41 SIDS victims

Seven of the 41 cases (17%) were classified as SIDS of category 1, whereas the remaining majority of 83% (34/41) showed minor pathological changes (e.g. infections of the respiratory system) and therefore were diagnosed as SIDS of category 2. Concordantly, none of these changes was considered to be causative for SIDS.

The gender distribution was not equal (39% females) but typical for SIDS cases. Age distribution showed a well-known peak of death between the second and fourth months (Fig. 1a). Seven SIDS victims (17%) had a premature birth. In 54% of all, delivery was uncomplicated (Fig. 1c). In all cases, including those with complicated delivery, the appearance–pulse–grimace–activity–respiration (APGAR) score at 10 min after birth was between 8 and 10 except for three out of the seven premature births (Fig. 1b). Medical problems in the neonatal period occurred in 15 of 41 cases (36%) (infectious diseases *n*=4, icterus neonatorum *n*=5, breathing problems *n*=3, alcohol embryopathy *n*=1, systolic cardiac murmur *n*=1 and withdrawal syndrome due to methadone dependency *n*=1). In two of the premature infants, acute life-threatening events were observed at the age of 3 months, and one infant showed apnoea phases in hospital and monitoring had been organized.

In two SIDS cases (~5%), family members had a borderline Q-T prolongation on surface ECG, thus suggesting transmitted LQTS as a potential, but not demonstrable cause of death. Both cases belong to SIDS category 2. In

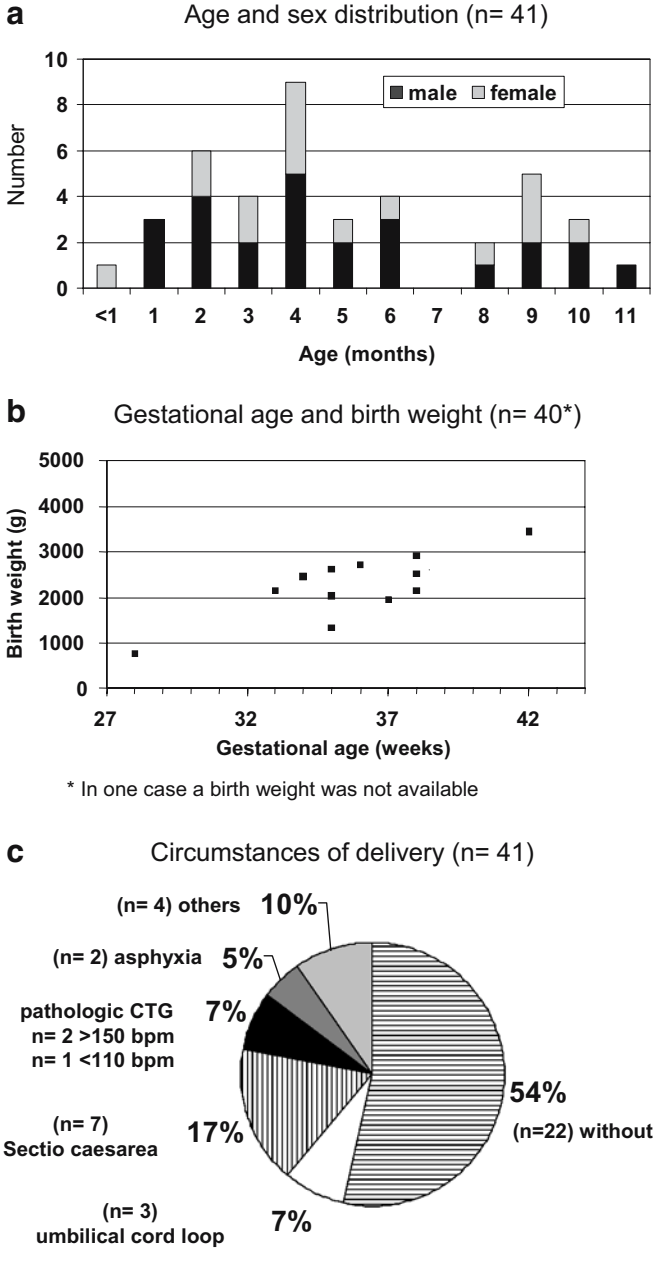

Fig. 1 **a**–**c** Characteristics of the investigated cases. **a** The typical age and gender distribution is shown. **b** The gestational age and birth weight and **c** the circumstances and complications during birth are presented

case 236, a brother was born 1 year after the SIDS victim. The surface ECG at 2 and 6 months showed a mild prolongation of the Q-T interval of 460 and 470 $ms^{1/2}$, respectively. However, during follow-up, QTc decreased to normal during the first year of life and has still remained there. In case 356, the 28-week-old female was found dead in a prone position; the ECG of the living dizygotic twin showed a QTc of 460 $ms^{1/2}$ at the age of 7 months and that of the mother of also 460 $ms^{1/2}$. Unfortunately, this family refused further clinical and genetic investigations. In both cases, there was no family history of sudden death.

Genetic analysis and restriction analysis

All exons of the LQT 1–3 and LQT 5–6 genes were investigated for the presence of mutations. The LQT4 and LQT7 genes were not screened in this study because mutations are obviously rare. Only in one SIDS case (145) a heterozygous A-to-T substitution (A314T) in exon 1 of the *KCNQ1* gene was identified, which leads to an amino acid exchange from histidine to leucine (H105L) in KvLQT1 isoform 1 (Genbank accession no. AF000571). Complete sequencing of all LQT genes demonstrated that this was the only alteration in this case. H105L creates a new restriction site (EcoNI), which was further used for restriction enzyme digestion. Absence of this restriction site in over 100 unrelated controls excluded a possible polymorphism for the variant. The alignment with *KCNQ1* cDNA sequences of different species showed that the H105L residue was evolutionarily conserved with the exception of *X. laevis* (N105) (Fig. 2).

In the other cases (25/41, 61%), several aberrant conformers in SSCP gels and subsequent DNA analyses were observed, which were located elsewhere in the LQT genes. Most of them were silent nucleotide changes, polymorphisms or intron variations. However, we identified some new nucleotide alterations in the LQT1 and LQT3 genes (e.g. F479F, T153T in *KCNQ1*, and D1810D and C1591C in *SCN5A*) and some intronic polymorphisms (IVS4-34G>A in *HERG* and IVS12 + 6delA, IVS13+36G>A, IVS15+32G>T in *KCNQ1*) (Table 1).

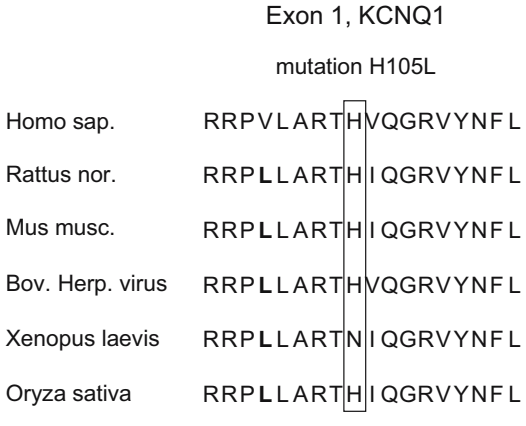

Fig. 2 Amino acid sequence alignments of the N-terminal part of the KCNQ1 α-subunit and orthologous potassium channels are shown in the *lower panels*. GenBank BLAST search reveal that the sequence is highly conserved during the evolution process

Biophysical properties of H105L mutant KCNQ1 channels

The consequences of the H105L mutation in *KCNQ1* on I_{Ks} currents were characterized by comparing the biophysical properties of functionally expressed wild-type ($KCNQ1_{wt}$) and mutant ($KCNQ1_{H105L}$) channels. For this purpose, oocytes from *X. laevis* as well as CHO cells were used as expression systems. Figure 3a shows original currents recorded from oocytes expressing either $KCNQ1_{wt}$ or $KCNQ1_{H105L}$ channel α-subunits. Figure 3b shows original current traces of $KCNQ1_{wt}$ or $KCNQ1_{H105L}$ channel α-subunits expressed together with the β-subunit minK. $KCNQ1_{wt}$/minK channels as well as $KCNQ1_{H105L}$ / minK channels passed currents that slowly activated and did not inactivate during the depolarizing test potentials. Analysis of the gating behaviour yielded significantly different activation midpoints of $KCNQ1_{wt}$ and $KCNQ1_{H105L}$ channel α-subunits (Table 2). However, activation midpoints of the respective complexes formed by the α- and β-subunits did not significantly differ. The maximal current size did not differ between either $KCNQ1_{wt}$ and $KCNQ1_{H105L}$ channel α-subunits nor between complexes formed by the respective α- and β-subunits (Fig. 4a,b). To confirm these results in a mammalian expression system, $KCNQ1_{wt}$/minK channels as well as $KCNQ1_{H105L}$/minK channels were also expressed in CHO cells. The parameter of the Boltzmann function as well as the current density still remained indistinguishable between wild-type and mutant channels (Fig. 4b).

Clinical presentation of the SIDS case with the *KCNQ1* H105L mutation

This female infant was born after an uncomplicated pregnancy at 40 weeks of gestation with a body weight of 3,810 g. She was the first child of a 20-year-old mother and a 22-year-old father. Despite the fact that during delivery, the umbilical cord was twined around the neck, the initial physical examination in the nursery was normal. APGAR scores were 10/10, the pH of the umbilical cord blood was 7.28 in the artery and 7.39 in the vein. At the second day of life, the newborn developed several episodes of cyanosis. After she was joggled, the cyanosis disappeared. A breathing monitor was used during the hospitalization, but no further episodes were observed and she was discharged from the hospital. Routine checkup showed no relevant illnesses besides a mild hypotonia and cough.

The last contact was on the day of death at 5.15 a.m. At 8.30 a.m. (age of the patient 9 months), the parents found her dead in her bed lying in a prone position. This position was observed for the first time (so-called inexperienced

Table 1 Summary of the identified LQT variants in 41 SIDS cases

Propositus	Age at death (months)	Gene	Exon	Mutation	Nucleotide exchange	Coding effect (Exon)		Intron	Domain	Reference
						Silent mutation	PP			
1	10.5	HERG	6-2	F513F	TTC>TTT	x	x		S3	[45, 48]
		SCN5A	28-3	D1810D	GAC>GAT	x			C-terminal	This study
2	1.5	HERG	8	Y652Y	TAT>TAC	x	x		S6	[45, 47]
		SCN5A	28-3	D1810D	GAC>GAT	x			C-terminal	This study
3	3	SCN5A	28-3	D1810D	GAC>GAT	x			C-terminal	This study
4	8	KCNQ1	11	F479F	TTC>TTT	x			C-terminal	This study
		KCNQ1	15	IVS15+32 G>T				x		This study
5	3	HERG	8	Y652Y	TAT>TAC	x	x		S6	[45, 47]
		SCN5A	23	G1406G	GGG>GGA	x			DIIIS5-S6	This study
		KCNQ1	15	IVS15+32 G>T				x		This study
6	9 days	SCN5A	28-3	D1810D	GAC>GAT	x			C-terminal	This study
7	2	HERG	8	Y652Y	TAT>TAC	x	x		S6	[45, 47]
8	10.5	HERG	6	I489I	C1467T, ATC>ATT	x	x		S2-S3	[45, 47]
		HERG	6-2	F513F	TTC>TTT	x	x		S3	[45, 48]
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
9	4	KCNQ1	2	T153T	G459A	x			S2	This study
10	2	KCNQ1	13	S546S	G1638A (TCG>TCA)	x	x		S6-C	[49]
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
11	9.5	KCNE1	3–1	S38G	AGT>GGT (A112G), het.		x			[50]
		KCNQ1	13	S546S	G1638A (TCG>TCA)	x	x		S6-C	[49]
		KCNQ1	13	IVS13+36G>A				x		This study
12	7	HERG	8	Y652Y	T1956C, TAT>TAC	x	x		S6	[45, 47]
		KCNQ1	13	IVS13+36G>A				x		This study
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
		KCNE1	3-2	D85N	GAT>AAT (G253A), het.		x			[46]
13	4.5	KCNQ1	13	S546S	G1638A (TCG>TCA)	x	x		S6-C	[49]
14	2.5	HERG	4-1	IVS4-34G>A				x		This study
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
		HERG	11	K897T	AAG>ACG		x		C-terminal	[45]
15	8	HERG	4-1	IVS4-34G>A				x		This study
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
16	2.5	HERG	11	K897T	AAG>ACG		x		C-terminal	[45]
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			This study
		SCN5A	23	G1406G	GGG>GGA	x			DIIIS5-S6	This study
17	9	KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
18	2	KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
19	2	HERG	4-1	IVS4-34G>A				x		This study
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
20	5	HERG	4-1	IVS4-34G>A				x		This study
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
21	4	KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
22	1	KCNQ1	12	IVS12+6delA				x		This study
		HERG	6-2	F513F	TTC>TTT	x	x		S3	[45, 47]
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
23	6	HERG	4-1	IVS4-34G>A				x		This study
		KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
24	2	KCNE1	3-1	S38G	AGT>GGT (A112G), het.		x			[50]
25	9	KCNQ1	1	H105L	CAC>CTC				N-terminal	This study
		SCN5A	27	C1591C	GGC>GGT	x			C-terminal	This study

For each variant, the exon number, the coding effect (pp = polymorphism) and the localization in the corresponding gene is shown. Furthermore, the references of the first description (if any) is given [45–50]

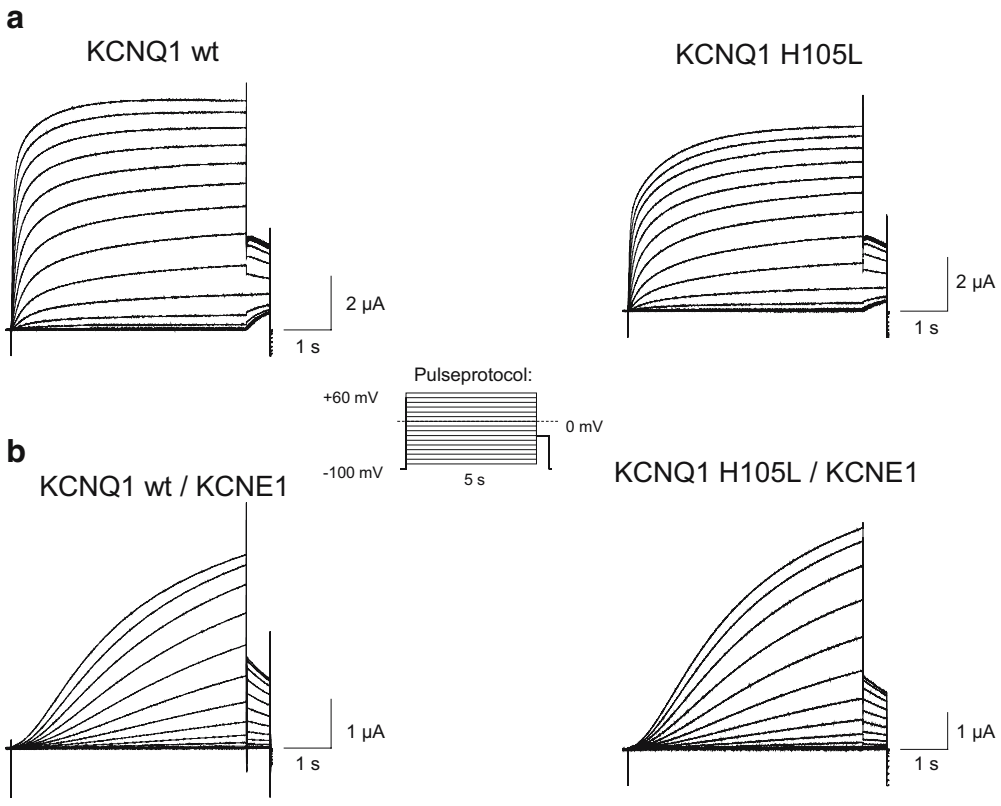

Fig. 3 **a**–**b** Original current recordings of **a** $KCNQ1_{wt}$ and $KCNQ1_{H105L}$ as well as of **b** $KCNQ1_{wt}$/minK and $KCNQ1_{H105L}$/minK expressed in oocytes from *X. laevis*

prone sleeper). She was covered by her sheet, rotated by about 180° and was all in sweat (room temperature 18°C). The autopsy showed only unspecific results; the toxicological, microbiological, virological and histological investigations were negative, which made the case eligible for the diagnosis of "typical SIDS" according to the previously described definition [31].

Table 2 Parameter of the Boltzmann functions and statistical comparison of the ion channels expressed in oocytes from *X. laevis* as well as in CHO cells

	$V_{0.5}$ (mV)	Slope (mV)	No. of experiments
Oocytes			
KCNQ1 wt	−24.81±0.42	10.23±0.55	4
KCNQ1 H105L	−22.20±0.84	10.15±0.55	5
t Test	$p<0.01$	$p>0.05$	
KCNQ1 wt/KCNE1	28.01±4.81	15.63±1.64	5
KCNQ1 H105L/KCNE1	29.46±6.06	14.90±1.79	5
t Test	$p>0.05$	$p>0.05$	
CHO cells			
KCNQ1 wt/ KCNE1	4.29±5.38	10.80±3.33	7
KCNQ1 H105L/KCNE1	5.38±7.98	10.52±1.25	8
t Test	$p>0.05$	$p>0.05$	

Shown are mean values±standard deviation

Discussion

The present study reports on epidemiological and genetic data of 41 autopsied SIDS cases, in which a thorough genetic and histopathological investigation was made to define potential contributors for sudden infant death. Because of recent evidence [6, 13, 15, 16], we investigated the major LQTS genes and were able to identify in 1 out of 41 cases (2.4%) a novel heterozygous LQT1 (*KCNQ1*) mutation (H105L). Subsequent electrophysiological analysis of the H105L variant, however, did not detect the potential disease mechanism despite the fact that two different expression systems were used that are commonly employed for LQT1 in vitro studies. Since other channel subunits and modulators for cardiac ion channels are continuously identified, one may speculate about a potential contribution and interaction of these for H105L mutant *KCNQ1* channels. However, one would expect that a mutation that leads to SCD in such a young age would have profound effects of the I_{Kr} current. Thus, the disease mechanism of the KCNQ1 mutant remained undetected so far.

Beside the finding of a novel amino acid change in the N-terminal region of *KCNQ1*, several other common and rare polymorphisms were found in the present study for which a causal relationship with SIDS is not obvious. The contribution of polymorphisms that influence the susceptibility for the development of cardiac arrhythmias has been discussed for a long time [32]. Studies that have investigated the frequency of genetic polymorphisms and mutations especially in LQT genes show similar low fre-

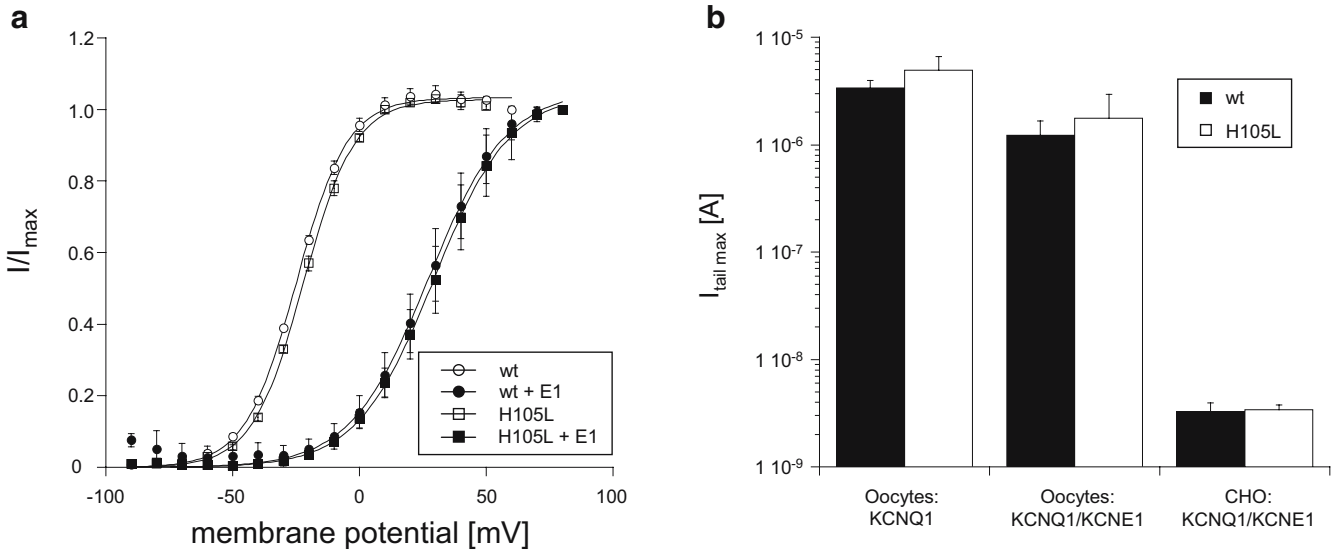

Fig. 4 **a**–**b** The tail current voltage relationships of $KCNQ1_{wt}$ and $KCNQ1_{H105L}$ as well as $KCNQ1_{wt}$/minK and $KCNQ1_{H105L}$/minK were described by Boltzmann functions. **a** The mutation caused a small but significant shift of the activation midpoint of KCNQ1 but not of the mutated KCNQ1 when co-expressed with minK. **b** The mutation in KCNQ1 did neither alter the current size of I_{Ks} when expressed in oocytes nor when expressed in CHO cells

quencies (5–12%) of 'forme fruste' mutations or functional polymorphisms in the studied population [33–37]. The significance of the identified polymorphisms, as well as the non-coding and intronic variations, is yet unknown and will need further clarification, e.g. screening large numbers of SIDS/LQT patients and controls with subsequent functional testing of these variants. At present, we do not know whether naturally occurring variants in these (and other) arrhythmia genes may serve as an additional cofactor that contribute to phenotypic disease expression. The same is true for mutations or polymorphisms in the promoter regions. For example, mutations in the cytokine interleukin-10 gene and/or the serotonin transporter protein gene were studied in SIDS by several groups [38, 39]. In both genes, polymorphisms may predispose infants to sudden death under unfavourable haplotypes. However, our intention for this study was to investigate the LQT genes for which the biggest body of evidence exists in causing arrhythmia and sudden death and not regulatory genes with yet unknown significance. Therefore, we did not screen promoter genes, since the structural components are not clearly identified.

In 2000, Schwartz et al. [6] demonstrated for the first time a relation between LQTS and SIDS in performing a post-mortem analysis and thereby identifying a de novo mutation in the cardiac sodium channel gene *SCN5A*. A causal role of inherited arrhythmias was also reported by Priori and colleagues [40] who found a missense mutation in the *SCN5A* gene in a family with transient ECG manifestation of Brugada syndrome. This is an allelic disorder and suggests that mutations in *SCN5A* indeed may lead to sudden death in infants. Early onset of a severe LQTS in newborns has been reported by several authors over the years, including the historic first case by Romano et al. [41] and Ward [42] in 1963. In previous reports, we also described infants with de novo mutations in the *SCN5A* gene that were associated with a severe phenotype and very long Q-T intervals, functional 2:1 block and life threatening arrhythmias [43, 44]. The first comprehensive genetic analysis of the LQT genes in SIDS victims was reported by Ackerman and colleagues [15, 16]. In this first report, only the LQT3 gene (*SCN5A*) was investigated in 93 consecutive SIDS cases. Two heterozygous mutations (A997S and R1826H) have been identified, and the frequency of *SCN5A* mutations in SIDS was estimated with ~2% [15]. In the further extended investigation of the other LQTS genes, the data indicate a prevalence of 4.3% which increases to 6.7% when the analysis is limited to Caucasians [16]. These reports together confirm that in some SIDS victims, mutations in cardiac ion channels may provide an arrhythmogenic substrate for malignant tachyarrhythmias.

The different reports raise an important issue to what extent LQTS is involved in SIDS. We thereby extended our post-mortem DNA analysis to the most relevant LQTS genes. The prevalence of prolongation of the Q-T interval in SIDS victims varies in several studies from low up to 50% [11, 13] and is still under considerable debate. Therefore, to provide further data, we analysed the major LQTS genes in 41 cases that are assumed to represent 55–60% of congenital LQTS.

In two families of our study population (~5%), LQTS was suspected due to the ECG recordings of living siblings, which was in one case a dizygote twin. In both cases, we did not find a strong support of a positive family history, since in one case, the Q-T interval prolongation was almost borderline, and in the other case, a transient Q-T interval prolongation in the first year of life was finally resolved to normal values. Furthermore, genetic investigation did not reveal an LQTS mutation.

The inability to identify further mutations may have several reasons: (1) some mutations were not detected despite a sufficient and robust SSCP protocol, (2) all genetic investigations do not include promoter regions or focus on gross chromosomal recombinations that may be causal and (3) the mutation frequency is indeed low because SIDS is a multifactorial and complex disorder (disease complex).

Taken together, our data suggest that LQTS mutations in SIDS cases have a lower incidence than it has been recently estimated. Furthermore, the pathophysiological link between SIDS and an identified LQTS mutation should be regarded with caution unless a pathophysiological relationship between SIDS and the LQTS mutation has been demonstrated by functional characterization of the mutated ion channel. We expect further pathophysiological heterogeneity, including other electrical heart diseases as well as other non-cardiac causes for SIDS.

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References

1. Dwyer T, Ponsonby AL (1995) SIDS epidemiology and incidence. Pediatr Ann 24:354–356
2. Statistisches Bundesamt Deutschland, Statistischer Informationsservice, Gustav-Stresemann-Ring 11, 65189 Wiesbaden. Gestorbene Säuglinge nach Alter und ausgewählten Todesursachen (ICD10: R95)
3. Fitzgerald K (2001) The “Reduce the risks” campaign, SIDS International, the Global Strategy Task Force and the European Society for Study and Prevention of Infant Death. In: Byard RW, Krous HF (eds) Sudden infant death syndrome. Problems, progress and possibilities. University Press, Oxford, pp 310–318
4. James TN (1968) Sudden death in babies: new observations in the heart. Am J Cardiol 22:457–506
5. Bajanowski T, Ortmann C, Teige K, Wedekind H, Zack F, Röse I, Brinkmann B (2003) Pathological changes of the heart in sudden infant death. Int J Leg Med 117:193–203
6. Schwartz PJ, Priori SG, Dumaine R, Napolitano C, Antzelevitch C, Stramba-Badiale M, Richard TA, Berti MR, Bloise R (2000) A molecular link between the sudden infant death syndrome and the long-QT syndrome. N Engl J Med 343:262–267
7. Schwartz PJ, Priori SG, Bloise R, Napolitano C, Ronchetti E, Piccinini A, Goj C, Breithardt G, Schulze-Bahr E, Wedekind H, Naftoli J (2001) Molecular diagnosis in a child with sudden infant death syndrome. Lancet 358:1342–1343
8. Schwartz PJ (1976) Cardiac sympathetic innervation and the sudden infant death syndrome. Am J Med 60:167–172
9. Maron BJ, Clark CE, Goldstein RE, Epstein SE (1976) Potential role of Q-T interval prolongation in sudden infant death syndrome. Circulation 54:423–430
10. Kukolich MK, Telsey A, Ott J, Motulsky AG (1977) Sudden infant death syndrome: normal Q-T interval in ECGs of relatives. Pediatrics 60:51–54
11. Southall DP (1983) Identification of infants destined to die unexpectedly during infancy: evaluation of predictive importance of prolonged apnoea and disorders of cardiac rhythm or conduction. Br Med J 286:1092–1096
12. Southall DP, Arrowsmith WA, Stebbens V, Alexander JR (1986) QT interval measurements before sudden infant death syndrome. Arch Dis Child 61:327–333
13. Schwartz PJ, Stramba-Badiale M, Segantini A, Austoni P, Bosi G, Giorgetti R, Grancini F, Marni ED, Perticone F, Rosti D, Salice P (1998) Prolongation of the QT interval and the sudden infant death syndrome. N Engl J Med 338:1709–1714
14. Schwartz PJ, Montemerlo M, Facchini M, Salice P, Rosti D, Poggio G, Giorgetti R (1982) The QT interval throughout the first 6 months of life: a prospective study. Circulation 66:496–501
15. Ackerman MJ, Siu BL, Sturner WQ, Tester DJ, Valdivia CR, Makielski JC, Towbin JA (2001) Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. JAMA 286:2264–2269
16. Ackerman MJ, Tester DJ, Bell CM, Towbin JA, January CT, Makielski JC, Sturner WQ (2002) Cardiac channel mutations in SIDS: a population-based molecular study in 93 SIDS victims. Circulation 106(suppl II):II–167
17. Christiansen M, Tonder N, Larsen LA, Andersen PS, Simonsen H, Oyen N, Kanters JK, Jacobsen JR, Fosdal I, Wettrell G, Kjeldsen K (2005) Mutations in the HERG K+-ion channel: a novel link between long QT syndrome and sudden infant death syndrome. Am J Cardiol 95:433–434
18. Brooks DR, Krous HF, Burton JL, McKinley J, McKinley W (1994) Results of a pilot test of the global strategy workshop’s international standardized autopsy protocol (SAP) in Georgia. In: Sudden infant death syndrome (SIDS), 3rd SIDS International Conference, Stavanger, Norway 31.07.-04.08.1994 Program and abstracts, p 155
19. Bajanowski T, Fürst P, Wilmers K, Beike J, Köhler H, Brinkmann B (2002) Dioxin in infants—an environmental hazard? Int J Leg Med 116:27–32
20. Beckwith J (1970) Discussion of terminology and definition of the sudden infant death syndrome. University of Washington Press, Seattle
21. Willinger M, James LS, Catz C (1991) Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development. Pediatr Pathol 11:677–684
22. Taylor EM, Emery JL (1990) Categories of preventable unexpected infant deaths. Arch Dis Child 65:535–539
23. Loddenkötter B, Becker K, Hohoff C, Brinkmann B, Bajanowski T (2005) Real-time quantitative PCR assay for the detection of Helicobacter pylori: no association with sudden infant death syndrome. Int J Leg Med 119:202–206
24. Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513
25. Higuchi R (1989) Simple and rapid preparation of samples for PCR. In: Ehrlich HA (ed) PCR-technology: principles and applications for DNA amplification, Chapter 4. Stockton Press, New York, pp 31–38
26. Chou Q, Russell M, Birch DE, Raymond J, Bloch W (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. Nucleic Acids Res 20:1717–1723
27. Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) “Touchdown” PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19:4008
28. Wang Q, Zhizhong L, Jiaxiang S, Keating MT (1996) Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics 34:9–16

29. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT (1998) Genomic structure of three long QT syndrome genes: KCNQ1, HERG, and KCNE1. Genomics 51:86–97
30. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SAN (1999) MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 3:175–187
31. Findeisen M, Vennemann M, Brinkmann B, Ortmann C, Röse I, Köpcke W, Jorch G, Bajanowski T, and the GeSID Group (2004) German study on sudden infant death (GeSID): design, epidemiological and pathological profile. Int J Leg Med 118:163–169
32. Moss AJ, Schwartz PJ (1982) Delayed repolarization (QT or QTU prolongation) and malignant ventricular arrhythmias. Mod Concepts Cardiovasc Dis 51:85–90
33. Paulussen AD, Gilissen RA, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJ, Schulze-Bahr E, Haverkamp W, Breithardt G, Cohen N, Aerssens J (2004) Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. J Mol Med 82:182–188
34. Kääb S, Schulze-Bahr E (2005) Susceptibility genes and modifiers for cardiac arrhythmias. Cardiovasc Res (in press)
35. Chevalier P, Rodriguez C, Bontemps L, Miquel M, Kirkorian G, Rousson R, Potet F, Schott JJ, Baro I, Touboul P (2001) Non-invasive testing of acquired long QT syndrome: evidence for multiple arrhythmogenic substrates. Cardiovasc Res 50: 386–398
36. Sesti F, Abbott GW, Wei J, Murray KT, Saksena S, Schwartz PJ, Priori SG, Roden DM, George AL Jr, Goldstein SA (2000) A common polymorphism associated with antibiotic-induced cardiac arrhythmia. Proc Natl Acad Sci U S A 97:10613–10618
37. Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M, Murray KT, Norris K, George AL Jr, Roden DM (2002) Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. Circulation 105:1943–1948
38. Eskdale J, Keijsers V, Huizinga T, Gallagher G (1999) Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. Genes Immun 1:151–155
39. Narita N, Narita M, Takashima S, Nakayama M, Nagai T, Okado N (2001) Serotonin transporter gene variation is a risk factor for sudden infant death syndrome in the Japanese population. Pediatrics 107:690–692
40. Priori SG, Napolitano C, Giordano U, Collisani G, Memmi M (2000) Brugada syndrome and sudden cardiac death in children. Lancet 355:808–809
41. Romano C, Gemme G, Pongiglione R (1963) Aritmie cardiace rare delleta pedriatica: accessi per fibrillazione ventricolare parossistica. Clin Pediatr 45:656–683
42. Ward OC (1964) A new familial cardiac syndrome in children. J Ir Med Assoc 54:103–109
43. Wedekind H, Smits JP, Schulze-Bahr E, Arnold R, Veldkamp MW, Bajanowski T, Borggrefe M, Brinkmann B, Warnecke I, Funke H, Bhuiyan ZA, Wilde AA, Breithardt G, Haverkamp W (2001) De novo mutation in the SCN5A gene associated with early onset of sudden infant death. Circulation 104:1158–1164
44. Schulze-Bahr E, Fenge H, Etzrodt D, Haverkamp W, Mönnig G, Wedekind H, Breithardt G, Kehl HG (2004) Long QT syndrome and life threatening arrhythmia in a newborn: molecular diagnosis and treatment response. Heart 90:13–16
45. Iwasa H, Kurabayashi M, Nagai R, Nakamura Y, Tanaka T (2001) Multiple single-nucleotide polymorphisms (SNPs) in the Japanese population in six candidate genes for long QT syndrome. J Hum Genet 46:158–162
46. Tesson F, Donger C, Denjoy I, Berthet M, Bennaceur M, Petit C, Coumel P, Schwarts K, Guicheney P (1996) Exclusion of KCNE1 (IsK) as a candidate gene for Jervell and Lange–Nielsen syndrome. J Mol Cell Cardiol 28:2051–2055
47. Larsen LA, Christiansen M, Vuust J, Andersen PS (1999) High-throughput single-strand conformation polymorphism analysis by automated capillary electrophoresis: robust multiplex analysis and pattern-based identification of allelic variants. Human Mutat 13:318–327
48. Akimoto K, Furutani M, Imamura S, Furutani Y, Kasanuki H, Takao A, Momma K, Matsuoka R (1998) Novel missense mutation (G601S) of HERG in a Japanese long QT syndrome family. Human Mutat Suppl 1:S184–S186
49. Lee MP, Hu RJ, Johnson LA, Feinberg AP (1997) Human KVLQT1 gene shows tissue-specific imprinting and encompasses Beckwith–Wiedemann syndrome chromosomal rearrangements. Nat Genet 15:181–185
50. Lai LP, Deng CL, Moss AJ, Kass RS, Liang C (1994) Polymorphism of the gene encoding a human minimal potassium ion channel (minK). Gene 151:339–340