Genetic analysis identifies the *SLC4A3* anion exchanger as a major gene for short QT syndrome

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BACKGROUND A variant in the *SLC4A3* anion exchanger has been identified as a novel cause of short QT syndrome (SQTS), but the clinical importance of *SLC4A3* as a cause of SQTS or sudden cardiac death remains unknown.

OBJECTIVE The purpose of this study was to investigate the prevalence of potential disease-causing variants in SQTS patients using gene panels including *SLC4A3*.

METHODS In this multicenter study, genetic testing was performed in 34 index patients with SQTS. The pathogenicity of novel *SLC4A3*-variants was validated in a zebrafish embryo heart model.

RESULTS Potentially disease-causing variants were identified in 9 (26%) patients and were mainly (15%) located in *SLC4A3*: 4 patients heterozygous for novel nonsynonymous *SLC4A3* variants—p.Arg600Cys, p.Arg621Trp, p.Glu852Asp, and p.Arg952His—and 1 patient with the known p.Arg370His variant. In other SQTS genes, potentially disease-causing variants were less frequent (2× in *KCNQ1*, 1× in *KCNJ2*, and *CACNA1C* each). *SLC4A3* variant carriers (n = 5) had a similar heart rate but shorter QT and J point to T wave peak intervals than did noncarriers (n = 29). Knockdown of *slc4a3* in zebrafish resulted in shortened heart rate–corrected QT intervals (calculated using the Bazett formula) that could be rescued by overexpression of the native human *SLC4A3*-encoded protein (AE3), but neither by the mutated AE3 variants p.Arg600Cys, p.Arg621Trp, p.Glu852Asp nor by p.Arg952His, suggesting pathogenicity of these variants. Dysfunction in *slc4a3*/AE3 was associated with alkaline cytosol and shortened action potential of cardiomyocytes.

CONCLUSION In about a quarter of patients with SQTS, a potentially disease-causing variant can be identified. Nonsynonymous variants in *SLC4A3* represent the most common cause of SQTS, underscoring the importance of including *SLC4A3* in the genetic screening of patients with SQTS or sudden cardiac death.

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Introduction

Short QT syndrome (SQTS) is a rare inherited cardiac disease associated with a high risk of ventricular and atrial arrhythmias and sudden cardiac death (SCD).[1,2] The disease often aggregates in families, but a disease-causing genetic variant is identified only in ≈20% of index patients.[3] This indicates that yet undetected causal genetic variants exist. Variants causing SQTS have been located in genes encoding cardiac cation channels. This is considered to cause an accentuated potassium efflux,[4–6] or an attenuated calcium influx,[7] which results in a shortening of the ventricular action potential as reflected by the shortening of the QT interval. We have recently identified a variant in the *SLC4A3* anion exchanger gene as a cause of SQTS.[8] *SLC4A3* encodes the Cl^-/HCO_3^- exchanger 3 (AE3), which transports Cl^- into the cardiomyocyte in exchange for HCO_3^-. The identified *SLC4A3* variant p.Arg370His reduces the Cl^-/HCO_3^- exchange over the cell membrane, leading to an increase in intracellular pH (pH_i), which in combination with a decrease in $[Cl^-]_i$ shortens the heart rate–corrected QT interval.[8] Since the discovery of *SLC4A3* as a cause of SQTS, genome-wide association studies have further linked *SLC4A3* to QT interval length as well as QT dynamics in response to exercise.[9,10] However, to date the prevalence of pathogenic variants in *SLC4A3* has not been reported and therefore the clinical importance of *SLC4A3* variants as a cause of SQTS or SCD remains unknown. We hypothesized that variants in the *SLC4A3* anion exchanger might be a previously overlooked cause of SQTS. To address this, we investigated the prevalence of potential disease-causing variants using gene panels including *SLC4A3* in a uniquely large German-Danish cohort of unrelated patients with SQTS and further investigated potentially disease-causing *SLC4A3* variants in a zebrafish embryo heart model.

Methods

Patient population with SQTS

This was a cross-sectional study from national tertiary centers in Germany (Münster) and Denmark (Aarhus and Copenhagen) with specialized functions in inherited cardiac diseases. We identified patients with suspected SQTS in our clinics and included all index patients fulfilling a clinical diagnosis of SQTS according to the European Society of Cardiology 2015 guideline criteria.[11] Thus, all included patients had a QT_c interval of ≤340 ms or a QT_c interval of ≤360 ms combined with at least one of the following criteria: (1) presence of a SQTS gene variant previously established as pathogenic, (2) a family history of SCD, or (3) a documented episode of ventricular tachycardia or ventricular fibrillation.

A 12-lead resting electrocardiogram (ECG) was obtained in all patients, and the R-R interval, QT interval, and J point to T wave peak interval were recorded. The QT interval was measured using the tangent method in the precordial lead presenting the highest T-wave amplitude, and the QT_c interval was calculated using the Bazett formula.[12] The J point was defined as the end of the QRS complex, and the T peak was measured at the highest point of the T wave.[12] Patient information was obtained from medical records.

The study complies with the Declaration of Helsinki. All study patients gave informed consent for genetic sequencing. The study was approved by the ethics committee in the Central Denmark Region (record no. 1-10-72-189-16) and local ethics committees. The zebrafish studies were performed in accordance with institutional guidelines.

Bioinformatic analysis

The DNA sequencing process is provided in the Online Supplement. Genes of interest were filtered and analyzed using the MOMA Heart Panel v4 (https://moma.dk/files/MOMA_Heartpanel.v4.2018-08-15.pdf) (n = 23 patients), the TruSight Cardio Sequencing Panel + *SLC4A3* (https://www.illumina.com/content/dam/illumina-marketing/documents/clinical/rgh/gene-list.xlsx) (n = 6 patients), or a customized SQTS gene panel comprising *KCNQ1*, *KCNH2*, *KCNJ2*, and *SLC4A3* (n = 5 patients) according to the clinical practice at each of the centers. Established (ie, disease-validated) genes for SQTS in the TruSight Cardio Sequencing Panel include *KCNH2*, *KCNQ1*, *KCNJ2*, and some genes suggested to be implicated in SQTS, although they have recently been disputed as being causative (*CACNA1C*, *CACNB2*, and *CACNA2D1*).[13] The MOMA NGS Heart Panel v4 includes all of the above plus *SLC4A3*. All variants were manually assessed and classified according to the American College of Medical Genetics (ACMG) criteria.[14]

Variants were classified as potentially disease causing when scored as pathogenic or likely pathogenic according to the ACMG criteria, but variants of uncertain significance in any of the SQTS genes were also considered as potentially disease causing despite the absence of additional information (eg, familial inheritance) that may upgrade those.

Zebrafish embryo experiments

All zebrafish experiments were performed at early developmental stages before they become recognized as experimental animals in agreement with EU Directive 2010/63/EU and according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Zebrafish were housed in accordance with published recommendations.[15] Details on animal housing and husbandry, animal model generation and validation, as well as phenotypic analyses are provided in the Online Supplement including Online Supplemental Figure S1.

Statistical analyses

Data are presented as mean ± SD, median (interquartile range), or number (percentage), and figure data are shown as individual data points with mean ± 1 standard error of the mean. All continuous variables were tested for normality, and the significance of difference between groups was tested using the Wilcoxon rank-sum test, Student *t* test, one-way analysis of variance with the Tukey posttest, χ^2 test, or Fisher exact test, as appropriate. Statistical analyses were performed using Stata/IC 13.1 (StataCorp LLC, College Station, TX) and GraphPad Prism 9 (GraphPad Software, San Diego, CA).

Results

Clinical data on patients with SQTS

We identified 47 index patients with suspected SQTS. Eleven patients had a "borderline" clinical presentation and did not meet the full European Society of Cardiology 2015 guideline criteria for SQTS and were therefore excluded. Furthermore, 2 patients were excluded because they have previously been reported in the discovery study of *SLC4A3*.[8] Among the remaining 34 index patients with SQTS, the mean QT_c interval was 333 ± 19 ms and 20 patients (59%) had a QT_c interval of ≤340 ms (Table 1). One patient with SQTS had an ECG with signs of early repolarization but no pathogenic variants were identified (sequenced using the MOMA NGS Heart Panel v4). All patients had normal echocardiograms except 1 patient with a secundum atrial septum defect and reduced left ventricular function.

A potentially disease-causing variant was identified in 9 patients (26%). Five variants were localized in *SLC4A3* (15% of patients) including 4 novel variants (c.1798C>T (p.Arg600Cys), c.1861C>T (p.Arg621Trp), c.2556G>C (p.Glu852Asp), and c.2855G>A (p.Arg952His)), which were classified as variants of uncertain significance according to the ACMG criteria. A detailed list of the 5 patients and corresponding variants is provided in Table 2 and Online Supplemental Table S1 (ECGs are provided in Online Supplemental Figures S2–S6). In the case of the Danish proband (Table 2, case 34), information from a brother was also available. This brother had a QT_c interval of 320 ms, atrial fibrillation and was found also to harbor the same c.2855G>A (p.Arg952His) variant. None of the patients with a potentially disease-causing variant carried ≥1 potentially disease-causing variant. Since rare missense variants are relatively common in *SLC4A3* (599 high-quality missense variants reported in the gnomAD (v2.1.1) allele frequency database at a frequency rate of ≤0.004%), we compared the rate of *SLC4A3* missense variants among patients with SQTS to 3528 in-house samples previously analyzed in our laboratory for hereditary diseases including various heart (≈5%), liver, kidney, neurological, and cancer diseases. There were 63 missense variants (1.7%) with an in-house allele frequency of <0.01, which was significantly lower than the rate of 5 (15%) observed in patients with SQTS ($P < .001$). Patients with *SLC4A3* variants had shorter QT and J point to T wave peak intervals than the remaining patients with SQTS. Other patient characteristics were comparable between patients with and without *SLC4A3* variants (Table 1).

In vivo analysis of novel *SLC4A3* variants

Loss of AE3 function by *slc4a3* knockdown in zebrafish embryos was previously reported to result in shortened QT_c interval and shortened systolic duration, which was rescued by overexpression of wild-type AE3 but not by the short QT interval–associated AE3 variant Arg370His.[8] We applied a similar strategy to determine the in vivo consequences of the 4 novel AE3 variants on cardiac function. Reverse transcription polymerase chain reaction analysis indicated efficient knockdown and similar in vivo stability of microinjected wild-type and variant AE3 messenger RNA 2 days postfertilization (Online Supplemental Figures S7A and S7B), suggesting that any potential loss of in vivo functionality compared to wild-type AE3 is not a result of impaired messenger RNA stability. All ECG recordings were blinded before analysis, and neither *slc4a3* knockdown nor co-overexpression of wild-type or variant AE3 affected the heart rate (Online Supplemental Figures S7C and S7G).

Table 1 Characteristics of patients with SQTS

Characteristic	All patients (N = 34)	*SLC4A3* variant present (n = 5)	*SLC4A3* variant absent (n = 29)	*P*
Age (y)	31 ± 12	30 ±9	31 ± 12	.88
Male sex	30 (88)	4 (80)	26 (90)	.49
Family history of sudden cardiac death	8 (25)	2 (40)	6 (22)	.58
Documented ventricular arrhythmia	19 (56)	3 (60)	16 (55)	>.99
ICD implanted	22 (65)	3 (60)	19 (66)	>.99
Heart rate (beats/min)	57 ± 12	63 ± 9	56 ± 12	.25
QT interval (ms)	346 ± 40	312 ± 11	352 ± 40	.0043
QT_c interval (ms)	333 ± 19	319 ± 20	335 ± 18	.075
Jp-Tp interval (ms)	217 ± 38	178 ± 26	224 ± 36	.022
NGS with coverage ≥30× (%)	99.5 (99.4–99.5)	99.3 (99.3–99.5)	99.5 (99.4–99.5)	.52

Values are presented as mean ± SD, median (interquartile range), or n (%).

Family history of sudden cardiac death <40 y was unknown in 2 patients. The Jp-Tp interval was missing in 6 patients. NGS with coverage ≥30× was missing in 2 patients.

ICD = implantable cardioverter-defibrillator; Jp-Tp = J point to T wave peak; NGS = next-generation sequencing; QT_c interval = heart rate–corrected QT interval (calculated using the Bazett formula); SQTS = short QT syndrome.

Table 2 Identified genetic variants

Case no.	Nationality	Sex (age at diagnosis, y)	FHx of SCD	Documented VT/VF (age, y)	HR	QT interval (ms)	QT_c interval (ms)	Comorbidity	Echocardiography	Medication	ICD implanted (age, y)	Gene	Reference sequence	Nucleotide	Protein change	MAF gnomAD v2.1.1	Coding effect	ACMG criteria*	Pathogenicity (ACMG)*	Sequencing panel
6	German	M (42)	No	No	58	320	317	None	N/A	None	No	*SLC4A3*	NM_201574.2	c.1861C>T	p.(Arg621Trp)	8.85×10^{-6}	Nonsynonynous	PM2 (supporting) PP3 (supporting)	VUS	MOMA Heart Panel v4
14	German	M (37)	No	Yes (37)	72	310	342	None	N/A	Chinidine 200 mg/d	Yes (37)	*KCNQ1*	NM_000218.2	c.1193A>G	p.(Lys398Arg)	0	Nonsynonynous	PM2 (moderate)	VUS	MOMA Heart Panel v4
18	German	F (18)	Yes	Yes (18)	50	320	292	None	Normal	None	Yes (18)	*SLC4A3*	NM_201574.2	c.2556G>C	p.(Glu852Asp)	0	Nonsynonynous	PM2 (moderate)	VUS	MOMA Heart Panel v4
23	German	M (25)	No	Yes (25)	70	300	324	None	Normal	Bisoprolol 2.5 mg/d	Yes (25)	*SLC4A3*	NM_201574.2	c.1109G>A	p.(Arg370His)	0	Nonsynonynous	PP1 (strong) PS3 (moderate) PM2 (moderate) PP4 (supporting) PP3 (supporting)	P	Custom SQTS panel
30	German	F (17)	Yes	Yes (17)	56	380	333	None	Normal	None	Yes (17)	*KCNQ1*	NM_000218.2	c.859G>A	p.(Ala287Thr)	2.18×10^{-4}	Nonsynonynous	PM1 (moderate) PS3 (moderate) PM2 (supporting) PP3 (supporting) BP5 (supporting)	VUS	Custom SQTS panel
31	German	F (8)	No	No	103	210	275	Early birth, psychomotoric retardation, low muscle tone	Normal	None	Yes (8)	*KCNJ2*	NM_000891.2	c.902T>G	p.(Met301Arg)	0	Nonsynonynous	PM2 (moderate) PS3 (moderate) PM5 (moderate) PP3 (supporting)	LP	Custom SQTS panel
32	German	M (32)	No	No	57	330	322	Autism, affective disorder, severe dental enamel defects	Normal	Chinidine 600 mg/d	No	*CACNA1C*	NM_000719.6	c.2399A>C	p.(Lys800Thr)	9.60×10^{-6}	Nonsynonynous	PS3 (moderate) PM2 (supporting)	VUS	Custom SQTS panel
33	German	M (20)	No	Yes (20)	66	300	315	None	Normal	Amiodarone 200 mg/d	Yes (20)	*SLC4A3*	NM_201574.2	c.1798C>T	p.(Arg600Cys)	0	Nonsynonynous	PM2 (moderate) PP3 (supporting)	VUS	TruSight Cardio Sequencing Panel
34	Danish	M (37)	Yes	No	71	320	349	None	Normal	None	No	*SLC4A3*	NM_201574.2	c.2855G>A	p.(Arg952His)	0	Nonsynonynous	PM2 (moderate) PP3 (supporting)	VUS	MOMA Heart Panel v4

ACMG = American College of Medical Genetics; F = female; FHx = family history of sudden cardiac death <40 y; HR = heart rate; ICD = implantable cardioverter-defibrillator; LP = likely pathogenic (according to the ACMG criteria); M = male; MAF = minor allele frequency; N/A = not available; P = pathogenic (according to the ACMG criteria); QT_c interval = heart rate–corrected QT interval (calculated using the Bazett formula); SCD = sudden cardiac death; SQTS = short QT syndrome; VF = ventricular fibrillation; VT = ventricular tachycardia; VUS = variant of unknown significance (according to the ACMG criteria).
*ACMG criteria were applied on the basis of the published evidence and not including the functional studies presented in this study.

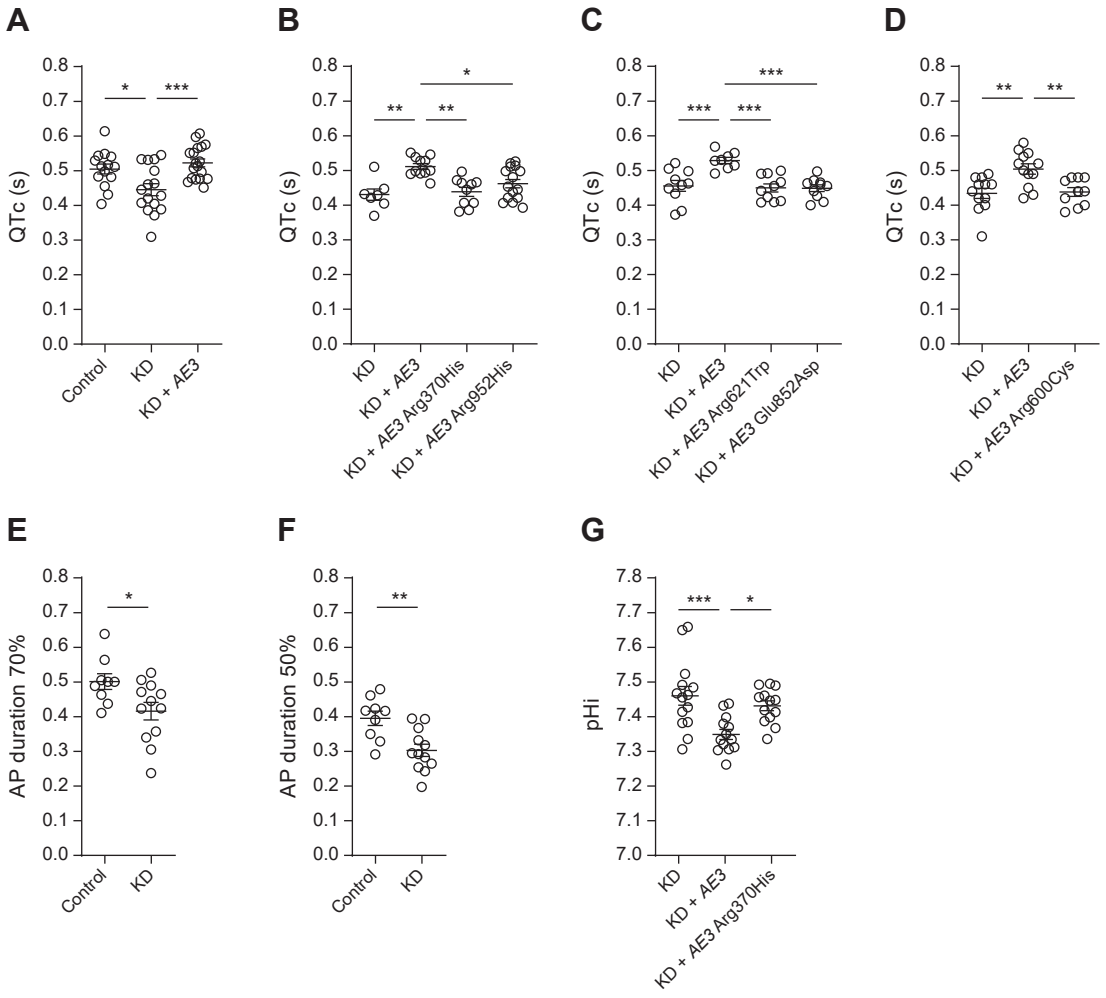

Figure 1 Short QT syndrome–associated *SLC4A3* variants are unable to normalize short heart rate–corrected QT (QT_c) interval in *slc4a3* knockdown zebrafish embryos. **A–D:** QT_c interval is shortened by *slc4a3* knockdown and normalized by coinjected messenger RNA (mRNA) encoding wild-type but not variant human AE3. Plotted data represent QT_c interval of individual zebrafish embryos microinjected with either standard control morpholino (Control), *slc4a3* targeted morpholino (KD), or *slc4a3* targeted morpholino in combination with mRNA encoding human wild-type or mutated AE3 as indicated. All electrocardiographic recordings were blinded before analysis. **A:** Control (n = 14; mean 0.5049 ± 0.0142), knockdown (n = 16; mean 0.4454 ± 0.0172), and knockdown + AE3 (n = 18; mean 0.5226 ± 0.0112). **B:** Knockdown (n = 7; mean 0.4305 ± 0.0163), knockdown + AE3 (n = 11; mean 0.5115 ± 0.0851); knockdown + AE3 Arg370His (n = 10; mean 0.4383 ± 0.0127), and knockdown + AE3 Arg952His (n = 15; mean 0.4622 ± 0.0121). **C:** Knockdown (n = 10; mean 0.4555 ± 0.0155), knockdown + AE3 (n = 8; mean 0.5288 ± 0.0088), knockdown + AE3 Arg621Trp (n = 10; mean 0.4497 ± 0.0114), and knockdown + AE3 Glu852Asp (n = 10; mean 0.4489 ± 0.0094). **D:** Knockdown (n = 12; mean 0.4342 ± 0.0146), knockdown + AE3 (n = 12; mean 0.5042 ± 0.0146), and knockdown + AE3 Arg600Cys (n = 10; mean 0.4380 ± 0.0124). **E and F:** Action potential (AP) duration is shortened by *slc4a3* knockdown. Plotted data represent AP duration of individual isolated embryonic zebrafish hearts. All AP recordings were blinded before analysis. **E:** $AP_{70\%}$. Control (n = 9; mean 0.5010 ± 0.0226) and knockdown (n = 12; mean 0.4160 ± 0.0255). **F:** $AP_{50\%}$. Control (n = 9; mean 0.3954 ± 0.0206) and knockdown (n = 12; mean 0.3030 ± 0.0176). **G:** AE3 Arg370His is unable to reduce intracellular pH (pH_i) in *slc4a3* knockdown embryos. Knockdown (n = 14; mean 7.456 ± 0.0276), knockdown + AE3 (n = 13; mean 7.344 ± 0.0146), and knockdown + AE3 Arg370His (n = 13; mean 7.427 ± 0.0140). Data in each plot were compiled from 3–4 independent experiments, each including all experimental groups. $*P < .05$, $**P < .01$, $***P < .001$ as determined by one-way analysis of variance with the Tukey posttest. Data are presented including mean ± 1 standard error of the mean.

However, knockdown of *slc4a3* resulted in shortened QT_c interval, which was rescued from the level of knockdown by overexpression of human wild-type AE3 ($P = .0008$) (Figure 1A and Online Supplemental Figure S7G), whereas the Arg370His variant was unable to rescue the shortened QT_c interval ($P = .98$) (Figure 1B and Online Supplemental Figure S7G) as previously reported.[8] Similarly, neither Arg952His ($P = .34$), Arg621Trp ($P = .98$), Glu852Asp ($P = .98$) nor Arg600Cys ($P = .98$) mutated AE3 variants rescued the short QT phenotype from the level of knockdown (Figure 1B and Online Supplemental Figure S7G). Action potential (AP) duration was reduced in *slc4a3* knockdown embryos ($AP_{50\%}$: $P = .0029$; $AP_{70\%}$: $P = .027$) (Figures 1E and 1F and Online Supplemental Figure S8), substantiating the QT_c duration phenotype. We previously reported increased pH_i in *slc4a3* knockdown zebrafish embryo hearts and that increased pH_i caused shortening of action potential duration in rabbit hearts.[8] To assess the correlation of variant AE3 with pH_i, we measured pH_i in *slc4a3* knockdown zebrafish hearts with and without

overexpression of wild-type or Arg370His-mutated AE3 (Figure 1G). Whereas overexpression of wild-type AE3 decreased pH_i from the level of *slc4a3* knockdown ($P = .0010$), the Arg370His variant was unable to do so ($P = .57$), suggesting increased pH_i as a cause of short QT_c interval associated with this variant. Taken together, these data suggest impaired function and pathogenicity of the assessed variants.

Discussion

In the present study, we performed targeted next-generation sequencing in order to investigate the yield of genetic testing in patients fulfilling a diagnosis of SQTS according to the current European Society of Cardiology guideline criteria. First, within this large SQTS cohort (n = 34), we were capable of identifying a potentially disease-causing gene variant in about a quarter of patients (26%). With a focus on the recently identified *SLC4A3* gene, we found a potentially disease-causing variant in this gene in 15% of all patients, including 4 novel nonsynonymous variants. Since rare *SLC4A3* variants are relatively common in the population, we functionally investigated the novel variants. Using genetic modulation, we created zebrafish embryo heart models for all 4 variants and demonstrated their inability to normalize shortened QT_c interval induced by lack of AE3, in contrast to the normalizing effect of injecting native AE3. Furthermore, increased pH_i resulted not only from AE3 deficiency but also from the patient-derived AE3 Arg370His variant, substantiating altered pH_i as a cause of the SQTS phenotype in agreement with previous observations.[8] Therefore, we estimate that variants in *SLC4A3* explain ≈15% of all SQTS cases (19% if the complete cohort of patients with SQTS was considered by including the 2 patients from the discovery study of *SLC4A3*[8]), indicating that *SLC4A3* is the gene most frequently involved in SQTS.

The majority of previous reports on genetic testing in SQTS have been published as case reports, but few studies have investigated the yield of genetic testing in smaller cohorts. Giustetto et al[1] studied 22 index patients with SQTS and reported a presumed disease-causing variant in 5 patients (23%), of whom 4 variants were located in *KCNH2* and 1 in *CACNB2b*.[1] Villafane et al investigated a pediatric population of index patients with a moderate-to-high probability of SQTS according to the Gollob criteria[16] and found a presumed disease-causing variant in 5 of 21 patients (24%) (2 variants in *KCNH2* and *KCNJ2*, respectively, and 1 in *KCNQ1*).[17] The largest cohort of index patients with SQTS to date was later published by Mazzanti et al,[2] who found a presumed disease-causing variant in 6 of 45 patients (13%) (2 located in *KCNH2*, 2 in *KCNJ2*, 1 in *KCNQ1*, and 1 in *CACNA1C*). Whereas these studies sequenced primarily potassium ion channel genes (*KCNH2*, *KCNJ2*, and *KCNQ1*), and to a lesser extent the questioned SQTS-related calcium channel genes (*CACNA1C* and *CACNB2b*), the present study is the first to include the anion exchanger gene *SLC4A3*. We demonstrate that the yield of sequencing this gene is significantly higher than the yield obtained from sequencing other SQTS genes, which establishes *SLC4A3* as the most common cause of SQTS to date and underscores the importance of including *SLC4A3* in the genetic screening of patients with SQTS or SCD. It should be noted that the variant rate in potassium channel genes was somewhat lower in our study than what has previously been reported by Giustetto et al[1] and Villafane et al.[17] However, Giustetto et al included already published discovery case reports in their study that might inflate the estimated yield and Villafane et al investigated a somewhat different population, which possibly contributes to the differences in estimates.

It is interesting to note that the QT interval was significantly shorter in *SLC4A3* variant carriers than in *SLC4A3* nonvariant carriers. The same observation has also been described in carriers of disease-causing SQTS gene variants in *KCNH2*.[1] One explanation may be a generally stronger contribution to QT interval length from Mendelian inherited disease-causing SQTS variants compared with a more polygenic contribution to QT interval length in noncarriers, a phenomenon that has been observed in other genetic conditions such as familial hypercholesterolemia.[18] Another possible explanation may be that *SLC4A3* causes SQTS through a different molecular mechanism where loss of protein function gives rise to a stronger impact on the QT interval. In prior reports, phenotypic variations have been observed among the SQTS subtypes, including a higher risk of atrial arrhythmias in *KCNQ1* variant carriers and a marked response to hydroxychloroquine treatment in *KCNH2* variant carriers,[1,19] and in that respect it is also interesting whether the *SLC4A3* SQTS phenotype differs from other SQTS subtypes. However, future longitudinal studies are needed in order to shed light on the outcome.

Despite the significant incremental yield obtained from sequencing *SLC4A3*, the SQTS phenotype remains unexplained in the majority of patients. This stands in contrast to the fact that familial aggregation of SQTS, and thus a likely genetic component in the pathogenesis of the disease, has been reported in approximately half of patients with SQTS.[2] These findings indicate the existence of yet unidentified SQTS genes and/or the presence of a polygenic component contributing to SQTS development. Prior identifications of novel SQTS-related genes have primarily been candidate gene driven,[4–7] suggesting a need for a broader approach to unravel disease-causing variants in unexplained SQTS cases. Given the phenotypic overlap of SQTS with Brugada syndrome,[20] they may potentially share a common genetic background. Moreover, a large number of genes associated with long QT syndrome (LQTS) have been identified,[21] and variants in genes associated with LQTS may also exhibit opposite effects leading to SQTS, as is the case for the 3 SQTS-related potassium channel genes (*KCNH2*, *KCNJ2*, and *KCNQ1*), where *loss-of-function* variants have been associated with LQTS whereas *gain-of-function* variants have been associated with SQTS.[21] However, this is not the explanation for the significant number of unexplained

SQTS probands in our study since the majority of patients were sequenced using panels covering already established genes associated with Brugada syndrome and LQTS. Recent genome-wide association studies of QT interval length have identified a number of novel candidate genes related to sodium, potassium, and calcium ion regulation as well as autonomic control of the QT interval that may also be involved in the development of SQTS.[9,10,22] Furthermore, these studies highlight the influence of a polygenic contribution to QT interval length. Thus, it is possible that the accumulation in 1 individual of several genetic variants that each causes a small shortening of the QT interval could also account for a proportion of the remaining unexplained SQTS cases (ie, the extreme of a quantitative trait distribution).

Limitations

The present study constitutes one of the largest studies to date investigating the genetic yield in SQTS, a rare arrhythmia phenotype associated with SCD, but a number of limitations deserve attention. All patients were recruited from tertiary referral centers, which may have introduced selection bias since less clinically affected patients with SQTS may have been underdiagnosed or refrained from referral. Except for the Danish patient with a *SLC4A3* variant, we only had access to index patient information, and therefore segregation data from affected family members were not available to support variant pathogenicity. However, our study has been strengthened by in vivo demonstration of the QT_c-shortening effects of the 4 novel variants. We used different sequencing panels among the study patients including 5 patients who were sequenced for only 4 SQTS genes. Using a wider gene panel might lead to the identification of additional disease-causing variants. However, despite the fact that the majority of patients were sequenced using panels covering genes of potential interest, we found no patient with a pathogenic or likely pathogenic variant outside the established SQTS genes, suggesting a low additional yield of sequencing patients with SQTS by currently established cardiac sequencing panels.

We acknowledge that measurements of QT interval and action potential duration in zebrafish embryos are not universally validated methods and are technically challenging. However, on the basis of the mutually substantiating observations of QT interval and action potential duration obtained by technically independent phenotyping methods, we are confident in the soundness of the reported phenotypic observations. Furthermore, while our data show that increased pH_i is a result not only of AE3 deficiency but also of the patient-derived AE3 Arg370His variant, this does not allow us to extend this conclusion to all AE3 variants included in this article. Additional studies are required to establish the molecular mechanism(s) of AE3 variants and associated SQTS.

Conclusion

We identified *SCL4A3* as a major cause of SQTS (variant detection rate 15%) and further established an experimental zebrafish AE3 (−/−) model for SQTS in which the novel *SLC4A3* variants showed a lack to normalize shortened QT interval. This takes the total proportion of patients with an identified genetic cause of SQTS to about a quarter and establishes *SLC4A3* as the most common cause of SQTS to date. Thus, our findings highlight the importance of other than cation channel–driven mechanisms in SQTS and underscore the importance of including *SLC4A3* in the genetic screening of patients with SQTS or SCD.

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Appendix

Supplementary Data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrthm.2023.02.010.

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