Long QT and Silver Russell syndrome: First case report in a 9-year-old girl

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Introduction

Long QT syndrome (LQTS) is a genetic-based cause of sudden cardiac death. A prolonged QTc interval on electrocardiogram (ECG) produces a potentially dangerous substrate for ventricular tachycardia and syncope or sudden death.[1,2]

The genetics of LQTS have been well documented over the past decade, with the identification of more than 15 genes corresponding to different disease subtypes.[3] LQT1, the most common form, is caused by loss-of-function of *KCNQ1* gene, which encodes a protein that forms a critical part of a voltage-gated potassium channel.[4] In LQT1 syndrome, syncope or sudden death is often triggered by emotional or physical stress such as diving and swimming.[2]

The locus of KCNQ1 falls within a region of chromosome 11p that contains a cluster of genes subjected to imprinting. Genomic imprinting is the monoallelic expression of genes, dependent on the parental origin of the chromosome, which is known to play a vital role in growth and development. Imprinting disorders originate from a disturbance in this monoallelic expression by disruption or epimutation of imprinted genes.[5,6] Aberrant genomic imprinting of the 11p15 region has a pivotal role in both Silver Russell syndrome (SRS) and the Beckwith-Wiedemann syndrome (BWS).[7] The molecular bases of both syndromes are complex and involve the altered expression of multiple growth regulatory genes in the 11p15.5 genomic region. The SRS is characterized by growth failure, typical craniofacial features such as a prominent forehead and/or triangular face, hemihypotrophy, and clinodactyly. On the other hand, BWS is an overgrowth syndrome, the clinical and molecular mirror of SRS.[8]

Although both LQT1 and SRS loci are in chromosome 11p, this is the first time a child manifesting features of both syndromes has been described. We describe a patient with LQT, an SRS phenotype, and a *de novo* 11p15 duplication and the investigation into the molecular cause of this combination of phenotypes.

Case report

A 9-year-old girl was referred to the pediatric cardiology clinic with a murmur. She was a 27-week gestational age preterm newborn and her birthweight was 1030 g, which corresponded to a 50th percentile using the Fenton WHO preterm growth chart (Supplemental Figure S1A). During the first years of her life, she was diagnosed with postnatal growth and length restriction falling below 2 standard deviation (SD) using WHO growth charts (Supplemental Figure S1B and C) and mild hypercalciuria. Her parents and brother are healthy and have no apparent phenotype. Moreover, there were no cases of sudden cardiac death in a 3-generation family history.

An SRS diagnosis was established as she met 4 of 6 NH-CSS criteria.[9] She had postnatal growth restriction with low height, relative macrocephaly at birth, prominent forehead and a triangular face, and feeding difficulties, with a body mass index below 2 SD at 24 months. We did not observe hemihypotrophy or clinodactyly. Her cardiovascular examination revealed an innocent murmur, and she had a structurally normal heart on echocardiography. However, a 491 ms QTc interval was observed in the ECG performed during the visit (Figure 1). No electrolyte disturbances were identified on initial blood sample analysis. This abnormal QTc was observed in several subsequent ECGs. Moreover, a standing test demonstrated a QTc interval of 508 ms at the

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KEY TEACHING POINTS

- Silver Russell syndrome (SRS) is characterized by growth failure, typical craniofacial features such as a prominent forehead and/or triangular face, hemihypotrophy, and clinodactyly.
- We describe the first case where a combination of an imprinting defect and disruption of the *KCNQ1* gene can cause the dual phenotype of SRS and long QT syndrome (LQTS).
- Ventricular arrhythmia risk appears to be similar to type 1 LQTS and responds well to beta-blocker therapy.

point of maximal QT-interval stretching and it remained prolonged on return to baseline heart rate.

Based on these abnormal findings, the patient was started on atenolol treatment at 1 mg/kg/day.

Initial genetic testing using next-generation sequencing and the analysis of single nucleotide variants in 13 genes associated with LQTS (*AKAP9*, *ANK2*, *CACNA1C*, *CAV3*, *KCNE1*, *KCNE2*, *KCNH2*, *KCNJ2*, *KCNJ5*, *KCNQ1*, *SNC4B*, *SNC5A*, *SNTA1*) found no pathogenic variants. Following that result, array comparative genome hybridization was performed and a 2.6 Mb duplication was found in 11p15 gene (11:223,538 – 2,849,629, hg37). The duplication spanned 87 genes, including imprinting control region 1 (ICR1), the *IGF2* and *H19* genes, and the long noncoding, reverse-transcribed RNA, *KCNQ1OT1*. A schematic overview of the 11p15 region, showing the position of the duplication, is shown in Figure 2A. The duplication was subsequently confirmed and refined to single-exon resolution by copy number variant analysis on a different targeted next-generation sequencing gene panel (Trusight Cardio Panel; Illumina, San Diego, CA), containing the *KCNQ1* and *HRAS* genes. This analysis showed that the duplication breakpoint disrupts the *KCNQ1* gene, with exons 1–12 included in the duplication (Figure 2B). The inheritance of single nucleotide polymorphisms within the duplicated 11p15 region was analyzed to check the parental origin of the patient's duplication allele. Single nucleotide polymorphism rs12628 was informative, showing a skewed allelic balance for the maternal A allele in the patient (G-to-A ratio of 0.35).

We performed methylation-sensitive multiplex ligation-dependent probe amplification (Salsa ME030-C3 and Coffalyser.net; MRC Holland, Amsterdam, Netherlands)[10] on DNA from the patient and her parents as well as 6 healthy control samples, in order to test the methylation status at ICR1 and ICR2 (Figure 3). The duplication was again clearly visible (Figure 3A) and methylation analysis showed hypomethylation at ICR1 and hypermethylation at ICR2 in the patient (Figure 3B). This is visible as the decreased percentage of methylation, from 50% in normal blood and in the patient's parents (Figure 3C and D) to an average of 37.75% in the patient (Figure 3B). Hypermethylation was apparent

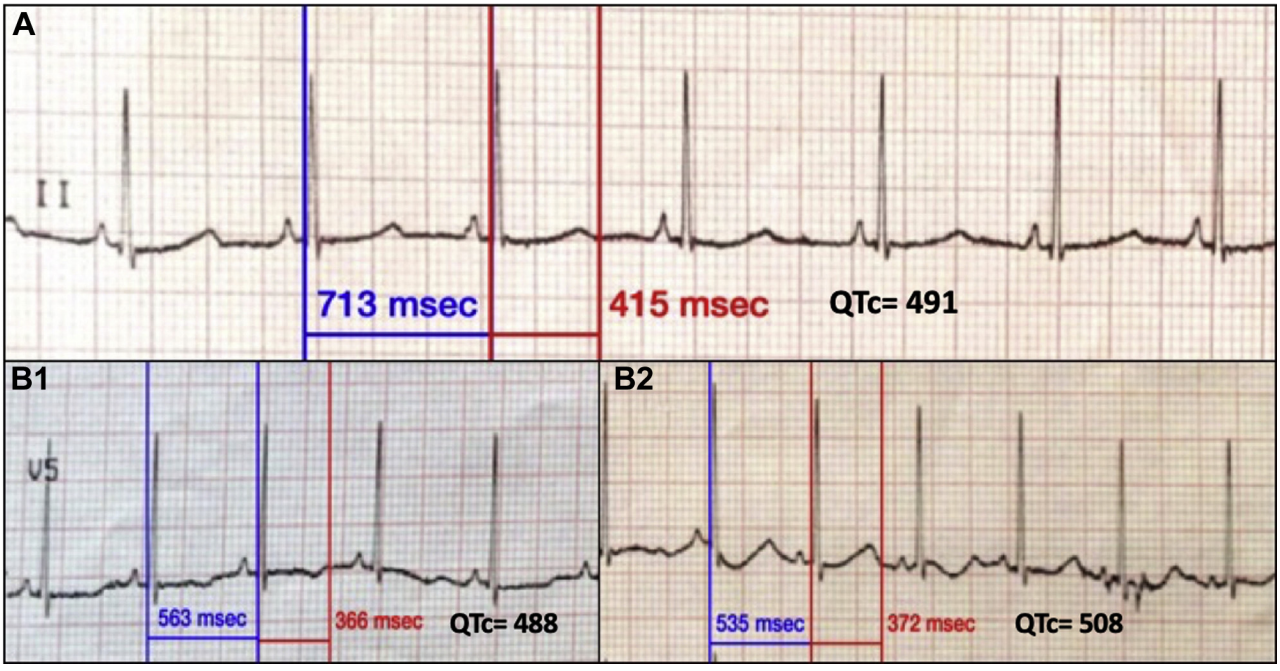

Figure 1 Baseline 12-lead electrocardiograms (ECG) and standing test demonstrating the point of maximal QT-interval stretching. **A:** The 12-lead ECG at baseline showed the R-R interval measured in lead II is 713 ms, the QT interval is 415 ms, and the corrected QT interval (QTc) is 491 ms. **B1:** Baseline standing test measured in V_5 showed that the R-R interval is 562 ms, the QT interval is 366 ms, and the QTc is 488 ms with a negative T wave. **B2:** The patient then stands up and at the point of maximal QT-interval stretching the R-R interval is 534 ms, the QT interval is 372 ms, and the QTc is 508 ms and the T wave has a different upright morphology.

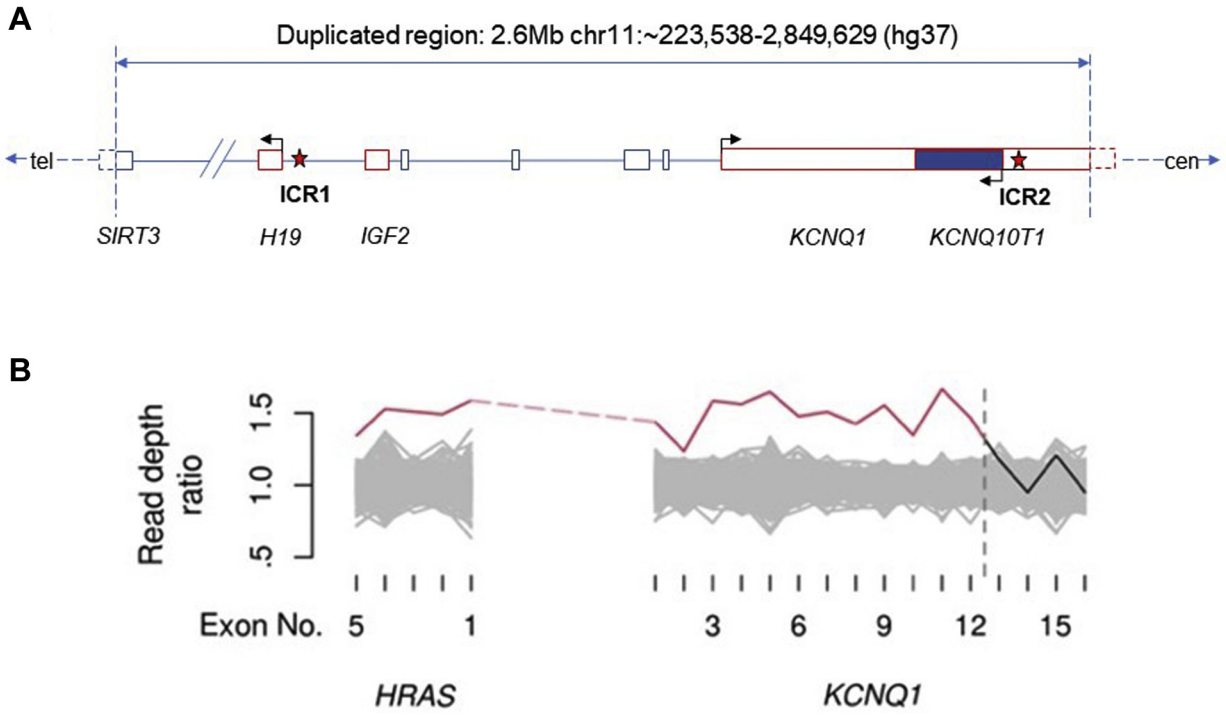

Figure 2 Schematic overview of the 11p15.5 region (A) and the result of copy number variant analysis after targeted next-generation sequencing (NGS) in the patient (B). The chromosome 11p15 duplication affecting the *HRAS* and *KCNQ1* genes, these being the only 11p15 genes on the targeted NGS panel, together with the duplication breakpoint in intron 12 are shown.

at ICR2, with an average of 72% methylation across the 4 probes in the patient (Figure 3B), as opposed to the normal 50% methylation shown in her parents (Figure 3C and D). Hypomethylation in the patient at ICR1 was further illustrated when the mean of the methylation ratios across 4 ICR1 probes in the control samples (SD $\leq$0.03) was compared with the values in the patient (Figure 3E). Despite the relatively low statistical power owing to the small sample number, if one assumes a normal distribution of the control values, the difference between the control and patient values is highly significant (SD $>$5, P =10e-6).

A loss-of-function of the *KCNQ1* gene, caused by the duplication breakpoint interrupting the gene, is consistent with the patient's phenotype on ECG. After that result, the patient was switched to nadolol owing to atenolol intolerance. No cardiac events have been reported during her follow-up. She was subsequently admitted to the emergency department presenting with syncope due to hypoglycemia. However, an implantable loop recorder was implanted to assist further monitoring as there were potentially multiple reasons for syncope/collapse. After 25 months follow-up the patient has had no other cardiac events and the implantable loop recorder remote monitoring has shown no cardiac arrhythmias.

Discussion

The 11p15 duplication spans one of the best-known, but most complex, imprinted regions in the genome. The imprinted 11p15 region is organized in 2 domains, each of them under the control of its own ICR: ICR1 for the *IGF2/H19* domain and ICR2 for the *KCNQ1OT1/CDKN1C* domain.[11] The telomeric ICR1 confers a differential chromatin structure to the 2 parental alleles. leading to a reciprocal expression of *H19* and *IGF2*.[12] The centromeric ICR2 is maternally methylated and controls the monoallelic silencing of the noncoding antisense transcript *KCNQ1OT1*, as well as the monoallelic expression of *CDKN1C* and *KCNQ1* in an as-yet-unknown manner.[12] This region, when subject to various types of mutation, including both genetic and epigenetic abnormalities, and depending on the parental inheritance, can be responsible for either BWS or SRS.[5] The most common cause of SRS (approximately 40% of cases) is hypomethylation of the ICR1. However, the exact mechanisms leading to ICR1 hypomethylation remain unknown. Uniparental disomy for chromosome 7 is the next most common cause of SRS (10% of patients). Duplications and other rearrangements are responsible for only 1%–2% of cases.[13] Our findings suggest that the *de novo* duplication on 11p15 on the maternal allele in our patient alters the methylation at both ICR1 and ICR2, thus causing the observed SRS phenotype.

At least 15 patients with duplications on 11p15 as the cause of an SRS-like syndrome have been described, with duplications ranging from 1.6 to 10 Mb [12]. Although at least two of these duplications appear to interrupt the *KCNQ1* gene.[11,14] LQTS has not been reported in these patients. The *KCNQ1* imprinted domain exhibits complex tissue-specific expression patterns at different stages of

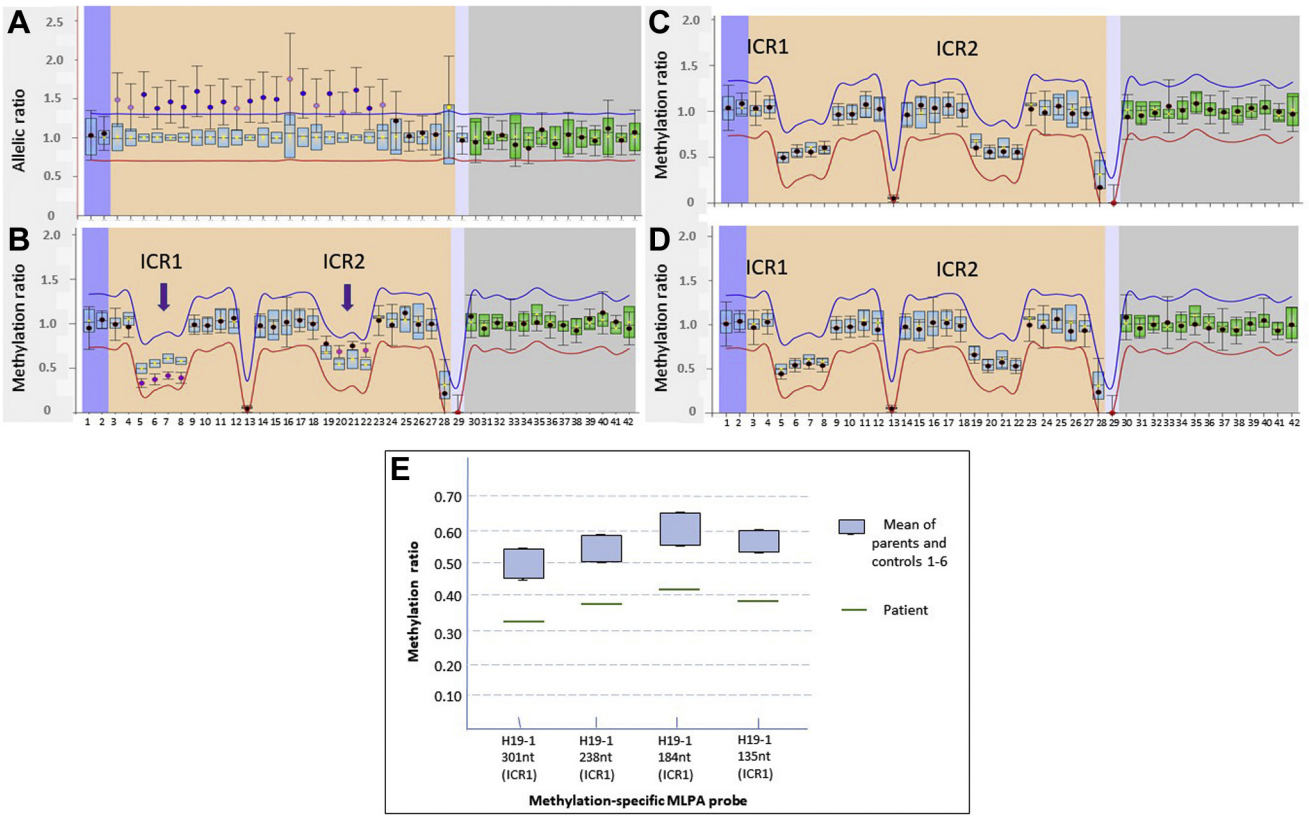

Figure 3 Methylation-sensitive multiplex ligation-dependent probe amplification (MLPA) analysis on chromosome 11p15 in the patient and her parents. **A:** Copy number MLPA showing the duplication on 11p15 in the patient (*purple dots*), spanning the *H19*, *IGFR2*, *KCNQ1*, *KCNQ1OT1*, and *CDKN1C* genes. **B–D:** Methylation MLPA in the patient (B) and her parents (C, D), showing the altered methylation status at imprinting control region (ICR) 1 and ICR2 in the patient, with hypomethylation at ICR1 and hypermethylation at ICR2 (*purple arrows*), and normal methylation patterns in her mother (C) and father (D). **E:** Mean methylation ratios in 6 healthy controls and the patient's parents (SD $\leq$0.03 for the 8 samples) plotted with the methylation ratios in the patient for ICR1. The reduced methylation at ICR1 in the patient was significantly different from that in the controls (>5 SD for all values, $P < 10e\text{-}6$). Lanes: 1: NSD1-21, 2: NSD1-23, 3: H19-5, 4: H19-1, 5-6: H19-1 (HhaI), 7-8: H19-up (HhaI), 9-11: H19-up, 12: IGH2-7, 13: IGH2-4 (HhaI), 14: KCNQ1-2, 15: KCNQ1-3, 16: KCNQ1-7, 17: KCNQ1-8, 18: KCNQ1-9, 19-22: KCNQ1OT1-1-ICR (HhaI), 23: KCNQ1-13, 24: KCNQ1-15, 25: KCNQ1-17, 26: CDKN1C-3, 27: CDKN1C-1, 28: CDKN1C-1 (HhaI), 29: ESCO2-1 (HhaI), 30–42: Reference C/M*.

development. The antisense noncoding RNA, *KCNQ1OT1*, is transcribed from the paternal allele, emerging from intron 11 of *KCNQ1* in an antisense direction. Experiments in mice have demonstrated that transcription of the paternally expressed *KCNQ1OT1* silences some neighboring genes in the embryo, while others are unaffected.[15] *KCNQ1* is imprinted and maternally expressed during early embryogenesis and transitions to biallelic expression in a tissue-specific manner. In the embryonic mouse heart, the transition to biallelic expression occurs at approximately E14.5 and appears to be quite consistent.[15] The epigenetic profile of *KCNQ1* expression during human cardiac development, however, has not yet been fully elucidated, and may well play a role in the extensive phenotypic variability shown in the expression of LQTS.[15]

In our patient, the duplication breakpoint is located in intron 12 of *KCNQ1*, and therefore disrupts *KCNQ1*, predicted to cause loss-of-function and the QTc prolongation. Interestingly, there are no reports in the literature of the occurrence of LQT accompanying SRS, but it is assumed that most of the SRS-causative defects do not disrupt the *KCNQ1* gene. The only cardiac defects reported in SRS were bicuspid aortic valve, subaortic stenosis, and ventricular septal defect. Until recently, there was a single report of a patient with mild BWS, an inherited maternal deletion in *KCNQ1*, affecting ICR2, and severe long QT.[16] The mother of the patient described in that paper (56-year-old woman) had no history of syncope attacks or palpitation and her Holter monitoring did not reveal any significant arrhythmia. She had a deletion on her paternal chromosome 11, SRS-like symptoms, and mild QT prolongation (QTc 463 ms) with flat T waves in V_2–V_3. More recently, an additional 3 patients were reported with both BWS and LQTS, with predicted *KCNQ1* haploinsufficiency and loss of methylation of IC2.[6] We therefore propose that the combination of an imprinting defect and disruption of the *KCNQ1* gene on the maternal allele in our patient has caused the dual phenotype of SRS and LQTS. We believe this is the first time the 2

syndromes have been described together but think this finding is important to raise awareness of the potential for LQTS in patients with SRS and BWS.

Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrcr.2020.06.003.

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