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Transcription alterations of *KCNQ1* associated with imprinted methylation defects in the Beckwith-Wiedemann locus

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Abstract

Purpose—The Beckwith-Wiedemann syndrome (BWS) is a developmental disorder caused by dysregulation of the imprinted gene cluster of chromosome 11p15.5 and often associated with loss of methylation (LOM) of the Imprinting Centre 2 (IC2) located in *KCNQ1* intron 10. To unravel the aetiological mechanisms underlying these epimutations, we searched for genetic variants associated with IC2 LOM.

Methods—We looked for cases showing the clinical features of both BWS and Long QT syndrome (LQTS) that is often associated with *KCNQ1* mutations. Pathogenic variants were

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Disclosure

The authors declare no competing interests.

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identified by genomic analysis and targeted sequencing. Functional experiments were performed to link these mutations to the imprinting defect.

Results—We found three rare cases in which complete IC2 LOM is associated with maternal transmission of *KCNQ1* mutations, two of which were demonstrated to affect *KCNQ1* transcription upstream of IC2. As a consequence of *KCNQ1* haploinsufficiency, these mutations also cause LQTS on both maternal and paternal transmission.

Conclusion—These results are consistent with the hypothesis that similarly to what demonstrated in the mouse, lack of transcription across IC2 results in failure of methylation establishment in the female germline and BWS later in development, and suggest a new link between LQTS and BWS which is important for genetic counselling.

Keywords

Beckwith-Wiedemann syndrome; imprinting disorders; genomic imprinting; Long QT syndrome; DNA methylation

Introduction

KCNQ1 (potassium voltage-gated channel subfamily Q member 1), the major gene involved in the Long QT syndrome (LQTS), is part of the imprinted gene cluster that is associated with the Beckwith-Wiedemann syndrome (BWS, OMIM #130650) at chromosome 11p15.5.

LQTS (prevalence 1:2,0001) is a cardiac arrhythmia characterized by prolongation of the electrocardiographic (ECG) QT interval and high risk of syncope, seizures, and sudden death. The genetic form of this disease is associated with more than 700 variants in 13 genes encoding ion channel subunits.2,3 Pathogenic *KCNQ1* variants (LQT1, OMIM #192500) represent 35% of the LQTS defects and are generally inherited as isolated cardiac defect within dominant pedigrees (also known as Romano-Ward syndrome), or in combination with other clinical features (e.g. deafness) as autosomal recessive trait (also known as Jervell and Lange-Nielsen syndrome).

BWS (prevalence 1:10,500) is a congenital genomic imprinting disorder, characterized by variable presence of macroglossia, abdominal wall defects, lateralized overgrowth, enlarged abdominal organs and increased risk to develop embryonal tumours.4,5 Heterogeneous molecular alterations affect expression of an imprinted gene cluster located at 11p15.5. The cluster is divided in two domains controlled by separate Imprinting Control Regions (ICRs). The ICR of the centromeric domain, known as *KCNQ1OT1*:Transcription Start Site (TSS) Differentially Methylated Region (DMR) (also known and herein indicated as Imprinting Centre 2 (IC2), is located within *KCNQ1* intron 10. IC2 corresponds to the promoter of the long non-coding RNA *KCNQ1OT1* and is methylated and inactive on the maternal chromosome. On the paternal chromosome, *KCNQ1OT1* is transcribed and represses in *cis* the flanking imprinted genes, including the growth inhibitor *CDKN1C*, which is normally transcribed from the maternal allele. In 50% of the BWS patients, loss of methylation (LOM) of IC2 leads to biallelic expression of *KCNQ1OT1* and biallelic silencing of *CDKN1C*. One-third of these cases also show methylation abnormalities at other ICRs (Multi-Locus Imprinting Disturbances, MLID). Further molecular lesions of BWS are: gain

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of methylation of the *H19/IGF2*: intergenic (IG) DMR (5-10%); 11p15 paternal uniparental isodisomy (20%), loss of function mutations of *CDKN1C* (5%) and 11p15.5 Copy Number Variants (CNVs, 1-4%).6 Clinical features, risk of recurrence and frequency and histotype of associated tumours differ significantly among the main molecular classes of BWS.7

The *KCNQ1* gene is 404 kb long, organized in 19 exons and harbours two major transcript isoforms arising from alternative use of promoters: isoform 1 from exon 1a and isoform 2 from exon 1b. Both isoforms are expressed in heart from both parental alleles. While isoform 2 is heart-specific, isoform 1 is expressed in several other tissues and imprinted with the paternal allele silenced during embryogenesis8,9, but expressed from both parental alleles in post-natal tissues including peripheral blood cells.10

Mouse studies indicate that transcription across ICRs is a prerequisite for establishing methylation imprints in the maternal germline.11,12 Since IC2 lies within the transcriptional unit of the *KCNQ1* gene, we hypothesized that maternally inherited mutations leading to defects of *KCNQ1* transcription might also result in loss of maternal IC2 methylation in BWS. We also expect that such mutations cause *KCNQ1* haploinsufficiency and possibly prolonged ECG QTc values and LQTS. In order to identify such rare cases, we looked for BWS patients with ECG alterations and IC2 LOM. We identified three patients who carried maternally inherited mutations of *KCNQ1.* Complete absence of methylation at the IC2 region was present in all three cases and defective transcription of the maternal *KCNQ1* allele could be demonstrated in two of them. These results indicate that genetic variants interfering with transcription across IC2 are associated with dramatic methylation loss at this region that is suggestive of defective establishment of imprint methylation in oocytes and may evolve in BWS later in embryo development.

Materials and Methods

Clinical reports

The proband of family 1 was a Dutch girl born from healthy and unrelated parents. Her mother became pregnant at 40 years of age. Amniocentesis was performed at 17 weeks of gestation because of advanced age and ultrasound abnormalities. The fetus showed features typical of BWS, omphalocele, organomegaly, macroglossia and polyhydramnios.5 The karyotype showed a balanced paternally derived Robertsonian translocation 45,XX,der(13;14)(q10;q10). AFP was normal [17.6 microgram/ml]. She was born prematurely at 33+2/7 weeks of gestation with a birth-weight of 2,565 grams (90^{th} percentile) and -length at the 97^{th} percentile. Clinical diagnosis of BWS was obtained at birth based on the presence of hypoglycaemia, macrosomia, macroglossia, naevus flammeus, ear creases and pits and organomegalia5. Electrocardiography showed a prolonged QTc interval of 471 msec indicative for LQTS13–15. Molecular diagnosis was obtained by MLPA (assays ME-030 and P144 for BWS and LQTS, respectively). The familial Robertsonian balanced translocation segregated in this family over five generations and did not have any effect on the phenotype. At age 18 months she had developed celiac disease. At 4.5 years, electrocardiography still showed a prolonged QTc interval (449 msec). At the clinical follow-up at 10 years, besides the BWS characteristics, LQTS and celiac disease, she had developed mild bilateral hearing loss.

The proband of family 2 was an American girl born from unrelated parents. BWS was clinically diagnosed at birth at the University of Florida Hospital, based on the presence of omphalocele, macroglossia and ear pits5. Molecular diagnosis of BWS with IC2 LOM was obtained by Southern blot analysis (Mayo Clinic, Rochester, MN, USA, data not shown). She also had a history of learning delay and a low frequency seizure disorder. At the age of nine years, an ECG, performed following an episode of chest pain, revealed prolonged QTc (492 ms), and LQTS was diagnosed. Asymptomatic LQTS was diagnosed by ECG in her mother (QTc = 473), who did not show any sign of BWS. No BWS sign and normal QTc values were found in her half-sister, maternal grandmother and maternal grandfather. The maternal aunt, not available to clinical and molecular analyses, had several miscarriages. The first fetus that was aborted at the twentieth week had large omphalocele, a clinical sign commonly found in BWS associated with molecular defects of the centromeric 11p15.5 domain. Her only full-term pregnancy was of two male twins. Of these, one infant was stillborn at scheduled c-section, while his brother had a heart defect that required surgery shortly after birth.

The BWS features of family 3, in which segregates a 160 kb duplication within *KCNQ1*, were previously reported.16 Two further family members, healthy cousins of the proband, who were not born at the time of the first study, were added to the present study. The proband was subjected to ECG within a screening for LQTS of BWS patients (see below). Her QTc value was of 478 ms at 7.8 years of age that is diagnostic of LQTS (Table S1).13–15 Following this finding, ECG was offered to all other carriers of the duplication of this family. All the individuals examined for ECG showed borderline prolonged QTc values (mother: 460 ms at 35 years, maternal uncle: 447 ms at 37 years, cousin: 440 ms at 2 years).

All genetic analyses of this manuscript have been performed after written informed consent was obtained from the patients or patients' parents. All research work has been carried out in accordance with the ethical principles and the legislation of Italy, The Netherland and The United States. The study was approved by the Ethical Committee of the University of Campania "Luigi Vanvitelli" (Approval number: 1135, October 13th, 2016)

ECG to BWS patients with IC2 LOM

Twenty-four patients with clinical and molecular diagnosis of BWS with IC2 LOM5 were enrolled in an ECG screening. The patients, aged between 1 month and 24 years, were subjected to clinical visit, baseline ECG with QTc measurement and family history collection. QTc interval was calculated using Bazett formula.13 QT values are reported in Table S1.

Results

Since the centromeric ICR of the BWS locus is located in intron 10 of the *KCNQ1* gene, which is also associated with LQTS, we wondered if abnormal QTc values in BWS patients with IC2 LOM could reveal the presence of mutations interfering with the imprinted methylation of this region. Thus, we looked for the presence of features of LQTS in BWS patients with IC2 LOM. Three cases were identified and are described below.

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LQTS and BWS with complete IC2 LOM associated with a transcription defect of *KCNQ1*

Unusually low (<5%) IC2 methylation levels were detected in the family 1 proband (Figure 1A) by MS-MLPA (ME-030 kit for BWS/SRS) (Figure 1B) and confirmed by pyrosequencing (Figure 1C), indicating absence of the epigenetic mosaicism (10-35% methylation) found in most BWS patients. Methylation levels comparable to values of healthy individuals were found at IC1 and other five ICRs in the proband, and at IC1 and IC2 in her parents (Figure 1C and Table S4). MLPA (P144 kit for LQTS) demonstrated a *de novo* heterozygous deletion of *KCNQ1* exons 1a-1b in the proband (II-1) (Figure 1D). By typing two SNVs (SNV2 and SNV3) falling in the intron between exon 1a and exon 1b, we demonstrated that the deletion arose on the maternal chromosome (Figure 1E).

A CGH-based chromosome array analysis confirmed the presence of the deletion in heterozygosity and demonstrated that it was 120 kb long and included the first two *KCNQ1* exons, the *KCNQ1* promoter and other three genes (*TRPM5*, *TSSC4* and *CD81*). The telomeric breakpoint was mapped between 2,383,764 and 2,397,123, and the centromeric breakpoint between 2,510,194 and 2,532,843 of chromosome 11, about 30 kb downstream of *KCNQ1* exon 1b (Reference genome: hg19. Figure S2). By sequencing the entire coding region, no additional mutations were detected, indicating *KCNQ1* haploinsufficiency as the most probable mechanism of LQTS in the proband.

To investigate the effect of the deletion on *KCNQ1* transcription, allele-specific expression analysis was performed on total RNA from peripheral blood leukocytes of the proband. Consistent with previous report,10 we observed biallelic *KCNQ1* expression in three age-matched healthy individuals (Figure S3). Transcription was derived from promoter 1a, while promoter 1b-specific transcript was not detected in blood (data not shown). Significantly, *KCNQ1* was expressed from only one allele in the proband, consistent with the loss of its promoters on the maternal chromosome (Figure 1F). These data demonstrate that a deletion removing both main promoters and affecting the expression of *KCNQ1* is associated with absence of IC2 methylation on the maternal chromosome.

LQTS and BWS with complete IC2 LOM associated with a splice-site variant and defective elongation of *KCNQ1* transcript

Similar to family 1, low IC2 methylation level (<5%) was found in the proband (III-1) of family 2 (Figure 2A-B). In contrast, methylation values comparable to healthy controls were found at IC1 and five other ICRs in the proband and at both IC1 and IC2 in her mother (II-2) and maternal grandparents (I-1 and I-2; see Figure 2B and Table S4). The maternal aunt (II-3) and her children (III-3 and III-4) were unavailable to molecular analysis. The molecular cause of LQTS was investigated by SNV-based CMA and targeted sequencing. No clinically relevant CNV was detected in the proband (data not shown). However, a novel SNV was identified in heterozygosity at the 5' donor site of the first intron of *KCNQ1* (*KCNQ1*-ENST00000155840: c.386+1G>C (IVS1+1G>C) (Fig 2C). This variant was absent in all available SNV databases (https://www.ncbi.nlm.nih.gov/projects/SNV; http://www.internationalgenome.org; http://gnomad.broadinstitute.org; http://exac.broadinstitute.org) and predicted to be potentially damaging by Human Splicing Finder (http://www.umd.be/HSF3/). The variant was also found in heterozygosity in the pobanda

mother (II-2) and maternal grandfather (I-1), but absent in her half-sister (III-2) and maternal grandmother (I-2) (Figure 2C).

To investigate the functional effect of the splice-site variant, we determined the transcription profile of *KCNQ1* on total RNA from blood leukocytes of the proband (III-1) and her half-sister (III-2), by genome RNA sequencing. This analysis indicated that, although the overall expression of *KCNQ1* was lower in the proband (III-1) than in her half-sister (Figure S5), the RNA level was not homogeneously distributed along the *KCNQ1* gene in III-1, with the most 5' 10 kb about 2-3-fold more abundant than the rest of the gene, while RNA level was homogeneously distributed in III2 (Figures 3A and 3B). Interestingly, a cluster of poly-A addition sites is present immediately downstream of the position of *KCNQ1* intron 1, in which the RNA level declines, suggesting a premature termination of transcription in III-1 (Figure 3A). Finally, consistent with IC2 LOM, *KCNQ1OT1* RNA level was increased in the proband with respect to her sister (Figure 3A).

To confirm the hypothesis of defective *KCNQ1* elongation in the proband, allele-specific expression of *KCNQ1* was analaysed in total RNA from peripheral blood leukocytes, by typing SNVs present in heterozygosity at different positions of the primary transcript (Figure 3C). By typing the intron 1 splice-site variant (SNV1), we found biallelic *KCNQ1* expression in the proband, as well as in II-2 and I-1 (Figure 3C, left panels). In contrast, transcription from only one allele was found in the same individuals, by typing SNVs in the 3' part of intron 1 (SNV7, middle panels) and in the 3' UTR (SNV15, right panels) of the *KCNQ1* gene. III-2 has not inherited the splice-site variant and therefore was not informative at this position. However, she showed biallelic *KCNQ1* expression in both intron 1 (SNV9, middle panel) and 3' UTR (SNV15, right panel). Similarly, *KCNQ1* was transcribed from both parental alleles in intron 1 (SNVs 6-9, middle panel of Figure 3C and left panel of Figure S3) and intron 14 (SNVs 13-14, right panels of Figure 3C and Figure S3) in three age-matched healthy individuals (Ctrl 1 -3) and four further BWS patients with IC2 LOM (BWS 1-4). To determine the parental origin of the full-length *KCNQ1* RNA in the individuals carrying the splice-site variant, we typed a SNV (SNV12) of intron 9 that is present in heterozygosity in the proband and in homozygosity in her mother (II-2). Only the G allele that is absent in the mother was amplified from III-2 RNA, indicating that the splice mutation is associated with the defective transcript (Figure 3D).

To better define the position of the *KCNQ1* gene in which expression shifts from two alleles to one allele in the individuals carrying the splice-site variant, we typed two further SNVs (4 and 5) that were located in the 5' part of intron 1 (Figure S4). Both SNV4 and SNV5 were informative in the maternal grandfather (I-1). Expression was found biallelic at SNV4 but monoallelic at SNV5 in this individual. Only one allele was transcribed at SNV5 also in the mother (II-2) and proband (III-1), while both parental alleles were expressed in two informative healthy individuals (Ctrl4 and 5). Consistent with the RNA seq data, these results demonstrated that expression shifts from two alleles to one allele between 6.6 (SNV4, chr11: 2473311) and 26 kb (SNV5, chr11: 2492629) downstream of the splice-site variant (Figure S4).

Overall, these data demonstrate defective expression of the *KCNQ1* transcript downstream of the splice-site variant and complete absence of methylation at IC2 on the maternal chromosome, suggesting that premature transcription termination upstream of IC2 did not allow to properly establish the methylation imprint in the germline of the proband's mother leading to an imprinting error in the embryo.

LQTS in a BWS family with a 160 kb *KCNQ1* duplication and complete IC2 LOM

To identify asymptomatic cases of LQTS in an Italian BWS cohort, ECG was evaluated in 24 patients with IC2 LOM. A prolonged QTc value diagnostic of LQTS was found in only one patient (patient 24 in Table S1). The proband (III-8 in Figure 4A) was part of a previously described family, including several other individuals with BWS features, and in which segregates a 160 kb *KCNQ1* duplication (Figure 4A and B, and Chiesa et al.16). Two apparently healthy children (III-4 and III-5) of II-3, not born at the time of the first study, were added to the present study. The duplication was confirmed in III-5, but excluded in III-4 (Figure S6). Further clinical examination of the family showed borderline prolonged QTc values in all maternal and paternal duplication carriers who accepted the ECG exam including III-5 (Figure 4A). This demonstrates that LQTS co-segregates with the duplication on both maternal and paternal transmission, while BWS does it only on maternal transmission. Importantly, the original study also showed that maternal transmission of the duplication was associated with complete loss of DNA methylation of the duplicated IC2, leading to activation of a truncated form of *KCNQ1OT1* and silencing of *CDKN1C.*16 To test if the methylation defect was extended to other ICRs or restricted to IC2 in this family, five loci that are frequently affected in MLID17 were analysed. The methylation values of all tested ICRs were comparable to the values of healthy individuals, in II-3, II-5, III-5 and III-8, indicating that the epigenetic defect was restricted to IC2 (Table S4).

These results demonstrate that a mutation within *KCNQ1* segregates with BWS and complete IC2 LOM on maternal transmission and with LQTS on both maternal and paternal transmission.

Discussion

In rare cases, DNA methylation defects in BWS occur secondary to genetic mutations acting *in cis* or *in trans* and are associated with higher recurrence risk.17–20 The identification of these genetic variants is therefore important for genetic counselling. However, identifying variants associated with IC2 LOM *in cis* is challenging, because of the large extension of the centromeric 11p15.5 imprinted domain. By looking for the presence of LQTS features within three BWS cohorts, we identified three cases in which complete IC2 LOM occurs secondary to *cis*-acting DNA mutations in the *KCNQ1* gene. In all three cases, the genetic variants cosegregate with BWS on maternal transmission and in at least two cases were demonstrated to affect *KCNQ1* transcription upstream of IC2, suggesting that lack of transcription across this ICR leads to LOM, likely because of defective *de novo* methylation in oocytes (Figure 5).

LQTS and BWS with complete IC2 LOM associated with a transcription defect of *KCNQ1*

The mutation found in the proband of family 1 removes the promoter and causes loss of both main *KCNQ1* transcript isoforms on the maternal chromosome. The complete absence of IC2 methylation in the proband indicates that this epimutation arose as a consequence of defective imprint establishment in the maternal germline. Consistent with the role of *Kcnq1* transcription in *de novo* IC2 methylation demonstrated in the mouse,11 it is possible that this imprinting defect derived from lack of *KCNQ1* transcription in the oocyte. Loss of expression of one allele also results in *KCNQ1* haploinsufficiency and this is the likely cause of LQTS in this individual. A deletion of similar extension on chromosome 11p15 but associated with more complex rearrangements has been recently described in a family with multiple offspring with large omphalocele and complete IC2 LOM which was incompatible with life.21 Similarly to our case, this epimutation could be originated as a consequence of defective *KCNQ1* transcription and imprint establishment.

LQTS and BWS with complete IC2 LOM associated with a splice-site variant and premature termination of *KCNQ1* transcript

Although several *KCNQ1* splice-site variants have been identified in LQTS22,23 (https://www.ensembl.org/Homo_sapiens/Transcript/ProtVariations?db=core;g=ENSG00000053918;r=11:2444684-2849110;t=ENST00000155840), our case 2 splicing defect is the only one, to our knowledge, that has ever been reported as associated with BWS. Often, abnormal splicing leads to premature translation termination and mRNA degradation by Nonsense Mediated Decay.24 However, in the case of this splice-site variant, the 2-fold reduction and the shift from two alleles to one allele in *KCNQ1* expression between the mutation and a cluster of polyA sites in intron 1 suggests a rather different mechanism, in which premature transcription termination occurs in the *KCNQ1* gene.

It is known that pre-mRNAs processing of eukaryotic genes including splicing occurs co-transcriptionally.25 Several studies have demonstrated that the splicing apparatus stimulates transcription elongation through direct interaction with elongation factors, suggesting that checkpoint mechanisms ensure that only spliced transcripts are efficiently elongated.26–28 Consistent with this hypothesis, knockdown of U1 snRNP results, in addition to splicing inhibition, in premature cleavage of pre-mRNAs at cryptic polyadenylation signals in introns located near the transcription start site of large genes.29–31 Intriguingly, in our case, the *KCNQ1* splice-site variant alters the 5’ splice site that normally pairs with the U1 snRNP, suggesting that weakening this binding causes termination, cleavage and polyadenylation of the promoter 1a-derived transcript upstream of IC2. Since 1a is an active *KCNQ1* promoter in most fetal tissues,8 we expect that the exon 1a-derived transcript is prematurely terminated in oocytes and this possibly results in failure of IC2 methylation establishment in the maternal germline. Further experiments, such as 3’-RACE, may be performed in the future to more precisely determine the transcription termination of *KCNQ1* relative to the cryptic polyA addition site in intron 1, in the present case.

In family 2, the BWS phenotype is associated with the splice-site variant on maternal transmission, while LQTS occurs on both maternal and paternal transmission. This is consistent with the maternal methylation of IC2 and the lack of imprinting of *KCNQ1* in

heart. *KCNQ1* haploinsufficiency is expected from incomplete elongation of the transcript caused by the splice-site variant. Consistent with our findings, exon 1a mutations have been reported associated with LQTS.22,32 The absence of BWS features including normal IC2 methylation in the maternal grandfather suggests, that the variant is present on his paternal chromosome, while the absence of LQTS in this individual is probably explained by incomplete penetrance of the mutation33. The multiple miscarriages, including one fetus with a large omphalocele, are reminiscent of what described in a family with *KCNQ1* deletion,21 and suggest that the proband's aunt is a silent carrier of the splice-site variant and that the phenotypes of the progeny are the result of combined *KCNQ1* haploinsufficiency and IC2 imprinting defect.

LQTS and BWS with complete IC2 LOM associated with a 160 kb *KCNQ1* duplication

The proband of family 3 scored positive for prolonged QTc value in an ECG screening offered to all BWS patients with IC2 LOM of an Italian cohort (Table S1). Following this result, three further members of this family were found to have prolonged QTc value. All these individuals carry a previously described 160 kb internal *KCNQ1* duplication segregating with BWS with IC2 LOM on maternal transmission.16 We now find that this mutation also causes overt LQTS or prolonged QTc value upon either maternal and paternal transmission. Unfortunately, the lack of informative SNVs in the 3' part of *KCNQ1* prevented investigation of allelic expression in this family. However, we originally demonstrated that one of the duplicated copies of IC2 is completely non-methylated on the maternal chromosome and that the duplication is inverted *in cis* (Figure 4B and Chiesa et al16). Here, we propose that *KCNQ1* transcription is arrested before reaching the duplicated IC2, because of the formation of a stem-and-loop secondary structure of the transcript caused by the inverted duplication and this in turn causes a failure of imprint establishment in the maternal germline, similarly to families 1 and 2 (Figure 4). Indeed, stem-and-loop-dependent mechanisms of transcription termination have been described in mammals.34 Further studies are needed, however, to directly demonstrate the role of this secondary structure in transcription termination of *KCNQ1*, in the present case. Interestingly, a case of BWS associated with a maternally inherited *KCNQ1* exon1c-1 duplication and complete IC2 LOM, which could also be caused by premature transcription termination, has been reported by Demars et al.35

This is the first case of LQTS associated with a *KCNQ1* duplication. The cardiac phenotype may be caused by either i) truncated KCNQ1 protein lacking the aminoacids encoded by last exon; or ii) degradation of the transcript lacking the normal polyA signal. Consistent with the first mechanism, a truncating mutation close to the last *KCNQ1* exon has been reported associated with LQTS36 (https://www.ensembl.org/Homo_sapiens/Transcript/ProtVariations?db=core;g=ENSG00000053918;r=11:2444684-2849110;t=ENST00000155840).

LQTS in BWS patients with IC2 LOM

Congenital disorders of the heart rhythm, such as LQTS, have been rarely reported in BWS and generally associated with maternal chromosome 11p15 rearrangements.37,38 In one of these cases,38 the proband carries a translocation with a breakpoint between *KCNQ1* exons

2 and 10 and shows IC2 LOM, which could indeed be caused by defective transcription across IC2. In this study, we identified three cases with combined BWS and LQTS related to three different genetic/genomic lesions leading to a common epigenotype derangement. Although further studies are needed to clarify the exact prevalence, the ECG screening performed in the Italian cohort indicates a low incidence of LQTS among the BWS patients with IC2 LOM, confirming the relatively rare overlap of these two diseases. The BWS guidelines recommend that physicians should be aware of the increased prevalence of cardiac anomalies in children with BWS and indicate annual evaluation and electrocardiogram in patients with genomic rearrangements involving the IC2 region. However, because of the incomplete penetrance of *KCNQ1* mutations and the associated high risk of major cardiac events in adult age,23,33 an ECG (and if positive further *KCNQ1* analysis) should be performed in all BWS patients with IC2 LOM.

In summary, this study proposes a new molecular mechanism, in which two diseases, LQTS and BWS, share the same genetic defect. We demonstrate that, by affecting *KCNQ1* transcription, genetic mutations may result in *KCNQ1* haploinsufficiency and LQTS, and at the same time we suggest these lesions may interfere with *de novo* IC2 methylation in the oocyte and cause BWS later in development. As consequence of the differential imprinting status of *KCNQ1OT1* and *KCNQ1*, BWS occurs only on maternal transmission of the mutations while no parent-of-origin-dependent effect is observed with LQTS (Figure 5).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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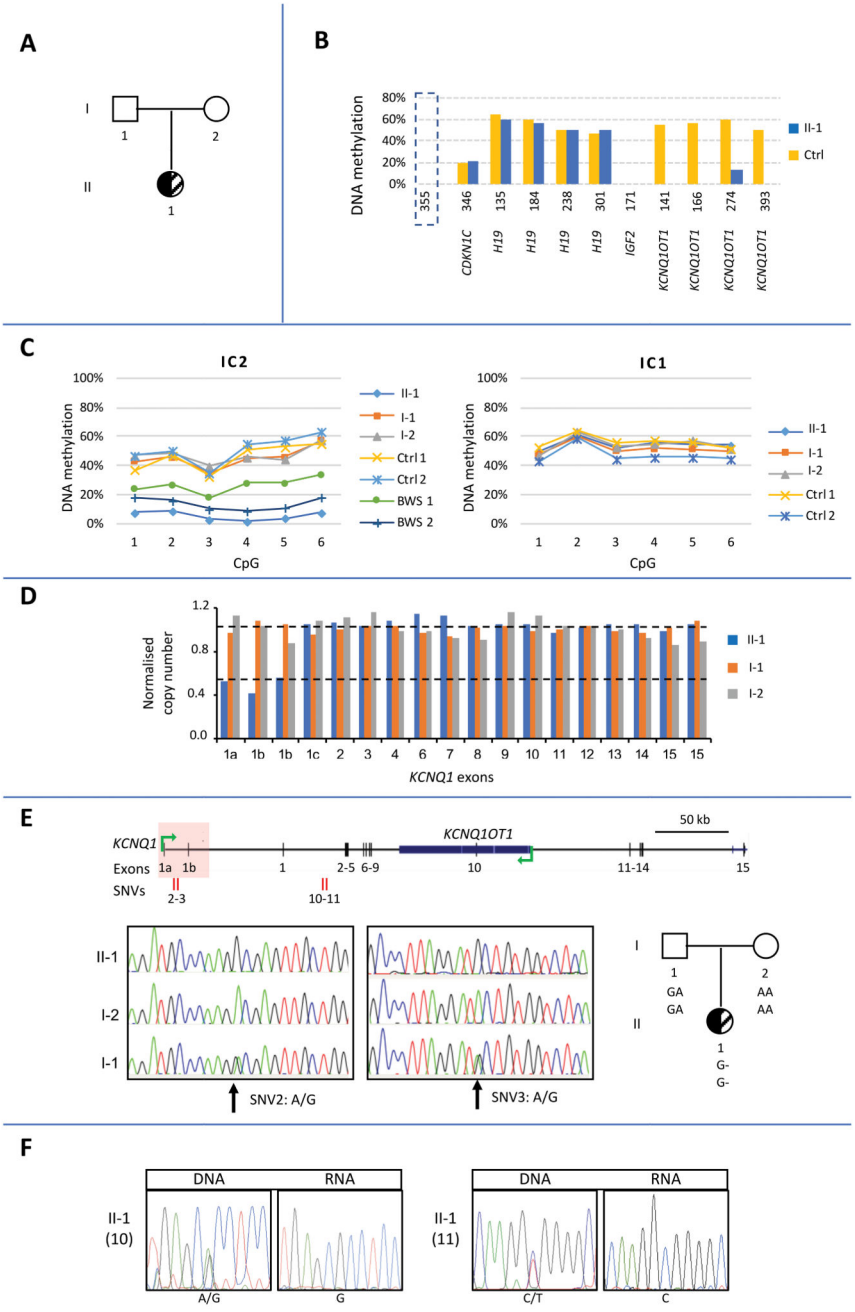

Figure 1. Characterization of a 120 kb deletion abolishing the *KCNQ1* promoter.

(A) Pedigree of family 1. The symbol partitioned in two halves with different filling indicate the proband (II-1) affected by BWS and LQTS1. (B) DNA methylation analysis of IC2 and IC1 by MS-MLPA. The histogram shows DNA methylation levels of the proband (II-1) and controls. Numbers indicate the length (nt) of the probes according to the instructions of the manufacturer (ME-030, MRC-Holland); 355 is a digestion control probe. (C) DNA methylation analysis of IC2 and IC1 by pyrosequencing. Each dot represents the methylation value of a single CpG. Ctrl1 and Ctrl2 are unrelated healthy individuals; BWS1 and BWS2,

two further BWS patients with IC2 LOM. (D) Copy number (CN) analysis of *KCNQ1* exons in the trio by MLPA. The histograms represent the normalized CN detected with 18 MLPA probes (SALSA MLPA 144 Long-QT probemix version A2) hybridizing within the *KCNQ1* exons. Exon numbering is according to Lee et al8. (E) Segregation in the trio of two SNVs (SNV2: rs2023818 and SNV3: rs800336) located in the deleted region, as determined by Sanger sequencing, and demonstrating the maternal origin of the deletion. A schematic diagram of the *KCNQ1* locus is reported. *KCNQ1* (represented with a black thin bar) and *KCNQ1OT1* (represented with a blue thicker bar) are transcribed on opposite strands. The pink rectangle highlights the region involved in the deletion. The vertical red lines indicate the location of SNVs 2 and 3 typed for segregation analysis and SNVs 10 and 11 reported in 1D. (F) Allele-specific expression analysis obtained by typing SNV10: rs2283168 A/G and SNV11: rs2283169 on total RNA from blood leukocytes of the proband. SNV10 and SNV11 are located between exon 1 and exon 2, downstream of the deletion and are present in heterozygosity in the proband. The nucleotides of the SNPs determined by Sanger sequencing in DNA and RNA are reported below each electropherogram. Note the monoallelic expression of *KCNQ1*.

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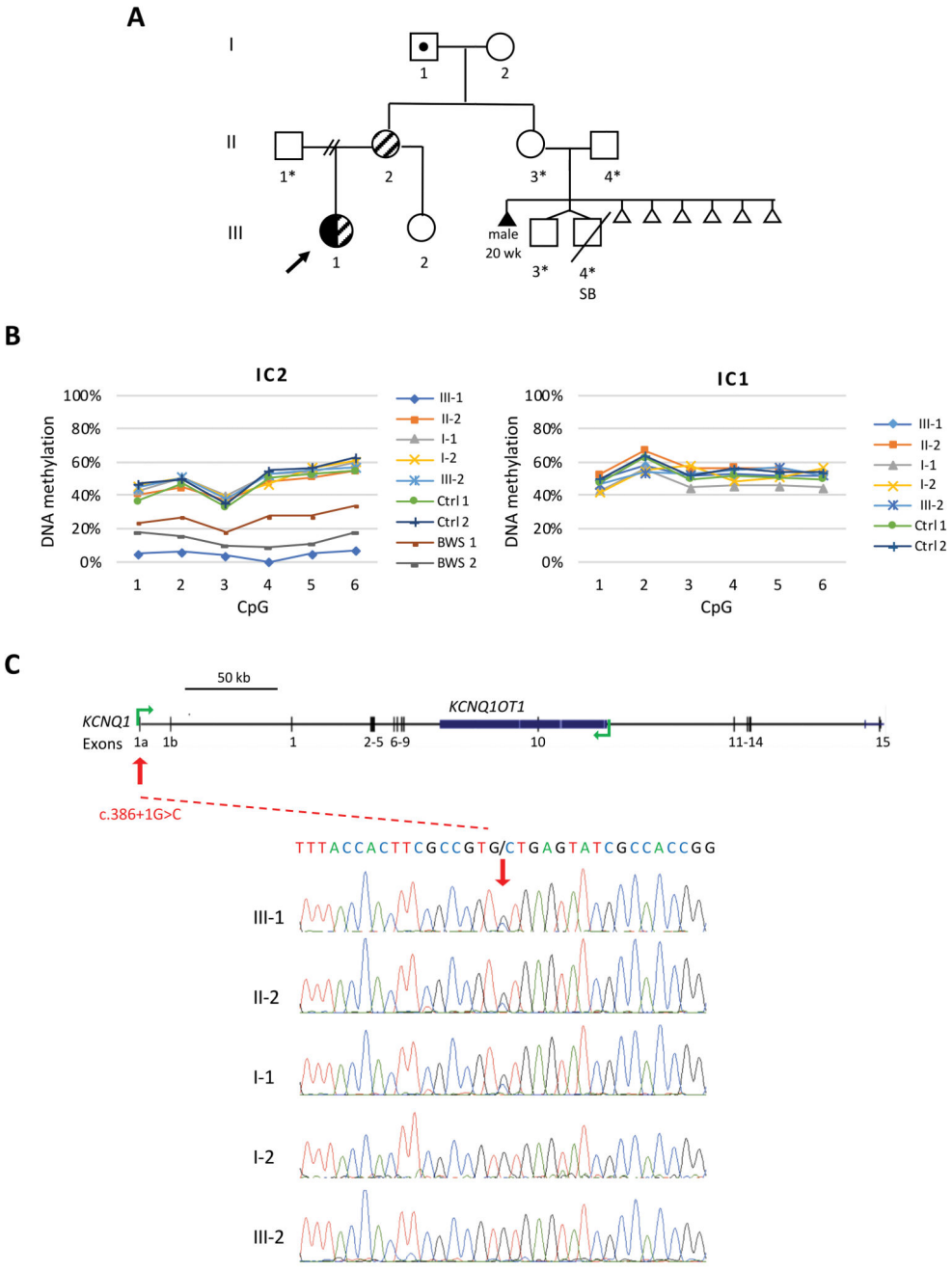

Figure 2. Clinical and molecular features associated with a splice-site variant in the 5' donor site of *KCNQ1* first intron.

(A) Pedigree of the family. The proband (III-1) affected by BWS and LQTS1 is indicated as in Figure 1. The symbol with stripes indicates the LQTS1 condition affecting the proband's mother (II-2), who is a carrier of the splice-site variant. The symbol with a dot in the middle indicates the silent carrier of the splice-site variant. Black triangle: male fetus with large omphalocele, demised in utero at 5 months of gestation. White triangles: spontaneous miscarriages. III-4: Stillborn (SB) twin. Asterisks: individuals unavailable to molecular analyses. (B) Methylation analysis of IC2 and IC1, as determined by pyrosequencing. Each

dot represents the methylation value of a CpG. Ctrl: unrelated healthy individual. (C) Electropherogram showing the novel variant (*KCNQ1*-ENST00000155840: c.386+1G>C (IVS1+1G>C), GRCh37/hg19 chr11: 2,466,715) located at the donor splice site of the first intron of *KCNQ1*. The splice-site variant was found in heterozygosity in III-1, II-2, I-1, but absent in III-2 and I-2.

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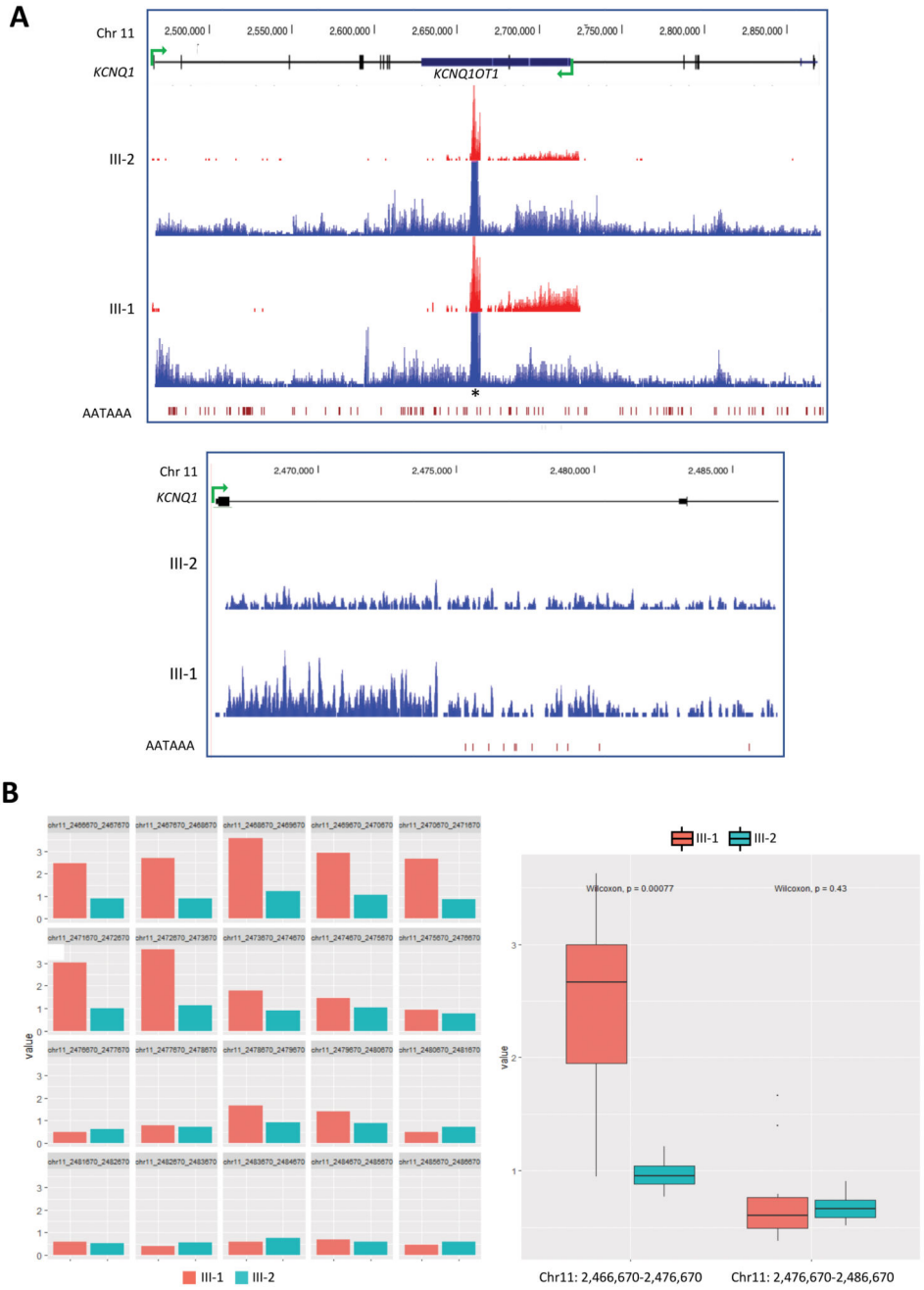

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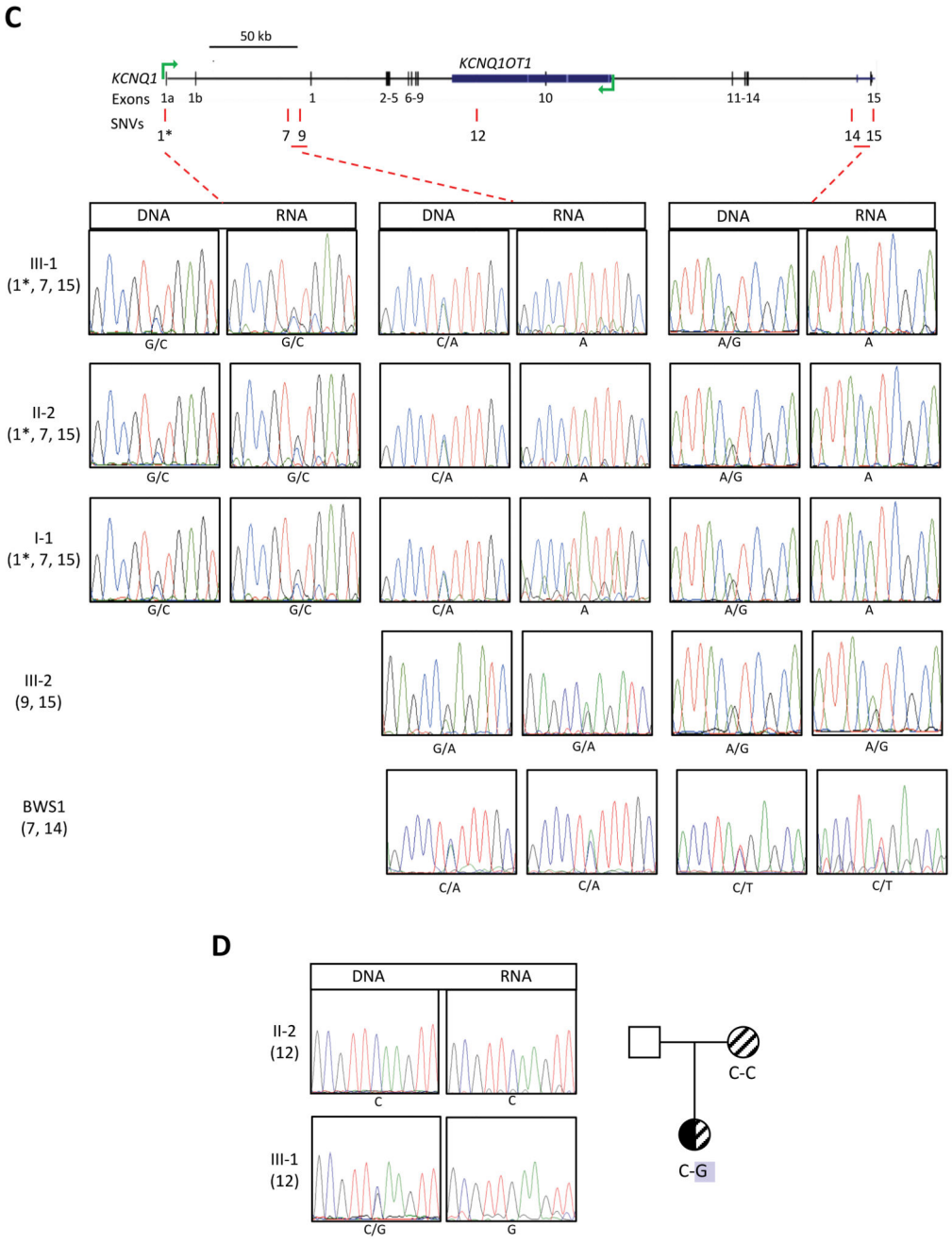

Figure 3. Effect of the splice-site variant on *KCNQ1* expression.

(A) Transcription profile of *KCNQ1* in III-1 and III-2 obtained by RNAseq on total RNA from blood leukocytes. Top: Screen shot from UCSC genome browser showing the RNA transcribed from the positive (blue) and negative (red) strands of the *KCNQ1* locus. A track showing the location of AATAAA polyA addition sites is reported below the RNA tracks. The peak of reads on both strands in the middle of the gene (indicated by an asterisk) corresponds to an L1 repeat. Bottom: Zoom in of the first 5' 20 kb of *KCNQ1*. (B) Observed increased expression of the most 5' 10 kb of *KCNQ1* in the proband (III-1) compared to her

sister (III-2). Expression profile of the most 5’ 20 kb (2466670-2486670) represented as normalized read count partitioned in windows of 1 kb (bar plots on the left) or normalized read count distribution in two windows of 10 kb (box plots on the right) of III-1 and III-2. Y-axis values in bar plots are absolute counts in millions normalized by the number of uniquely mapped reads. Box plots show a statistically significant enrichment of the coverage in the first 10 kb (2466670-2476670) of III-1 compared to III-2, but similar coverages in the following 10 kb (2476670-2486670). (C) Allele-specific expression analysis of *KCNQ1* in III-1, II-2, I-1, III-2 of family 2 and an unrelated BWS case with IC2 LOM, analysed as in Figure 1F. A schematic diagram of *KCNQ1* is reported in the top to indicate the positions of the analysed SNVs (red vertical lines). SNV codes are listed in Table S2. The electropherograms are organized in three panels corresponding to SNVs located in different part of the *KCNQ1* gene. The numbers in brackets at the left of the electropherograms represent the SNVs analysed in each individual. The splice-site variant described in Figure 2C is indicated by an asterisk. (D) Allele-specific expression analysis of *KCNQ1* to determine the parental origin of the transcribed allele in III-1. The typed SNV (SNV12= rs7942590 G/C) falls in the part of the *KCNQ1* gene that is expressed from only one allele. The boxed G represents the only allele present in the RNA of III-1.

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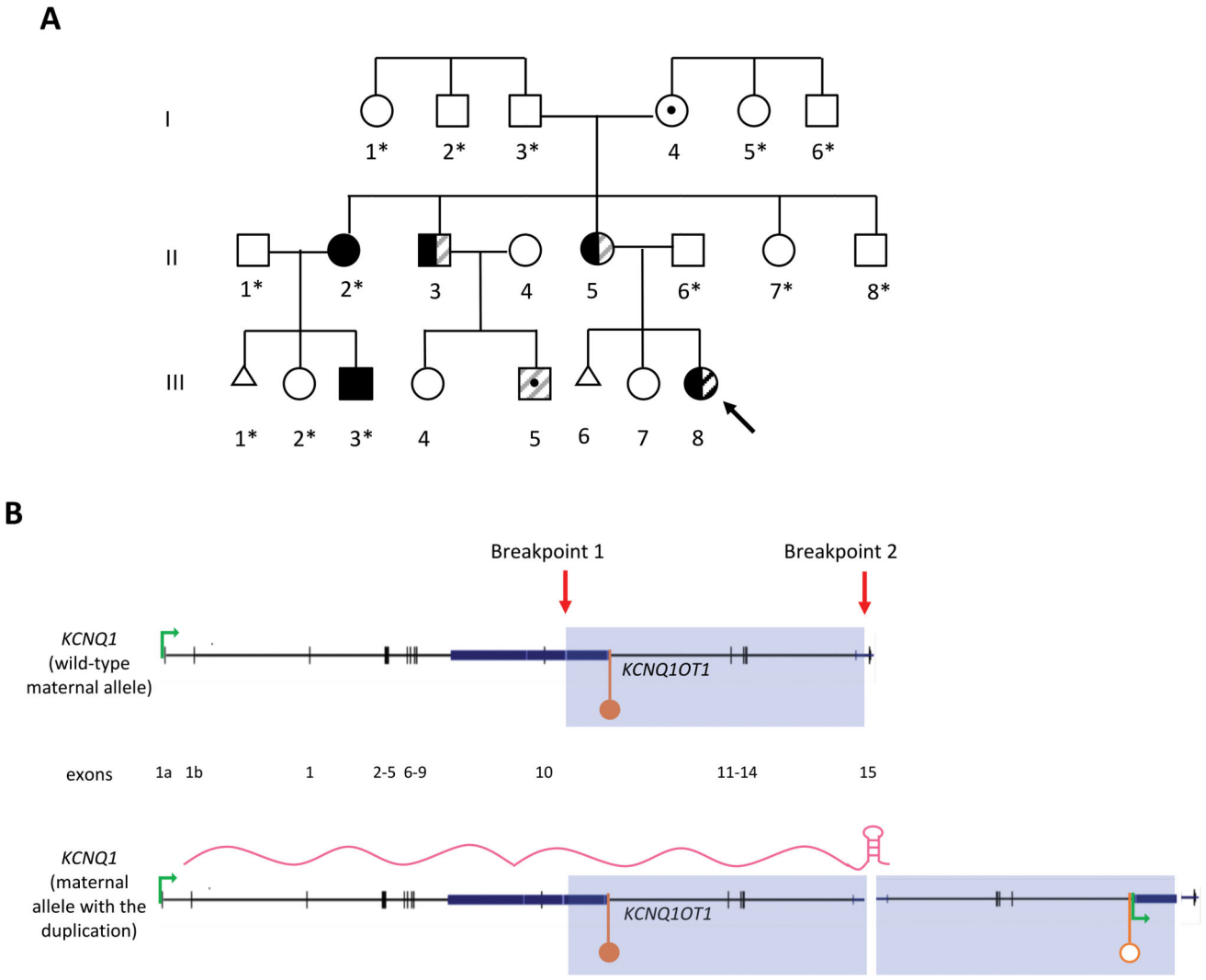

Figure 4. Segregation of LQTS and BWS phenotypes in a family with a 160 kb *KCNQ1* duplication.

(A) Pedigree of family 3, modified from Chiesa et al.16 The proband (III-8) affected by both BWS and LQTS1 is indicated as in Figure 1. The grey strips in II-3, II-5 and III-5 indicate borderline prolonged QTc values. Asterisks: family members unavailable to molecular analysis. (B) Schematic representation of the 160 kb *KCNQ1* duplication. The light blue rectangle highlights the region involved in the duplication. Breakpoint 1 is telomeric; breakpoint 2, centromeric. The position of the inverted duplication in the centromeric breakpoint and the the stem-and-loop structure in the *KCNQ1* transcript are proposed to explain the molecular data.

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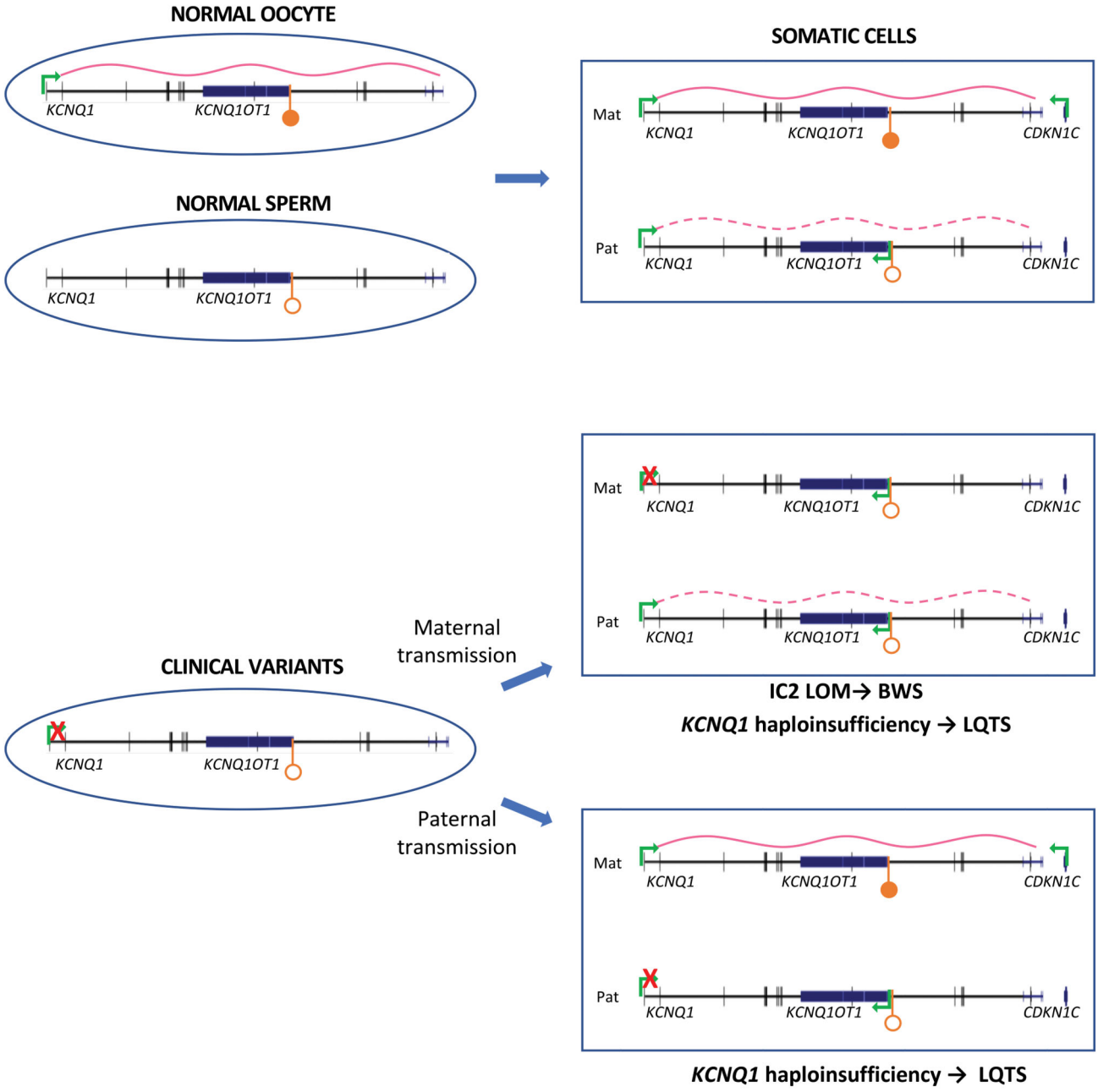

Figure 5. Model of how a single genetic *KCNQ1* variant may determine both LQTS and BWS.

In the female germline, the *KCNQ1* promoter drives a transcript across IC2, which is required for *de novo* methylation of this ICR. Differential IC2 methylation regulates *KCNQ1OT1* and *CDKN1C* imprinting in somatic cells. Genetic variants causing loss or premature termination of *KCNQ1* transcription result in i) BWS due to defective IC2 methylation establishment, biallelic *KCNQ1OT1* expression and *CDKN1C* silencing, and LQTS due to *KCNQ1* haploinsufficiency, on maternal transmission; ii) LQTS due to *KCNQ1* haploinsufficiency on paternal transmission. Active promoters and transcription orientation are indicated by bent green arrows. Methylated IC2 is indicated by filled lollipop,

unmethylated IC2 by open lollipop. The *KCNQ1* transcript is depicted as a pink curved line. The dashed curved line indicates the paternal *KCNQ1* transcript that is expressed in heart and adult tissues and imprinted in embryo. Genetic variants causing loss of *KCNQ1* transcription are indicated by a red cross. The *KCNQ1* gene is represented non-imprinted in somatic cells, according to the results obtained in blood leukocytes.

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