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LETTER

Looking for *CDKN1C* enhancers

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Gurrieri *et al*[1] recently described a familial case with mild Beckwith–Wiedemann Syndrome (BWS) and mild Silver-Russell Syndrome (SRS) phenotypes due to opposite parental origin of a 198 kb deletion on chromosome 11p15.5. Our group identified another deletion (of 60 kb) that eliminates a subfragment of the region missing in the 198 kb deletion on the paternal chromosome, but is associated with severe Intra-Uterine Growth Restriction (IUGR).[2] Careful analysis of the epigenetic marks reported by the ENCODE Project for this genomic region provides insights into the location of the *cis*-acting regulatory elements of the 11p15.5 region and a possible explanation for the phenotypic differences between the two clinical cases.

Altered expression of imprinted genes on chromosome 11p15.5 is associated with the growth disorders BWS (MIM #130650) and SRS (MIM #180860).[3] The 11p15.5 imprinted gene cluster is divided into two distinct domains, each including an own imprinting control region (ICR). BWS and SRS are characterized by opposite growth phenotypes, and DNA methylation defects in the ICRs account for 60% of their molecular abnormalities. However, in a significant number of patients (>30%) no molecular defect has been identified yet.

The centromeric domain of the cluster is 800 kb long and includes several imprinted genes. Its ICR (ICR2) is located within *KCNQ1* intron 10, it is methylated on the maternal chromosome and encompasses the promoter for the non-coding RNA *KCNQ1OT1* (antisense to *KCNQ1*). Transcription of *KCNQ1OT1* on the non-methylated paternal chromosome results *in cis* bi-directional silencing of the flanking imprinted genes. Among these genes, there is *CDKN1C* that encodes a cyclin-dependent kinase inhibitor and has growth suppressing properties. Decreased *CDKN1C* activity due to the loss of ICR2 methylation and maternal *KCNQ1OT1* activation has been associated with BWS,[4] whereas increased *CDKN1C* activity due to maternal 11p15.5 duplication has been involved in SRS.[5]

John *et al*'s[6] work in the mouse indicates that, in addition to ICR2, *Cdkn1c* is regulated by distant enhancers likely located within the *Kcnq1* gene. Enhancer sequences are identified as conserved non-coding regions (>100 bp, which are >70% identical between human and

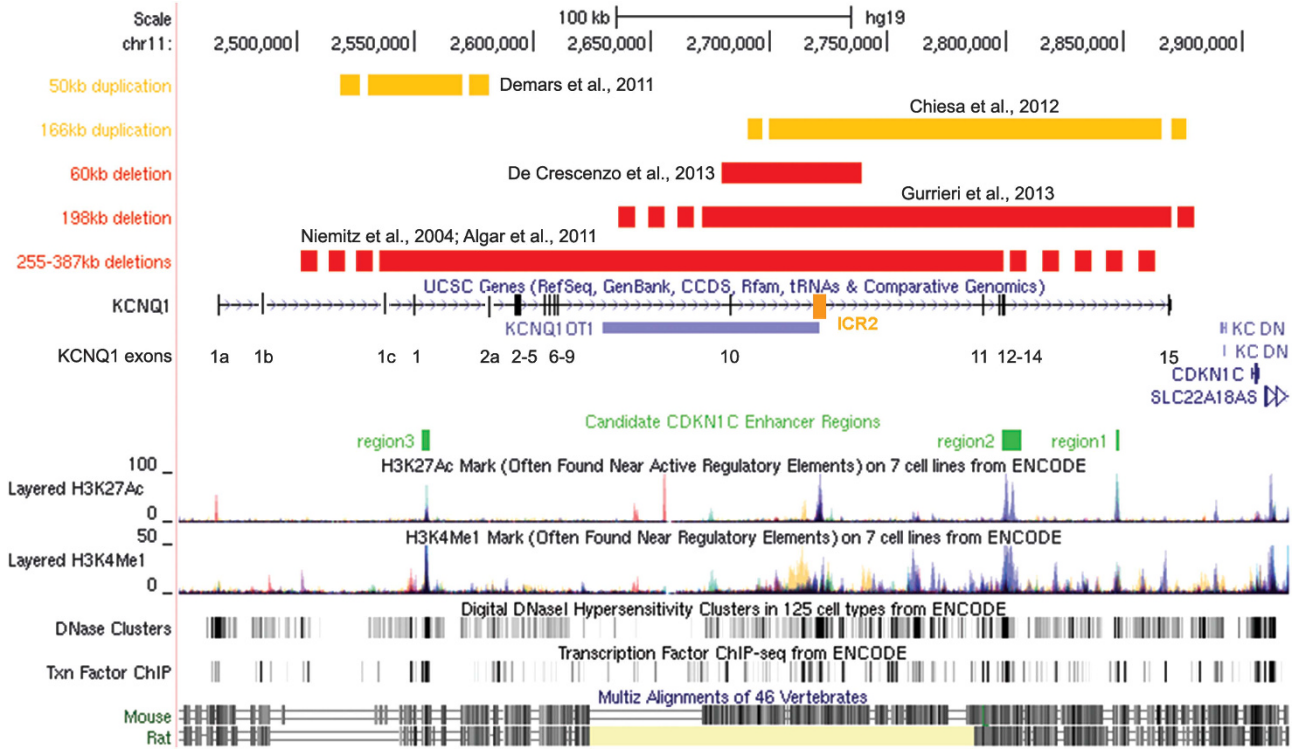

Figure 1 Identification of candidate *CDKN1C* enhancer regions through integration of data of chromosome imbalances, chromatin features and sequence conservation at 11p15.5. The image shows an UCSC Genome Browser screen shot including the most relevant information used to locate the putative enhancers. Three deletions associated with BWS/SRS or severe IUGR[1,2,10,11] and two duplications associated with BWS[12,13] are indicated by red and yellow lines, respectively. Dots indicate undefined borders of duplications/deletions. The exons of the *Kcnq1* gene (NM_000218) are numbered.[15] ICR2 in *KCNQ1* intron 10 is shown as an orange box. Green boxes indicate the candidate enhancers (region 1, chr11: 2 846 000–2 849 000; region 2, chr11: 2 798 000–2 807 000; region 3, chr11: 2 552 500–2 556 500 – GRCh37/hg19, February 2009 assembly). No sequence information is available in the rat genome (rn4, November 2004) for the region corresponding to human chr11: 2 622 000–2 788 000. KCDN, KCDNQ1DN. Note that regions 1–3 correspond to evolutionary conserved intronic regions that are enriched in H3K27Ac, H3K4Me1, DNase hypersensitive sites and transcription factors binding as reported by ENCODE (http://genome.ucsc.edu Chr11: 2 450 000–2 920 000; GRCh37/hg19, February 2009 assembly).

rodents) that are enriched in specific histone modifications (ie, H3K27acetylation, H3K4monomethylation), DNase hypersensitive sites and transcription factor binding.[7,8] Based on the conservation between human, mouse and rat genomes[9] and the epigenetic marks deposited by the ENCODE project,[8] we found three sequences meeting the features of enhancers within the *KCNQ1* gene region (Figure 1, green boxes and Supplementary Figure 1). Two of these sequences are located within *KCNQ1* last intron (regions 1 and 2) and one in intron 1 immediately 3′ to the exon 1 boundary (region 3). Some of the characteristic features of the enhancers are also present in the ICR2/*KCNQ1OT1* promoter which, however, also displays H3K4Me3 marks (not shown).

In human, rare chromosome imbalances involving ICR2 and different portions of the *KCNQ1* gene have been reported associated with BWS/SRS phenotypes and severe IUGR (Figure 1). A deletion of 255–387 kb removing most of *KCNQ1* (from intron 1b to intron 14) was first described.[10,11] This deletion results in *CDKN1C* downregulation and BWS when maternally inherited, but in normal phenotype on paternal transmission. More recently, a 198 kb deletion extending from *KCNQ1* intron 9 to exon 15 was found associated with mild BWS when maternally inherited and mild SRS when paternally inherited.[1] Finally, a paternal deletion of 60 kb resulting in *KCNQ1OT1* silencing and *CDKN1C* activation was associated with severe and recurrent IUGR.[2] The existence of multiple *CDKN1C* enhancers was originally proposed to explain the phenotypes of the 255–387 kb and 198 kb deletions.[1,11] To reconcile the phenotypes of all three deletions, we now propose that these enhancers are all (or mostly) located within the 255–387 kb deletion, in part within the 198 kb deletion, but none within the 60 kb deletion (Figure 1). So, by eliminating ICR2 but keeping the enhancers on the paternal chromosome, the 60 kb deletion would abolish the silencing effect of the *KCNQ1OT1* RNA and lead to *CDKN1C* activation and severe growth restriction. In contrast, by eliminating one or more enhancers, the larger deletions would result in weaker (198 kb deletion) or no activation (255–387 kb deletion) of the paternal *CDKN1C*. Interestingly, all the candidate enhancer sequences indicated by the ENCODE data lie outside the 60 kb deletion and likely within the 255–387 kb deletion, but only two of them are within the 198 kb deletion, thus meeting the characteristics proposed for the *CDKN1C* enhancers. These regions may also be involved in imprinting control, as they are included in two maternal duplications (Figure 1) associated with ICR2 hypomethylation and BWS.[12,13] Intriguingly, region 3 also harbors strong binding sites for CTCF (Supplementary Figure 1), which have been shown to interact with other CTCF binding sequences near *CDKN1C*, possibly forming higher-order chromatin structures or insulators that may be disrupted by the duplication and result in ICR2 imprinting alteration.[14]

In conclusion, by integrating information on chromosome rearrangements and chromatin features, we have identified three sequences meeting the features predicted for *CDKN1C* enhancers that help explain the phenotypes associated with different 11p15.5 deletions. Further experimental work in animal models (transgenic enhancer assays) and in the patients (mutation screening) will possibly confirm their functional role.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)