## In-class assignment: Chiesa et al. (2012) paper

Group: Martin Thygesen, Dina Von Werder, Ardalan Hendi and Ida Vinggaard Kjeldsen.

1. Based on the article, what are the known causes of SRS and BWS? Which of these causes are genetic, and which are epigenetic?

BWS: Loss of methylation of the maternal ICR2 leads to bi-allelic activation of KCNQ1OT1, which acts in cis to silence the genes controlled by the ICR2. This is an epigenetic cause.

SRS: Duplication of ICR2 on the maternal chromosome. This is a genetic cause.

1. Consult Figure 8 to remind yourself of what/where ICR2 is, then consider the data shown in Figure 5B. What do they show, and what do they tell us about the methylation state of the ICR2 region in individuals I-4, II-4 and III-6?

Figure 5B shows the methylation levels of the ICR2 regions in the three individuals.

Individual I-4: the duplicated paternal allele is not methylated on either ICR2 region, and the maternal allele is methylated on ICR2.

Individual II-4 and III-6: Both individuals have a non-methylated ICR2 on their paternal chromosome (not duplicated). The duplicated region on the maternal allele contains two ICR2 regions, where one is methylated and one region is non-methylated.

1. Notice how I-4, II-4 and III-6 all have the same number and methylation pattern of ICR2 ‘loci’. How can their difference in terms of having *vs*. not having BWS be explained?

The duplication containing a non-methylated ICR2 acts in cis to regulate the expression of CKNQ1 and CDKN1C. If this duplication is on the paternal chromosome, where the ‘original’ ICR2 is already non-methylated, there is no effect and no phenotype (individual I-4). If the duplication is on the maternal chromosome, where the original ICR2 is methylated, it leads to bi-allelic expression of KCNQ1OT1. The additional expression of KCNQ1OT1 silences the maternal allele of the genes (KCNQ1 and CDKN1C) that would normally be maternally expressed. This silencing leads of the BWS phenotype.

1. Explain what Figure 7B shows and how you interpret the data.

The level of paternal KCNQ1OT1 RNA binding to the DNA is the same in all four individuals. There is no significant difference to the control.

The level of maternal KCNQ1OT1 binding to DNA is much higher in BWS patients. This indicates that the duplication containing the non-methylated ICR2 region in BWS P1 and P2 leads to maternal expression of KCNQ1OT1 that binds to DNA. The same is the case with the unrelated BWS patient (P4).

1. One of the authors’ hypothesis is that many of the physical phenotypes associates with the BSW patients are due to reduced expression of CDKN1C. Propose two possible mechanisms that would explain how the duplication of ICR2 in these patients causes a reduction in the expression of CDKN1C. Based on what you know about *Airn, Igf2r,* and *slc22a3*, which of the two hypotheses is most likely and why?

Based on our knowledge of the *Airn* locus regulative activity, we considered two possibilities:

* 1. Active transcription of KCNQ1OT1 silences the neighboring genes (CDKN1C). This is less likely, since the two genes don’t overlap and have transcription in the same direction (not opposite directions and overlap, like *Airn* and *Igf2r*).
	2. The KCNQ1OT1 transcript silences the CDKN1C gene by binding and preventing transcription. This is more likely, considering the data in figure 7, showing that the lncRNA from KCNQ1OT1 has DNA-binding activity.
1. After reading this paper, how do you think clinical papers describing just a few patients can contribute to our understanding of the regulation of developmentally relevant genes?

Knockdowns/knockouts of genes in humans is not a possibility in research. Naturally occurring mutations, such as duplications in this case, enables us to study the effects of loss of function or gain of function mutations in humans.