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

You are welcome to work on these questions individually or as part of a group. If you work in a group, your group will submit one set of answers for the whole group. Please ensure that all group members participate in the development of the answers.

The answers are to be submitted by email (as an attachment) by the end of class.

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

SRS is caused by an inverted duplication of the 11p15.5 cluster on the maternal chromosome, which is a genetic cause.

BWS is most commonly caused by loss of maternal-specific ICR2 methylation, which is an epigenetic cause that results in bi-allelic activation of KCNQ1OT1 and bi-allelic silencing of the centromeric domain genes, including CDKN1C. In addition, uniparental disomy at the 11p15.5 cluster is a genetic cause of BWS, but is less common.

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?

The figure shows methylation of ICR2 regions on both paternal and maternal chromosomes in different individuals.

Patients with a duplicated paternal region in their maternal chromosome have one copy unmethylated and the other copy methylated with occasional unmethylated dinucleotides (which resembles the control – for the methylated copy).

I-4 (normal phenotype) – duplicated paternal region but inside the paternal chromosome – same methylation pattern as above (difference is the copy on the paternal chromosome)

Impaired imprinting results from having the unmethylated copy incorporated into the maternal chromosome.

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 difference is in the imprinting as a result of the truncated duplication of KCNQ1OT1 on the maternal chromosome, which is not methylated. Since the duplicated region is not methylated, the truncated KCNQ1OT1 gene can be transcribed, which thus inhibits transcription of CDK1NC from the maternal chromosome in cis. Since transcription of CDK1NC from the paternal chromosome is already normally repressed, repression of CDK1NC on the maternal chromosome as well leads to insufficient CDK1NC transcription overall and BWS.

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

The figure shows the interaction of KCNQ1OT1 RNA with chromatin at both chromosomes. On the paternal chromosome interaction of RNA in the control individual and in the patients are similar – SD error bars overlap. On the maternal chromosome interaction of RNA in the patients increases several fold compared to the control individuals (with WT phenotype). The interaction levels between individual patients differ greatly as well.

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?
2. The duplication of KCNQ1OT1 on the maternal chromosome is not methylated. Since the duplicated region is not methylated, the duplicated KCNQ1OT1 gene can be transcribed, which thus inhibits transcription of CDK1NC from the maternal chromosome in cis. Since transcription of CDK1NC from the paternal chromosome is already normally repressed, repression of CDK1NC on the maternal chromosome as well leads to insufficient CDK1NC transcription overall.
3. The duplication of ICR2 includes a silencer element to which regulatory proteins can bind and inhibit transcription of CDKN1C.

Hypothesis a) is more likely based on how the paper shows that the duplicated ICR2 is not methylated, whereas there is less support for hypothesis b) because it is unknown if a silencer element that acts on CDKN1C is actually included in the duplication

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?

By studying humans in clinical studies, the information obtained is more relevant compared to information obtained using animal models. Processes in animal models may deviate from the processes observed in human counterparts. Therefore, clinical studies using humans can help to confirm that the findings observed in animal models can also extend to humans in order to increase the confidence of conclusions derived from studies using models.