**Monday March 10th in-class “assignment”:**

**studying SRS and BWS patients as a way to elucidate regulatory mechanisms of imprinted loci**

Assigned paper: Chiesa et al. (2012) The KCNQ1OT1 imprinting control region and non-coding RNA: new properties derivedfrom the study of Beckwith–Wiedemann syndromeand Silver–Russell syndrome cases

You are welcome to work on this set of questions collaboratively or individually, and to request help as/if needed. You will hand in your answers at the end of class (one set of answers per group if you work collaboratively).

1. Compare and contrast the phenotypes of the SRS vs. BWS patients (you will need to look at Table 1). Do you notice any trends? Knowing that all the patients studied in this article have mutations in the same imprinted cluster, what could explain the differences in phenotypes?

-More facial morphology defects in SRS than BWS, pre-natal growth failure in SRS but not BWS patients. The BWS babies tend to be heavier than normal (except patient 1). SRS patient is below normal weight at pre-natal weight and at present weight.

-Umbillical hernia seem to be associated with BWS but not SRS

-some nervous system problems are observed in SRS, but not BWS

-Even though all the patients in this article have mutations in the same imprinted cluster, the mutation is not the same. That is, the duplication of entire cluster resulted in phenotype of SRS and the partial duplication resulted in phenotype of BWS. This differences in phenotypes may be because the different level of transcription of different genes as a result of the different types of duplication which have different level of methylation of ICR2.

1. The authors report using OMIM to obtain some information for their research.Take a few minutes to look up *Kcnq1ot1* on OMIM and see what information you get.
* overlaps with KCNQ1, associated with BWS and multiple cancers, expressed from the paternal allele
* KCNQ1OT1 promoter is a repressor of neighboring genes. However, highly conserved repeats in the 5' end of the KCNQ1OT1 transcript is not necessary for imprinting
* KCNQ1OT1 is transcribed in all tissues but its role in imprinting is tissue specific
1. Look at the pedigree in Figure 1.
2. What can immediately be concluded about BWS (even without knowing who inherits the mutant allele from whom)?

-The inheritance is autosomal inheritance, not sex-linked. Both male and female could have BWS.

1. II-2 and II-4 both have BWS, and both have one child with BWS and one child without BWS. Briefly explain how this is possible.

Since the inheritance in autosomal, the chance of passing the mutant chromosome/alleles from mom to child is 50% for each child. If the mutant maternal copy is passed to child, then child have BWS. If WT maternal copy is passed to the child, then the child is normal.

1. Briefly describe the mutation detected in the BWS patients and the mutation detected in the SRS patient, and their respective effects at the molecular, cellular, and organismal levels (use figures 2, 3, and 8, as well as your answer to Question 1).

NOTE: molecular level = DNA sequence, DNA methylation, gene expression; cellular = proteins present in the cell, potential effects on the cell; organismal = effects on the entire organism.

BWS: The mutation is an inverted duplication of ICR2 and a small portion of 5‘ of KCNQ1OT1 in cis. When this mutation in inherited from mother, only one of the maternal ICR2 allele is methylated while the additional allele is not. Also, the paternal allele is not methylated. The additional partial KCNQ1OT1 is transcribed because of lack of methylation so the total amount of KCNQ1OT1 transcribed is higher than WT. The transcribed KCNQ1OT1 interacts and represses CDK1NC expression so there is lower overall level of growth inhibitor CDK1NC (essentially, the paternal KCNQ1OT1and transcribed truncated maternal KCNQ1OT1 both repress CDK1NC), which could affect growth at molecular level and macroscopic level so that the patient have higher birth weight.

 If mutation is inherited from father, then there is no deviation from WT cellularly or macro- phenotype. The methylation pattern is normal: methylated for maternal allele, and non-methylated for the paternal allele (and the duplicated allele). But the expression of protein IGF2 might be higher than normal.

SRS: The inverted duplication of entire 11p15.5 cluster in cis is detected. Both maternal copies are methylated. Methylated ICR2 inhibits the transcription of KCNQ1OT1 and this result in the higher transcription of KCNQ1 and expression of this protein. Also there is higher level transcription of CDK1NC, and low relative level of IGF2, a growth factor. This high level of CDK1NC protein and low level of IGF2 protein (relatively) affects downstream genes/ protein expression which ultimately may result in the slow growth of the patient at organismal level.

1. Explain what the data in Figure 7 show, and how you interpret them.

Fig 7 a): Experimental results after performing qPCR analysis of ChRIP-purified RNA from lymphoblast cell lines. Using primers located 500bp downstream of transcription start site, the researcher showed that KCNQ1OT1RNAS interacts with chromatin in all four cell lines. In BWS patients, KCNQ1OT1 RNA was detected to be much higher than in control. This is true for BWS patients that had maternal duplication of ICR2, or patients with ICR2 hypomethylation & no microduplication. This high level of KCNQ1OT1 RNA transcript detected in BWS patients likely due to the lack of ICR2 methylation.

Fig 7b): The researchers uses SNPs to detect whether the KCNQ1OT1 RNA from (a) is the paternal allele or maternal allele. They showed that similar level of paternal allele is detected for control and all BWS patients. But while no maternal allele is detected for control, the BWS patients have significantly higher maternal allele KCN1OT1 RNA in the cell lines. This part of figure showed that BWS patients’ high KCNQ1OT1 RNA detected in (a) is likely due to the high transcription level of maternal allele, not the paternal allele.

Fig 7c): The ChRIP RNAs from the control and BWS patients were retrotranscribed and sequenced. The sequence clearly shows that the BWS patients had a dual RNA type expression while control only have one allele transcribed. They also sequenced the control’s DNA to demonstrate that the control have heterozygosity genetically, so the single allele detection at ChRIP RNA is not because of having two copies of same allele but because only one allele was transcribed.

1. What valuable fundamental information was gained about imprinting control regions through the study of these patients?

It's possible that the sequence of the ICR itself is not sufficient for establishing imprinted methylation. Orientation of the ICR sequence and it's methylation pattern may play a significant role in imprinting.

1. Please list any questions and points of confusions relative to imprinting and the regulation of *Hox* genes (if nothing much comes to mind, please think about it and post on FB or on the Connect Discussion board). We will take the time to clear up your questions before starting on X inactivation.
2. Bonus: do you think the phenotypes of BWS patients 1-3 are representative of BWS? justify.
* BWS patients 1-3 do not represent BWS phenotype as whole because of the wide range genetic variations that result in BWS and it's correct diagnosis is still under study. In this paper, it is only a 160kb duplication that seems to result in BWS in the three patients, it's possible the duplication can have different sizes and occur in different locations of the 11p15.5 locus.