A clinical paper on imprinting

Questions

1. Please list the names of all the group members who participated:
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2. General question: 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 a duplication of the entire 11p15.5 imprinted gene cluster and then the duplicated imprinted gene cluster is inverted and then inserted into the 3' end of the maternal imprinted gene cluster (genetic cause). This duplication event resulted in partial ICR1 hypomethylation and hypermethylation of ICR2 and these epigenetic changes result in the SRS phenotype (epigenetic cause).

BWS is caused by a duplication event in which ICR2 and the 5' 20 kb of *KCNQ1OT1* is inverted and inserted into the maternal chromosome (genetic cause). As a result, maternal the ICR1 locus is hypermethylated and ICR2 is hypomethylated and a truncated version of *KCNQ1OT1* is transcribed and these epigenetic changes result in the BWS phenotype (epigenetic cause).

Other known causes of BWS include loss of function defect for a trans-acting factor (possibly a genetic cause in the gene for the trans acting factor, but from the perspective of the gene it is acting on, this could be an epigenetic cause), uniparental disomy (genetic cause in that there may have been duplication, and epigenetic cause if this was an imprinted loci), mutation in CDKN1C (genetic cause).

3. Consult Figure 8 to remind yourself of what/where ICR2 is. Then consider the data shown in Figure 5B.

   A. What did the authors do?
   B. What were the results?
   C. What do the data tell us about the methylation state of the ICR2 region in individuals I-4, II-4 and III-6?

A. Authors performed a sodium bisulphite sequencing experiment to determine the location of methylated cytosines along the ICR2 sequence and to visualize ICR2 allele specific DNA methylation (paternal versus maternal imprinted gene locus).

B. BWS: Individual I-4 shows paternal allele duplication and in addition, more CpG dinucleotides are methylated on the I-4's paternal allele than II-4 and III-6 paternal alleles. Maternal alleles is more methylated than paternal alleles. In individuals II-4 and III-6, there is a difference in methylation between the duplicated paternally derived allele and the maternally derived allele.

C. The paternal ICR2 regions of II-4 and III-6 are highly unmethylated and have similar level of methylation compared to the control paternal allele. Individuals II-4 and III-6 have a highly methylated ICR2 regions as well as a second ICR2 region that is highly unmethylated. The duplicated ICR2 region of I-4 shows a slightly higher methylation pattern than the paternal alleles of patients II-4 and III-6. The maternal ICR2 region in I-4 is highly methylated and is similar to the methylation pattern of one of the ICR2 regions in II-4 and III-6.

4. 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 paternal ICR2 is not methylated and expresses *KCNQ1OT1* which silences the centromeric domain genes in cis. The maternal ICR2 is normally methylated and *KNCQ1OT1* not expressed and the centromeric genes are expressed from the maternal allele. In individual I-4, the paternal allele was duplicated and unmethylated ICR2 plus a truncated version of *KCNQ1OT1* is present in the paternal chromosomes. In the paternal chromosome, the centromeric domain is already being silenced by *KCNQ1OT1* therefore the duplication event does not affect the expression of the centromeric domain gene. In individuals II-4 and III-6, the duplicated paternal ICR2 and *KCNQ1OT1* is inverted and inserted into the maternal allele. Although the paternally derived ICR2 is on the maternal chromosome it does not receive the same methylation mark as the maternally derived ICR2 region and it maintains it's unmethylated state. Thus on the maternal chromosome, a truncated version of *KCNQ1OT1* is expressed and can act in cis on the maternal centromeric genes. Thus the expression of the maternal centromeric genes is affected. In individuals II-4 and III-6, the centromeric domain genes on both the paternal and maternal chromosomes are silenced. The maternal chromosome transcription of the truncated KCNQ10T1 can downregulate the expression of *CDKN1C*. With biallelic silencing of CDKN1C, the cell growth inhibitor, there will be too much cell growth resulting in the BWS phenotype.

5. Consider Figures 7A and 7B.

A. What did the authors do?

To test whether the truncated KCNQ10T1 RNA interacted with chromatin in cells with 160 kb duplication the authors performed chromatin RNA immunopurification (ChRIP) and then qPCR analysis to determine fold enrichment of the KCNQ10T1 RNA. The authors used four different lymphoblastoid cell lines: (i) normal control, (ii) BWS patient 2, (iii) BWS patient 1, and (iv) an unrelated BWS patient with ICR2 hypomethylation but no microduplication (BWS patient 4). Anti-histone H3 antibodies were used to pull down the cross-linked RNA. Anti-rabbit IgG antibodies were used as a control.

To assess the global amount of KCNQ10T1 associated with chromatin, they did qPCR using primers 500 bp downstream of the transcription start site of KCNQ10T1.

To assess the allelic origin of the KCNQ10T1 transcript associated with chromatin, the authors used allele-specific primers to an SNP located 4kb downstream of the KCNQ10T1 transcription start site.

B. What were the results?

Figure 7A
Total KCNQ10T1 RNA (from all alleles) in the immunoprecipitated chromatin was more enriched in the BWS patients compared to the normal control.

Figure 7B
There were similar enrichment levels of ChRIP-purified KCNQ10T1 RNA derived from the paternal allele in all four cell lines. The maternally derived transcript in the immunoprecipitated chromatin was higher in the BWS cell lines than in the control.

C. What can we directly conclude?

The maternally transmitted 160 kb duplication is sufficient to cause enrichment of the KCNQ10T1 transcript in the immunoprecipitated chromatin. Proper methylation of the maternally transmitted ICR2 is necessary to prevent enrichment of KCNQ10T1 in immunoprecipitated chromatin. (Patient 3 non-duplication)

Maternally transmitted 160kb duplication is sufficient to cause enrichment of the maternally derived KCNQ10T1 transcript in the immunoprecipitated chromatin. Proper methylation of the maternally transmitted ICR2 is necessary to inhibit enrichment of maternally derived KCNQ10T1 transcript in the immunoprecipitated chromatin. (Patient 3).

D. Provide an interpretation for the results.

The KCNQ10T1 transcript interacts with chromatin to silence the centromeric domain genes. Normally only the paternal allele is expressed because ICR2 is unmethylated. Normally the maternal allele is not expressed because ICR2 is methylated. The 160 kb duplication contains a third copy of ICR2 and the 5' part of the KCNQ10T1 gene resulting in a truncated transcript which is still able to interact with chromatin.

6. One of the authors' hypothesis is that many of the physical phenotypes associated 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 imprinted loci, which of the two mechanisms is most likely and why?

In this paper they link CDKN1C expression to the physical phenotype of BSW patients. CDKN1C expression was studied in BSW patients and was observed to be reduced in diseased versus non-diseased individuals. Therefore, the 160 kb duplication of ICR2 and *KCNQ1OT1* seems to regulate maternal CDKN1C expression. The *KCNQ1OT1* transcript silences imprinted genes on the paternal chromosome in a cis regulatory mechanism. Authors in this paper found that in BWS patients, the maternal non-duplicated ICR2 is methylated, whereas the maternal paternally derived, duplicated ICR2 region is

unmethylated. Thus a truncated version of *KCNQ1OT1* is produced and regulates CDKN1C expression. Regulation of CDKN1C by truncated *KCNQ1OT1* could occur via one of two mechanisms. Firstly, *KCNQ1OT1* transcription could interfere with *CDKN1C* expression by destabilizing transcription machinery from producing *CDKN1C* transcript. Secondly, the truncated *KCNQ1OT1* transcript may act in cis to silence *CDKN1C* expression. In this latter case, *KCNQ1OT1* may recruit proteins that causes *CDKN1C* expression to be reduced compared to individuals who do not have BSW. Of these two mechanism, we believe that the second is most likely to be regulating *CDKN1C* expression. Based on what we know about the paternal chromosome imprinted gene cluster, *KCNQ1OT1* acts in cis to silence imprinted genes. The paternal, imprinted *CDKN1C* genes is not expressed and in normal individuals without BSW, the maternal chromosome expresses *CDKN1C* whereas individuals with BSW do not produce *CDKN1C* from their maternal chromosome. The authors of this paper were able to demonstrate using ChRIP that the maternal truncated *KCNQ1OT1* transcript interacts with chromatin. Moreover, the *KCNQ1OT1* and *CDKN1C* genes are not overlapping therefore it seems unlikely that transcription of *KCNQ1OT1* is regulating *CDKN1C* expression. Taken together, it seems more likely that the truncated *KCNQ1OT1* transcript interacts with *CDKN1C* and regulates expression via a cis regulatory mechanism.

7. Time permitting: what do you think is the value of clinical studies? Who are they valuable for?

Clinical studies are valuable for researchers (and by extension, the general public) as they allow us to study rare disorders. By studying rare disorders we can identify mechanism that have been disrupted in diseased individuals. Thus, clinical studies give us an opportunity to understand cellular mechanisms and the role of certain molecules for proper cell function. Data collected from clinical studies is also valuable because this information can be used to devise therapies for individuals with the disease. Additionally, clinical studies are valuable to prospective parents who are concerned with passing along a disease phenotype to their offspring. Since it is unethical to create knockout genes to see the phenotypic effects in humans, clinical studies are highly valuable for studying the human genome by enrolling patients with genetic disorders.