Analysis of a cohort of Silver Russell patients for 11p15 epigenetic changes

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Silver Russell Syndrome

Major
• Pre- and post-natal growth retardation without growth catch-up after two years
• Proportionately short stature
• Classical facial features: triangular face with prominent forehead

Minor
• Clinodactyly V
• Skeletal asymmetry: facial, limb or body
• Muscular hypotrophy/tonia
• Cafe au lait patches
• Relative macrocephaly

11p15 = imprinted region, contains genes implicated in growth

- **H19** – maternally expressed, possible tumour suppressor
- **IGF2** – paternally expressed fetal growth factor
- **KCNQ1** – maternally expressed, forms part of a potassium channel
- **CDKN1C** – maternally expressed tumour suppressor, potential negative growth regulator
- **KCNQ1OT1** – antisense transcript, silencing of KCNQ1 and CDKN1C
Chromosome 11 involvement in SRS:

mat dup11p15 - 4 growth retarded patients (2 with SRS-like features)  
(Kosaki 2000, Fisher 2002)

- 2/46 SRS patients with mat dup 11p15 (Eggerman 2005)

Epimutation studies

H19DMR partial hypomethylation in 5/9 patients  
(Gicquel 2005)

H19DMR demethylation in 16/51 patients  
(Eggerman 2005)

Complete H19 hypomethylation in 2 SRS patients  
(Bliek 2006)
Decreased methylation in a further 2 patients – SRS-like features

KvDMR methylation was normal in all patients
Project Aim

1. To screen the largest cohort of UPD7/SRS patients to date for epigenetic changes at the H19DMR and KvDMR

2. Methylation sensitive PCR not southern blotting

3. To compare any epigenetic changes with previous studies

4. To compare the frequency of results with those of previous studies

   - more advantageous to screen chromosome 11 in SRS patients than chromosome 7?
Patient source

- 94 patients referred to WRGL for UPD7 and SRS.

Method

Methylation sensitive PCR currently used for BWS screening

Bisulphite treated genomic DNA

Triplicate PCR for H19DMR, KvDMR region for ABI3100 analysis
Methylation Analysis

H19DMR

<table>
<thead>
<tr>
<th>Normal</th>
<th>BWS Positive</th>
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Increased ratio = Gain of methylation
Decreased ratio = Loss of methylation

KvDMR

| Normal | BWS positive |

Increased ratio = Loss of methylation
Decreased ratio = Gain of methylation

Control for individual variation - ratios were normalised against the average of a panel of normals PCRd simultaneously with the patients.
94 patients screened in total:

- 5 fails

- 70 patients had normal ratios

- 11 patients ratios below 0.8 indicating loss of methylation $\Rightarrow$ overexpression of H19 tumour suppressor $\Rightarrow$ **Growth retardation**

- 8 patient ratios above 1.2 indicating gain of methylation $\Rightarrow$ biallelic expression of IGF2 (growth factor) $\Rightarrow$ **Overgrowth!! BWS epimutation!!**
No changes previously found at KvDMR

- 3 fails
- 67 patients had normal ratios
- 7 abnormally low ratios indicating gain of methylation ⇒ Overexpression of tumour suppressor (CDKN1C) ⇒ Growth retardation
- 17 abnormally high ratios indicating loss of methylation ⇒ Loss of expression of CDKN1C ⇒ Overgrowth!! BWS epimutation!
94 patients referred for growth retardation

2μg gDNA bisulfited

Triplicate PCR for H19DMR, KvDMR region for 3100 analysis

Normal

abnormal

No further work

2nd bisulphite

PCR's in triplicate for 3100 analysis

Three categories of patients:
Normal
BWS Positive

**Category one**
H19DMR Hypomethylation
↑ Growth suppressor expression

3 patients

**Category two**
H19DMR Hypermethylation
↑ Growth factor expression!

1 patient
Normal
BWS Positive

Category three
KvDMR hypermethylation
† Growth suppressor expression

2 patients
Categories of results:

H19DMR Hypomethylation (↑ Growth suppressor) 3/94
KvDMR Hypermethylation (↑ Growth suppressor) 2/94 NEW FINDING
H19DMR Hypermethylation (↑ Growth Factor) 1/94 NEW FINDING

= ~6% of patient cohort
Technical Conclusions

• Important to base methylation results on at least two separate bisulphite reactions

• Methylation sensitive PCR may be more sensitive than southern blotting for detecting epigenetic changes - especially KvDMR changes

• Test for epigenetic changes at 11p15 in any patients referred for short stature and asymmetry, full SRS, or SRS-like features.
Further work

Trace clinical referrals for the abnormally methylated patients to see if they fit the SRS criteria

Confirm the results using pyrosequencing
  More sensitive?
  Same results - different region amplified?

CA repeat analysis on abnormals and their parents if available – localised UPD?

Test other chromosomal imprinted regions eg chr 6
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