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

Anne-Marie Coupe
Wessex Regional Genetics Laboratory
Salisbury

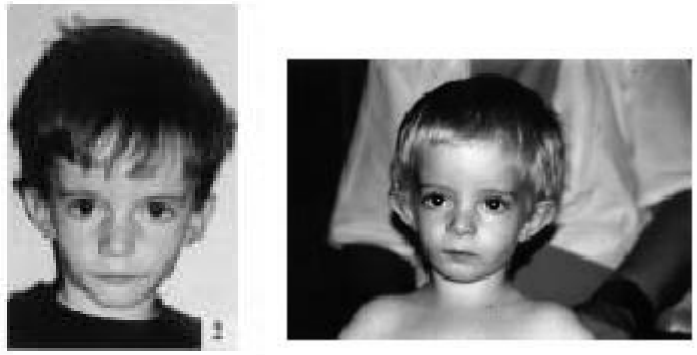
Silver Russell Syndrome

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

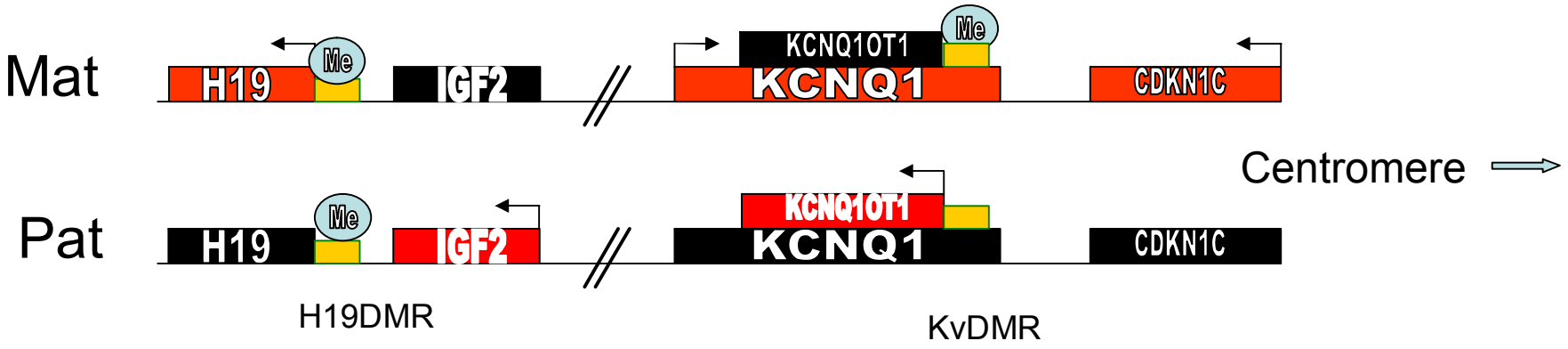
Minor

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



Reproduced from the **Price SM**, R Stanhope, C Garrett, M A Preece, R C Trembath. paper 'The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria'**.** *J Med Genet* 1999;**36:**837-842 (November)

11p15



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?

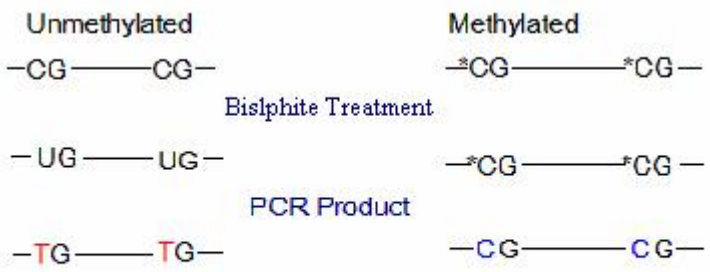
Patient source

- 94 patients referred to WRGL for UPD7 and SRS.

Method

Methylation sensitive PCR currently used for BWS screening

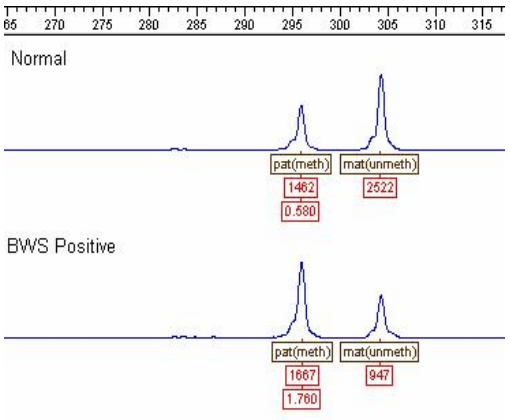
Bisulphite treated genomic DNA



Triplicate PCR for H19DMR, KvDMR region for ABI3100 analysis

Methylation Analysis

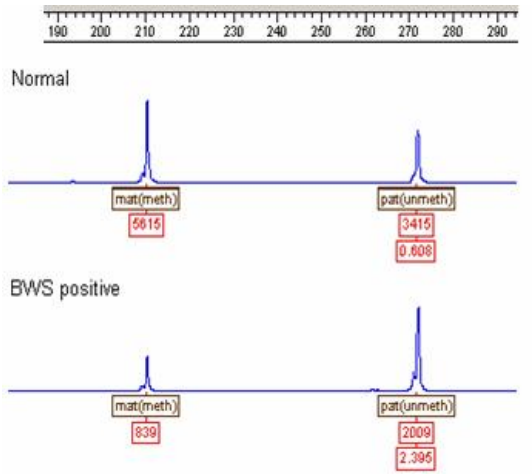
H19DMR



H19 – Meth(P)/Unmeth(M)

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

KvDMR

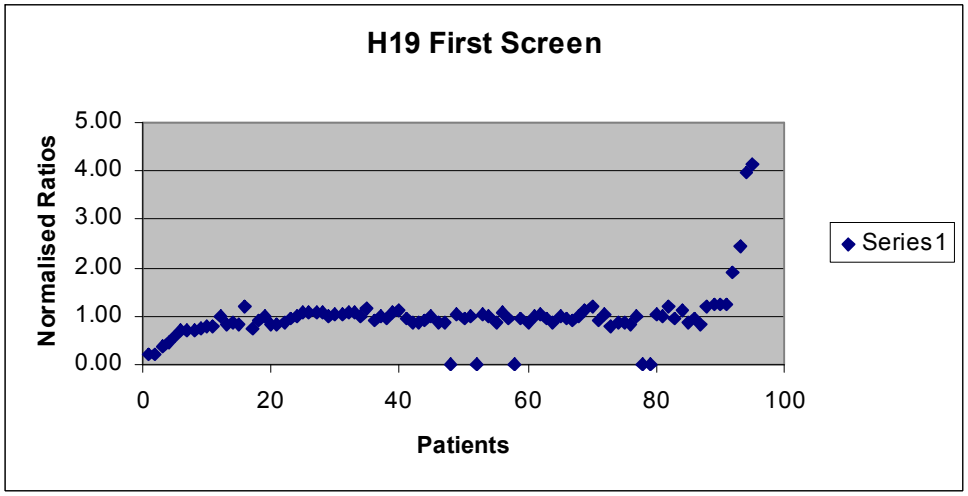


KvDMR – Unmeth(P)/meth(M)

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.

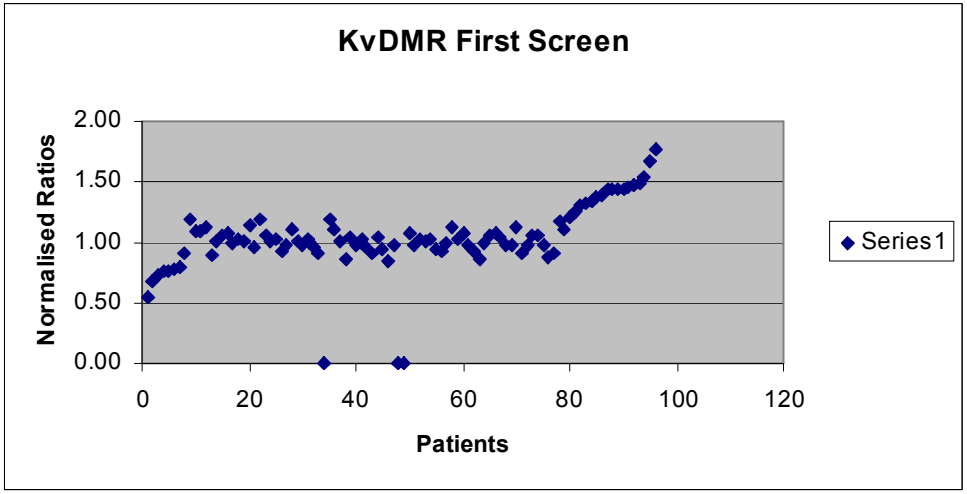
ABI 3100 H19 Results



94 patients screened in total:

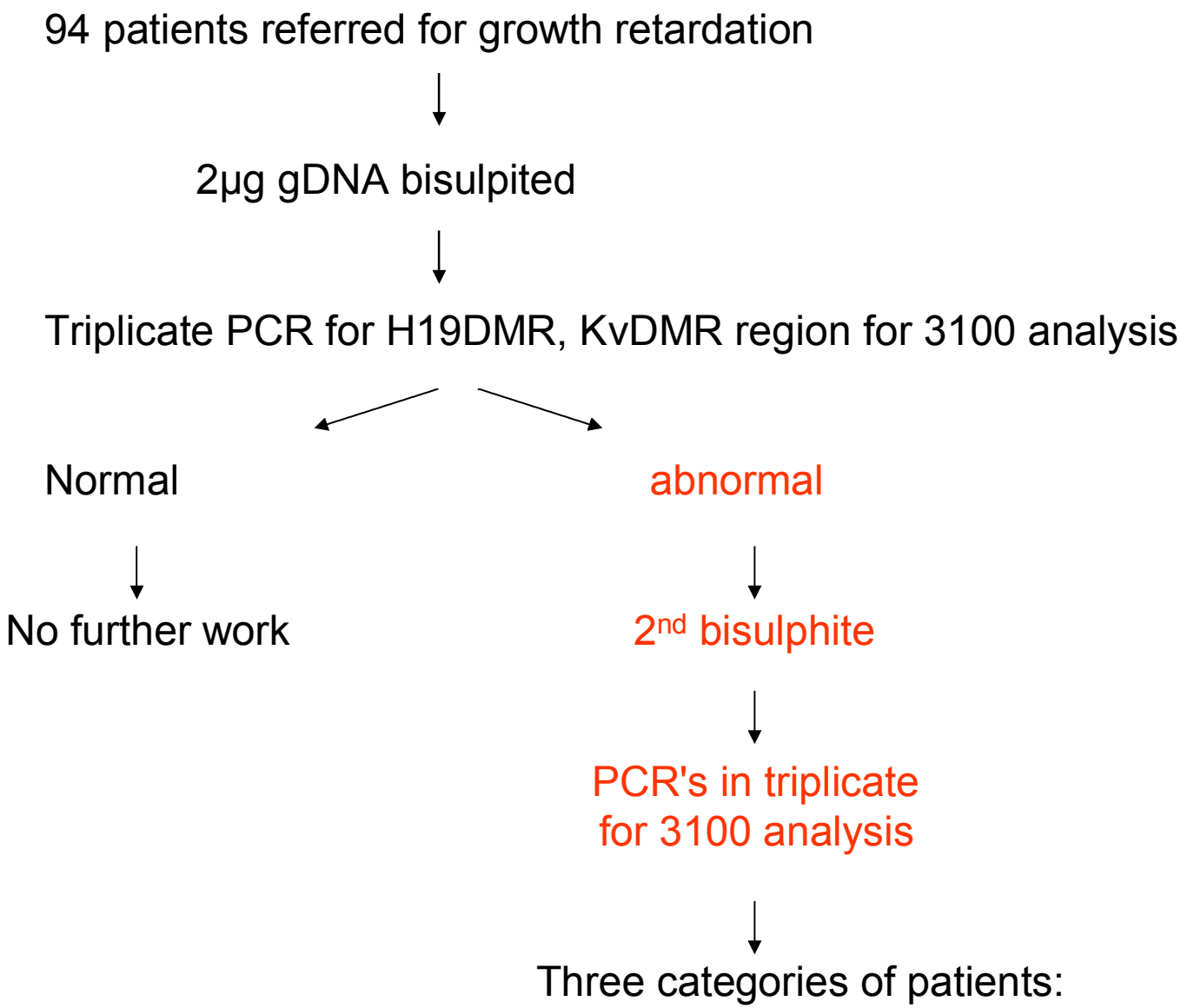
- 5 fails
- 70 patients had normal ratios
- 11 patients ratios below 0.8 indicating loss of methylation ⇒ overexpression of H19 tumour suppressor ⇒ **Growth retardation**
- **8** patient ratios above 1.2 indicating gain of methylation ⇒ biallelic expression of IGF2 (growth factor) ⇒ **Overgrowth!! BWS epimutation!!**

ABI 3100 KvDMR Results

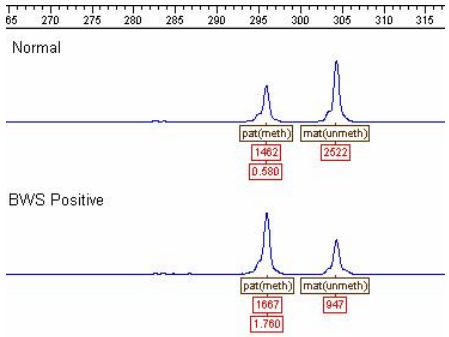


No changes previously found at KvDMR

- 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 bisulpited
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:

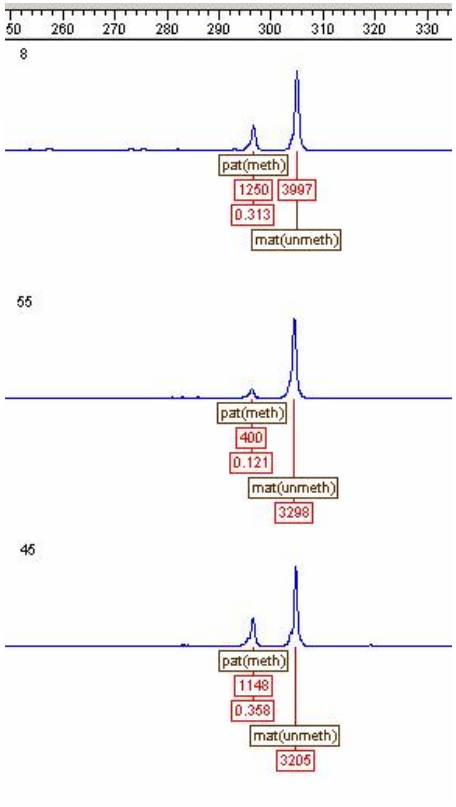
Normal
BWS Positive



Category one

H19DMR Hypomethlation

⇑ Growth suppressor expression

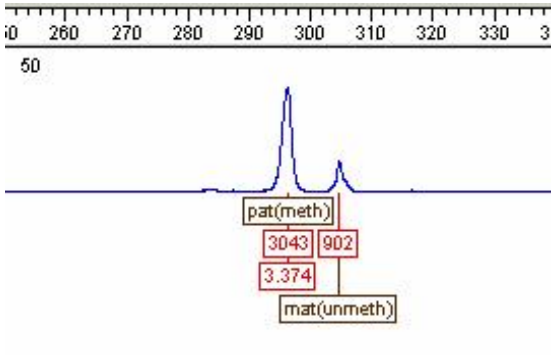


3 patients

Category two

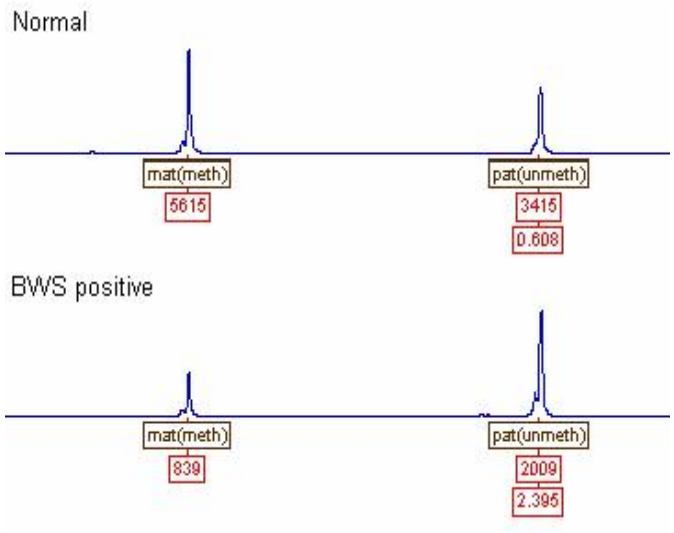
H19DMR Hypermethylation

⇑ Growth factor expression!

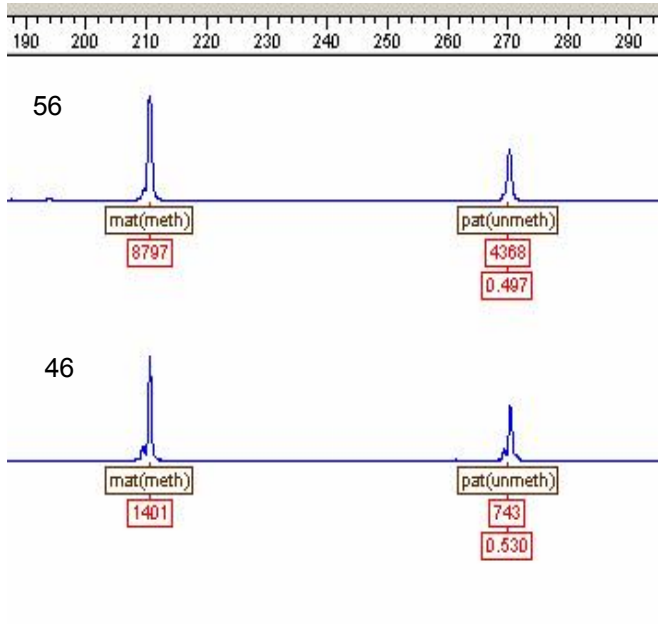


1 patient

Normal
BWS Positive

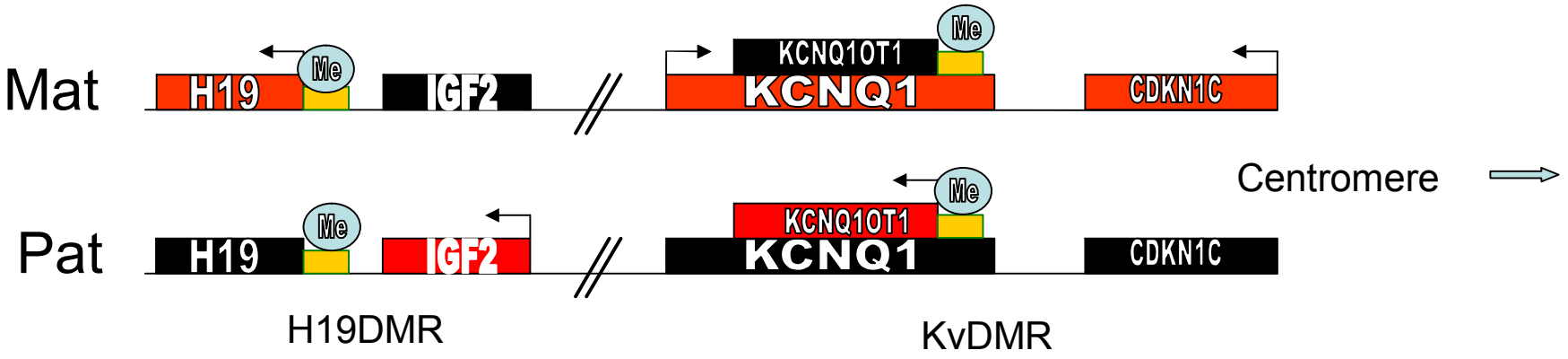


<u>Category three</u>
KvDMR hypermethylation
⇑ Growth suppressor expression



2 patients

Results Summary



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

Acknowledgements

Wessex Regional Genetic Laboratory

- Simon Thomas
- Hilary Bulman

Wessex Research Department

- Deborah Mackay

National Reference Genetics Laboratory (Wessex)

- Helen White
- Vicky Durston