Supplementary Figure 1: HIL Patient cluster analysis. Clustering of 5 TND-HIL samples with 221 control samples and 5 BWS-HIL with 245 samples respectively, while including and excluding probes from X and Y chromosomes. A) and C) show samples clustered with all chromosomes for TND-HIL and BWS-HIL respectively (cluster by gender). B) and D) show clustering of TND-HIL and BWS-HIL patient groups after the removal of X and Y chromosome probes (samples no longer cluster by gender).

Supplementary Figure 2: Detection of DNA methylation within PLAGL1 locus in patients with BWS and TND.

A) Modified screen grab from UCSC genome browser, representing the PLAGL1 gene. The sub-regions highlighted in the panels below are marked by red double-ended arrows. B) methylation of PLAGL1 imprinting control region measured using Illumina 450k array in patients versus controls. Solid lines denote M-values (left axis: methylation level expressed as a logarithmic ratio, with +4, 0, and -4 equivalent to hypermethylation, hemimethylation and hypomethylation respectively). Dashed lines represent P-values of methylation difference between patients and controls (right axis). Black line represents 221 normal controls; blue lines represent averaged methylation of five BWS patients; red lines represent averaged methylation of five TND patients. C) DNA methylation of PLAGL1 non-imprinted CpG island. As above, solid lines represent M-values, and dashed lines represent P values of methylation difference between patients and controls, with black, blue and red lines representing control, BWS and TND patients respectively.

Supplementary Figure 3: DNA methylation analysis of PPIEL in patients with BWS and TND.

A: screen grab from UCSC genome browser, representing the PPIEL locus and imprinted locus. The sub-region highlighted in the panel below is marked by a red double-ended arrow. Note that PPIEL is transcription from right to left with respect to genomic orientation. B: divergent DNA methylation between normal controls and patients, detected by methylation array. Solid lines denote M-values (left axis). Dashed lines represent P-values of methylation difference between patients and controls (right axis). Black line represents normal controls; blue lines represent averaged methylation of five BWS patients; red lines represent averaged methylation of five TND patients. C: illustrative electrophorogram from methylation-specific PCR experiment, showing difference in DNA methylation between a single patient and control. Amplicons derived from methylated and unmethylated DNA are marked by red and blue lines, respectively. D: summary of bisulphite cloning and sequencing experiment comparing patients and controls. The circles represent CpG dinucleotides within a sequence amplified after bisulphite modification, with filled and empty circles representing methylated and unmethylated DNA sequences respectively. The number to the right indicates the number of times that sequence...
was detected in individual clones. In no case were methylated and unmethylated CpG dinucleotides detected within a single clone.

**Supplementary Figure 4:** DNA methylation analysis of *WRB* in samples from individuals with trisomy 21.

The images in the left panels are illustrative electropherograms from one methylation-specific PCR experiment, showing the differences in DNA methylation between control DNA samples and groups of samples from individuals with trisomy 21 (T21). Amplicons derived from methylated and unmethylated DNA are marked by red and blue lines, respectively. A: normal controls, B and C, DNA of individuals diagnosed with T21, showing hypermethylation and hypomethylation respectively.

The figures to the right represent normalised ratios of unmethylated to methylated peak heights. Individual DNA samples were tested in duplicate, the results averaged, and then normalised to the average methylation across seven normal controls. The ratio of the unmethylated and methylated amplicons reflects that of the source DNA, such that a twofold change of peak height ratio is equivalent to a twofold excess of its source DNA. Of 34 samples from individuals diagnosed with T21, 31 showed partial hypermethylation, 2 partial hypomethylation, and one showed methylation equivalent to controls (not shown).

**Supplementary Figure 5:** Parent of origin methylation analysis of *NHP2L1* differentially-methylated region

Panels A and B show sequencing electropherograms from the mother, father and child of a trio, with the child showing heterozygous inheritance of rs6519270 (A/G: MAF 0.38). For the offspring, the upper electropherogram illustrates (heterozygous) genomic sequence, and the lower electropherogram shows DNA amplified after restriction with BstUI, which digests methylated DNA. Panel A: maternally-inherited G allele unaffected by BstUI digestion, indicating maternal methylation; Panel B: maternally-inherited A allele unaffected by BstUI digestion, indicating maternal methylation; DNA methylation is associated with parent of origin, not snp allele.

**Supplementary Figure 6:** Parent of origin methylation analysis of *PPIEL* differentially-methylated region

Panels A and B show sequencing electropherograms from the mother, father and child of a trio, with the child showing heterozygous inheritance of rs138909742 (G/-: MAF 0.15). For the offspring, the upper electropherogram illustrates (heterozygous) genomic sequence, and the lower electropherogram shows DNA amplified after restriction with McrBc, which digests unmethylated DNA. Panel A: paternally-inherited (deleted) allele unaffected by McrBc digestion, indicating maternal methylation; Panel B: paternally-inherited G allele unaffected by McrBc digestion, indicating maternal methylation; DNA methylation is associated with parent of origin, not snp allele.
Supplementary Figure 1: HIL Patient cluster analysis.

A. Clustering samples by all CpG loci:
   - TND-HIL and control samples
   - Hclust (*, "average")

B. Clustering samples by all CpG loci excluding X & Y:
   - TND-HIL and control samples
   - Hclust (*, "average")

C. Clustering samples by all CpG loci:
   - BWS-HIL and control samples
   - Hclust (*, "average")

D. Clustering samples by all CpG loci excluding X & Y:
   - BWS-HIL and control samples
   - Hclust (*, "average")
Supplementary Figure 2: Detection of DNA methylation within *PLAGL1* locus in patients with BWS and TND.

A

B 1: PLAGL1 imprinted CpG

C 2: PLAGL1 non-imprinted CpG
Supplementary Figure 3: DNA methylation analysis of *PPIEL* in patients with BWS and TND.

**A**

[Graph showing genomic location and methylation analysis.]

**B**

[Graph showing M-values and P-values for genomic locations.]

**C**

[Graph comparing control and patient DNA methylation patterns.]

**D**

[Graph showing number of methylation events.]
Supplementary Figure 4: DNA methylation analysis of *WRB* in samples from individuals with trisomy 21.

<table>
<thead>
<tr>
<th>methylated</th>
<th>unmethylated</th>
<th>Methylation</th>
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<tbody>
<tr>
<td>A</td>
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<td>Control DNA</td>
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<td></td>
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<tr>
<td>B</td>
<td></td>
<td>T21</td>
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<tr>
<td></td>
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<td>Partial hypermethylation</td>
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<tr>
<td></td>
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<tr>
<td>C</td>
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<td>T21</td>
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<td>1.46 ± 0.12 (N=2)</td>
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</table>
Supplementary Figure 5: Parent of origin methylation analysis of *NHP2L1* differentially-methylated region

A

Father 1: A/A

Mother 1: G/G

Proband 1: A/G

+BstUI: G (maternal)

B

Father 2: G/G

Mother 2: A/G

Proband 2: A/G

+BstUI: A (maternal)
Supplementary Figure 6: Parent of origin-specific methylation analysis of PPIEL differentially-methylated region

A

Father 1: -/delG

Mother 1: -/-

Proband 1: -/delG

+McrBc: delG (paternal, unmethylated)

Mother 2: -/delG

Father 2: -/

B

Proband 1: -/delG

+McrBc: - (paternal, unmethylated)