Allele frequency (f) determination for LOI – f/(1-f) (Chen et al. 2002 – manuscript)

Allele frequencies were normalized against heterozygous controls. Specifically, we let C_1 and C_2 be the PCR Ct values [average of 3 replicate runs] with allele-specific primers 1 and 2, and C_1' and C_2' [average of 3 replicate runs] be those for a heteroduplex sample used for calibration of allele specificity. Then, the cycle difference is $\Delta Ct = [C_1 - C_2]-[C_1' - C_2']$, and the allele frequency is given by:

$$\mathbf{f = 1 / [2^{\Delta Ct} + 1]}$$

The standard deviation in ΔCt, $\sigma_{\Delta Ct}$, is calculated from the weighted root mean square of the standard deviations of the contributions from the replicate sample and heterozygote measurements.

Calibration curve for RNA stability determination

Total cDNA concentration from 3 placentas was determined using a Nanodrop ND-1000 (NanoDrop Technologies). We constructed a duplicate RT-PCR calibration curve for 18S rRNA with concentrations of 5, 10, 15, 20 and 30 ng, bracketing the typical cDNA concentrations used for qASPCR. The non-parametric Wilcoxon signed rank test for two related samples confirmed that the duplicate assays were not significantly different, and the Wilcoxon rank-sum test on the averaged duplicate samples confirmed that the three placental samples were not different. Final averaged values were then plotted in a regression model using the SPSS software (Release 15.0.0 for Windows™, September 6, 2006, Chicago, IL, USA). Given the experimental condition of 40 amplification cycles, an arbitrary value of 0.001 ng for starting cDNA corresponding to a Ct value of 40 cycles was introduced, leading to a regression curve of:

$$\ln(Ct) = \ln(13.048) – 0.162 * \ln(ng)\ (R^2 = 1.000,\ p < 0.001).$$

The RNA stability time course data in Table 2A were derived from analysis of Ct values for 6 genes at each time point plotted in a regression model consistent with the 18S rRNA calibration curve:

$$\ln(Ct) = \ln(b_0) - b_1 * \ln(ng)$$

in order to obtain a weighted Ct value average over the three samples for each gene at each time point.

Supplementary Table 1. Primer set designed for PCR amplicon synthesis

Gene	Primers	
	Forward	Reverse
Paternally Expressed		
IGF2	5'-CGAAGAGTCACCACCGAGC-3'	5'-CTCAGGACTGGGCTCTCTGG-3'
PEG1/MEST	5'-TGACCACATTAGCCACTATCCA-3'	5'-CCTGCTGGCTTCTTCCTATACA-3'
PEG3/PW1	5'-ACATTTCTGGTGTTTGAGGAGTT-3'	5'-AGACCAGGTTCCGGTAATTCT-3'
PEG10	5'-AAATTGCCTGACATGAAGAGGAGTCTA-3'	5'-AAGCCTAGTCACCACTTCAAAACACACTAAA-3'
ZAC/PLAGL1	5'-CATATTTGCATGTTAGAAGAATCAGC-3'	5'-TGAGTCAGTTAGGTCAGTGTAGAGAGA-3'
DLK1	5'-AGCTGCACCCCCAACC-3'	5'-CTGCTGGCGCAGTTGGTC-3'
Maternally Expressed		
GTL2/MEG3	5'-GTTTCTGGACTGTGGGCTGT-3'	5'-CAACAGCAACAAAACTCAGAACATTCA-3'
H19	5'-ATTTGCACTAAGTCATTTGCACTG-3'	5'-CAGTCACCCGGCCCAGAT-3'
TP73	5'-AGGCAGGTGGGCCAATG-3'	5'-TGGGAGATGTTAGTAGGGGAAGC-3'
Not-Imprinted		
GNAS1	5'-GCCATGAGCAACCTGGTG-3'	5'-GAAGTCAAAGTCAGGCACGTT-3'
ATP10A	5'-GCAAAGACAGTGTGTCCAGTTACC-3'	5'-GCTTTGGTATGTGCCATGCAG-3'
OSBPL5	5'-CTGGGCACCTGCAAGC-3'	5'-GGGAACAGGTCTTGGTCTGG-3'
KCNQ1	5'-CGCAGAGAAGTGACGGTTC-3'	5'-CTCATTAAAACACAGATCCAAATCACCAC-3'
PPP1R9	5'-TGTTGACCTTGCTACTGCTGTGT-3'	5'-AGGAGAGGAAACCTGAAAATGGTG-3'
Other Genes		
TXK	5'-GGCCGCCCTACATTTGC-3'	5'-TGGGTTGGCATTCTGTTTCC-3'
Reference Genes		
ACTB	5'-ACTGGAACGGTGAAGGTGAC-3'	5'-GTGGACTTGGGAGAGGACTG-3'
18S rRNA	5'-TCAACACGGGAAACCTCACC-3'	5'-CAGACAAATCGCTCCACCAA-3'

Supplementary Table 2. Primer set designed for qASPCR

Gene	Primers[(a)]	
	Forward	Reverse
Paternally Expressed		
IGF2	5'-CGAAGAGTCACCACCGAGC(C/T)-3'	5'-CTCAGGACTGGGCTCTCTGG-3'
PEG1/MEST	5'-TGTATTACCTCCCCTACTCCCTTAT(G/C)-3'	5'-CCTGCTGGCTTCTTCCTATACA-3'
PEG3/PW1	5'-TTCAGCCCAGAGGAACTTAG(T/C)-3'	5'-AGACCAGGTTCCGGTAATTCT-3'
PEG10	5'-AAATTGCCTGACATGAAGAGGAGTCTA-3'	5'-TTTAAACATGCAAGAATAAGAGC(A/G)-3'
ZAC/PLAGL1	5'-TGCATGTTAGAAGAATCAGCCT-3'	5'-GTTCCCCAGTCTGTTTTTGGA(C/T)-3'
DLK1	5'-GGCGTCTGCACTGACAT(T/C)-3'	5'-CTGCTGGCGCAGTTGGTC-3'
Maternally Expressed		
GTL2/MEG3	5'-TGTGTACCTTGGTTGGTGACTCAGTGAGAA-3'	5'-CAACAGCAACAAAACTCAGAACATTCA(C/T)-3'
H19	5'-ATTTGCACTAAGTCATTTGCACTG-3'	5'-CACTCACGCACACTCG(T/C)-3'
TP73	5'-AGGCAGGTGGGCCAATG-3'	5'-ACCACACATACCCTAGGCAG(G/A)-3'
Not-Imprinted Genes		
GNAS1	5'-GCCATGAGCAACCTGGTG-3'	5'-GCACGTTCATCACACTCAG(G/A)-3'
ATP10A	5'-AAGACAGTGTGTCCAGTTACC-3'	5'-TTACGGAAGTGCCCT(G/C)-3'
OSBPL5	5'-CTGGGCACCTGCAAGC-3'	5'-GTCTTGGTCTGGGTGGAC(G/A)-3'
KCNQ1	5'-GGGTTCCTTCTGGGCATTACA-3'	5'-CTCATTAAAACACAGATCCAAATCACCAC-3'
PPP1R9	5'-CAACACAGTTCCTCTTCCCCC(T/C)-3'	5'-AAATGGTGGTTTCCTTAAAAATCAATTTC-3'
Other Genes		
TXK	5'-GGCCGCCCTACATTTGC(C/G)-3'	5'-TGGGTTGGCATTCTGTTTCC-3'

[(a)]: Allele-specific primers appear with the alternative allele-specific nucleotide at the 3' end in parentheses

Supplementary Table 3. Genes tested from the Otago/Geneimprint Databases

Not-Expressed in Placenta			Readout SNP with MAF < 20%[(a)]			Not-Imprinted in Placenta			Enrolled		
Gene	NCBI ID	Expressed Allele	Gene	NCBI ID	Expressed Allele	Gene	NCBI ID	Expressed Allele	Gene	NCBI ID	Expressed Allele
CPA4	51200	M	*INS*	3630	P	*OSBPL5*	114879	M	*IGF2*	3481	P
IGF2AS	51214	P	*ASCL2*	430	P	*ATP10A*	57194	M	*PEG1*	4232	P
KCNQ1OT1	10984	P	*PHLDA2*	7262	M	*GNAS1*	2778	M	*PEG3*	5178	P
KCNQ1DN	55539	M	*CDKN1C*	1028	M	*PPP1R9A*	55607	M	*PEG10*	23089	P
SLC22A18	5002	M				*KCNQ1*	3784	M	*ZAC*	5325	P
UBE3A	7337	M							*DLK1*	8788	P
ZNF264	9422	M							*GTL2*	55384	M
L3MBTL	26013	P							*H19*	283120	M
									TP73	7161	M

[(a)]: MAF = Minimum Allele Frequency