Postnatal Survival of Mice with Maternal Duplication of Distal Chromosome 7 Induced by a \textit{Igf2/H19} Imprinting Control Region Lacking Insulator Function

Li Han\textsuperscript{1*}, Piroska E. Szabó\textsuperscript{1}, Jeffrey R. Mann\textsuperscript{1,2,3*}

\textsuperscript{1} Division of Biology, Beckman Research Institute, City of Hope National Medical Center, Duarte, California, United States of America, \textsuperscript{2} Department of Zoology, The University of Melbourne, Melbourne, Victoria, Australia, \textsuperscript{3} Laboratory and Community Genetics Theme, Murdoch Childrens Research Institute, The Royal Children’s Hospital, Parkville, Victoria, Australia

Abstract

The misexpressed imprinted genes causing developmental failure of mouse parthenogenones are poorly defined. To obtain further insight, we investigated misexpressions that could cause the pronounced growth deficiency and death of fetuses with maternal duplication of distal chromosome (Chr) 7 (MatDup.dist7). Their small size could involve inactivity of \textit{Igf2}, encoding a growth factor, with some contribution by over-expression of \textit{Cdkn1c}, encoding a negative growth regulator. Mice lacking \textit{Igf2} expression are usually viable, and MatDup.dist7 death has been attributed to the misexpression of \textit{Cdkn1c} or other imprinted genes. To examine the role of misexpressions determined by two maternal copies of the \textit{Igf2/H19} imprinting control region (ICR)—a chromatin insulator, we introduced a mutant ICR (ICR\textsuperscript{A}) into MatDup.dist7 fetuses. This activated \textit{Igf2}, with correction of \textit{H19} expression and other imprinted transcripts expected. Substantial growth enhancement and full postnatal viability was obtained, demonstrating that the aberrant MatDup.dist7 phenotype is highly dependent on the presence of two unmethylated maternal \textit{Igf2/H19} ICRs. Activation of \textit{Igf2} is likely the predominant correction that rescued growth and viability. Further experiments involved the introduction of a null allele of \textit{Cdkn1c} to alleviate its over-expression. Results were not consistent with the possibility that this misexpression alone, or in combination with \textit{Igf2} inactivity, mediates MatDup.dist7 death. Rather, a network of misexpressions derived from dist7 is probably involved. Our results are consistent with the idea that reduced expression of \textit{IGF2} plays a role in the aetiology of the human imprinting-related growth-deficit disorder, Silver-Russell syndrome.

Introduction

Parthenogenetic mouse embryos usually die before 6½ days post coitum (dpc). Occasionally they develop to the 25 somite forelimb bud stage or approximately 9½ dpc [1–5]. Parthenogenones possess two maternal-derived genomes and would be expected to possess abnormal levels of transcript of all known imprinted genes, that is, lack of expression of paternally expressed genes (two inactive copies), and over-expression of maternally expressed genes (two active copies). Their death is likely a composite effect of at least some of these misexpressions, although those involved are not well defined. Defining the causes is important for improving understanding of the aetiology of genomic imprinting [6–9] and the prevalence of sexual anomalies. Only three of these are associated with peri- or prenatal death, these being maternal duplication of proximal Chr 6 (MatDup.prox6)—prior to 11½ dpc [14], maternal duplication of distal Chr 7 (MatDup.dist7)—late fetal death [15], and maternal disomy of Chr 12—perinatal death, probably attributable to the distal region [16]. Second, knockouts of imprinted genes and imprinting control regions (ICRs) have provided information on the effects of disregulation of imprinted genes, for example, [17–21]. To better define the causes of failed parthenogenetic development, and learn more of how imprinted genes at dist7 work together to regulate normal development, we have examined some of the misexpressions of imprinted genes thought to contribute to the abnormal development of MatDup.dist7 conceptuses. These display a pronounced growth deficit of the fetus and placenta and die at the late fetal stage, or possibly at birth. Live MatDup.dist7 young have never been observed [13,15] (J. Mann, unpublished data).

Citation: Han L, Szabó PE, Mann JR (2010) Postnatal Survival of Mice with Maternal Duplication of Distal Chromosome 7 Induced by a \textit{Igf2/H19} Imprinting Control Region Lacking Insulator Function. PLoS Genet 6(1): e1000803. doi:10.1371/journal.pgen.1000803

Copyright: © 2010 Han et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by National Institutes of Health (USA) <http://www.nih.gov> grant no. 2R01GM48103 and by National Health and Medical Research Council (Australia) <http://www.nhmrc.gov.au> grant nos. 350216 and 350217. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: jeff.mann@mcri.edu.au

\textsuperscript{*} Current address: Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, United States of America
Dist7 is an important region in terms of genomic imprinting, containing over 20 imprinted genes [13,22]. At least three of these are regulated by the Igf2/H19 imprinting control region (ICR), these being 'insulin like growth factor 2' (Igf2)—paternally expressed and encoding a mitogen important for embryonic growth [23,24], 'insulin II' (Ins2)—paternally expressed in yolk sac [25], and the non-coding 'H19 fetal liver mRNA' (H19) gene—maternally expressed [26]. Other non-coding transcripts have been described, these being Mir483, contained within an intron of Igf2 [27] and for which imprinting status is unknown, Mir675, contained with an H19 exon and therefore likely to follow the imprinting pattern of the host gene [28,29], and antisense transcripts within Igf2 [30]. The targets of the Mir483 and Mir675 miRNAs are unknown. The maternally-derived Igf2 allele is inactive due to the hypo-methylated maternal Igf2/H19 ICR functioning as a 'CCCTC-binding factor' (CTCF)-based chromatin insulator. This lies between the Igf2 promoter and the shared Igf2-H19 enhancers, preventing their interaction. The maternal H19 promoter lies on the same side of the insulator as the enhancers, therefore interaction occurs. On the paternal Chr the ICR is hyper-methylated, preventing CTCF binding and insulator formation and allowing for paternal Igf2 promoter and enhancer interaction. The paternal H19 promoter, just distal to the methylated ICR, also becomes methylated, and is inactive. The Ins2 gene is located just distal to Igf2. The Ins2 parental alleles are affected in the same way as their Igf2 counterparts, but only in yolk sac. Ins2 is expressed biallelically in pancreas [25,31–33].

Telomeric or distal to the Igf2/H19 ICR domain is a large cluster of imprinted genes under regulatory control of the Kv differentially methylated region (DMR)-1 [KvDMR1] ICR. The active state of maternally-derived genes within this cluster is coincident with maternal-specific ICR methylation and the inactive state of the promoter of the KCNQ1 overlapping transcript 1 (Kcnq1ot1) gene contained within the ICR. The paternal ICR is hypo-methylated, and paternal-specific elongation of the Kcnq1ot1 transcript is coincident with silencing in cis of genes within the cluster [17,34,35]. One of the genes regulated by this ICR is the 'cyclin-dependent kinase inhibitor 1C (P57)' (Cdkn1c) gene encoding a protein facilitating reduced cell proliferation, increased apoptosis and delayed cell differentiation [36,37].

MatDup.dist7 fetuses are maternally duplicated for the hypo-methylated Igf2/H19 ICR and hyper-methylated KvDMR1 ICR regions, as well as for other imprinted transcripts at dist7. This epigenetic configuration is highly similar to that associated with the human imprinting-related growth deficit disorder, Silver-Russell syndrome (SRS) (OMIM 180860). More than half of cases are associated with hypo-methylation of the Igf2/H19 ICR, also known as 'ICR1'. The disease is also associated with maternal duplication of the KvDMR1 ICR region, also known as 'ICR2', and maternal duplication of the 11p15.5 Chr region encompassing both ICRs. It is strongly suspected that SRS is caused by downregulation of Igf2, and, in a minority of cases, excess CDKN1C or other imprinted genes regulated by ICR2. However, empirical evidence is lacking [38–40].

The death of MatDup.dist7 fetuses has been difficult to decipher. Available evidence suggests that maternal duplication of the Igf2/H19 ICR regulatory domain alone is insufficient to explain the total phenotype observed. Mice with paternal inheritance of a tandem duplication of a chicken β-globin CTCF-based chromatin insulator, substituted for the endogenous Igf2/H19 ICR, are similar to MatDup.dist7 mice in having a fully functional hypo-methylated insulator on both parental Chr. They lack Igf2 activity, have at least twofold over-expression of H19, with both parental alleles probably active, and would be expected to lack Ins2 activity in yolk sac. Nevertheless, their phenotype—dwarfism combined with postnatal viability—is essentially identical to Igf2 mutants [41]. Mice homozygous for this genetic modification, in a mix of strains 129S1/SvImJ and outbred Swiss CF-1, showed normal fecundity and were maintained as a random-bred line for several years (J. Mann, unpublished data). Further, lack of Igf2 activity is unlikely to be the sole cause of reduced growth in MatDup.dist7 fetuses. At 17.5 dpc, their weight is approximately 40% of wild-type [42] (J. Mann and Walter Tsark, unpublished observations) compared to 50–60% of wild-type for Igf2 mutants and mice maternally inheriting the chicken insulator [41]. Overall, these observations indicate that the MatDup.dist7 phenotype of fetal growth deficit and death involves the misexpression of imprinted genes outside the influence of the Igf2/H19 ICR, and this has previously been suggested [42].

Available evidence also indicates that maternal duplication of the KvDMR1 ICR regulatory domain alone is insufficient to explain the total phenotype observed. Mice with paternal inheritance of a deletion of this element exhibit biallelic expression of adjacent imprinted genes. These mice, in a mix of mouse strains 129S4/SvJae and C57BL/6J, are postnatally viable. They show some reduction in size, and it has been indicated that this is caused by over-expression of Cdkn1c [35]. Reduced growth has also been observed in Cdkn1c-BAC transgenic mice. While these displayed high frequency perinatal mortality in strain 129/Sv, high postnatal viability was obtained in a mix of strains 129/Sv and outbred Swiss MF1 [43]. These observations indicate that MatDup.dist7 late fetal death, occurring in the context of mixed strains including outbred Swiss, involves the misexpression of imprinted genes outside the influence of the KvDMR1 ICR. Overall, these observations have led to suggestions that MatDup.dist7 death could be a composite effect of misexpressions derived from both imprinted domains, for example, Igf2 inactivity combined with Cdkn1c over-expression [43].

To define the role of imprinted genes regulated by the Igf2/H19 ICR in the MatDup.dist7 phenotype, we evaluated the effects of introducing a mutated Igf2/H19 ICR [ICR3] which cannot bind CTCF and form an insulator [44]. MatDup.dist7 fetuses carrying ICR3 would be expected to be corrected in terms of the number of active alleles of Igf2—activation of one of two inactive alleles,
H19—repression of one of two active alleles, and Ino2—activation of one of two inactive alleles in yolk sac. MatDup.dist7 fetuses carrying ICR\textsuperscript{A} were significantly rescued in terms of growth and were able to survive to adulthood. These results demonstrate that the aberrant phenotype of MatDup.dist7 fetuses is highly dependent on the presence of two maternally-derived Igf2/H19 ICR chromatin insulators.

**Results**

**Maternal Inheritance of ICR\textsuperscript{A} Rescues Growth in Igf2 Null Mutants**

Maternal inheritance of ICR\textsuperscript{A} results in activation of Igf2 in cis such that total Igf2 RNA is 1.7 and 2.1 times the normal level in the liver and kidney of 17.5 dpc fetuses, respectively, and also repression of H19 in cis, such that total H19 RNA is 0.2 and 0 times the normal level in these same tissues, respectively [44]. This configuration of expression—two active Igf2 and two inactive H19 alleles—is coincident with increased growth, an effect thought to be due to the former misexpression [18,45,46]. Lack of H19 RNA alone has no effect on Igf2 expression or imprinting and results in no discernible phenotype [47]. Maternal inheritance of ICR\textsuperscript{A} would also be expected to result in activation of Ino2 in yolk sac.

To confirm that maternal inheritance of ICR\textsuperscript{A} can mediate normal growth, we tested its function in mice paternally inheriting a null mutation of Igf2 (Igf2\textsuperscript{−/−}). Mice of genotype (ICR\textsuperscript{A/+}, Igf2\textsuperscript{+/−}) are small due to lack of Igf2 activity, with the maternal allele inactive, and the paternal allele null [24]. Results are shown in Figure 1. Experimental young of genotype (ICR\textsuperscript{A/+}, Igf2\textsuperscript{+/−}), in which the maternally-derived Igf2 allele is activated in cis by ICR\textsuperscript{A}, were not significantly different in weight to control (ICR\textsuperscript{+/+}, Igf2\textsuperscript{+/+}) mice at 6 weeks of age (females, \( P = 0.271 \); males, \( P = 0.035 \)). Thus, a single maternally derived copy of ICR\textsuperscript{A} induces sufficient Igf2 activity for achieving normal postnatal growth. We note that, in respect to growth with one versus two active Igf2 alleles, experimental (ICR\textsuperscript{A/+}, Igf2\textsuperscript{+/−}) animals with one active allele (maternal), were not significantly different in weight to (ICR\textsuperscript{A/+}, Igf2\textsuperscript{+/-}) animals with two active alleles (females, \( P = 0.378 \); males, \( P = 0.089 \)). Further, (ICR\textsuperscript{+/+}, Igf2\textsuperscript{+/+}) females with one active allele (paternal) were not significantly different in weight to (ICR\textsuperscript{+/+}, Igf2\textsuperscript{+/−}) females with two active alleles (\( P = 0.04 \)). However, in males, mice with one active allele (paternal) were lighter than mice with two active alleles, as expected (\( P = 0.002 \)). Given the borderline probability values obtained, greater numbers of animals need to be analysed to accurately determine the relative growth rates of mice of the various genotypes.

**ICR\textsuperscript{A} Rescues Growth and Viability in MatDup.dist7 Fetuses**

MatDup.dist7 zygotes were produced in intercrosses of mice carrying the reciprocal translocation T(7;15)9H (T9H). Such intercrosses give rise to a high proportion of unbalanced zygotes, and litter size is small. Of balanced zygotes, only one in seven are expected to be MatDup.dist7, these identified by the dist7 marker, albino (\( c \)), a mutation of the ‘tyrosinase’ (\( Tyrc \)) gene [15]. The ICR\textsuperscript{A} mutation was introduced into female T9H/+ parents and was inherited by MatDup.dist7 zygotes (Figure 2). Expected allelic activity of Igf2 and Cdkn1c in the three possible MatDup.-dist7 genotypes is shown (Figure 2B). ICR\textsuperscript{A}-induced activation of Igf2 was confirmed in 13.5 dpc MatDup.dist7 fetuses obtained in (T9H/+), Tyrc\textsuperscript{+/−}, ICR\textsuperscript{A/+} Q×T9H/+), Tyrc\textsuperscript{+/-}, ICR\textsuperscript{A+/-} Q intercrosses. The level of Igf2 transcript in MatDup.dist7 ICR\textsuperscript{A−/−} fetuses was the same as in control ICR\textsuperscript{+/+} fetuses with one active allele, while it was almost double the normal amount in MatDup.dist7 ICR\textsuperscript{A−/−} fetuses with probably two active alleles (Figure 3A and 3B). Increased total Igf2 RNA was also seen in mice which maternally inherit ICR\textsuperscript{A} and have an active maternal and paternal allele of Igf2 (Figure 3A and 3B). Also, MatDup.dist7 fetuses of all genotypes contained at least double the amount of Cdkn1c RNA relative to controls, probably because of two active alleles (Figure 3A and 3B). These intercross matings were allowed to proceed to term and we immediately began to observe viable albino or MatDup.dist7 young which were of overtly similar size to agouti littersmates. A MatDup.dist7 animal and its two littersmates at 10 days post-partum is shown (Figure 4). All MatDup.dist7 young obtained were of genotype ICR\textsuperscript{+/+} or recombinant ICR\textsuperscript{A−/−}. Seven of 52 mice born were MatDup.dist7 which is similar to the expected frequency, indicating that ICR\textsuperscript{A} was always able to increase growth and rescue viability. In age- and litter-matched animals, a significant weight deficit of approximately 17% in
MatDup.dist7 animals became apparent at 6 weeks of age when compared with controls carrying an equivalent number of active Igf2 alleles, that is, MatDup.dist7 ICR\(^{+/+}\) with control ICR\(^{+/+}\) (one active allele each) and MatDup.dist7 ICR\(^{+/-}\) recombinant with control ICR\(^{+/-}\) (probably two active alleles each) (Figure 5A).

**Figure 2. Production of MatDup.dist7 fetuses.** (A) Quadrivalents at meiosis I occurring in (T9H/+, Tyr\(^{+/-}\), ICR\(^{+/-}\) \(\times\) T9H/+, Tyr\(^{+/-}\), ICR\(^{+/-}\)) matings. Female reciprocal translocation heterozygote (T9H/+\) parent is homozygous for the dist7 marker albino (Tyrc\(^{+/-}\)) and carries ICR\(^{+/-}\) (ICR\(^{+/-}\)), while the male T9H/+\) parent is wild-type at both of these loci (Tyrc\(^{+/-}\), ICR\(^{+/-}\)). (B) Genotypes of MatDup.dist7 fetuses obtained from the union of unbalanced complementary gametes. MatDup.dist7 individuals are readily identified as albinos from 12\(^{+/-}\) days of age. Many of these are ICR\(^{+/-}\) although a high frequency of homzygous recombinants, ICR\(^{+/-}\) and ICR\(^{+/-}\), are also obtained. Chrs depicted are actually paired chromatids. Underneath is depicted allele-specific expression—MatDup.dist7 ICR\(^{+/-}\) and ICR\(^{+/-}\) fetuses have one and probably two activated Igf2 alleles, and should have one and two repressed H19 alleles, respectively.

doi:10.1371/journal.pgen.1000803.g002

**Figure 3. Expression of imprinted genes in 13\(^{+/-}\) dpc MatDup. dist7 fetuses.** (A) Northern blots for the imprinted genes Igf2 and Cdkn1c, and for the housekeeping Gapdh gene for normalization. Each lane is an individual fetus. ICR genotype is given immediately above the lanes. (B) Northern blots in (A) were quantitated to show relative RNA levels. Values for Igf2 and Cdkn1c were normalized to Gapdh RNA, calibrated to control ICR\(^{+/-}\) values, and adjusted to a mean of 1.0. Values are mean\(\pm\)s.d. with (n) as shown in (A). Mating scheme to breed these animals was as described in the legend to Figure 2.

doi:10.1371/journal.pgen.1000803.g003

This result is not consistent with the idea that MatDup.dist7 death results only from the combined action of the Cdkn1c and Igf2 misexpressions.

**Involvement of Cdkn1c Expression in MatDup.dist7 ICR\(^{+/-}\) Postnatal Growth Deficit**

To test for a role of Cdkn1c over-expression in the growth deficit at 6 weeks of age of rescued postnatal MatDup.dist7 ICR\(^{+/-}\) animals, we introduced Cdkn1c\(^{-}\) into MatDup.dist7 fetuses such that they were of genotype (ICR\(^{+/-}\), Cdkn1c\(^{-}\)). This genotype should be normalized for the number of active alleles of imprinted genes regulated by the Igf2/H19 ICR, and also be normalized for Cdkn1c\(^{-}\). In (T9H/+, Tyr\(^{+/-}\), ICR\(^{+/-}\), Cdkn1c\(^{-}\) \(\times\) T9H/+, Tyr\(^{+/-}\), ICR\(^{+/-}\), Cdkn1c\(^{-}\)) matings, viable MatDup.-dist7 ICR\(^{+/-}\), Cdkn1c\(^{-}\) young were obtained and these did not display a significant weight deficit at 6 weeks of age—with the caveat that the weight measurements are relative to control young obtained in the previous matings (Figure 5B). Their weights could not be compared to littermates as, given the mating scheme, agouti littermates were always positive for ICR\(^{+/-}\) and therefore possessed two active copies of Igf2. In any event, these results are consistent with the possibility that biallelic expression of Cdkn1c does contribute to a reduction in postnatal growth in MatDup.dist7 ICR\(^{+/-}\) or ICR\(^{+/-}\), Cdkn1c\(^{-}\) animals.

Maternal Duplication of Distal Chr 7 in Mice

CDKN1C may antagonize the growth promoting effects of IGFI [17,48], and it has been suggested that excess CDKN1C may combine with lack of IGFI to cause MatDup.dist7 death [43]. To test this possibility, we introduced a null allele of Cdkn1c (Cdkn1c\(^{-}\)) into MatDup.dist7 fetuses to enforce its monoallelic expression. In (T9H/+, Tyr\(^{+/-}\), ICR\(^{+/-}\) Cdkn1c\(^{-}\), Tyrc\(^{+/-}\), ICR\(^{+/-}\) Cdkn1c\(^{-}\)) matings, all of 35 young obtained were agouti controls, that is, at least six albino MatDup.dist7 (ICR\(^{+/-}\), Cdkn1c\(^{-}\)) pups were expected, but none were observed.
transgenic mice with ectopic over-expression [49–52]. Biallelic or
First, for H19
tions of the effects of misexpression of each imprinted gene alone.
postnatal viability [41]. Further evidence is provided by observa-
mice in respect to this region, yet these animals have normal
derived chicken insulator substituted for the
Igf2
insulators—a maternally-derived
Igf2
growth.
also, in this same strain mix, we maintained a Igf2
null mutation [56] revealed that lack of IGF2 in strain C57BL/6J
results in death at birth. This effect was not peculiar to this second
knockout allele as homozygous mutants can be obtained in strain
129 (M. Constancia, personal communication). In the present
study, MatDup.dist7 young were a mix of strains 129/SvEv[,CF-1, C57BL/6J and CBA/Ca. In this mix, lack of IGF2 activity is
highly likely to be compatible with survival. Given these various
lines of evidence, the present experiments strongly suggest that
misexpressed imprinted genes, as regulated by the IGF2/H19 ICR,
work in combination with misexpressions derived outside of this
region of influence in causing the total MatDup.dist7 phenotype.
The significant rescue in growth probably mediated by IGF2
activation may also be directly related to MatDup.dist7 survival in
that it could compensate for negative effects derived from outside
the IGF2/H19 ICR region. Nevertheless, we cannot rule out the
possibility that Inso inactivity in yolk sac, excess H19 RNA, or the
misexpression of non-coding RNAs regulated by the IGF2/H19
ICR make a contribution to the lethal effect. These possibilities
could be investigated through correction of their misexpression in
MatDup.dist7 fetuses, then determining growth and survival. For
example, correction of H19 over-expression could be achieved by
introducing a deletion of the transcript region only.

The imprinted genes operating outside the influence of the IGF2/
H19 ICR that contribute to MatDup.dist7 death would be expected to require maternal-, rather than paternal-specific
imprinting or methylation for attaining differential expression in
the normal context. This is because for full-term development,
there is apparently no other requirement, aside from IGF2/H19
ICR methylation, for paternal imprinting at dist7 [57]. The cluster
of genes requiring maternal-specific methylation of the KvDMR1
ICR for activity fulfills this criterion. While the introduction of a
null mutation of Cdkn1c, and hence enforced monoallelic
expression of this gene, did not rescue MatDup.dist7 fetuses, this
does not rule out the possibility that CDKN1C excess has a role in
causing MatDup.dist7 death. In MatDup.dist7 (ICR
Cdkn1c
null) fetuses, Cdkn1c
RNA levels were found to be more than three times
that of controls, suggesting that each maternally-derived Cdkn1c
allele was upregulated 1.5-fold. Therefore, CDKN1C could still be
in excess in MatDup.dist7 (ICR
Cdkn1c
null) animals. Also, there
remains the possibility that excess Cdkn1c
RNA may contribute as
part of a network of misexpressions derived from the cluster
regulated by the KvDMR1 ICR. For example, biallelic expression
of the ‘pleckstrin homology-like domain, family A, member 2’
(Phlda2) gene results in placental growth retardation and marginal
fetal growth restriction [50], and upregulation of PHLD42 is

Discussion
We have shown that maternal introduction of a mutant IGF2/
H19 ICR, which lacks chromatin insulator activity, into MatDup.dist7 fetuses substantially alters their abnormal phenot-
type—small size and death at the late fetal stage—to one of near
normal growth rate and survival to adulthood. This result clearly
demonstrates the dependence of this phenotype on a misexpres-
ion of imprinted genes caused by the presence of two active
paternally-derived IGF2/H19 ICR chromatin insulators. As this
ICR is known to regulate the expression of at least three dist7
imprinted genes—H19, Inso, IGF2, and a number of non-coding
transcripts—correction in the misexpression of one or more of
these was probably responsible for the result obtained. Activation
of IGF2 was likely an important correction, this being the only
alteration in expression induced by ICRΔ expected to affect
growth.

The survival of MatDup.dist7 mice with ICRΔ is more difficult
to decipher. As discussed in the Introduction section, it is unlikely
that the IGF2/H19 ICR-derived misexpressions are solely respon-
sible for their death, as mice with two functional chromatin
insulators—a maternally-derived IGF2/H19 ICR, and a paternally-
derived chicken insulator substituted for the IGF2/H19 ICR,
possess the same combination of misexpressions as MatDup.dist7
mice in respect to this region, yet these animals have normal
postnatal viability [41]. Further evidence is provided by observa-
tions of the effects of misexpression of each imprinted gene alone.
First, for H19, no overt effect on phenotype is observed in
transgenic mice with ectopic over-expression [49–52]. Biallelic or
over-expression of H19 has been suggested to cause perinatal
death of mice produced by combining a non-growing oocyte
genome (ng, carrying a deletion of the distal Chr 12 IG-DMR
ICR Δ12), with a fully grown oocyte genome (fg)—ngΔ12/fg mice
[53]. However, these mice would be predicted to have the
equivalent expression profile of imprinted genes as mice with
maternal inheritance of the chicken insulator substitution. The
latter mice are viable, despite twofold over-expression of H19 [41].
Therefore, the perinatal death of ngΔ12/fg mice may result from the
combined action of H19 RNA excess—or possibly IGF2 RNA
absence—and small imperfections in expression derived from the
non-growing oocyte genome, for example, as related to the IG-
DMR ICR deletion. Second, for Inso, mice lacking in expression
of this gene are viable [54]. Third, for IGF2, mice lacking expression
are dwarfed and have impaired lung development [55], but are
usually viable. High postnatal survival frequency of IGF2 mutants
is seen in inbred strain 129/SvEv [23,24] although in this strain we
have observed a low level of perinatal death [J. Mann, unpublished
observations]. In the present study, in a mix of strains 129/SvEv
and outbred Swiss CF-1, we observed high frequency survival.
...
Figure 5. Weight gain in post-partum MatDup.dist7 mice rescued by ICR

(A) Weight gain in litter matched MatDup.dist7 (ICR\textsuperscript{A,} Cdkn1c\textsuperscript{+/+}, or (ICR\textsuperscript{A,} Cdkn1c\textsuperscript{++/++}) young obtained from matings of (T9H/+, Tyr\textsuperscript{+/+}, ICR\textsuperscript{A,} Cdkn1c\textsuperscript{+/+}, Cdkn1c\textsuperscript{++/++} and (T9H/+, Tyr\textsuperscript{+/+}, ICR\textsuperscript{A,} Cdkn1c\textsuperscript{+/+}, Cdkn1c\textsuperscript{++/++}). Each graph represents a single litter where at least one control with the same number of active Igf2 alleles was obtained. Each bar represents a single animal. The paired-sample t-test was used to determine the probability that the weight of MatDup.dist7 and control mice was equal. For three-animal litters, the pairs used in the statistical test are indicated by the bracket above the bars. The P value was 0.01 for 6 week old mice, indicating a significant difference, while there was no significant difference in weight at 1, 2, and 3 weeks of age. (B) All weight gain data collected from the two sets of matings as described in (A) regardless of littermate matching. Bars are mean±s.d., with (n) given above the 1 wk bars. The two-sample t-test was used to determine the probability that two 6 week samples (identified as paired squares) were equal. Key: alleles are maternal/maternal in derivation for MatDup.dist7 and maternal/paternal in derivation for controls.

doi:10.1371/journal.pgen.1000803.g005
correlated with growth retardation in humans [59,60]. Also, it has been suggested that excess expression of the ‘achaete-scute complex homolog 2 (Drosophila)’ (Asg2) gene could cause the MatDup.dist7 lethal effect [42]. The phenotype of MatDup.dist7 fetuses could also involve misexpressions of dist7 imprinted genes lying outside of the influence of the two known ICRs. For example, ‘adenosine monophosphate deaminase 3’ (Amphd3)—maternally expressed in placenta, and identified in a transcriptome analysis of MatDup.dist7 conceptuses [22], ‘inositol polyphosphate-5-phosphatase F (Imp5f)—an isoform paternally expressed in brain [61], and ‘cathepsin D’ (Ctld)—possible paternal-specific expression [62].

The postnatal weight deficit of approximately 17% in MatDup.dist7 young at 6 weeks of age was similar to that in mice paternally inheriting a deleted KvDMR1 ICR. This deletion results in biallelic expression of imprinted genes regulated by this ICR, including Cdkn1c [17,34]. Indeed, excess Cdkn1c has been indicated as the cause of the weight deficit [35]. Consistent with this possibility is that the weight of MatDup.dist7 ICRΔ−, Cdkn1c−/− young was normal at 6 weeks of age. However, we note that MatDup.dist7 neonates displayed no significant weight deficit until reaching adulthood, while in mice paternally inheriting the deleted KvDMR1 ICR, the weight deficit is present in fetuses and persists throughout postnatal development [17]. More data regarding weight gain in relation to the inheritance of ICRΔ−, in MatDup.dist7 young and otherwise, is required to confirm these observations.

In terms of MatDup.dist7 death, additional experiments are required to determine exactly which combination of misexpressions are involved. The total MatDup.dist7 phenotype has been ascribed to the very distal portion of Chr 7 as defined by the reciprocal translocation T(7;11)65H (T65H) [42]. This translocation has a breakpoint far more distal on Chr 7 relative to the T9H translocation used in this study, although it is still proximal to the two clusters of imprinted genes regulated by the Igf2/H19 and KvDMR1 ICRs. However, some caution should be exercised in ascribing the total effect to this region. While it was shown that T65H- and T9H-MatDup.dist7 fetuses are of similar morphology [42], the postnatal viability of the former was not investigated. If T65H-MatDup.dist7 fetuses are also inviable, then the composite lethal effect is likely to be contained within the two aforementioned clusters of imprinted genes. Evidence that the KvDMR1 cluster contributes to the effect could be obtained by determining the viability of MatDup.dist7 fetuses carrying a deletion of this whole cluster. This would result in enforced monoallelic expression of all genes under regulation of the KvDMR1 ICR, including Cdkn1c, and these mice and would be expected to be postnatally viable, although small because of Igf2 inactivity. Such a deletion, made through truncation of Chr 7 at a point distal to the Iac2 gene, has been described [63]. A complication with this possible experiment is the existence of imprinted genes at dist7 which are not regulated by either ICR. Another experiment could be to breed mice with paternal inheritance of the chicken β-globin insulator substituted for the Igf2/H19 ICR [41] combined with paternal inheritance of the KvDMR1 ICR deletion [17]. These would misexpress all imprinted genes under regulatory control of both ICRs. If these were the only misexpressions involved in the MatDup.dist7 phenotype, then the phenotype should be reproduced.

MatDup.dist7 fetuses provide an epigenetic model of a subtype of human Silver-Russell syndrome (SRS) involving maternal duplication of the orthologous Chr region, 11p15.5, which encompasses ICR1 and ICR2. In these fetuses, we have shown that abrogation of ICR1 insulator function was able to restore Igf2 expression, concomitant with restoration of growth and survival. The most common subtype of SRS, that involving hypomethylation of ICR1, is perhaps better modelled in mice maternally inheriting the chicken insulator in place of ICR1. These animals provide information on the effects of the presence of two functional insulators at the Igf2/H19 region as the only epigenetic lesion. In these fetuses, we previously showed that DNA methylation was abrogated while insulator function remained intact. This resulted in reduced Igf2 activity and growth retardation [41]. Both of these findings support the idea that reduced expression of Igf2 during fetal development is causal in the development of SRS. They also support the suggestion that the failure to detect low concentrations of serum IGF2 in SRS patients is related to downregulation of Igf2 by this stage [38]. Further genetic manipulation in these mouse models should provide additional information for the human disease.

Our experiments suggest that misexpression of imprinted genes caused by two maternal copies of the Igf2/H19 ICR constitute one component of a composite barrier to parthenogenetic development that was not previously predicted. The lethal effect in MatDup.dist7 fetuses may be specific to later stages of development, and not normally occur in parthenogenones given their peri-implantation death. Nevertheless, high-level paternal- and maternal-specific expression of Igf2 and H19, respectively, is present shortly after implantation, at least by 6.5 dpc [64]. Therefore, it cannot be ruled out that these misexpressions, and others regulated by the Igf2/H19 ICR, play a role in what probably is a complex composite lethal effect involving a network of misexpressed imprinted genes. Indeed, the fact that parthenogenones fail earlier in development than embryos with maternal duplication of any single Chr region, indicates that misexpressions of imprinted genes from different regions are cumulative or synergistic in their deleterious effects. Further, at the molecular level, it has been shown that disregulation of the imprinted genes ‘pleiomorphic adenoma gene-like 1’ (Plagl1) and H19 can affect the expression of other imprinted genes in an imprinted gene expression network [65,66].

Previous observations have shown that the normal activity of imprinted genes regulated by the Igf2/H19 ICR are one of a small number of developmentally critical expression profiles provided exclusively by imprinting through the male germ line, provided that most if not all other imprinted genes are not misexpressed [57]. The present results raise the possibility that full-term parthenogenetic development could be achieved by correcting the misexpressions of only a few imprinted genes in order to repair the total expression network. One necessary condition would be to activate the ‘paternally expressed 10’ (Peg10) gene. Lack of expression of this gene results in death by 10.5 dpc, and this misexpression alone would be expected to present a barrier to parthenogenesis. It would be expected to contribute to, or could be solely responsible for, the embryonic death of MatDup.prox6 mice, which occurs prior to 11.5 dpc [20].

Materials and Methods

Mouse Lines

Line no.; genotype; strain; source, how produced, or reference: Line-1; 129S1/SvImJ (129S1); Tyr+/-; The Jackson Laboratory, stock no. 002448. Line-2; outbred Swiss CF-1; Tyr+/-; Charles River Laboratories. Line-3; T9H/T9H, Tyr+/-; mix of C75BL/6j (B6) and CBA/Ca (CB); The Jackson Laboratory, stock no. 001752. Line-4; T9H/T9H, Tyr+/-; mix of B6, CB and CF-1; made by mating line-2 with -3, then intercrossing. Line-5; Tyr+/-, ICRΔ−; mix of CF-1 and 129S1; made by mating ICRΔ− mice [44] with line-2, then intercrossing. Line-6; Tyr+/−, Cdkn1c−/−; mix
of 129S7/SvEvBrd (129S7), B6 and CF-1; made by mating Cdkn1c+/+ mice [37] with line-2, then intercrossing. Line-7; T9H/ T9H, T9+/+, Cdkn1c+/+; mix of strains B6, CF-1, and 129S7; made by mating line-4 with -6, then intercrossing. Line-6; Igg2/#/--; 129/SvEv [23].

Matings

Production of experimental (ICR\textsuperscript{A}\textsuperscript{+}, Igg2/#/) mice (Figure 1): Female parents (ICR\textsuperscript{A}\textsuperscript{+}, Igg2/#/) were bred in (line-5 x line-1)\textsuperscript{C} matings. Male parents (ICR\textsuperscript{A}\textsuperscript{+}, Igg2/#/) were of line-6. Young were a mix of strains 129, B6, CF-1, and 129S7; made by mating line-4 with -6, then intercrossing. Line-8; Igg2/#/--; T9H+/+, ICR\textsuperscript{A}\textsuperscript{+}-, Cdkn1c+/+ were bred in (line-5 x line-4)\textsuperscript{C} matings. Male parents (T9+/+, T9+/+, ICR\textsuperscript{A}\textsuperscript{+}+/+) were bred in (line-3 x line-1)\textsuperscript{C} matings. Young were a mix of strains 129S1, CF-1, B6, and CB. Production of MatDup.dist7 Cdkn1c+/+ young; attempted: Female parents (T9+/+, T9+/+, Cdkn1c+/+) were bred in (line-5 x line-6)\textsuperscript{C} matings. Male parents (T9+/+, T9+/+, ICR\textsuperscript{A}\textsuperscript{+}+/+) were bred in (line-3 x line-1)\textsuperscript{C} matings. Young were a mix of strains 129, B6, and CF-1. Production of MatDup.dist7 Cdkn1c+/+ young (Figure 5B): Female parents (T9+/+, T9+/+, ICR\textsuperscript{A}\textsuperscript{+}/+) were bred in (line-5 x line-7)\textsuperscript{C} matings. Male parents (T9+/+, T9+/+, ICR\textsuperscript{A}\textsuperscript{+}/+) were bred in (line-3 x line-1)\textsuperscript{C} matings. Young were a mix of strains 129S1, 129S7, B6, and CF-1.

Genotyping

For the ICR, two pairs of primers were used. The first pair was specific for the mutant ICR, identical to a pair previously described [41]: 5\textsuperscript{'}- GCCCC ACCA GCTG CTAG CGATC -3\textsuperscript{'} and 5\textsuperscript{'}- CCTA GAGA ATTC GAGG GACC TTAAT AAC -3\textsuperscript{'}; 240 bp ampiclon identified ICR\textsuperscript{A}\textsuperscript{+} and ICR\textsuperscript{A}\textsuperscript{A} animals. The second pair was specific for ICR\textsuperscript{C}, with primers binding to sequence positions that were modified in ICR\textsuperscript{A}\textsuperscript{[44]}: 5\textsuperscript{'}- AACG AGGG AACG GATG CTAC CG -3\textsuperscript{'} and 5\textsuperscript{'}- GCAA TGAT CACA CCAC -3\textsuperscript{'}.

References

1. Kaufman MH, Barton SC, Surani MA (1977) Normal postimplantation development of mouse parthenogenetic embryos to the termal limb bud stage. Nature 265: 53–55.
2. Barton SC, Surani MA, Norris ML (1984) Role of paternal and maternal genomes in mouse development. Nature 311: 374–376.
3. Mann JR, Lovell-Badge RH (1984) Two maternally derived X chromosomes contribute to parthenogenetic inviability. Development 104: 129–136.
4. McGrath J, Solter D (1984) Completion of mouse embryogenesis requires both maternal and paternal chromosomes. Cell 37: 179–183.
5. Mann JR, Lovell-Badge RH (1988) Two maternally derived X chromosomes contribute to parthenogenetic inviability. Development 104: 129–136.
6. Hurst LD, McVean GT (1998) Do we understand the evolution of genomic imprinting? Curr Opin Genet Dev 8: 701–708.
7. Wilkins JF, Haig D (2003) What good is genomic imprinting? The function of parent-specific gene expression. Nat Rev Genet 4: 359–368.
8. Coan PM, Burton GJ, Ferguson-Smith AC (2005) Imprinted genes in the placenta: a review. Placenta 26 Suppl A: S10–20.
9. Wood AJ, Osley K (2006) Genomic imprinting in mammals: emerging themes and established theories. PLoS Genet 2: e147.
10. Avic J (2008) Clonality: The genetics, ecology, and evolution of sexual clonality in vertebrate animals. New York: Oxford University Press.
11. Cattanach BM, Beechy CV (1997) Genetic imprinting in the mouse: possible final analysis. In: Reik W, Surani A, eds. Genomic imprinting. Oxford: IRL Press. pp 118–145.
12. Cattanach BM, Beechy CV, Peters J (2004) Interactions between imprinting effects in the mouse. Genetics 168: 397–413.
13. Williamson CM, Blake A, Thomas S, Beechy CV, Hancock J, et al. MRC Harwell, Oxfordshire. World Wide Web Site - Mouse Imprinting Data and Resources - http://har.mrc.ac.uk/research/genomic imprinting/.
14. Beechy CV (2000) Iggl/Myelloc locate distal to the currently defined imprinting region on mouse proximal chromosome 6 and identifies a new imprinting region affecting growth. Cytogenet Cell Genet 90: 309–314.
15. Searle AG, Beechy CV (1996) Genome imprinting phenomena on mouse chromosome 7. Genet Res 56: 237–244.
16. Tavendale M, Watkins M, Rasberry C, Cattanach B, Ferguson-Smith AC (2006) Analysis of mouse concepts with uniparental duplication/deliciency for distal chromosome 12: comparison with chromosome 12 uniparental disomy and imprinting for genomic imprinting. Cytogenet Genome Res 113: 213–222.
17. Fitzpatrick GV, Soloway PD, Higgins MJ (2002) Regional loss of imprinting and growth deficiency in mice with a targeted deletion of Dnmt1. Nat Genet 32: 428–431.
18. Leighton PA, Ingram RS, Grgurinovitch J, Efratiadis A, Tilghman SM (1995) Disruption of imprinting caused by deletion of the H19 gene region in mice. Nature 374: 34–39.
19. Lin SP, Youngson N, Takada S, Seitz H, Reik W, et al. (2003) Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtfl2 imprinting cluster on mouse chromosome 12. Nat Genet 35: 97–102.
20. Ono R, Nakamura K, Inoue K, Naruse M, Usami T, et al. (2006) Deletion of Pig/I, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. Nat Genet 38: 101–106.
21. Peters J, Williamson CM (2008) Control of imprinting at the Gnas cluster. Adv Exp Med Biol 628: 16–26.
22. Scholz R, Menheniot TR, Woodfine K, Wood AJ, Choi JD, et al. (2006) Chromosome-wide identification of novel imprinted genes using microarrays and uniparental disomies. Nucleic Acids Res 34: e88.
23. DeChiara TM, Efraimtadis A, Robertson EJ (1990) A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 345: 78–80.
24. DeChiara TM, Robertson EJ, Efraimiadis A (1991) Parental imprinting of the mouse insulin-like growth factor 2 gene. Cell 64: 449–459.
25. Goldspie JJ, King CD, Harman KW, Flood JE, Carnaghi LR (1994) Allele specific inactivation of insulin 1 and 2, in the mouse yolk sac, indicates imprinting. Nat Genet 6: 310–313.
26. Bartolomei MS, Zemel S, Tilghman SM (1991) Parental imprinting of the mouse H19 gene. Nature 351: 153–155.
27. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, et al. (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129: 1401–1414.
28. Mineo J, Okamoto S, Audo T, Sato M, Choue H, et al. (2006) The expression profile of microRNAs in mouse embryos. Nucleic Acids Res 34: 1765–1771.
29. Cai X, Cullen BR (2007) The imprinted H19 noncoding RNA is a primary microRNA precursor. RNA 13: 315–316.
Moore T, Constancia M, Zuhair M, Bailleul B, Feil R, et al. (1997) Multiple imprinted sense and antisense transgenes, differential methylation and tandem repeats in a putative imprinting control region upstream of mouse Igf2. Proc Natl Acad Sci U S A 94: 12509–12514.

Bell AC, Felsenfeld G (2000) Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature 405: 482–485.

Hark AT, Schenider CJ, Katz DJ, Ingram RS, Levorse JM, et al. (2000) CTCF mediates methylation-sensitive enhancer-blocking activity at the H19 Igf2 locus. Nature 405: 486–489.

Szabo P, Tang SH, Rentsendorj A, Pfeifer GP, Mann JR (2000) Maternal-specific footprints at putative CTCF sites in the H19 imprinting control region give evidence for insulator function. Curr Biol 10: 607–610.

Mancini-Dinardo D, Steele SJ, Levorse JM, Ingram RS, Tilghman SM (2006) Elongation of the Kingfisher transcript is required for genomic imprinting of neighboring genes. Genes Dev 20: 1285–1292.

Binder G, Seidel AK, Weber K, Haase M, Wollmann HA, et al. (2006) IGF-II serum levels are normal in children with Silver-Russell syndrome who frequently carry epimutations at the IGF2 locus. J Clin Endocrinol Metab 91: 4709–4712.

Shin JY, Fitzpatrick GV, Higgins MJ (2008) Two distinct mechanisms of silencing by the KvDMR1 imprinting control region. EMBO J 27: 168–178.

Eggermann T (2009) Epigenetic regulation of growth: lessons from Silver-Russell syndrome. Endocr Dev 14: 10–19.

Zhang P, Liegeois NJ, Wong C, Finegold M, Hou H, et al. (1997) Altered cell differentiation and proliferation in mice lacking p57Kip2 indicates a role in Beckwith-Wiedemann syndrome. Nature 387: 151–158.

Eggermann T (2009) Epigenetic regulation of growth: lessons from Silver-Russell syndrome. Endocr Dev 14: 10–19.

Binder G, Seidel AK, Weber K, Haase M, Wollmann HA, et al. (2006) IGF-II serum levels are normal in children with Silver-Russell syndrome who frequently carry epimutations at the IGF2 locus. J Clin Endocrinol Metab 91: 4709–4712.

Abu-Amero S, Monk D, Frost J, Preece M, Stanier P, et al. (2008) The genetic aetiology of Silver-Russell syndrome. J Med Genet 45: 193–199.

Eggermann T (2009) Epigenetic regulation of growth: lessons from Silver-Russell syndrome. Endocr Dev 14: 10–19.

Szabo P, Tang SH, Reed MR, Silva F, Tsark WM, et al. (2002) The chicken β-globin insulator element conveys chromatin boundary activity but not imprinting at the mouse Igf2/H19 domain. Development 129: 897–904.

Beechey CV, Ball ST, Townsend KM, Jones J (1997) The mouse chromosome 7 distal imprinting domain maps to G-bands F4/F5. Mamm Genome 8: 236–240.

Andrews SC, Wood MD, Tunster SJ, Bartov SC, Surani MA, et al. (2007) Gdabal-1 (p57Kip2) is the major regulator of embryonic growth within its imprinted domain on mouse distal chromosome 7. BMC Dev Biol 7: 53.

Szabo P, Tang SH, Silva F, Tsark WM, Mann JR (2004) Role of CTCF binding sites in the Igf2/H19 imprinting control region. Mol Cell Biol 24: 4791–4800.

Ripoche MA, Kress C, Poirier F, Dandolo L (1997) Deletion of the H19 transcription unit reveals the existence of a putative imprinting control element. Genes Dev 11: 1596–1604.

Eggenschwiler J, Ludwig T, Fisher P, Leighton PA, Tilghman SM, et al. (1997) Mouse mutant embryos overexpressing IGF-II exhibit phenotypic features of the Beckwith-Wiedemann and Simpson-Golabi-Behmel syndromes. Genes Dev 11: 3120–3142.

Jones BK, Levorse JM, Tilghman SM (1998) Igf2 imprinting does not require its own DNA methylation or H19 RNA. Genes Dev 12: 2200–2207.

Caspary T, Cleary MA, Perelman EJ, Zhang P, Ellenberger SJ, et al. (1999) Oppositely imprinted genes p57Kip2 and Igf2 interact in a mouse model for Beckwith-Wiedemann and Silver-Russell syndromes. Genes Dev 13: 3158–3174.

Pfeifer K, Leighton PA, Tilghman SM (1996) The structural H19 gene is required for transgene imprinting. Proc Natl Acad Sci U S A 93: 13070–13083.

Ainscough JF, Koidze T, Tada M, Barton S, Surani MA (1997) Imprinting of Igf2 and H19 from a 130 kb YAC transgene. Development 124: 3621–3632.

Eleon DA, Bartolomei MS (1997) A 5′ differentially methylated sequence and the 3′-flanking region are necessary for H19 transgene imprinting. Mol Cell Biol 17: 5584–5591.

Carr MS, Getek KA, Levorse JM, Schmidt JV (2006) Expression of a modified H19 RNA does not cause embryonic lethality in mice. Mamm Genome 17: 5–13.

Kawahara M, Wu Q, Ferguson-Smith AC, Kono T (2007) Appropriate expression of imprinted genes on mouse chromosome 12 extends development of bi-maternal embryos to term. FEBS Lett 581: 5178–5184.

Dorville B, Cordoumain N, Doutour L, Dandoy-Dron F, Irié JM, et al. (1997) Phenotypic alterations in insulin-deficient mutant mice. Proc Natl Acad Sci U S A 94: 5137–5140.

Silva D, Vennila M, Gao WH, Lopez MF (2006) IGF2 deficiency results in altered lung development at the end of gestation. Endocrinology 147: 5304–5310.

Murrell A, Heessen S, Bowden L, Constancia M, Dean W, et al. (2001) An intragenic methylated region in the imprinted Igf2 gene augments transcription. EMBO Rep 2: 1101–1106.

Kawahara M, Wu Q, Takahashi N, Morita S, Yamada K, et al. (2007) High-frequency generation of viable mice from engineered bimaternal embryos. Nat Biotechnol 25: 1045–1050.

Salas M, John R, Saxena A, Barton S, Frank D, et al. (2004) Placental growth retardation due to loss of imprinting of PHLDb2. Mech Dev 121: 1199–1210.

McMinn J, Wei M, Schupf N, Cusmai J, Johnson EB, et al. (2006) Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta 27: 540–549.

Apostolidou S, Abu-Amero S, O’Donoghue K, Frost J, Olafsdottir O, et al. (2007) Elevated placental expression of the imprinted PHLDA2 gene is associated with low birth weight. J Mol Med 85: 379–387.

Choi JD, Underkoffler LA, Wood AJ, Collins JN, Williams PT, et al. (2005) A novel variant of Igf2 is imprinted in brain, and its expression is correlated with differential methylation of an internal CpG island. Mol Cell Biol 25: 5314–5322.

Laidi PP, Hartemink AJ, Jirtle RL (2005) Genome-wide prediction of imprinted murine genes. Genome Res 15: 875–884.

Oh R, Ho R, Mar L, Gertsenstein M, Paderova J, et al. (2008) Epigenetic and phenotypic consequences of a truncation disrupting the imprinted domain on distal mouse chromosome 7. Mol Cell Biol 28: 1092–1103.

Szabo P, Mann JR (1995) Allele-specific expression and total expression levels of imprinted genes during early mouse development: implications for imprinting mechanisms. Genes Dev 9: 3097–3108.

Varrau A, Guerydan C, Delalbre A, Bellmann A, Houssami S, et al. (2006) Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell 11: 711–722.

Gabory A, Ripoche MA, Le Digarcher A, Watrin F, Ziyyat A, et al. (2009) H19 acts as a trans regulator of the imprinted gene network controlling growth in mice. Development 136: 3413–3421.

McLaughlin KJ, Szabo P, Haegele H, Mann JR (1996) Mouse embryos with paternal duplication of an imprinted chromosome 7 region die at midgestation and lack placental spongiotrophoblast. Development 122: 265–270.

Szabo P, Mann JR (1994) Expression and methylation of imprinted genes during in vitro differentiation of mouse preimplantation and androgenetic embryonic stem cell lines. Development 120: 1651–1660.
