Unregulated Expression of the Imprinted Genes *H19* and *Igf2r* in Mouse Uniparental Fetuses

Running Title: Imprinted Genes in Mouse Uniparental Fetuses

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Abstract

The present study shows that the \textit{H19} and \textit{Igf2r} genes, which are imprinted and expressed solely from maternal alleles, are expressed unregulatorily in mouse uniparental, androgenetic and parthenogenetic fetuses at day 9.5 of gestation. In the androgenetic fetuses, the \textit{H19} and \textit{Igf2r} genes were respectively expressed at 12\% and 40\% of the levels in biparental fetuses. In addition, the expression of both genes was excessive (1259\% and 482\%, respectively) in the parthenotes. These expressions of the imprinted genes were not regulated by methylation in the regulatory regions. Moreover, the expression of the antisense \textit{Igf2r} RNA (\textit{Air}) was also excessive and was not correlated with \textit{Igf2r} gene expression in the uniparental fetuses. Taken together, these results indicate that the parental-specific expression of imprinted genes is not maintained in particular genes in uniparental embryos, which in turn suggests that both parental genomes are required to establish maternal-specific expression of the \textit{H19} and \textit{Igf2r} genes by trans-acting mechanisms.

Key Words: Imprinted expression, Expression level, \textit{H19}, \textit{Igf2r}, Mouse, Parthenogenetic embryos, Androgenetic embryos

Introduction

Imprinted genes are genes that are expressed solely from one parental allele and are reciprocally suppressed in the other parental allele. The parental-specific expression of the genes is governed by epigenetic genome modification (genomic imprinting), which is thought to involve the methylation of cytosine in CpG dinucleotides (1-5), as well as by conformational change of chromatin structure (2, 6) and a time lag in DNA replication (6). However, the precise mechanism of the parental-specific expression remains unknown.

It is generally known that parental allele-specific expressions of maternally and paternally expressed imprinted genes are maintained in mouse parthenogenetic/gynogenetic and androgenetic embryos (7). Because these uniparental embryos are constructed from only the maternal or paternal genome, they are often used in the study of imprinting mechanisms as models of the parental expression of imprinted genes. Recently, an attempt was made to identify novel imprinted genes by estimating differences in imprinted gene expression between parthenogenetic and androgenetic embryos using the subtraction method (8, 9) and fluorescent...
differential display screening (10). However, no study has examined in detail whether or not parental-specific expressions of imprinted genes are always maintained in uniparental embryos.

In the present study, to confirm the parental-specific expression of imprinted genes in uniparental embryos, we screened the expression of the maternally expressed imprinted genes \( H19 \) (11), \( Igf2r \) (12), and \( P57^{KIP2} \) (13) and the paternally-expressed imprinted genes \( Snrpn \) (14, 15), \( Ndn \) (16), \( Zfp127 \) (16), \( Peg1/Mest \) (8, 17), and \( Peg3 \) (18) by semi-quantitative RT-PCR. The results showed that the \( H19 \) and \( Igf2r \) genes were irregularly expressed in paternal alleles of androgenetic embryos at the respective levels of 12% and 40% of the control biparental fetuses, although androgenetic fetuses have only the paternal alleles of the \( H19 \) and \( Igf2r \) genes, in which the alleles are naturally silenced in biparental embryos. In addition, we performed a quantitative analysis of the expressions of these genes in individual uniparental fetuses using real time quantitative RT-PCR (19, 20), and attempted to clarify the gene regulation mechanism by methylation analysis of the gene regulatory regions.

**Materials & Methods**

Production of fetuses at day 9.5 of gestation

B6CBF1 (C57BL/6N x CBA) mice were used as oocyte donors to produce embryos. They were superovulated with injections of 5 IU of equine chorionic gonadotrophin (eCG; Peamex, Sankyo, Ltd., Tokyo, Japan) and 5 IU of human chorionic gonadotrophin (hCG; Puberogen, Sankyo, Ltd.) given 48 hours apart. Oocytes at metaphase II were released from the oviducts 13-16 hours after the hCG injection, and the cumulus cells were removed by digestion with 300 units/ml hyaluronidase in M2 medium (21). To produce diploid parthenogenetic embryos by artificial activation, oocytes were cultured for 2 hours in a drop of Ca\(^{2+}\)-free M16 medium (22) containing 10 mM SrCl\(_2\) (Sr\(^{2+}\)) (23). The oocytes were then cultured in M16 medium supplemented with 0.4% BSA for 4 days in an atmosphere of 5% CO\(_2\), 5% C\(_2\), and 90% N\(_2\) at 37°C. During the culture in the Sr\(^{2+}\) medium and following the first 4 hours of culture in the M16 medium, 5µg/ml cytochalasin B was added to these media to induce diploidy by inhibiting a second-polar body extrusion. Androgenetic embryos were produced by *in vitro* fertilization (IVF) of enucleated oocytes, according to the procedure previously reported (24). When required,
pronuclear transfer was performed to produce diploid androgenones. Control biparental embryos were produced by IVF.

To identify the expressed alleles, biparental and androgenetic embryos were constructed using mice that had a polymorphism in their genomic DNA sequences. For the \( H19 \) gene, B6.Spretus-\( H19 \) congenic mice (established in our laboratory) having a restriction enzyme (Bgl I) fragment length polymorphism (RFLP) were used. For the \( Igf2r \) gene, JF1 mice having the full-length polymorphism (LP) were used.

\( H19 \) gene knock out (\( H19\)-KO) mice (25), in which the 3-kb transcription unit of the \( H19 \) gene was deleted, were used to identify the expression leakage of the \( H19 \) gene from the paternally suppressed allele in the absence of the maternally expressed allele. Fertilized embryos were constructed from \( H19\)-KO mice oocytes and B6CBF1 mice sperm by IVF.

The obtained blastocysts were transferred to the uterine horns of CD-1 females on day 3 of pseudopregnancy (2.5 days post coitum), and the fetuses were recovered at day 9.5 of gestation. For the following procedures, fetuses that had more than 15 somites and possessed a heartbeat were selected and introduced to RNA extraction.

Analysis of gene expression (RT-PCR)

Total RNA was isolated from the parthenogenetic, androgenetic, and control biparental fetuses, one by one, at day 9.5 of gestation using an SV Total RNA Isolation kit (Promega Co., Ltd., Madison, WI). After quantitative measurement of the total RNA extracted from each embryo using a GeneQuant Pro spectrum analyzer (Amersham Pharmacia Biotech, Buckinghamshire, UK), the fetuses from which more than 1 µg RNA was extracted were subjected to cDNA synthesis. First strand cDNA was synthesized from 1 µg of the total RNA from each embryo using a Superscript reverse transcriptase II kit (Gibco-BRL, Ltd., Gaithersburg, MD) according to the manufacturer’s instructions. To determine the biological activity in each sample, the expression of the beta-actin gene (26) was first analyzed before the analysis of the imprinted gene expression. For semi-quantitative analysis of imprinted gene expression, the cDNA was diluted to concentrations of 1/1, 1/10, 1/100 and 1/1000 before being subjected to PCR reaction. PCR was carried out in a 50 µl reaction buffer containing 1.25 U of Taq DNA polymerase (Takara Shuzo Co., Ltd., Kyoto, Japan) in a GeneAmp PCR system (Perkin-Elmer Co., Ltd., Norwalk, CT). The following primer sets were
used: 5’-TGTAAACCTCTTTGGCAATGCTGCC-3’ and 5’-TATTGATGGACCCAGGACCTCTGGT-3’ for the H19 gene; 5’-GGGTACTTTGCTTTTGGGTA-3’ and 5’-TTCGACCTATAAGAAGCCTT-3’ for Igf2r; 5’-ATACTGGCATTGCTCGTG-3’ and 5’-TGGAGGAGGCTTGCTCTACGC-3’ for Snrpn; 5’-GTCTTTCTTCTCTGATGAC-3’ and 5’-CACAAGTTAACAAGTGCAC-3’ for Znf127; 5’-ATTGCAACAATGACGC-3’ and 5’-TGAGGTGGACTATTGTGTCACC-3’ for Peg1/Mest; 5’-GTGCAGGTGAAGGTGAGGTGG-3’ and 5’-GTTCTTCCTTCTACCCAACTTAC-3’ for Znf127; 5’-ATTTGATGGACCCAGGACCTCTGGT-3’ and 5’-TTCGACCTATAAGAAGCCTT-3’ for Igf2r. For the RFLP analysis in H19, this specific primer set was used: 5’-TGCTCCAAGGTGAAGCTGAAAG-3’ and 5’-GTAGGGCATGTTGAACACTTTATG-3’.

Quantitative analysis of gene expression was performed by means of real time quantitative PCR (19, 20). After adding a fluorescent reagent, SYBER Green I, to the PCR reaction, the quantity of PCR products was detected by monitoring the luminous intensity with a LightCycler (Roche Diagnostics, Ltd., Tokyo, Japan). From the resulting indexes, the initial quantity of the cDNA/RNA of interest was estimated. The primers used were 5’-CATGTCTGGGCCTTTGAA-3’ and 5’-TTGGCTCCAGGATATGGATGT-3’ for the H19 gene, 5’-CGGGCGTGTCCTACAAGTA-3’ and 5’-CGGCCTGAGTGAACTTTCAC-3’ for the Igf2r gene, and 5’-GTGGATTCAGGTTTCATG-3’ and 5’-GGCCCAGATATAAGGTGAGCTGAC-3’ for the antisense Igf2r RNA (Air). As control samples, the fetuses from fertilized embryos were analyzed as well.

Analysis of methylation in the H19 and Igf2r genes

In the CpG island sequences of the H19 and Igf2r (regions II) genes of androgenetic, parthenogenetic (only for the Igf2r gene) and normal fetuses, the methylation pattern was analyzed by Southern hybridization using genome DNAs that had been digested by methyl-sensitive restriction enzymes. To obtain DNA fragments containing the CpG islands, extracted genome DNAs were digested by BamH1 for the promoter region of the H19 gene, and by Pvu II for region II of the Igf2r gene. The digested DNAs were then digested by Hpa II and applied to electrophoresis (1.2% agarose). The DNAs were transferred to nylon membrane (Hybond-N+; Amersham Pharmacia Biotech, Ltd.) and then subjected to Southern hybridization. Hybridization was performed for 15 hours at 56°C using AlkPhos DIRECT Labeling Reagent (RPN3680;
Amersham Pharmacia Biotech, Ltd.). The probes used were a 2.6Kbp-BamH 1 fragment for the
H19 gene (27) and PCR products obtained using the primer set 5’-AACCCTCGGAACCCTGCCCTT-3’ and 5’-GAGGGTAGGATTCCGTTGCAAGG-3’ for region II of the Igf2r gene; all probes were alkaline-phosphatase labeled by the reagent. The hybridized
membrane was treated with CDP-Star (RPN3682; Amersham Pharmacia Biotech, Ltd.), and the
intensity of the band was measured by an LAS1000 image analyzer (Fuji Photo Film Co., Ltd.,
Tokyo, Japan).

Results
To confirm that the parental expressions of imprinted genes are maintained in uniparental
embryos, we first examined the expression of three maternally expressed genes, H19, Igf2r and
p57KIP2, and five paternally expressed genes, Snrpn, Zfp127, Peg1/Mest, Peg3 and Igf2, in
each individual parthenogenetic and androgenetic fetus at day 9.5 of gestation by semi-
quantitative RT-PCR (Fig. 1). The Igf2r and H19 genes were unexpectedly found to be expressed
in androgenetic fetuses. The expression levels of these genes were roughly 10% to 50% of those
in the control fetuses. The other genes maintained their parental expression levels in each of the
uniparental fetuses.

Quantities of the unregulated expression of the H19 and Igf2r genes in androgenetic
fetuses were further analyzed by real time quantitative RT-PCR. The results showed that
androgenetic embryos expressed the H19 gene at a mean of 12% (ranging from 0.2 to 55%,
n=12), and the Igf2r gene at a mean of 40% (ranging from 3 to 84%, n=12) of the level in the
control fetuses (n=12) (Fig. 2a and b). The expression alleles were accessed using androgenetic
and biparental fetuses constructed from B6CBF1 and congenic (for the H19 gene)/J F1 (for the
Igf2r gene) mice genomes, which contain polymorphic sequences in the exon region between the
strains (Fig. 3a and b). The results showed that both genes were expressed from both alleles in
the androgenetic fetuses. It was also confirmed that both genes were not expressed from the
maternal allele in the biparental fetuses. Moreover, to access the leakage of expression from the
paternal allele in the biparental fetuses, the quantity of H19 gene expression in H19-KO x
B6CBF1 fetuses was analyzed by real time quantitative PCR. The results showed that the
paternal allele of the H19-KO x B6CBF1 fetuses (n=11) greatly suppressed the H19 gene (0.1%
of B6CBF1 x H19-KO fetuses (n=11), Fig. 3c). These observations suggest that the regulation mechanism for parental-specific suppression of the H19 and Igf2r genes should be relaxed in androgenetic fetuses.

The quantitative analysis in parthenogenetic fetuses also showed unregulated expression of the H19 and Igf2r genes. The quantity of expression of the maternally expressed imprinted genes in parthenogenetic fetuses was thought to be roughly twice that of biparental fetuses, since two maternal alleles exist in the parthenogenetic fetuses. However, the parthenogenetic fetuses expressed the H19 gene at a mean of 482% (ranging from 317 to 742%, n=12), and the Igf2r gene at a mean of 1259% (ranging from 347 to 2692%, n=12), of the level in control fetuses (n=12) (Fig. 2a and b). This suggests that the expression of the H19 and Igf2r genes was not controlled by the dose-regulation mechanism.

Generally, parental expression of imprinting genes is closely related to the methylation level of the CpG islands (1-4). The H19 gene on the paternal genome is silenced while the regulatory region of the paternal allele in the CpG island is hypermethylated (28). Conversely, the Igf2r gene on the maternal allele is activated when the region of the maternal allele is hypermethylated (4, 29). To investigate whether this phenomenon is seen in the gene expressions in androgenetic fetuses, the methylation levels in the promoter region of the H19 gene and the intronic CpG island of the Igf2r gene were analyzed by Southern blot analysis (Fig. 4a and b) using a methylation-sensitive endonuclease HpaII. The results show that the HpaII sites in the regions of the H19 gene were partially hypomethylated in the androgenetic fetuses, suggesting that the unregulated expression is caused by this incomplete methylation (Fig. 4c). On the other hand, all of the Hpa II sites in the Igf2r gene were completely hypomethylated in the androgenetic fetuses, despite considerable expression of the gene (Fig. 4d). This finding suggests that other regulation factors may be involved in the disruption of the regulated expression of the Igf2r gene in androgenetic fetuses. The Hpa II sites in the H19 and Igf2r genes were hypomethylated in half doses in the biparental fetuses, and those in the Igf2r gene were hypermethylated in parthenogenetic fetuses, reflecting the maintenance of maternal expression.

To gain further insight into the unregulated expression of the Igf2r gene, the expression of antisense Igf2r RNA (Air) (29), which down-regulates the expression of the Igf2r gene, was examined. The results showed that expression of the Air was also unregulated in the
androgenetic and parthenogenetic fetuses. The mean expression of the Air was 1432% (ranging from 469 to 2255%, n=7) and 217% (ranging from 104 to 342%, n=7) of that in the control fetuses (n=7) in the androgenetic and parthenogenetic fetuses, respectively (Fig. 5). This suggests that parental-specific regulation in this region is disrupted in the uniparental fetuses.

Discussion

Assuming that the parental-specific expression of imprinted genes is maintained in uniparental fetuses, it is estimated from the number of expressed alleles that the expression in parthenogenetic and androgenetic fetuses will be not detected or twice that in the biparental ones. In the present study, however, the maternally expressed genes H19 and Igf2r were unexpectedly expressed in androgenetic fetuses and over-expressed in parthenogenetic fetuses. Recently, we also observed the unregulated expression of the U2afbp-rs paternally expressed gene in maternal alleles in parthenogenetic fetuses (30). These findings indicated that some imprinted genes are unable to maintain their imprinted expression status in mouse uniparental embryos.

We previously reported that the expression of the paternally expressed gene Igf2 in androgenetic fetuses was slightly accelerated to one-and-a-half times the expected value (31). Surprisingly, however, in the present study the expressions of the H19 and Igf2r genes in parthenogenetic fetuses were more than two and six times the expected values, respectively, and the expressions were far different from that of the Igf2 gene in androgenetic fetuses. This shows that expression dosages from single maternal alleles of the H19 and Igf2r genes in parthenogenetic fetuses are not regulated like those in biparental fetuses. The losses of gene regulation were assumed to arise from the lack of the paternal genome in parthenogenetic fetuses. It has been reported that fetuses carrying maternal duplication/paternal deficiency for distal chromosome 7 (MatDi7) express the H19 gene at twice the dose of normal fetuses, thus showing that the dose per maternal allele is maintained in the absence of a paternal allele and of the neighboring DNA sequence of the H19 gene (32). This suggests that the abundant expression of the H19 gene in parthenogenetic fetuses is likely caused by an abnormality in the regulation of a gene located outside of the disomy region of MatDi7 in chromosome 7 or the other chromosome.

This study confirmed that biparental fetuses maintain maternally specific expressions of
the \textit{H19} and \textit{Igf2r} genes, while the androgenetic fetuses unexpectedly expressed the \textit{H19} and \textit{Igf2r} genes at 12\% and 40\%, respectively, of the levels in biparental fetuses, in which the unregulated expressions resulted from both paternal alleles. Using a sensitive RT-PCR protocol to specifically quantify primary transcripts, Sasaki et al. (1) previously reported that the expression of the paternally expressed \textit{Igf2} gene in parthenogenetic fetuses was approximately 4\% of the level in controls. They speculated that this expression represented leakage from two silenced maternal alleles in diploid parthenogenetic fetuses, and was twice that of the maternal allele in the biparental fetuses. The abundant expression of the \textit{H19} and \textit{Igf2r} genes in androgenetic fetuses is, however, at a very different level from the leakage expression seen in the \textit{Igf2} gene. This suggests that there might be a regulatory element, which is mediated with the maternal genome, for establishing and/or maintaining the paternal-specific expression of the genes.

The differentially methylated region (DMR) of the \textit{H19} gene that is required for paternal-specific silencing is located between 2 and 4 Kb upstream from the start of transcription, and that of the paternal allele is normally methylated in the fetuses at day 9.5 of gestation (33, 34). In this study, we analyzed the methylation status of the CpG island region near the DMR (28) in the androgenetic fetuses and found that the region was partially undermethylated. It could be speculated that the unexpected expression of the \textit{H19} gene in androgenetic fetuses arises from this incomplete methylation. It was previously reported that the normally repressed paternal allele of the \textit{H19} gene is activated (loss of imprint) when the DMR deletion allele is transmitted to the progeny from the father, suggesting that the DMR contains a cis-acting silencer element of the gene (35). Thus the unregulated expression might also be attributed to the fact that the silencer on the paternal allele does not work in androgenetic fetuses. This, in turn, suggests that the maternal allele must interfere to establish the repression of the \textit{H19} gene from the paternal allele.

The \textit{Igf2r} gene has two DMRs, DMR1, which is located in the promoter region, and DMR2, which is in the intronic 3kb CpG island (4). It is known that DMR2 in the maternal allele begins to be methylated in the late stage of oogenesis. This methylation is required for the activation of the maternal allele (4, 36, 37), and the methylation of the 113bp DNA sequences in the DMR2 involving the HpaII-site 4 in region II governs the maternal-specific expression (38). We therefore investigated the methylation status of the DMR2 in androgenetic fetuses, and showed that DMR2s on two paternal alleles were undermethylated despite their high-level expression. In
contrast, one allele (this would be a maternal allele) in the biparental fetuses and two maternal alleles in the parthenogenetic fetuses were normally hypermethylated in correspondence to the expression from the maternal alleles. This suggests that another gene regulation region or regulation mechanism that has a coefficient with the methylation is necessary to maintain the imprinted expression of the \textit{Igf2r} gene.

Expression of the \textit{Air} is correlated with \textit{Igf2r} repression on the paternal alleles. While the DMR2, which is also the promoter of \textit{Air}, is undermethylated, the \textit{Air} is expressed and the \textit{Igf2r} gene is repressed (39). One of the mechanisms of \textit{Igf2r} repression by DMR2 could occur through the action of the \textit{Air} in, e.g., transcriptional interference or repressor binding (40). The present study showed that the \textit{Igf2r} gene and \textit{Air} were expressed reciprocally between the androgenetic and parthenogenetic fetuses, with an abundant expression of \textit{Igf2r} occurring in the parthenogenetic fetuses and an abundant expression of \textit{Air} occurring in the androgenetic fetuses. In the parthenogenetic fetuses, the \textit{Air} was expressed at twice the level as in the biparental fetuses despite the fact that DMR2 was methylated. These results suggest that transcriptional activity is up-regulated in the region of the \textit{Igf2r} gene and \textit{Air}, and that a common cofactor, which is necessary for the establishment of regulated expression of the \textit{Igf2r} gene and \textit{Air}, could be absent and/or insufficient in the uniparental fetuses.

In the present study and in previous work (30), we defined three imprinted genes, U2af1-rs1, \textit{H19}, \textit{Igf2r}, and antisense \textit{Igf2r} RNA (\textit{Air}), which did not maintain the imprinted expression in uniparental mouse fetuses at day 9.5 of gestation. These findings suggest that, at least in some imprinted genes, some kinds of transcripts, such as those that require an interaction between the male and female genomes, play an important role in establishing the gene regulation mechanism for parental-specific/imprinted expression and for the expression dosage. However, the substance of the imprinting mechanism is still unknown. To gain an explanation for the disruption of the imprinted status in uniparental embryos, further investigation of the system of imprinted gene regulation is needed.

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References


Fig. 1. Semi-quantitative analysis of the expression of control non-imprinted genes; beta-actin; the maternally expressed imprinted genes H19, Igf2r and p57KIP2; and the paternally expressed imprinted genes Snrpn, Ndn, Zfp127, Peg1/Mest and Peg3 in control biparental (C), androgenetic (A), and parthenogenetic (P) fetuses by RT-PCR. PCR products were amplified from cDNA samples that were transcribed from 100 ng (lanes 1, 5, and 9), 10 ng (lanes 2, 6, and 10), 1 ng (lanes 3, 7, and 11) and 0.1 ng (lanes 4, 8, and 12) of the total RNA.
Fig. 2. Quantitative analysis of the H19 (a) and Igf2r (b) gene expressions in control biparental (C), androgenetic (A) and parthenogenetic (P) fetuses by real time RT-PCR. Twelve samples were examined for each of these three groups. The expression level of each fetus is indicated with a dot. The average of the expression levels in the control fetuses taken as a standard (100%). Shaded boxes indicate the average expression level in each group (H19 and Igf2r genes: 12% and 40%, respectively, in androgenetic fetuses, and 482% and 1259%, respectively, in parthenogenetic fetuses).
Fig. 3. Analysis of allele-specific expression of the H19 and Igf2r genes in the biparental and androgenetic fetuses at day 9.5 of gestation. (a) RFLP analysis of the H19 gene expression using B6CF1 and B6.Spretus-H19 congenic (Cong.) mice. In the B6CF1xCong. and Cong.xB6CF1 biparental fetuses, expressions were theoretically detected only in the maternal B6CF1 (521bp) and Cong. (384bp) mouse alleles, respectively. In the androgenetic fetuses (A: B6CF1 + Cong.), gene expressions were detected in both alleles. (b) LP analysis of the Igf2r gene expression using B6CF1 and JF1 mice. In the B6CF1xJF1 and JF1xB6CF1 fetuses, expressions were theoretically detected only in the maternal B6CF1 (240bp) and JF1 (220bp) mouse alleles, respectively. In the androgenetic fetuses (A: B6CF1 + JF1), gene expressions were detected in both alleles. (c) Quantitative analysis of H19 gene expression by real time RT-PCR in biparental fetuses, of which the male and female alleles were derived from B6CF1 and H19-deficient mice (F1xKO), and from H19-deficient and B6CF1 mice (KOxF1). Eleven samples were examined for each of the two groups. The expression level of each fetus is indicated with a dot. The average of the expression levels in the F1xKO fetuses was taken as a standard (100%). Shaded boxes indicate the average expression level in each group (KOxF1 fetuses: 0.1%).
Fig. 4. Southern analysis of the DNA methylation patterns of the \textit{H19} and \textit{Igf2r} genes in control biparental (C), parthenogenetic (P), and androgenetic (A) fetuses at day 9.5 of gestation. (a) Methylation status of the \textit{H19} gene. In the control fetus, about half the sequences in the CpG island regions (BamHI-digested fragments, 2512 bp) were digested into 991-bp fragments by HpaII in correspondence with the gene expression. In the androgenetic fetuses, some of the sequences were partially digested by HpaII (open arrowhead, approximately 2 Kbp), which showed the methylation status related to the gene expression. (b) Methylation status of the \textit{Igf2r} gene. After the treatment by HpaII, about half of the sequences in the region 2 (PvuII-digested fragments, 2932 bp) were digested into 962- and 571-bp fragments in the control fetuses, and no sequence was digested in the parthenogenetic fetuses, which findings corresponded to the gene expression patterns. In contrast, the sequences in the androgenetic fetuses were completely digested by HpaII, indicating that undermethylation occurred irrespective of the gene expression.
Fig. 5. Quantitative analysis of the Air expressions in the control biparental (C), androgenetic (A) and parthenogenetic (P) fetuses by real time RT-PCR. Seven samples were examined for each of these three groups. The expression level of each fetus is indicated with a dot. The average of the expression levels in the control fetuses was taken as a standard (100%). Shaded boxes indicate the average expression level in each group (1432% in androgenetic fetuses, and 217% in parthenogenetic fetuses).
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