Development 124, 4897-4904 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
DEV4894

Roles of the imprinted gene *Igf2* and paternal duplication of distal chromosome 7 in the perinatal abnormalities of androgenetic mouse chimeras

K. John McLaughlin[1,*], Helga Kochanowski[1], Davor Solter[1], Georg Schwarzkopf[2], Piroska E. Szabó[3] and Jeffrey R. Mann[3]

[1]Department of Developmental Biology, Max Planck Institute for Immunology, P. O. Box 1169, 79108 Freiburg, Germany
[2]Pathology Institute, Albert Ludwigs University, Albertstrasse 19, 79104 Freiburg, Germany
[3]Division of Biology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, California 91010, USA
*Author for correspondence (e-mail: mclaughlin@immunbio.mpg.de)

SUMMARY

Mouse chimeras made with androgenetic (two paternal genomes) ova or embryonic stem cells frequently die at the perinatal stage and exhibit a range of defects, the most noticeable being a pronounced overgrowth of rib cartilage. Excess concentrations of IGFII, a potent mitogen, has been suggested to play a major role in these defects, as androgenetic cells possess two active paternal copies of the imprinted *Igf2* gene, rather than one inactive maternal and one active paternal copy as in normal cells. Here, we show that chimeras made with androgenetic embryonic stem cells, homozygous for an *Igf2* null mutation, do not develop rib cartilage hyperplasia, demonstrating the dependence of this defect on *Igf2* activity produced by androgenetic cells. In contrast, in these same chimeras, many other defects, including whole body overgrowth and perinatal death, are still prevalent, indicating that the abnormal expression of one or more imprinted genes, other than *Igf2*, is also capable of inducing most of the defects of androgenetic chimeras. Many of these genes may reside on distal chromosome 7, as we also show that perinatal chimeras made with embryonic stem cells possessing paternal duplication of distal chromosome 7 exhibit a range of defects similar to those of androgenetic chimeras. The relevance of these findings for the human imprinting-related disorder, Beckwith-Wiedemann syndrome, is discussed.

Keywords: androgenetic, Beckwith-Wiedemann syndrome, chimera, imprinting, insulin-like, growth factor 2, mouse

INTRODUCTION

Diploid androgenetic (AG) zygotes have two paternally derived pronuclei or genomes, and can be produced experimentally by pronuclear transplantation. They usually die at the periimplantation stage, although rarely they can develop to early somite stages (Barton et al., 1984; McGrath and Solter, 1984). The development of androgenetic chimeras is considerably more successful. Those with a high contribution of androgenetic cells can survive to the perinatal stage, although only chimeras with a low contribution can survive postnatally (Mann et al., 1990; Barton et al., 1991; Mann and Stewart, 1991). The highly lethal effect of androgenetic cells in perinatal and postnatal androgenetic chimeras is associated with various skeletal malformations, most noticeably a pronounced overgrowth of rib cartilage as well as whole body overgrowth (Mann et al., 1990; Barton et al., 1991; Mann and Stewart, 1991; Fundele et al., 1995).

Excess concentrations of insulin-like growth factor II, IGFII, may contribute significantly to the various defects in AG chimeras. This is suggested by a number of observations. (1) IGFII is a potent embryonic mitogen (DeChiara et al., 1990) and stimulates the growth of many cell types in vitro, including cartilage cells (Humbel, 1990). (2) Embryoid bodies derived from AG embryonic stem (ES) cells in culture express high levels of *Igf2* RNA (Allen et al., 1994; Szabó and Mann, 1994), therefore it is likely that AG cells in chimeras also produce excess *Igf2* RNA. Presumably, this overexpression results from the presence of two active paternal copies of *Igf2*, rather than one inactive maternal and one active paternal copy of this imprinted gene in normal or wild-type (WT) cells. (3) *Igf2*-transgenic mice appear to die in utero, suggesting that overexpression of *Igf2* is deleterious (Efstratiadis, 1994). (4) Chimeras in which one component is composed of cells with paternal duplication of the chromosome region harboring *Igf2*, i.e. distal chromosome 7, exhibit whole body overgrowth, and excess *Igf2* activity has been suggested as the cause (Ferguson-Smith et al., 1991). This suggestion has also been made for AG chimeras, which also exhibit whole body overgrowth during embryogenesis (Barton et al., 1991). (5) The human imprinting-related disorder, Beckwith-Wiedemann syndrome, is characterized by various congenital malformations, including overgrowth and a higher incidence of perinatal lethality. It is often associated with paternal duplication of an *IGF2*-containing chromosome region, and excess *IGF2* expression is widely believed to play a major role in the pathology (Junien, 1992; Witte and Bove, 1994).

To investigate the role of *Igf2* expression in the phenotype of perinatal AG chimeras, we have derived AG ES cell lines homozygous for a null mutation of the *Igf2* gene, and examined the phenotype of perinatal chimeras derived from them. The defect of rib cartilage overgrowth, as obtained in standard AG chimeras, was no longer observed. However, other defects, including whole body overgrowth and perinatal death, were still obtained. *Igf2* expression in AG cells is therefore a prerequisite for at least one, but not all defects of perinatal AG chimeras.

In a further experiment, we investigated the possibility that abnormal expression of imprinted genes on distal chromosome 7 might play a causal role in the abnormal development of AG chimeras. This was achieved by examining the development of chimeras derived from ES cells which, rather than possessing paternal duplication of the whole genome as do AG ES cells, carried paternal duplication of a subset of the genome, distal chromosome 7. The possible involvement of this region was considered likely, as with most of the genome now investigated for the effects of paternal duplication, distal chromosome 7 is one of only two regions which, when paternally duplicated, causes death in utero (Beechey and Cattanach, 1997). The results were consistent with the possibility that perinatal chimeras derived from ES cells carrying paternal duplication of distal chromosome 7, PatDup.d7, possess a range of defects similar to that seen in AG perinatal chimeras.

MATERIALS AND METHODS

Derivation of ES cell lines

AG blastocysts for derivation of ES cell lines were produced by pronuclear transplantation (Mann et al., 1990), using 129/Sv males heterozygous for a null mutation of *Igf2* (DeChiara et al., 1990). This strategy yielded AG *Igf2*+/+ control and AG *Igf2*–/– experimental ES cell lines in the same experiments. Blastocysts for derivation of PatDup.d7 and WT ES cell lines were obtained from intercrosses of mice heterozygous for the T(7;15)9H reciprocal translocation, using a *Pst*I polymorphism to identify genotypes. These cell lines were a mixture of C57BL6J, T(7;15)9H and 129/Sv genetic backgrounds. (McLaughlin et al., 1996). Cell culture conditions for derivation of the cell lines were as previously described (Robertson, 1987; Buzin et al., 1994).

Production of chimeras

Chimeras were produced by the injection of ES cells into blastocysts (Stewart, 1993). The latter were derived by flushing morulae from superovulated (C57BL/6×CBA)F_1 females, mated to C57BL/6 males, at 2.5 days post coitus (d.p.c.) (Hogan et al., 1994), then culturing them overnight in medium CZB plus glucose, minus EDTA (Chatot et al., 1989). All AG and PatDup.d7 ES cell lines were homozygous for the allele encoding the A electrophoretic form of glucose phosphate isomerase-1, GPI-1, whereas blastocysts were homozygous for the allele encoding the B form. This allowed assessment of chimerism by GPI-1 isozyme electrophoresis (Hogan et al., 1994).

Specimen preparation

Histology

Whole embryos were fixed in a solution of 60% (v/v) ethanol, 30% (v/v) chloroform, and 10% (v/v) acetic acid. Fixative was changed after 3 and 24 hours depending on the size of the embryo. Incisions were made in the body cavity, and the head removed to ensure adequate fixation. Samples were dehydrated in isopropanol, embedded in paraffin, and sections stained with hematoxylin and eosin.

Skeleton whole mounts

Animals were soaked overnight in water followed by a 3 minute incubation at 65°C. After removal of viscera and skin, carcasses were fixed in 95% (v/v) ethanol for 3 days. Samples were stained with alizarin red and/or alcian blue (McLeod, 1980). Tissue was cleared with a 1% (w/v) potassium hydroxide solution and samples stored in glycerol until photographed.

RNA analysis

Whole embryos, neonates and organs were frozen in liquid nitrogen, then pulverized with a mortar and pestle in liquid nitrogen. The powder was dissolved in RNAzol-B (Tel-Test Inc., Friendswood, Texas, USA), and total RNA was extracted according to the manufacturer's protocol. Samples were run on denaturing formaldehyde gels and transferred with phosphate buffer to GeneScreen nylon membrane (NEN Research Products) according to the manufacturer's protocol. Probes recognizing transcripts of *Igf2*, *H19*, and the glyceraldehyde 3-phosphate dehydrogenase gene, *Gapd*, and hybridization conditions were as described by Szabó and Mann (1994). The hybridization probe for transcripts of the p57 cyclin kinase inhibitor protein 2 gene, $p57^{KIP2}$, was derived by RT-PCR from total embryonic RNA, and was a 386 base pair fragment spanning nucleotides 1040-1425 (Matsuoka et al., 1995).

RESULTS

Derivation of ES cell lines

AG ES cell lines: Fifty one androgenetic zygotes were produced by pronuclear transplantation from which twenty two lines were derived. Of these lines, four were *Igf2*–/–, five were *Igf2*+/+, and the remainder were *Igf2*+/–. T(7;15)9H/+ intercross ES cell lines: Sixty one lines were derived, of which five were PatDup.d7, 10 were MatDup.d7 (maternal duplication of distal chromosome 7), and the remainder were WT. All lines used in chimera production had a modal number of 40 chromosomes, and were of an XY sex chromosome constitution.

Characterization of perinatal defects in AG ES cell chimeras

Two AG *Igf2*+/+ (hereafter termed AG) cell lines were used for chimera production. The degree of AG contribution to perinatal chimeras at 18.5 d.p.c., one day before birth, is shown in Table 1. These chimeras exhibited overgrowth compared with non-chimeric littermates, skeletal defects, and a high frequency of perinatal death as previously described (Mann et al., 1990; Barton et al., 1991; Mann and Stewart, 1991; Fundele et al., 1995). Also, we found a number of other external defects not previously described; eyelid fusion failure, reduced limb extension, postaxial polydactyly, umbilical hernia and abdominal swelling (Table 1; Fig. 1A-C).

Two AG *Igf2*–/– ES cell lines were used for chimera production. At 18.5 d.p.c., the degree of chimerism obtained with AG *Igf2*–/– cell lines was similar to that obtained with AG ES cells (Table 1). 18.5 d.p.c. AG *Igf2*–/– chimeras (Fig. 1D,E) derived from both cell lines exhibited most of the the defects seen in their AG counterparts (Table 1) except for consistent differences in the skeletal phenotype (four observations were made, i.e., n=4) including rib cartilage hyperplasia, a predominant feature of AG chimeras (Fig. 2B,C). Both AG (n=4) and

Table 1. Frequency of defects in 18.5 d.p.c. WT, AG, AG *Igf2*–/– and PatDup.d7 chimeras

	ES cell type							
	WT	*n*	AG	*n*	AG-*Igf2*-/-	n	PatDup.d7	*n*
Mean % chimerism (s.d.)	61(19)	24	23(12)	10	27(15)	12	42(16)	16
Incomplete eyelid fusion	2	49	2	17	2	25	31	39
Umbilical hernia	0	49	2	17	8	25	11	39
Abdominal swelling	0	49	6	17	4	25	19	39
Cranial asymmetry	0	49	2	17	2	25	6	39
Limb extension	0	49	5	17	2	25	12	39
Polydactyly	0	49	2	17	3	25	5	39
Sternum defect	0	20	4	4	6	6	13	14
Cartilage hyperplasia	0	20	4	4	0	6	0	14
Tail bend	0	12	nd		nd		8	13
Mean weight chimera vs. littermates (s.d.)	1.01(0.1)	23	1.58(0.24)	6	1.31(0.19)	37	1.33(0.19)	22

Values for each type of chimera represent the combined numbers for two cell lines. *n* = number of fetuses analysed for each defect. Level of chimerism was estimated by GPI-1 isozyme analysis of tail tissue. The weight of each chimera is a value relative to that of the non chimeric littermates (weight = 1). The mean weight chimera vs. littermates is the average value for the relative weights of all chimeras of each type. Chimeras with a higher level of chimerism usually had more defects, and those defects ocurring at the lowest frequency were usually present in the highest level chimeras. Most defects were evaluated at time of recovery from the mother, or after partial dissection as for the sternum defect. Cartilage hyperplasia was scored as present when this defect was evident prior to dissection.

AG *Igf2*–/– chimeras exhibited hypoossification as seen in midline skeletal elements. However, the AG *Igf2*–/– chimeras (Fig. 2G,K,O) were in all instances less ossified than were the AG chimeras (Fig. 2F,J,N). In both types of chimera, this was most pronounced in the area surrounding the fourth lumbar vertebrae and the xiphoid process. In these two regions ossification centers exhibited the most pronounced lack of fusion. Examination of the skull in both AG and AG *Igf2*–/– chimeras revealed many defects associated with hypoossification. This was particularly evident in the midline elements forming the sphenoid bones, the palate and membranous parietal bones (Fig. 2N,O).

Histological analysis of AG chimeras revealed that the abdominal swelling was largely due to enlargement of the liver. This enlargement was at least partly the result of sinusoidal dilation caused by blood congestion (Fig. 3B). Livers of AG *Igf2*–/– chimeras were not as abnormal as AG chimera livers, but blood vessels were midly dilated (Fig. 3C). A lack of abdominal wall muscle and lack of mature brown fat was seen in both AG and AG *Igf2*–/– chimeras.

Developmental potential of PatDup.d7 ES cell chimeras

With WT ES cells derived from the T(7;15)9H/+ intercross, it was possible to obtain a 90% ES cell-derived contribution in chimeras up to 18.5 d.p.c. With PatDup.d7 ES cells derived from the same intercross, 70% contribution was attained up to 18.5 d.p.c., although at the earliest stage examined, 9.5 d.p.c., some higher level chimerism was obtained with one of the PatDup.d7 cell lines used. These observations indicate either that PatDup.d7 chimeras with more than ~70% chimerism die after 9.5 d.p.c., or that the PatDup.d7 cells in the chimera are subject to negative selection pressure between 9.5 d.p.c. and 18.5 d.p.c. With respect to chimeras surviving to at least one day post partum, WT chimeras were up to 90% ES cell-derived, while PatDup.d7 chimeras were at the most 40% ES cell-derived. This indicates that PatDup.d7 pups with greater than ~40% chimerism die perinatally. Eight surviving postnatal PatDup.d7 chimeras were mated, and at least three litters were bred from six males. No germline transmission of the PatDup.d7 component of these chimeras was obtained.

Range of perinatal defects in PatDup.d7 ES cell chimeras resembles that in AG ES cell chimeras

Perinatal 18.5 d.p.c. PatDup.d7 chimeras possessed a higher ES cell-derived contribution than perinatal AG chimeras (Table 1). There was no bias of contribution of PatDup.d7 cells to any of the peri- and postnatal stage organs tested (Table 2). High level PatDup.d7 chimeras were often unable to maintain steady breathing compared to other non-chimeric littermates. The most consistent phenotypic feature of PatDup.d7 chimeras was large size (Table 1). Other defects often observed included abdominal swelling, eyelid fusion-failure of variable expressivity, and retarded limb extension, most noticeably affecting the hind limbs (Fig. 4A-F). Less common defects, seen most frequently in higher level chimeras, included umbilical hernia, postaxial polydactyly, cranial asymmetry, and slight tail kink (Table 1; Fig. 4F). As seen in sagittal sections of 40% to 60% level PatDup.d7 chimeras, in all instances, the heart and the liver dominated the space within thoracic and abdominal cavity respectively (Fig. 4D,H). Further examination of the heart (Fig.4H) and liver (Fig. 3D) revealed that both had an increased number and size of blood sinusoids and veins. Sinusoids within the ventricular myocardium were often only a few cell layers from the pericardium, suggesting a possible lack of integrity of the ventricular wall. It is possible that defects in the heart, in particular the right ventricle, increased blood retention in the liver, thereby causing enlargement of the hepatic veins and sinusoids. The lungs failed to inflate and did not exhibit the same distribution within the thoracic cavity as observed in WT chimeric littermates (Fig. 4H). The anterior body wall was underdeveloped and lacked the inner muscle layer (Fig. 4J). This latter defect, together with the enlarged liver, possibly caused the umbilical hernias.

Skeleton preparations of 10% to 70% level PatDup.d7 chimeras consistently revealed the presence of defects (Table 1). There was evidence of cartilage hyperplasia (Fig. 4H), although even in the highest level PatDup.d7 chimera this

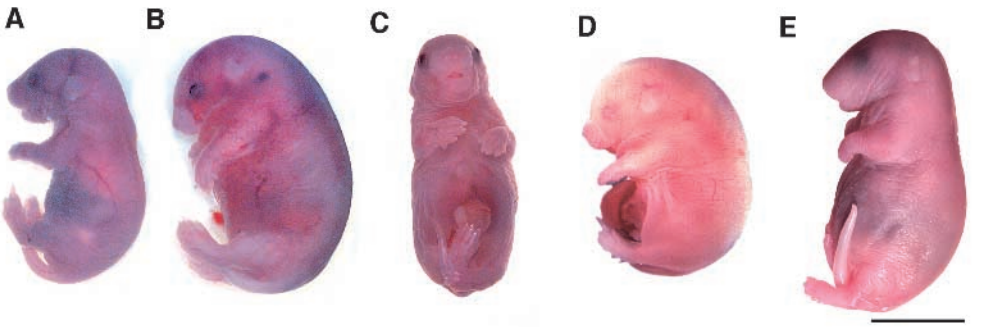

Fig. 1. Views of chimeras and a WT animal (A) at 18.5 d.p.c. showing various examples of phenotypes observed in AG (B,C) and AG *Igf2–/–* chimeras (D, E). Examples shown include an extreme phenotype seen only with higher level, 40-50% chimerism (B,D), and a less pronounced phenotype seen with lower level chimerism (C, E). Several features can be observed in the chimeras including the abnormal limb extension (B-E), abdominal swelling (C,E), postaxial polydactyly in the forelimbs (C), umbilical hernia (D), slight tail kink (E) subcutaneous contusions (B), thin abdominal wall (C), unfused eyelids (B,C).

defect was less severe than previously observed in any AG chimeras. PatDup.d7 chimeras exhibited hypoossification, but this was less pronounced than in the AG chimeras. Cartilage elements along the vertebral column and the sternum were not fused (Fig. 2H,L). This resulted in a disorganization, and occasionally bifurcation, of the xiphoid process (Fig. 2H), and a lack of ossified element fusion in the dorsal vertebral column centered around the fourth lumbar vertebra (Fig. 2L). The vertebral column often exhibited a scoliosis in the antero-posterior axis. Skulls of these chimeras exhibited defects of

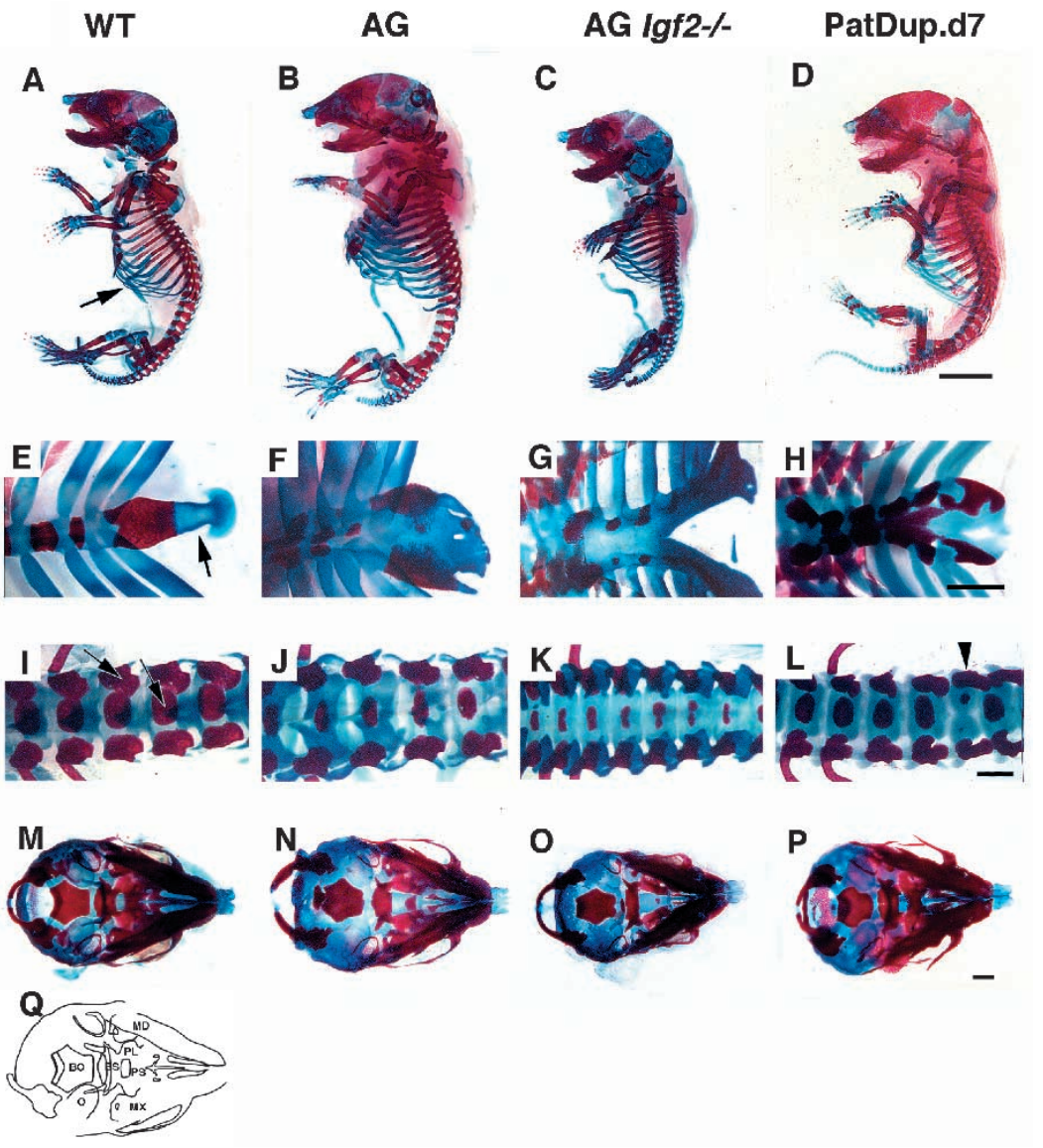

Fig. 2. Skeleton whole mounts of 18.5 d.p.c. chimeras. Red and blue areas represent bone and cartilage, staining with alizarin red and alcian blue respectively. (A-D) Whole skeleton; (E-H) xiphoid process; (I-L) vertebral column in upper lumbar region, dorsal view; (M-P) skull, posterior view. (A,E,I,M) WT chimera; (B,F,J,N) AG chimera; (C,G,K,O) AG *Igf2–/–* chimera; (D,H,L,P) PatDup.d7 chimera. Q, schematic, skull, posterior view, indicating the following structures; BO, basisoccipital; BS basisphenoid; MD, mandible; MX, maxilla; O, otic capsule; PL, palatine; PS, presphenoid; T, tympanic. In A the arrow indicates the costal margin of the ribs. This region is typically hyperplastic in AG chimeras (see this region in B). In L the arrow indicates the region where lack of fusion of central ossification elements was most commonly observed. Scale bar; A-D, 5 mm; E-H, 1 mm; I-L, 1 mm; M-P, 1 mm.

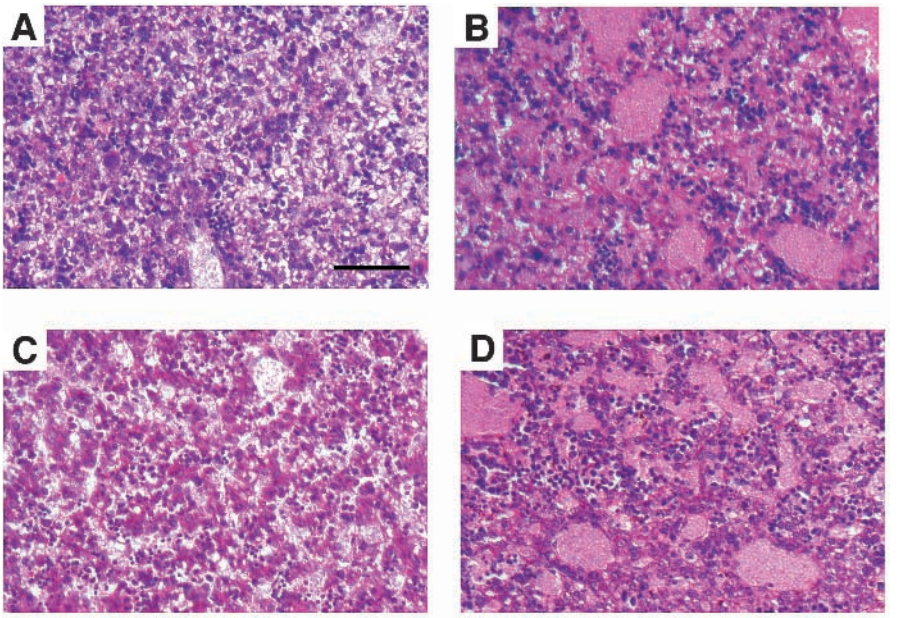

Fig. 3. Liver sections of 18.5 d.p.c. chimeras. Sections are stained with hematoxylin and eosin, and are (A) WT; (B) AG; (C) AG *Igf2*–/–; (D) PatDup.d7. Scale bar 0.2 mm.

hypoossification as were observed in AG chimeras, but were less pronounced (Fig. 2M,N,P).

In comparing the defects in AG and PatDup.d7 chimeras, we point out that the genetic backgrounds of AG and PatDup.d7 ES cells were not identical (see Materials and Methods), and therefore might be the basis for some differences, and even some similarities. Nevertheless, it has been noted that the developmental potential of embryos with uniparental duplications is generally not influenced by genetic background (Cattanach and Beechey, 1990).

Expression of distal chromosome 7 imprinted genes in PatDup.d7 and AG chimeras

In high level PatDup.d7 (~60%) and AG (~40%) chimeras, total *Igf2* RNA levels would be expected to be greater than normal, due to the presence of two active copies of *Igf2* in the PatDup.d7 and AG components, while RNA levels of $p57^{KIP2}$ and *H19* would be expected to be lower than normal, due to the presence of two inactive copies of these genes in these same components. The observed RNA levels were in accord with expectation (Fig. 5). These findings are consistent with the possibility that high and low levels of IGFII and P57KIPII, respectively, are causally related to the defects. Low levels of *H19* RNA would not be expected to play a role, as *H19* null mutant mice are essentially normal (Leighton et al., 1995).

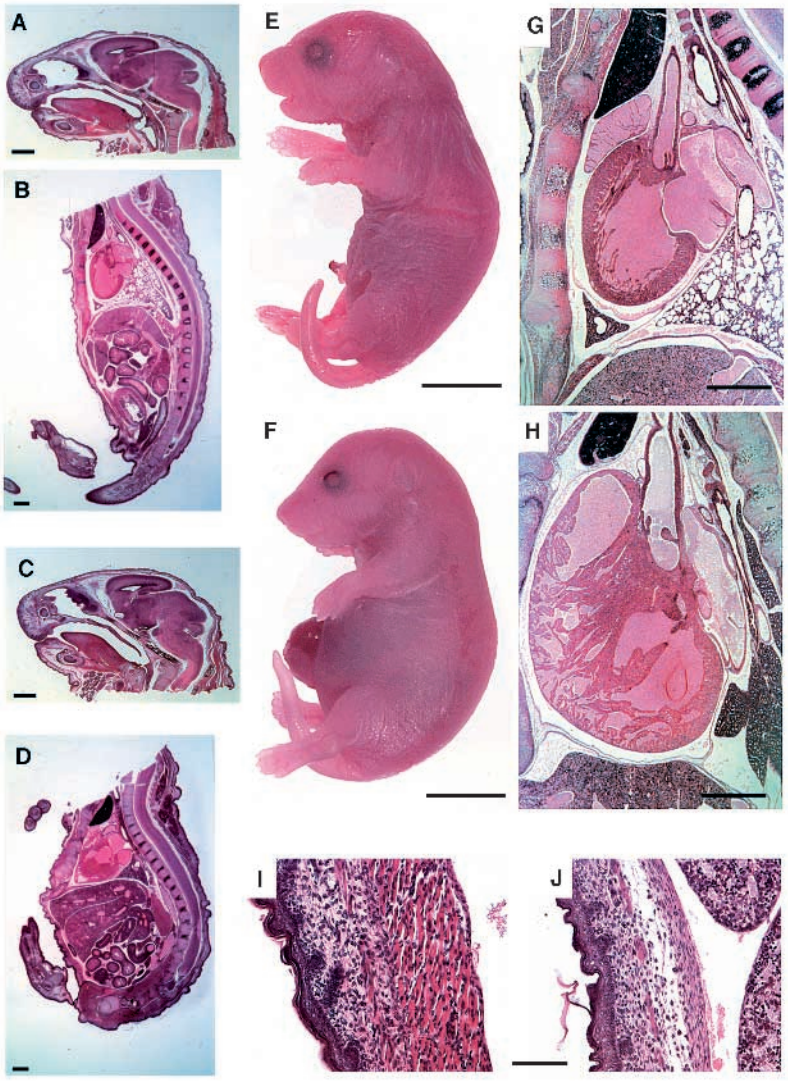

Fig. 4. Defects in 18.5 d.p.c. PatDup.d7 chimeras. Sagittal sections, stained with hematoxylin and eosin: WT, non chimeric littermate; (A) head; (B) whole body; (G) thoracic cavity; (I) abdominal wall. PatDup.d7 chimeras; (C) head; (D) whole body; (H) thoracic cavity; (J) abdominal wall. External appearance: (E) WT; (F) PatDup.d7 chimera. Scale bars: (A-D) 1 mm; (E-F) 5 mm; (G,H) 1 mm; (I,J) 0.05 mm.

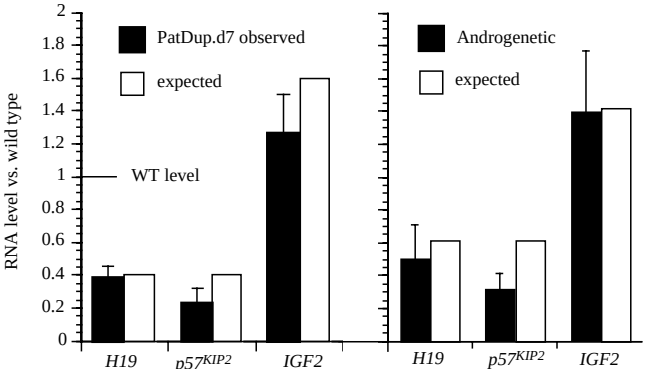

Fig. 5. Relative RNA levels of imprinted genes in 18.5 d.p.c. chimeras. Radioactive bands on northern blots were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, California, USA), and counts per minute (c.p.m.) obtained with probes for imprinted genes. These were standardized by dividing by c.p.m. obtained with the probe for *Gapd* RNA. Standardized c.p.m. for four PatDup.d7 chimeras with 50%-60% contribution and two AG chimeras with ~40% contribution were then divided by the mean standardized c.p.m. of two WT embryos to provide a relative RNA level (relative WT level =1). Expected values are based on a presumed 60% and 40% contribution of PatDup.d7 and AG cells in chimeras respectively.

DISCUSSION

We have described experiments designed to genetically dissect the basis for the perinatal defects of AG chimeras. First, we examined the development of chimeras made with AG ES cells homozygous for an *Igf2* null mutation. Second, we studied the development of chimeras made with ES cells which, as distinct from AG ES cells, carried paternal duplication of only a subset of the genome, distal chromosome 7.

The most striking defect in perinatal AG chimeras, rib cartilage hyperplasia, was lost in their *Igf2*–/– counterparts, showing that *Igf2* expression in the AG component is indeed an integral part of the mechanism which leads to this abnormality. It was previously suggested that *Igf2* expression may not be involved in this defect (Fundele et al., 1995), as no skeletal defects of any kind were observed in fetal chimeras made with PatDup.d7 blastocyst inner cell masses (Ferguson-Smith et al., 1991). The relative lack of cartilage hyperplasia in PatDup.d7 chimeras, which we have also observed here, indicates that expression of *Igf2* from both alleles in one component of a chimera is not sufficient by itself to generate this defect. This conclusion assumes that the activity of each paternal *Igf2* allele in relevant PatDup.d7 cells is of similar intensity to that in their AG counterparts. Given this assumption, in AG cells, the induction of the defect would therefore appear to involve the action of *Igf2* expression in concert with the aberrant expression of at least one other imprinted gene located outside the distal chromosome 7 region. One possibility may be a lack of interaction between IGFII and insulin-like growth factor 2 receptor, IGFIIR, in critical regions of the rib cartilage. IGFIIR is encoded by the imprinted *Igf2r* gene located on chromosome 17, is expressed from only the maternal allele, and acts to sequester IGFII ligand (Filson et al., 1993; Ludwig et al., 1996).

Aside from rib cartilage hyperplasia and the size and number of the liver blood vessels, perinatal AG *Igf2*–/– chimeras exhibited many other defects found in their AG counterparts. This finding does not show that *Igf2* expression in the AG component is incapable of inducing these defects, but does inidicate that the aberrant expression of one or more other imprinted genes in AG cells is capable of invoking them. We note that many of the defects occurring in perinatal AG chimeras also occur in mice lacking a functional *Igf2r* gene (Lau et al., 1994; Wang et al., 1994). In these latter mice, the defects are caused by the presence of excess IGFII ligand, which is in turn caused by a lack of sequestering IGFIIR activity (Filson et al., 1993; Ludwig et al., 1996). Thus, while the defects in AG *Igf2*–/– chimeras cannot be related to IGFII activity derived from the AG *Igf2*–/– component, the similarity with the *Igf2r* null phenotype is suggestive of the action of imprinted genes with a similar function to *Igf2*, i.e., to influence the rate of cell division.

To assess if the imprinted genes involved in the phenotype of perinatal AG chimeras might reside on distal chromosome 7, we studied the effects of PatDup.d7 cells in chimeras. Consistent with this possibility was that the range of defects observed in PatDup.d7 chimeras was remarkably similar to that in AG

Table 2. Contribution of ES cell-derived component in organs of PatDup.d7 and WT chimeras

Age	Coat	Blood	Intestine	Spleen	Liver	Kidney	Lung	Heart	Muscle	Thymus	Brain	Gonad	Skin
PatDup.d7 chimeras													
61 d.p.p.	3	2	0	3	4	2	2	2	2	2	2	0	–
78 d.p.p.	2	2	2	2	2	2	1	2	1	1	2	1	–
101 d.p.p.	2	2	2	2	0	1	1	2	2	2	1	1	–
10 d.p.p.	2	2	2	2	2	1	1	1	0	0	0	1	–
10 d.p.p	1	0	1	1	1	1	1	1	1	1	1	1	–
18.5 d.p.c.	-	–	3	–	3	–	3	3	3	–	–	3	3
18.5 d.p.c.	-	–	4	–	3	–	2	2	2	–	–	4	4
WT chimeras													
45 d.p.p.	3	–	4	3	–	4	1	2	3	2	2	1	–
67 d.p.p.	4	2	3	2	–	4	3	4	4	2	2	4	–
50 d.p.p.	2	–	1	1	2	2	1	2	4	1	2	3	–
89 d.p.p.	4	1	3	4	3	1	3	4	1	2	4	4	–
140 d.p.p.	2	–	3	4	3	2	1	4	1	4	4	3	–

The contribution in each organ was determined by GPI-1 isozyme analysis and is expressed on a scale of 0 to 4: 0, 0%; 1, 1%-25%; 2, 26%-50%, 3, 51%-75%, 4, 76%-100%. –= not done.

chimeras. As the PatDup.d7 cells were partially 129/Sv in contrast to the AG cells which were inbred 129/Sv, there may have been differences due to the genetic background. The critical region for the PatDup.d7 phenotype involves only the very distal portion of chromosome 7, determined with the reciprocal translocation T(7;11)65H (Beechey and Cattanach, 1995). Of the known imprinted genes located in this region, *p57^{KIP2}*, which is expressed in many tissues throughout development (Hatada and Mukai, 1995; Matsuoka et al., 1995), and *Igf2*, may play significant roles. This is supported by the phenotype observed in mice lacking a functional *p57^{KIP2}* gene. These mice possess many, but not all, of the defects observed in PatDup.d7 and AG chimeras. The defects in common include sternum disorganization, palate defects, neonatal death, umbilical hernia and body wall muscle dysplasia (Yan et al., 1997, Zhang et al., 1997). Explanation of other defects in AG chimeras could be achieved by further genetic analyses similar to those we have described here. For example, it would be of interest to examine the effects in chimeras of cells which carry paternal duplication of distal chromosome 12, the only other region aside from distal 7 for which paternal duplication results in embryonic death.

Human Beckwith-Wiedemann syndrome (BWS) is often associated with paternal duplication of a region which harbors *IGF2*, *p57^{KIP2}*, and other imprinted genes (Hoovers et al., 1995; Lalande, 1997). Many of the defects we have described in AG, AG *Igf2–/–*, and PatDup.d7 chimeras, oomphalocoele, whole body overgrowth, organomegaly, and perinatal death, are also observed in the human syndrome (Wiedemann, 1964; Beckwith, 1969; Junien, 1992). Although the etiology of this syndrome has not been traced to a single gene, excess *IGF2* expression is widely believed to play a significant role (Hedborg et al., 1994; Witte and Bove, 1994; Olshan, 1995). However, the persistence of many BWS-like defects in mouse AG *Igf2–/–* chimeras as shown in the present study adds weight to the case for the involvement of paternal duplication of other imprinted genes, such as *p57^{KIP2}* (Hatada et al., 1996; Matsuoka et al., 1996) and *KVLQT1* (Lee et al., 1997), in the human syndrome. Indeed, the phenotype of mice lacking *p57^{KIP2}* expression suggests that lack of expression of this gene in humans explains a large subset of BWS defects. This is also consistent with the fact that BWS is often a combination of features of overgrowth and at the same time, lack of differentiation. The simplest hypothesis for such a phenotype would be the overexpression of a growth promoting gene such as *IGF2* and lack of expression of a gene involved in differentiation such as the *p57^{KIP2}*. Determination of the exact role of particular genes in the BWS phenotype will probably be best addressed using a mouse models displaying a BWS phenotype such as those described here.

We thank S. Kuschert for technical assistance. This work was supported in part by Public Health Service Grant No. 1RO1 GM 48103-01 to J. R. M. This paper was written while D. S. was a Scholar-In-Residence at the Fogarty International Center for Advanced Study in the Health Sciences, National Institutes of Health, Bethesda Maryland, USA.

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(Accepted 16 September 1997)