Time of initiation and site of action of the mouse chromosome 11 imprinting effects

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Summary

Previous studies have shown that mice with paternal disomy for chromosome 11 are consistently larger at birth than their normal sibs, whereas mice with the maternal disomy are consistently smaller. An imprinting effect with monoallelic expression of some gene/s affecting growth was indicated. Here we show that the size differences become established prior to birth and are only maintained subsequently, indicating that the gene repression is limited to prenatal development. Fetal analysis was limited to 12·5–17·5 days post coitum. However by extrapolating the data backwards it could be calculated that both the maternal and paternal size effects might commence as early as 7 days post coitum, although possibly slightly later. It may be deduced that initiation of expression of the gene/s responsible may occur at about this time in development. The two disomy growth rates were mirror-images of each other, suggesting that expressed gene dosage is the underlying cause. Differential growth of the placentas of the two disomies was also found, and extrapolation of these data backwards suggested that the placental size differences were initiated later in development than those for the fetuses. The differential placental growth of the maternal and paternal disomies may therefore have developed independently or emerged as a consequence of the differential fetal growth. In either event it would seem that the expression of the responsible gene occurs in the fetus itself to cause the anomalies of growth. The data therefore provide information on the temporal and tissue specificity of the gene/s responsible for the chromosome 11 imprinting effects. Possible candidate genes are discussed.

1. Introduction

It is now well established that germ line modification, or imprinting, leads to functional differences in expression of certain autosomal regions/genes in the zygote according to their parental origin (Solter, 1988; Cattanach, 1991; Cattanach & Jones, 1994; Surani, 1994). Thus, mouse genetic studies using Robertsonian translocations to generate uniparental disomies, and reciprocal translocations to generate uniparental duplications of selected regions, have demonstrated that the imprinting may be limited to only 10 or perhaps 11 regions of the genome distributed over six or seven chromosomes: proximal and distal Chr 2; proximal Chr 6; proximal, central and distal Chr 7; proximal Chr 11; distal Chr 12; proximal and distal Chr 17 (Cattanach & Jones, 1994; Beechey & Cattanach, 1995), and seemingly now some region of Chr 18 (Oakley *et al.* 1995). Mice with two maternal and no paternal, or two paternal and no maternal copies of these regions (maternal and paternal duplication, respectively) show phenotypic abnormalities (imprinting effects) despite having a normal balanced chromosome constitution. Including the new Chr 18 examples (Oakley *et al.* 1995), a total of 17 such effects have been identified, ranging from early embryonic lethalities, through fetal lethalities and growth effects, to various developmental abnormalities affecting behaviour and growth after birth (Cattanach & Jones, 1994). Each of these effects is therefore likely to be attributable to one or more genes located within each region.

Fifteen genes that are subject to imprinting have been identified and of these all but one (*Ins1*), which is expressed only in the yolk sac (Giddings *et al.* 1991), have been located within the imprinting regions. A number of other genes have been considered good candidates for the imprinting effects based on their map position and their possible roles in development (Cattanach & Jones, 1994). However, only *Igf2r* in

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proximal Chr 17 and *Snrpn* in central Chr 7 have been identified in this way (Barlow *et al.* 1991; Leff *et al.* 1992). The others have been recognized either by chance (*H19* and *Igf2*), by their proximity to these known imprinted genes (*Mas*, *Ins2*, *Mash2* and $p57^{KIP2}$), by screening for methylation differences (*D11Ncvs75*, formerly *U2afbp-rs*), by parthenogenetic/normal subtractive hybridizations (*Mest* and *Apoc2*), or by a combination of such approaches (*Znf127* and *Dn34*) (Bartolemei *et al.* 1991; DeChiara *et al.* 1991; Ferguson-Smith *et al.* 1991; Giddings *et al.* 1994; Hatada *et al.* 1993; Hayashizaki *et al.* 1994; Villar & Pederson, 1994; Guillemot *et al.* 1995; Hatada & Mukai, 1995; Kaneko-Ischino *et al.* 1995; Jones & Cattanach, unpublished). A further approach, however, might be achieved through the phenotypic characterization of the imprinting effects. This might distinguish the tissue and temporal specificities of the responsible gene action and so potentially aid identification of the genes themselves.

This communication is concerned with the phenotypic characterization of the growth effect phenomenon associated with chromosome 11 (Chr 11) imprinting (Cattanach & Kirk, 1985). The original work had shown that mice with paternal disomy (PatDi) generated using the Rb(11.13)4Bnr Robertsonian translocation were invariably larger (130%) than their normal sibs at birth, and subsequently grew into oversized but otherwise normal, fertile adults. Similarly, mice with Chr 11 maternal disomy (MatDi) were invariably smaller (70%) than their normal sibs at birth and subsequently grew into small but otherwise normal, fertile adults. Further analysis using the T(2;11)30H reciprocal translocation established that only the region proximal to the translocation breakpoint at band B1 was involved, establishing that the responsible gene/s must lie in this region of the chromosome. Subsequent studies (Cattanach, 1986) suggested that the postnatal growth rates of the PatDi, MatDi and normal sibs were similar. Here we present detail of these data to confirm the conclusions and add new information on the prenatal growth rates. Statistical analysis of the data suggests that the size differences are initiated at about 7 days post coitum (dpc), increase throughout prenatal development, and may be mediated through the fetus rather than through the placenta. On the basis of these findings candidate genes for the imprinting effects are discussed.

2. Materials and methods

For the postnatal studies, PatDi and MatDi animals were generated by intercrossing heterozygotes for the Rb(11.13)4Bnr Robertsonian translocation. They were identified by making one parent homozygous for the Chr 11 marker gene, vestigial tail (*vt*) and the other homozygous for the wild-type (+) allele (Cattanach & Kirk, 1985). Any *vt* progeny therefore had received two copies of Chr 11 from the marked parent, these being either MatDi or PatDi according to which parent carried the *vt* marker. With this method the frequency of occurrence of each marked disomy has approximated 1–2% (Cattanach, 1986). It should be noted that an equal low frequency of unmarked disomies may be expected to occur among the wild-type (+) sibs (Fig. 1). As the probability of marked and unmarked disomies occurring in any one litter is low, the chance of the latter distorting any disomy–normal comparisons is small. Weights of *vt* and unmarked sibs were taken at birth, at weaning (21 days) and in early adulthood (42 days) to give a measure of size. All animals derived from a normal breeding regime.

To reduce the numbers of females required in the subsequent prenatal studies, a monobrachial homology system (Gropp *et al.* 1975) was used as this gives a higher frequency of disomies. Double heterozygotes for two Robertsonian translocations having a Chr 11 arm in common, Rb(11.13)4Bnr and Rb(10.11)8Bnr, were therefore intercrossed and the *vt* Chr 11 marker was again used to identify PatDi and MatDi progeny produced. An important feature of the *vt* marker is that it can be classified from as early as 12·5 dpc (Grüneberg, 1957), permitting recognition of the disomies at all stages of development from 12·5 dpc to birth. From preliminary cytological studies on the non-disjunction frequencies in Rb4Bnr/Rb8Bnr compound males (Beechey, 1989) the expected incidence of the disomies with this system was calculated to be 9·33%.

Because of the higher incidence of disomy production in the prenatal studies using the monobrachial system, the chances of marked and unmarked types occurring in the same litters is relatively high. Therefore allowance for this had to be considered when comparing the marked (*vt*) disomy and unmarked (+) normal classes. Timed matings were also used in these studies. Thus, the females were checked daily for vaginal plugs, taken as evidence of mating, and autopsied at 12·5–17·5 dpc (counting the day of plug as day 0·5). The fetuses were classified for *vt* versus + under a dissecting microscope, and all fetuses and their placentas from uteri containing at least one *vt* animal were carefully trimmed free of fetal membranes and weighed to give a measure of size.

Weight comparisons of disomic mice and their normal sibs were assessed on the basis of weight ratios (*vt* marked PatDi versus + and *vt* marked MatDi versus +), as this eliminates weight differences between different litters of the same age attributable to varying litter sizes. In the prenatal studies, placental growth was similarly evaluated.

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3. Results

(i) *Incidence of PatDi and MatDi young*

In the postnatal studies using the single Robertsonian (Rb4Bnr) system the frequencies of marked MatDi and PatDi young observed were 1·8% (15/817) and 2·3% (39/1693), respectively. In the prenatal studies using the monobrachial (Rb4Bnr/Rb8Bnr) system higher incidences were observed, as expected. Thus, from the crosses of wild-type R4B +/R8B + females with R4B *vt* /R8B *vt* males 630 live fetuses of varying ages were recovered. Among these, 53 (8·41%) showed the marker gene phenotype and therefore represented the PatDi class. This frequency is in good accord ($P = 0{\cdot}62$ on a two-tailed exact test) with the 9·33% expected from the non-disjunction frequencies detected in compound heterozygote males (Beechey, 1989). From the crosses of R4B *vt*/R8B *vt* females with R4B +/R8B + males, however, there appeared to be shortage of the MatDi class. Thus, from among the 382 live fetuses recovered, only 17 (4·45%) showed the *vt* phenotype indicative of the MatDi class – a significantly lower figure than expected ($P = 0{\cdot}0097$). Despite this, the incidences of the disomy classes detected using the monobrachial method clearly exceeded those obtained using the single Robertsonian system. The present analysis therefore confirms the findings regarding postnatal disomy 11 mice described previously (Cattanach & Kirk, 1985; Cattanach, 1986).

(ii) *Postnatal growth*

The weight ratios (disomy/+) of the postnatal PatDi and MatDi young are shown as a function of age in Table 1. Within the PatDi group all the ratios were significantly greater than 1, showing that at all postnatal ages the PatDi young were larger/heavier than their + sibs. Likewise, all ratios within the MatDi group were significantly less than 1, showing that at all ages the MatDi young were smaller/lighter than their + sibs. In the PatDi group, there was no significant evidence to suggest that the ratios were increasing further with age ($t_{77} = -2{\cdot}00$; $P = 0{\cdot}976$ on a one-tailed test). Indeed there was, if anything, a slight tendency for the ratios to decrease. Likewise, in the MatDi group, there was no significant evidence to suggest that the ratios were decreasing further ($t_{70} = 1{\cdot}10$; $P = 0{\cdot}86$ on a one-tailed test). Thus in neither group was there any indication that over the 2–42 day period studied the weights of the marked offspring diverged further from those of their + sibs. The size differences were attained prenatally. The results agree with those of postnatal studies described previously (Cattanach & Kirk, 1985; Cattanach, 1986).

(iii) *Fetal growth*

The weight ratios of 12·5–17·5 dpc fetuses are shown in Table 2. As observed postnatally, the PatDi group were significantly larger than their + sibs while the MatDi group were significantly smaller. However, in both groups the weight ratios progressively changed throughout the prenatal period studied. In the PatDi group they tended to increase with age, indicating that these fetuses grew progressively more rapidly than their + sibs. On a semilogarithmic plot (Fig. 1) this increase could be satisfactorily fitted by a straight line [$F(4,76) = 1{\cdot}96$; $P = 0{\cdot}11$], the slope [$(4{\cdot}26 \pm 1{\cdot}28) \times 10^{-2}$] being statistically significant ($t_{80} = 3{\cdot}33$; $P = 0{\cdot}0013$). In contrast, the MatDi prenatal weight ratios showed a tendency to decrease with age,

Table 1. *Postnatal weight ratios of marked* (*vt*) *PatDi and MatDi compared with unmarked* (+) *sibs*

Genotype/ age in days	No. of litters	No. of: *vt*	No. of: +	Offspring: Weight ratios ±SE	Offspring: Significance of ratio (*P*)
PatDi*					
2	21	21	40	1·372±0·037	$6{\cdot}0 \times 10^{-8}$
21	19	21	38	1·188±0·033	$2{\cdot}8 \times 10^{-8}$
42	9	11	15	1·287±0·053	$4{\cdot}5 \times 10^{-8}$
MatDi†					
2	23	24	36	0·608±0·022	$4{\cdot}0 \times 10^{-20}$
21	21	22	33	0·591±0·023	$3{\cdot}5 \times 10^{-20}$
42	8	8	11	0·679±0·042	$3{\cdot}8 \times 10^{-8}$

* At 2, 21 and 42 days after birth animals were sexed but no significant differences were found between the ratios of males and females [$F(3,73) = 0{\cdot}408$; $P = 0{\cdot}75$]. The data were therefore pooled

† At 2, 21 and 42 days after birth animals were sexed but no significant differences were found between the ratios of males and females [$F(3,66) = 2{\cdot}19$; $P = 0{\cdot}097$]. The data were therefore pooled.

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Table 2. *Prenatal weight ratios of marked (vt) PatDi and MatDi compared with unmarked (+) sibs*

Genotype/ gestational age in days	No. of uteri	No. of: *vt*	No. of: +	Fetuses: Weight Ratios ±SE	Fetuses: Significance of ratio (*P*)	Placentas: Weight Ratios ±SE	Placentas: Significance of ratio (*P*)
PatDi							
12·5	5	6	15	1·166±0·059	0·0033	1·052±0·121	0·66
13·5	3	3	10	1·491±0·100	$7·2\times10^{-8}$	1·120±0·170	0·46
14·5	2	2	5	1·265±0·104	0·0054	1·175±0·218	0·39
15·5	14	17	29	1·385±0·044	$1·1\times10^{-15}$	1·288±0·092	0·0013
16·5	7	7	17	1·517±0·068	$5·6\times10^{-14}$	1·257±0·127	0·031
17·5	5	5	13	1·472±0·077	$2·0\times10^{-10}$	1·491±0·177	0·0021
MatDi							
12·5	6	8	20	0·778±0·050	0·00020	0·870±0·064	0·079
13·5	4	4	14	0·695±0·055	$1·8\times10^{-5}$	0·709±0·065	0·0012
14·5	3	3	8	0·781±0·072	0·0095	0·735±0·079	0·0094
15·5	9	9	19	0·699±0·039	$1·6\times10^{-8}$	0·781±0·050	0·0010
16·5	2	2	7	0·586±0·064	$5·5\times10^{-6}$	0·557±0·070	0·00015
17·5	2	2	3	0·567±0·071	$2·3\times10^{-5}$	1·027±0·149	0·85

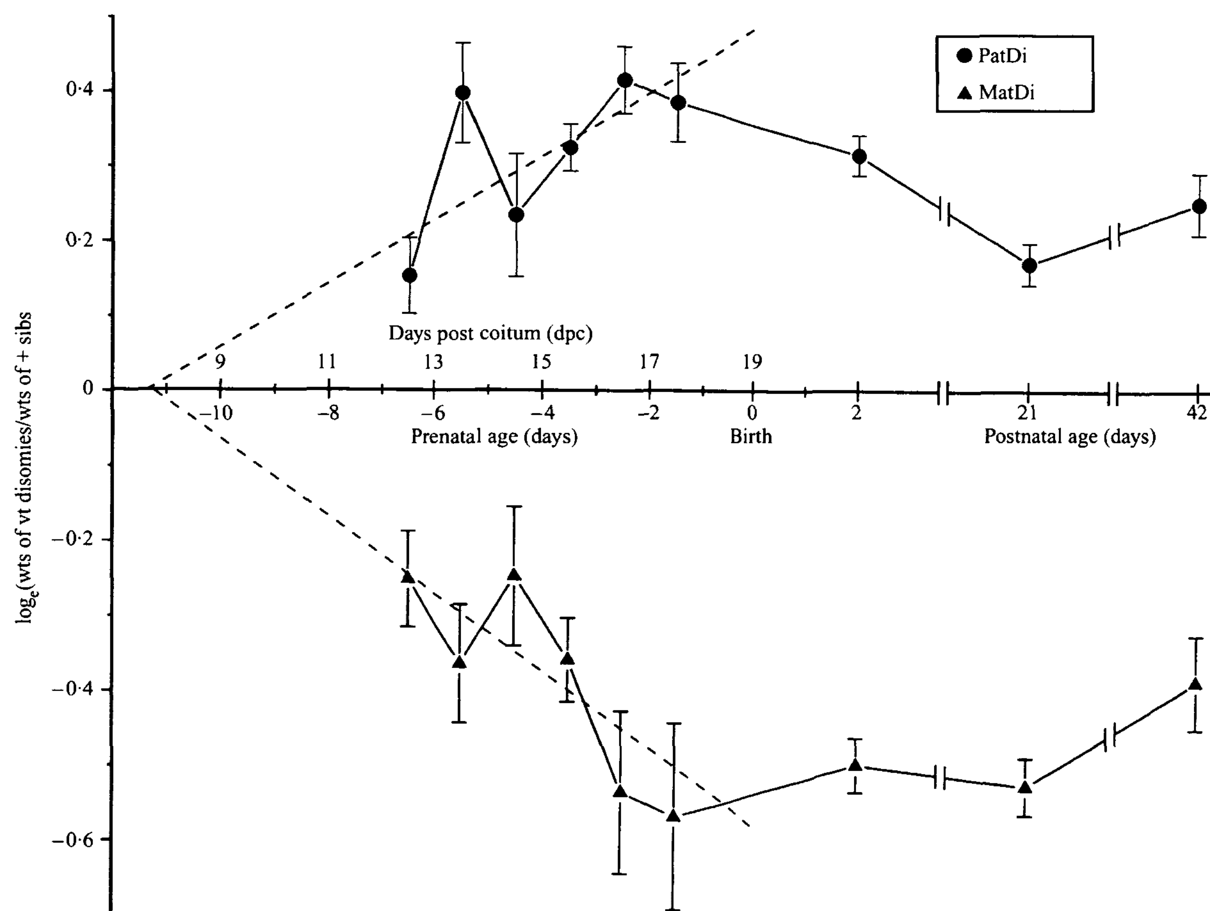

Fig. 1. Graph of the logarithms of the ratios, [weights of marked disomic (*vt*) fetuses and offspring]÷[weights of normal siblings], against age in days. Birth is taken as day 0. Ratios of weights are averaged over all litters or uteri at each age.

indicating that these fetuses grew progressively more slowly than their + sibs (Fig. 1). This decrease could likewise be satisfactorily fitted by a straight line [$F(4,69) = 0·833$; $P = 0·51$] and once again the slope [$-(5·15\pm2·04)\times10^{-2}$] was statistically significant ($t_{73} = 2·52$; $P = 0·014$).

(iv) *Origin and relationship of the fetal disomy growth patterns*

Since the fetal PatDi and MatDi weight ratios differed significantly from 1 even at the earliest age studied (12·5 dpc, Table 2), the age at which the weights of the

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disomy animals first began to differ from those of their + sibs cannot be identified directly. An indirect estimate can be deduced, however, from the semilogarithmic straight lines of best fit to the weight ratios in each group. Thus, the straight line of best fit to the PatDi ratios, when extrapolated backwards (Fig. 1), crossed the age axis at $7{\cdot}6 \pm 2{\cdot}4$ dpc, whilst the corresponding point for the MatDi group was $7{\cdot}8 \pm 2{\cdot}8$ dpc. The similarity of these two independent estimates is remarkable and, if it is assumed that the relative growth rates of the different classes of fetus remain the same throughout development, the data could suggest that a gene responsible for control of prenatal growth comes into play soon after implantation.

To elucidate the role of imprinting in the growth differences described, the growth patterns of the PatDi and MatDi fetuses were compared. Striking similarities were observed. The two patterns appeared to be mirror-images of each other (Fig. 1). Thus, not only did the two straight lines of best fit have similar intercepts on the age axis, but their slopes, while opposite in sign, were very similar in magnitude [$(4{\cdot}26 \pm 1{\cdot}28) \times 10^{-2}$ in the PatDi group; $-(5{\cdot}15 \pm 2{\cdot}04) \times 10^{-2}$ in the MatDi group], showing that that the PatDi fetal weights increased, relative to those of their + sibs, at much the same rate as the relative MatDi weights decreased. Further evidence of a mirror-image relationship was obtained by multiplying each weight ratio (Table 2) in the PatDi group by the corresponding ratio in the MatDi group. The products were all found to be close in value to 1, and the mean product, averaged over all age groups, was $0{\cdot}943 \pm 0{\cdot}035$, which does not differ significantly from 1 ($t_{150} = 1{\cdot}58$; $P = 0{\cdot}12$). There was therefore a clear suggestion that the enhanced growth of the PatDi fetuses during late development (12·5–17·5 dpc) was the reciprocal of the reduced growth of MatDi fetuses.

On testing this hypothesis, it was found that the deviation of the two weight ratios from a mirror-image relationship was indeed not statistically significant [$F(2,153) = 1{\cdot}20$; $P = 0{\cdot}30$]. The two best-fitting mirror-image lines jointly provided an acceptable fit to the observed fetal weight ratios [$F(10,145) = 1{\cdot}23$; $P = 0{\cdot}27$]. Their slopes, namely $\pm(4{\cdot}22 \pm 1{\cdot}22) \times 10^{-2}$, were highly significant ($t_{155} = 3{\cdot}76$; $P = 0{\cdot}00024$) and when extrapolated backwards, the lines jointly crossed the age axis at $7{\cdot}0 \pm 2{\cdot}2$ dpc. The latter therefore provides a combined estimate of the age in development at which the PatDi and MatDi fetal growth rates might first begin to diverge from those of their + sibs.

The overall inference from the above findings is that a gene affecting fetal growth is switched on at some early stage of post-implantation development, and continues to influence growth up until birth, but not beyond. The reciprocally different growth rates of the PatDi and MatDi fetuses would then reflect the imprinting of this gene, possibly by being repressed in the MatDi fetuses and over-expressed in the PatDi fetuses.

(v) *Placental growth*

The placental weight ratios are also shown in Table 2 and it may be seen that, as with the fetal weight ratios, those for the PatDi group were all greater than 1 and showed a tendency to increase with age, whereas those for the MatDi group were (with one exception) less than 1 and showed a tendency to decrease with age. On a semilogarithmic plot (Fig. 2) the increase in the PatDi ratios was well fitted by a straight line [$F(4,30) = 0{\cdot}161$; $P = 0{\cdot}96$] with a slope [$(6{\cdot}11 \pm 2{\cdot}69) \times 10^{-2}$] that was statistically significant ($t_{34} = 2{\cdot}27$; $P = 0{\cdot}029$]. When the line was extrapolated backwards it crossed the age axis at $11{\cdot}7 \pm 1{\cdot}7$ dpc (Fig. 1). This suggests that the PatDi placental weights begin to diverge from those of their + sibs at about this time. Consistent with this conclusion is the fact that the weight ratios at 12·5–14·5 dpc do not differ significantly from 1 (Table 2). The placental growth differences of PatDi fetuses therefore appear to develop later than those of the fetuses themselves.

The MatDi placental weight ratios showed a tendency to decrease with fetal age, as seen with the fetuses themselves (Table 1). However, the decrease with age was less well fitted by a straight line [$F(4,20) = 3{\cdot}46$; $P = 0{\cdot}026$] (Fig. 2), but the poor fit seemed largely attributable to what appeared to be an anomalous high ratio at 17·5 dpc. This was based on only two litters in which the control (+) placental weights were noted to be unusually low, probably as a consequence of a sampling artefact. When the data at 17·5 dpc were omitted, the fit became much more acceptable [$F(3,20) = 2{\cdot}07$; $P \doteq 0{\cdot}14$]. The line had a slope [$(5{\cdot}60 \pm 2{\cdot}93) \times 10^{-2}$] that was marginally significant ($t_{23} = 1{\cdot}91$; $P = 0{\cdot}069$) and, when extrapolated backwards, it crossed the age axis at $9{\cdot}4 \pm 2{\cdot}7$ dpc, again rather later than the crossing point for the fetuses themselves.

As observed with the fetuses, the PatDi and MatDi placental growth rates appeared to be reciprocals of each other. Thus, the straight lines of best fit to the two groups of placental weight ratios showed no significant deviation from a mirror-image relationship. This was true whether the outlying MatDi ratio at 17·5 dpc was included [$F(2,58) = 1{\cdot}14$; $P = 0{\cdot}33$] or excluded [$F(2,57) = 1{\cdot}49$; $P = 0.23$] From this we may infer that, as in the case of the fetal weight ratios, there is a reciprocal relationship between the PatDi and MatDi placental weight ratios. Again, when the outlying MatDi ratio is excluded, the PatDi/MatDi mirror-image lines of best fit jointly provide an acceptable fit to the observed placental weight ratios [$F(9,50) = 0{\cdot}838$; $P = 0{\cdot}59$]. Their slopes, $\pm(4{\cdot}88 \pm 1{\cdot}92) \times 10^{-2}$, were highly significant ($t_{59} = 2{\cdot}54$; $P = 0{\cdot}014$), and when extrapolated backwards, the lines jointly crossed the age axis at $9{\cdot}9 \pm 2{\cdot}1$ dpc.

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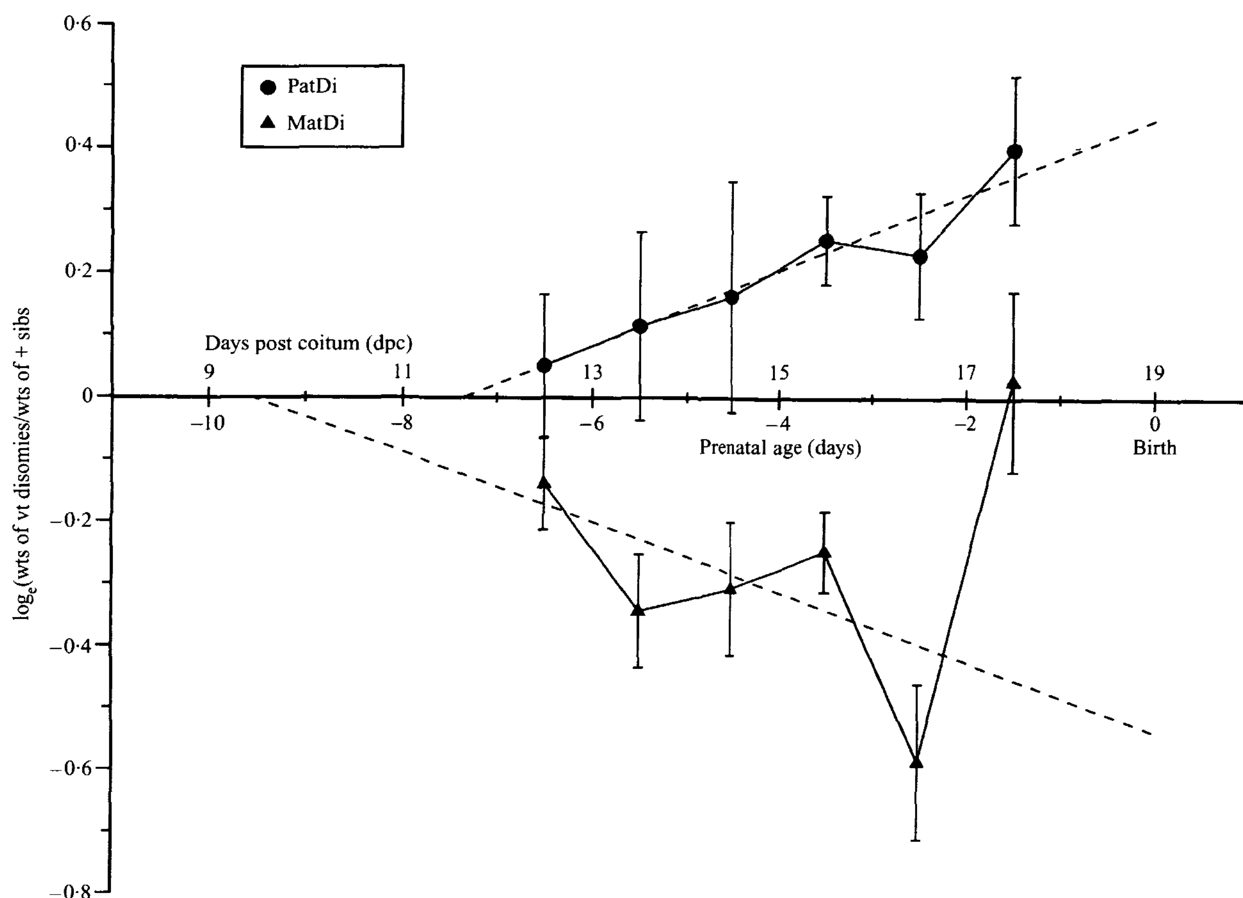

Fig. 2. Graph of the logarithms of the ratios, [weights of placentas from disomic (*vt*) fetuses] ÷ [weights of placentas from normal siblings], against age *in utero* in days. Birth is taken as day 0. Ratios of weights are averaged over all uteri at each age. (NB: In the case of the MatDi data, the ratio at 17·2 dpc has been excluded from the straight line of best fit.)

This therefore provides a combined estimate of the time in development when the PatDi and MatDi placental growth rates first begin to diverge from those of their + sibs.

Although there is uncertainty over the MatDi placental data and there is no background information on the relative growth rates of fetuses and placentas in the early post-implantation period , all the observations suggest that the differential placental growth rates of the disomic fetuses start later (9–11 days) than the differential growth rates of the disomic fetuses themselves (about 7 days). However, as the slopes of the lines of best fit for the combined fetal ratios were not significantly different from those of the PatDi placental ratios ($t = 0{\cdot}296$; d.f. $= 101{\cdot}4$; $P = 0{\cdot}77$ on an Aspin–Welch t-test), it would seem likely that, despite their (possibly) later start, the *vt* placentas grow at the same rate relative to their + sibs as their corresponding fetuses from 12·5 dpc to birth.

4. Discussion

The data presented here and their analyses provide some evidence on the timing and site of action of the gene/s responsible for the growth effects attributable to Chr 11 imprinting. A first observation of significance was that, postnatally, there was no indication of differential growth rates between the MatDi and PatDi mice and their + sibs. Thus, although the PatDi mice were larger and the MatDi mice were smaller at birth, the weight ratios did not diverge further subsequently. It may be concluded from this that the responsible gene action occurs prior to birth. Any screening of candidate genes for imprinting in juvenile or adult mice may therefore be expected to be uninformative.

Differential growth rates were, however, observed prenatally. Thus, the weight ratios for the PatDi fetuses increased progressively and those for the MatDi fetuses decreased progressively over the period of development studied (12·5–17·5 dpc), indicating that this period may be the time of the responsible gene action. This should therefore be a time when a screening operation for candidate genes may be effective.

Through extrapolation backwards of the semilogarithmic straight lines of best fit to the PatDi and MatDi fetal weight ratios, it was estimated that the imprinting believed to be responsible for the growth differences (Cattanach & Kirk, 1985) could commence around 7 dpc, i.e. shortly after implantation. However, for at least two reasons this conclusion can only be accepted with caution. Firstly, consideration must be given to the effects of contamination of the + sibs by

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the unmarked disomies. The unmarked disomies comprise the reciprocal types to the marked disomies (e.g. unmarked MatDi young are found in the same crosses as the marked PatDi young and vice versa). They will therefore have had the effect of making the observed weight ratios in the PatDi group larger than they ought to be, and those in the MatDi group smaller. Moreover, the factor by which they are larger or smaller will increase with fetal age. However, allowance can be made for this (see Appendix). It can then be estimated that mirror-image straight lines of best fit to the corrected weight ratios, when extrapolated backwards, would cross the age axis at about 7·1 dpc, only marginally later in development than the uncorrected estimate. Contamination of the + fetal group with a disomic class has therefore not appreciably affected the statistical conclusion.

A second reason for caution in accepting 7 dpc as the age of onset of the imprinting effect concerns the legitimacy of the extrapolation itself. It is known that fetal growth rates are not constant throughout development (McLaren, 1975). Thus, there is rapid growth between 6 dpc and 11 dpc, after which growth slows. The extrapolation has therefore been made from the period of slow growth to the period of faster growth. Insofar as the estimates of growth used here were based on weight ratios, this should not in itself be an important consideration. However, if the relative growth rates (weight ratios) of the PatDi and MatDi mice increase/decrease at different rates before and after 12·5 dpc, the age of onset of the growth differences may be underestimated (with more rapid change before 12·5 dpc) or overestimated (with slower change). As one might expect the growth differences to commence only after the embryos implant, it would seem most likely that the time of onset of expression of the gene responsible would occur at or soon after 7 dpc.

There is also the question of the site of gene expression raised by the observation that the PatDi and MatDi placentas also show the growth differences. It might have been expected that the fetal growth differentials would be mediated through the differential placental growth and, on this basis, the expectation would be that the responsible gene/s act in this tissue, rather than directly in the fetus. However, extrapolation of the placental weight ratios backwards suggested the reverse. Thus, the disomy placental weight ratios crossed the age axis later in development than the fetal weight ratios. While the extrapolation might be flawed if the fetuses and placentas grow at different rates prior to 12·5 dpc, it may be noted that the PatDi placental weight ratios did not differ significantly from 1 at the earliest ages studied (12·5–14·5 dpc). The findings therefore suggest that in disomic mice, which present an exceptional genetic situation, the placental weight changes emerge as a consequence, rather than the cause, of the fetal weight changes. This hypothesis would imply that the expression of the gene responsible for the imprinting effects occurs within the fetus. It is, however, also possible that that the fetal and placental weight changes develop independently. In this case, it would be expected that the responsible gene is expressed in both fetal and placental tissue, perhaps with expression in the latter occurring later than in the former.

A further observation of potential significance is that the PatDi and MatDi fetal weight ratios were mirror-images of each other. As PatDi and MatDi mice differ only in the parental origin of their Chr 11s, it would be expected that, as a result of imprinting, expressed gene dosage is the underlying cause of the growth differences. Thus, normal mice could have only one functional (paternal) copy of some growth-controlling gene. The PatDi class would then have two copies, resulting in their increased size, and the MatDi class disomies would have none, resulting in impaired prenatal growth. Such a model is based on the knowledge that imprinted genes are expressed exclusively from one allele in the tissues affected. That growth occurs in the absence of a functional copy of the gene implies that the gene triggers an enhancement of growth, rather than providing an essential condition for post-implantation growth. A similar enhancement can be deduced for the insulin-like growth factor 2 gene (*Igf2*). Thus, as a consequence of imprinting of the maternal *Igf2* allele, intrauterine growth retardation occurs in mice that lack a functional paternal allele (DeChiara *et al.* 1991), and overgrowth occurs in chimaeric fetuses possessing two paternal alleles in some of their cells (Ferguson-Smith *et al.* 1991). The stage in development at which these *Igf2* growth differences emerge is not known however.

A number of known genes on Chr 11 could be considered interesting candidates for the imprinting effect described (Fig. 3) – the growth hormone genes in particular. The long-known Ames dwarf (*df*) mutation (Fig. 3) was initially suggested as a candidate (Cattanach & Kirk, 1985) but was quickly discarded on the basis that the mutant is of normal size at birth despite a severe absence of pituitary hormones (Bartke, 1965). A second Chr 11 growth hormone gene *Gh* can now also be excluded on the grounds that it lies well outside the region proximal to the T30H breakpoint associated with the imprinting effect (Fig. 3). The nerve growth factor receptor-bound proteins (*Grb2* and *Grb7*) and nerve growth factor receptor (*Ngfr*) could also have been considered candidate genes, but each of these also lies outside the imprinting region (Fig. 3).

The epidermal growth factor receptor (*Egfr*) was initially regarded as a good candidate gene (Cattanach & Jones, 1994) as it is widely expressed in the embryo and maps within the Chr 11 imprinting region (Fig. 3). However, it has since been identified as the waved2 (*wa2*) locus which affects hair growth and lactation, and RT-PCR analyses of whole embryos and placentas have so far failed to provide any evidence that it is

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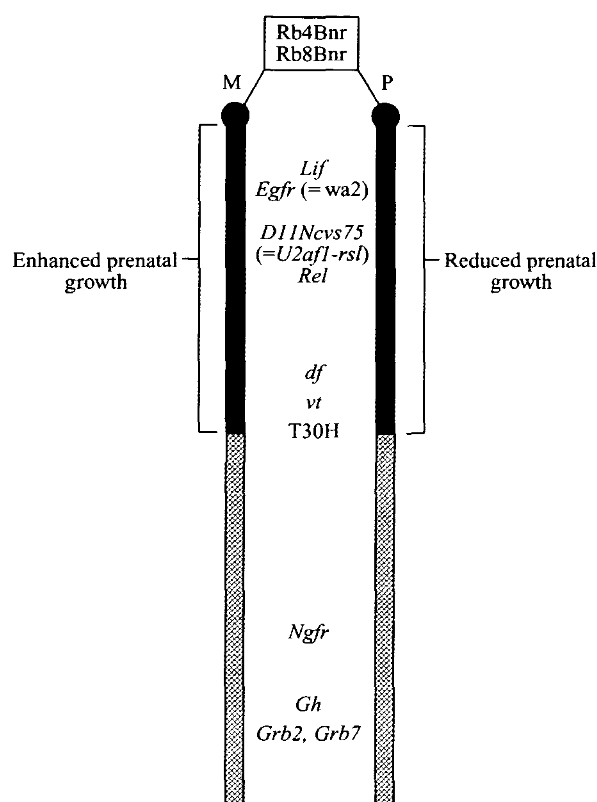

Fig. 3. Diagram of Chr 11 showing the imprinting region (black), defined by the T30H breakpoint (bold), and the imprinting effects seen in maternal (**M**) and paternal (**P**) disomies/duplications. Approximate positions of possible candidate genes (italics) are indicated as well as that of the one proven imprinted gene, *D11Ncvs75*, in the region (bold italics).

imprinted (Cattanach & Jones, 1994). Parallel studies on the closely linked leukaemia inhibitory factor (*Lif*) also failed to show evidence of imprinting.

A more specific candidate in the sense that it has been shown to be imprinted and to lie within the Chr 11 imprinting region (Fig. 3) is the recently discovered gene *D11Ncvs75* (formerly known as *U2afbp-rsl*), which shows significant homology to the human U2af splicing factor. However, the gene has been shown to be expressed and imprinted in every adult tissue studied (Hayashizaki *et al.* 1994; Hatada *et al.* 1993; Jones *et al.* unpublished), and the imprinting (maternal repression) has been demonstrated as early in development as the 2-celled embryo (Hatada *et al.* 1995). It would therefore seem doubtful that *D11Ncvs75* is specifically involved with the prenatal fetal growth effects described here. Nevertheless, in so far as imprinted genes appear to be clustered in domains (e.g. *Igf2/H19/Ins2/Mash/p57*^{KIP2} in distal Chr 7; *Snrpn/Dn34/Znf127* in central Chr 7; see Beechey & Cattanach, 1995), the location of *D11Ncvs75* provides a target site for other imprinted genes which might have direct roles in regulating fetal growth. Yet the reticuloendothelial oncogene (*Rel*), which lies approximately 1 cM distal to *D11Ncvs75* (Lyon & Kirby, 1995), does not seem to be imprinted as judged by RT-PCR studies of whole fetuses and placentas (Cattanach & Jones, 1994).

It may be concluded that at this time there is no obvious candidate gene/s for the paternal and maternal disomy Chr 11 imprinting effects. While *D11Ncvs75* may continue to identify a target region, it should be recognized that the currently defined imprinting region is very large, comprising about one-third of the whole chromosome. There is therefore ample scope for more than a single imprinting domain within it, as is well demonstrated by the existence of three imprinting domains in Chr 7 (Searle & Beechey; 1990; Cattanach *et al.* 1994; Jones and Cattanach, unpublished). To address this problem studies are in progress with a new translocation stock, T(11;13)41Ad (Adler *et al.* 1994), which has its Chr 11 breakpoint within the current imprinting region, the primary object being to reduce the size of the known imprinting region. In this connection it will be interesting to see whether the reduced region spans the imprinted *D11Ncvs75* locus. This would suggest the existence of a single Chr 11 imprinting domain. Were the region to lie elsewhere, a second imprinting region in the chromosome would be indicated.

Appendix. Correction for unmarked disomies

Animals/fetuses belonging to a given litter will in general be of three kinds, namely (i) + animals, whose mean weight is (say) w_+; (ii) *vt* marked disomies, whose mean weight is kw_+, where k is the 'true' *vt*/+ weight ratio; and (iii) unmarked disomies of the reciprocal type to the *vt* marked disomies, whose mean weight is therefore assumed to be w_+/k. Note that $k > 1$ for litters belonging to the PatDi group, and $k < 1$ for litters belonging to the MatDi group.

Animals belonging to class (iii), because they are unmarked, are indistinguishable from their + sibs, and will therefore be classified as +; suppose they comprise a fraction f of all animals classified as + (so that only a fraction $1-f$ of all animals classified as + are truly +). The mean weight of all animals classified as + is therefore

$$(1-f)\, w_+ + f\frac{w_+}{k}.$$

Thus the mean weight of the *vt* marked disomies, expressed as a ratio of the mean weight of all animals classified as +, is

$$\frac{kw_+}{(1-f)w_+ + f\frac{w_+}{k}} = \frac{k^2}{f+(1-f)k} \qquad \text{(A1)}$$

By equating this uncorrected ratio to the observed ratio at a given age, and solving the resulting quadratic equation for k, an estimate of the corrected ratio, k, at each age can be obtained.

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The cytological estimates (Beechey, 1989) indicate that approximately 9% of all animals classified as + may belong to the unmarked disomy class; the value $f = 0{\cdot}09$ was therefore substituted in expression (A1) to obtain the corrected weight ratios.

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