Targeted disruption of the \textit{Wnt2} gene results in placentation defects

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SUMMARY

\textit{Wnt} genes have been implicated in a range of developmental processes in the mouse including the patterning of the central nervous system and limbs. Reported here for the first time is the expression of \textit{Wnt2} in the early heart field of 7.5-8.5 dpc (days post-coitum) mouse embryos, making \textit{Wnt2} a potentially useful gene marker for the early stages of heart development. Expression was also detected in the allantois from 8.0 dpc and at later stages in the placenta and umbilicus. Mice deficient in \textit{Wnt2}, generated by gene targeting, displayed runting and approximately 50\% died perinatally. Histological analysis revealed alterations in the size and structure of placentas from these mice from 14.5 dpc. The placental defects were associated primarily with the labyrinthine zone and included oedema and tissue disruption and accumulation of maternal blood in large pools. There was also an apparent decrease in the number of foetal capillaries and an increase in the amount of fibrinoid material in the \textit{Wnt2} mutant placentas. These results suggest that \textit{Wnt2} is required for the proper vascularisation of the mouse placenta and the placental defects in \textit{Wnt2}-deficient mice result in a reduction in birthweight and perinatal lethality.

Key words: \textit{Wnt}2, mouse, embryogenesis, allantois, gene targeting, placental pathology, perinatal death, IUGR

INTRODUCTION

Members of the \textit{Wnt} gene family have been implicated in a number of developmental processes in the mouse as well as in a range of other species. The first \textit{Wnt} gene identified, \textit{Wnt1}, was isolated as a candidate oncogene involved in mammary tumours of mice (van Ooyen and Nusse, 1984). The \textit{Drosophila} orthologue of \textit{Wnt1} is the segment polarity gene \textit{wingless} (\textit{wg}), a gene known to play a number of important roles during the development of the \textit{Drosophila} embryo (Rijsewijk et al., 1987; Chu-LeGraff et al., 1993; Couso et al., 1993; Wu et al., 1995; Skaer et al., 1992). Human \textit{WNT2} was identified by chance during a chromosome walk while searching for the gene defective in cystic fibrosis (Wainwright et al., 1988). This was followed soon after by the homology cloning of the mouse \textit{Wnt2} (\textit{mirp}) cDNA (McMahon and Mcmahon, 1989). To date at least 20 distinct \textit{Wnt} genes have been identified in \textit{Drosophila}, zebrafish, \textit{Xenopus}, chick, mouse and man.

While \textit{Wnt} genes are expressed in a range of sites in the developing mouse embryo, the most common sites of expression are the developing limb and/or the developing central nervous system (Gavin et al., 1990; Parr et al., 1993; Christiansen et al., 1995) In some cases, the expression patterns of certain \textit{Wnt} genes coincide, suggesting that there may be a degree of functional redundancy between genes with overlapping expression (McMahon and Bradley, 1990; McMahon et al., 1992). Null mutations have been generated for a number of \textit{Wnt} genes and, in all cases, the genes have been demonstrated to be essential for the correct development of the mouse embryo. \textit{Wnt1} ‘knockout’ mice have defects in the cerebellum often with the complete loss of the midbrain structures and these mice rarely survive birth (McMahon and Bradley, 1990; Thomas and Capocchi, 1990). \textit{Wnt3A} mutants exhibit defects in tail development, including absence of the caudal somites, a disrupted notochord and failure of tailbud formation as well as CNS defects such as incomplete closure and ‘kinking’ of the neural tube (Takada et al., 1994). In \textit{Wnt4} mutant mice, the kidneys fail to develop normally and as a result these mice die soon after birth (Stark et al., 1994). Mutation of the \textit{Wnt7A} gene results in a dorsal-to-ventral transformation of the limbs of these mutant mice with foot pads and other ventral structures appearing on the dorsal surface of the limbs (Parr and McMahon, 1995).

Despite the fact that \textit{Wnt2} was the second \textit{Wnt} gene identified, its function is still poorly understood. Apart from mice and humans, the only other species from which possible \textit{Wnt2} orthologues have been reported are rat (Levay-Young and Navre, 1992), sea urchin (partial cDNA with a low level of homology (Sidow, 1992)) and leech for which a \textit{Wnt2} orthologue has been reported are rat (Levay-Young and Navre, 1992), sea urchin (partial cDNA with a low level of homology (Sidow, 1992)) and leech for which a \textit{Wnt} cDNA fragment was obtained that demonstrated higher homology with \textit{Wnt2} than any of the other \textit{Wnt} genes (Kosterik and Weisblat, 1992). There is very little known about the function of either human or mouse \textit{Wnt2}. \textit{Wnt2}, like all \textit{Wnt} genes, is expressed in a restricted pattern during mouse embryogenesis.
(McMahon and McMahon, 1989; Bellusc i et al., 1996). It has also been implicated in normal mammary gland differentiation (Buhler et al., 1993) and has been indirectly implicated in the formation of mouse mammary tumours in vivo (Roelenk et al., 1992).

In order to gain some understanding of the role played by Wnt2 in the development of the mouse we have analysed the embryonic expression of Wnt2 in embryos from 7.5 dpc to 10.5 dpc using whole-mount in situ hybridisation. In addition to confirming the pattern of Wnt2 expression reported previously (McMahon and McMahon, 1989), we observed that Wnt2 is expressed in the precardiac mesoderm from 7.5 dpc and in the foetal side of the developing placenta from 8.5 dpc until at least 11.5 dpc. The functional role played by Wnt2 during mouse embryogenesis was addressed by generating mice that possess a targeted disruption in the Wnt2 gene. This mutation, which is predicted to result in the absence of any functional WNT2 protein, causes structural defects in the placenta, which in turn result in runting and perinatal death.

MATERIALS AND METHODS

Whole-mount in situ hybridisation

Whole-mount hybridisation and subsequent sectioning of embryos was performed according to the procedure described in Christiansen et al. (1995). An adaptation of this method was used for the whole-mount in situ of placentas where the proteinase K treatment was increased 10-fold to allow efficient probe penetration. The efficiency of probe penetration using this method has been established using probes that detect cells throughout the depth of the placenta (D. J. Pennisi unpublished). For the detection of Wnt2 expression, two Wnt2 probes were subcloned from a near full-length Wnt2 cDNA clone isolated from a C57Bl6/CBA adult mouse lung library. One probe was from within the coding region and the other from the 3′ untranslated region. Both probes gave the identical staining pattern and so only the probe that gave the strongest signal was used in subsequent experiments. Wnt2 sense probes were used as a negative control and no staining was observed with these probes (not shown).

Digoxigenin in situ hybridisation of placental sections

In situ hybridisation to placental sections were performed as detailed in Schaeren-Wiemers and Gerfin-Moser (1993).

Electroporation of ES cells

Electroporation of ES cells was performed essentially according to the method of Wurst and Joyner (1993). Colonies were picked between 7 and 10 days after G418 and FIAU selection.

Morula aggregation

Morula aggregation was performed essentially according to the tetraploid aggregation method of Nagy and Rossant (1993) with the exception that only one 8-cell diploid embryo was used per aggregation with a clump of 10-15 ES cells. 8-cell embryos obtained from superovulated CD1 females were cultured and transferred to host CD1 pseudopregnant females as described in Nagy and Rossant (1993).

PCR genotyping

Two sets of primers were used in PCR genotyping reactions. One set amplified the DNA from within the neo coding sequence. The other set of primers amplified the region within exon 2 of the Wnt2 gene across the site of the neo insertion. Only DNA that does not contain a neo gene in exon 2 is amplified by these primers. PCR reactions were performed in accordance with the protocols supplied by the manufacturers of the Taq polymerase used (Boehringer-Mannheim). Primers employed were: neo primers: 5′-ATCTCTGTGATCCTACCTTGCG-3′ and 5′-CAAAGCTCTTCGACATATCAGG-3′. Wnt2 primers: 5′-GGTTATGCATAGGACTCTAGG-3′ and 5′-CTACTCGGAGGAGGACC-3′. The amplification conditions were 30 cycles at 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute.

RT-PCR

3 µg of total or cytoplasmic RNA was incubated with 100 U of MMLV reverse transcriptase (BRL) in 10 mM Tris pH 8.3, 50 mM KCl, 5 mM MgCl2, 0.01% Tween-20, 0.01% NP-40 in the presence of 1 mM dNTPs for 10 minutes at 25°C followed by 1 hour at 42°C. 1/5 of this reaction was heated to 95°C for 5 minutes then PCR amplified in the same buffer containing 2 µM of each primer and 1.5 U of Taq polymerase. Three separate primer pairs were used, one pair to amplify within the Hprt cDNA as a control (5′-CTCTGGATGATACATTAAAGCACTG-3′ and 5′-GCTAAAGGCCATATCCACAAAC-3′) and two Wnt2 gene-specific primer pairs: exon 1-3 (5′-GGGATAGACCTCCTCTCCTG3′ and 5′-TCTTGGCTGTAACAGGCTTG-3′) and exon 3-4 (5′-CGGGCTTGTATACGCCATC-3′ and 5′-TGAATACAGTAGTCGTGAGAA-3′). The amplification conditions were 35 cycles at 94°C for 30 seconds, 57°C for 45 seconds and 72°C for 1 minute.

Caesarean section and fostering

Caesarean sections were performed on 18.5 dpc pregnant females essentially according to the method of Nagy and Rossant (1993). Following dissection of neonates from the uterus and removal of foetal membranes, the umbilicus was cut and a segment was retained for genotyping. The neonates were then placed under a heat lamp on a damp tissue and were prodded gently at intervals until they had begun to breath normally. They were then fostered with a female that had given birth in the past three days and observed at regular intervals for the next 10-12 hours.

Histology

Both embryos and other tissues (eg placenta) were fixed overnight at 4°C in 4% paraformaldehyde in PBS. The tissue/embryo was then embedded in paraffin and either 7 µm (for tissues) or 10 µm (for embryos) sections were cut. Haematoxylin and eosin was used to stain placental sections.

RESULTS

Embryonic expression of Wnt2 by whole-mount in situ hybridisation

No Wnt2 expression was detected prior to about 7.5 dpc (pre-headfold) using whole-mount in situ hybridisation (data not shown). At 7.5 dpc Wnt2 expression was detected in a faint crescent-shaped pattern, which corresponds to the region of the heart primordium (Fig. 1A). At approximately 7.75 dpc when the headfold has become elevated, the foregut involutes and the differentiation of the heart precursor cells has begun, Wnt2 expression had increased and was seen in a crescent surrounding the foregut involution (Fig. 1B). Sectioning of these embryos showed Wnt2 expression was restricted to the mesodermal component of the heart field (Fig. 1D,E). Strongest expression in these embryos was detected in the lateral arms of the crescent, the prospective sinus venosus, and was weaker and more diffuse in the medial part of the crescent. Expression of Wnt2 in 8.0 dpc embryos continued to be
Wnt2 is required for placenta development

Detected in the crescent shaped cardiac mesoderm (Fig. 1C). At this stage, significant structural rearrangements have begun to occur at the midline where an anterior protrusion consists of differentiating myocardial and endocardial cells. Wnt2 expression was weaker in this region of differentiating cells and strongest in the lateral arms the crescent. At later stages of development, Wnt2 expression continued in the regions posterior to the heart such as the septum transversum and sinus venosus but was much weaker in the heart proper (Fig. 2A-C).

Other sites of Wnt2 expression include tissues involved in foetal maternal communication. At 8.0 dpc, Wnt2 expression was faintly detected in the allantois (Fig. 1C) and by 8.5 dpc (around the time that the allantois fuses with the chorion to form the chorioallantoic plate) the level of expression in this region increased (Fig. 2A). Wnt2 expression is also detected in the chorioallantoic plate region once allantoic fusion has occurred (Fig. 2A). The Wnt2 expression in the placental region at 9.5 dpc and 10.5 dpc continues to be confined to the chorioallantoic plate region (Fig. 2B,C). Whole-mount in situ hybridisation of 11.5 dpc placenta demonstrates that Wnt2 is also expressed in the foetal blood vessels of the placenta (Fig. 2D,E). No expression was seen in any of the placenta that is derived from foetal trophoblasts.

Targeted disruption of the Wnt2 gene in ES cells

A targeting vector was constructed to allow disruption of the Wnt2 gene in ES cells by homologous recombination. The strategy outlined in Fig. 3A utilises a positive/negative replacement targeting construct (Mansour et al., 1988) that contains approximately 10 kb of Wnt2 genomic sequence including exons 1-3. Following homologous recombination at the Wnt2 locus, exon 2 is disrupted by 1.8 kb of neomycin phospho-transferase (neo) gene driven by the phosphoglycerate kinase-1 (pgk) promoter. The insertion of the pgk promoter and neo gene in exon 2 introduces at least one in frame stop codon preventing the production of full-length WNT2 protein from the targeted locus.

Following electroporation of the linearised targeting vector into R1 ES cells, G418- and FIAU-resistant ES cell colonies were screened for homologous recombination by Southern blotting. The screening strategy involved probing SacI-digested genomic ES cell DNA with a 3′ external Wnt2 probe (probe 1) shown in Fig. 3A. A representative Southern blot used to screen ES cell colonies is shown in Fig. 3B. Of the 96 ES cell colonies picked and screened, 8 were targeted at the Wnt2 locus. SacI digests of genomic DNA from these 8 clones and some of the non-targeted clones were also screened with an internal probe (probe 2, Fig. 3A) and, in all cases, the same band pattern was seen as for the external probe (data not shown) indicating that no random, additional insertion of the targeting vector had occurred in any of these ES cell clones.

Generation of Wnt2-deficient mice

Six of the eight targeted ES cell lines were successfully used to generate chimeric mice using diploid morula aggregation. 3 male and 1 female chimera (each derived from different targeted ES cell line) when crossed to CD1 mates transmitted their ES-derived genome to their offspring as determined by coat colour. Wnt2 heterozygous offspring from these matings were identified by a PCR genotyping strategy, which showed complete agreement with that used for Southern analysis (data not shown). From 21 intercross matings, 194 offspring have been genotyped at weaning (Table 1) and of these 22 (12 males and 10 females) were Wnt2−/−. There were no deaths observed either before or after weaning in any of these litters. The distortion in the segregation of genotypes is highly significant (P<0.0001) for these intercrosses and is significant (P=0.03) for the Wnt2+/− × Wnt2−/− crosses. No significant (P≥0.1 and P=1) segregation distortion was seen for litters genotyped prior to birth (Table 1) indicating that the Wnt2−/− mice died between birth and when the litter was first observed 12-24 hours after birth. Given that the number of Wnt2−/− expected according to Mendelian inheritance should be the same as the number of wild-type animals (59) in the case of the intercrosses or the same as the number of Wnt2+/− in the case of the Wnt2+/− animals, the survival rate for the Wnt2−/− animals is approximately 50%.

As a number of Wnt2−/− animals subsequently survived to adulthood, we examined the possibility that functional Wnt2 transcripts were being made from the targeted allele. Reverse transcriptase PCR (RT-PCR) was performed on RNA extracted from the lungs and hearts of wild-type, Wnt2+/+ and Wnt2−/− adult mice. Wnt2 primer pairs were used to amplify across two regions of the Wnt2 mRNA and HPRT gene primers were used for each of the RNA samples as positive controls (Fig. 3C). HPRT control primers amplified bands in all samples as did the Wnt2 exon 3-4 primer pair. The Wnt2 exon 1-3 primer pair only amplified a 401 bp product corresponding to wild-type Wnt2 transcripts are being made that contain the neo gene or that exon skipping has removed exon 2 (and neo) gene (data not shown). Southern blotting indicated this was a non-specific amplification track containing the exon 1-3 amplification reaction. Southern blotting indicated this was a non-specific amplification product not derived from the Wnt2 gene (data not shown). This results indicates that no wild-type Wnt2 transcript was present in the tissues from Wnt2−/− animals. The presence of transcripts containing exon 3 and 4 in Wnt2−/− tissues indicates either that Wnt2 transcripts are being made that contain the neo in exon 2, or that transcription is initiating downstream of the neo gene or that exon skipping has removed exon 2 (and therefore the neo insertion). In all cases, given the highly

<table>
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<tr>
<th>Table 1. Genotype of offspring from Wnt2+/− × Wnt2+/− and Wnt2+/− × Wnt2−/− matings</th>
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<tr>
<td>Wnt2 genotype</td>
</tr>
<tr>
<td>28 days</td>
</tr>
<tr>
<td>+/+ × +/− mating</td>
</tr>
<tr>
<td>+/− × −/− mating</td>
</tr>
<tr>
<td>≤ 18.5 dpc</td>
</tr>
<tr>
<td>+/− × +/− mating†</td>
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<td>+/− × −/− mating†</td>
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* Ages ranging from 9.5-18.5 dpc
† Ages ranging from 14.5-18.5 dpc

P values calculated using χ² goodness of fit test for actual genotype segregation versus predicted.
conserved nature and functional importance of cysteine residues distributed throughout WNT proteins, it is highly unlikely that such transcripts would produce functional protein.

Perinatal death and runting of Wnt2\(^{−/−}\) mice

To observe animals at and soon after birth when it appeared some lethality was occurring, litters were delivered from their mothers at 18.5 dpc by Caesarean section. The details of the genotypes of the 37 animals delivered by Caesarean section in these experiments are presented in Table 2. Although all Wnt2\(^{−/−}\) neonates survived Caesarean section, they took longer to settle into a regular breathing pattern than their littermates, remaining grey for many minutes. Some after continual prompting turned pink and began breathing regularly but two of these when placed with foster mothers did not appear to suckle, and died soon after. Of 9 Wnt2\(^{−/−}\) mice delivered, only 5 survived the first 24 hours. Although mutant animals on dissection appeared anatomically normal, with subsequent histological examination showing no gross abnormalities, they were often runted (Fig. 4). Animals were weighed at various ages both before and after birth and Table 3 shows the mean weights of Wnt2\(^{−/−}\) animals and controls. The difference in the average weights of the two groups becomes apparent at 14.5 dpc but does not become significant until 17.5 dpc. At birth the average weight of the Wnt2\(^{−/−}\) mutants was 67% of the average weight of the controls and at weaning age both the male and female Wnt2 mutants weighed 85% of their normal littermates. There was no difference between the average weights of wild-type animals and Wnt2 heterozygotes.

To date 63 Wnt2\(^{−/−}\) mice have been observed from birth to adulthood. None of these outbred mutant homozygote (31 male or 32 female) animals have exhibited any apparent physiological or anatomical defects. The oldest of these, which at writing are nearly 2 years old, remains apparently healthy and fertile. All 8 male and 8 female Wnt2\(^{−/−}\) mutants tested were fertile and, in matings to heterozygotes, the phenotype of control and mutant offspring was indistinguishable to that of animals produced from intercross matings. Dissections of 2 adult Wnt2\(^{−/−}\) animals have revealed no obvious abnormalities in terms of gross anatomy, organ size or organ appearance. Similarly when the Wnt2\(^{−}\) mutation was crossed on to 129/Sv background although a similar level of perinatal lethality occurred a proportion of homozygous animals survived to adulthood and appear normal.

Fig. 1. Analysis of Wnt2 expression in 7.5-8.0 dpc embryos. (A-C) Whole-mount in situ analysis of a 7.5 dpc embryo (A), a 7.75 dpc embryo (B) and an 8.0 dpc embryo (C). Arrowheads, precardiac mesoderm; asterisk, foregut involution; open arrow, allantois; arrow, protrusion of differentiating cardiac cells. (D,E) Transverse section through 7.75 dpc (D) and 8.0 dpc (E) Wnt2 whole-mount embryo. Arrowheads, splanchnic mesoderm; arrow, myocardial cells; A, allantois; H, head.
Defects in the placentas of $Wnt2^{-/-}$ mice

During Caesarean sections, it was observed that some of the $Wnt2$ mutant placentas exhibited visible haematomas on the foetal side of term placentas (Fig. 4D). To investigate possible defects in placentation, the placentas from both $Wnt2$ mutant and control mice were collected at various stages throughout development and were examined histologically. No significant abnormalities were observed in 12 mutant placentas prior to 14.5 dpc. Between 14.5 dpc and 18.5 dpc, a number of defects were observed although the severity of pathology observed varied between mutant placentas. In a pairwise blinded comparison to placentas from control siblings, approximately 85% of 22 mutant placentas showed at least one defect including oedema and general disruption in the labyrinthine zone, the presence of abnormally large and/or numerous maternal blood pools and/or a reduction in the size and proportion of the labyrinthine zone and the chorioallantoic plate region (Fig. 5). An increase in the incidence of blood pools and a decrease in the incidence of oedema was observed as the placentas became successively older (compare Fig. 5B and I). In some of the more severe cases, there was often an increase in the amount of acellular material (often containing dead cells), possibly fibrinoid, in the labyrinthine zone (Fig. 5G,L). There was an also apparent reduction in the number of foetal capillaries when compared with control placentas (compare Fig. 4B,C). There was no evidence of necrosis or apoptosis in any of the placentas studied. The severity of pathology in the nine 18.5 dpc placentas of mutant animals delivered by Caesarean sections showed a close correlation with the degree of runting and length of survival after birth.

Fig. 2. Whole-mount in situ analysis of $Wnt2$ expression in 8.5 dpc (A), 9.5 dpc (B), 10.5 dpc (C) embryos, 11.5 dpc placenta (D). (E) A section of the placenta shown in D showing expression localised to the blood vessels of the foetal circulation. The 8.5 dpc embryo in A is still attached to the decidua (d) via the allantois (white arrow). Arrowhead, heart; white arrow, allantois/umbilicus; red arrow, chorioallantoic plate; d, decidua; t, tail; h, head.
Placentas from Wnt2−/− animals have a normal number and distribution of trophoblasts

To examine the effect of the Wnt2 mutation on the general organisation of the mouse placenta, we used in situ hybridisation to detect two different populations of placental trophoblasts in both control and mutant placentas. *Mouse placental lactogen II* (*mpl II*) was used to detect the trophoblast giant cells while clone 4311 hybridises specifically to spongiotrophoblast cells. Placental sections from various developmental stages from 10.5 to 16.5 dpc were used and, while there was no discernible difference in the pattern of 4311 expression between mutant and control placentas at any stage (Fig. 6A-D and data not shown), there were slight differences in the distribution of the *mpl II*-expressing giant cells. This was first observable at 14.5 dpc where in mutants placentas *mpl II*-positive cells were detected all the way to the foetal side of the placenta (Fig. 6G,H). In control placentas however there are very few *mpl II*-positive cells towards the foetal side of the placenta and none in the region of the chorioallantoic plate (Fig. 6E,F).

DISCUSSION

**Wnt2 in heart and lung development**

Wnt2 is expressed in a temporally and spatially restricted pattern during embryogenesis but, unlike all other Wnt genes thus far identified, it is not expressed in either the developing limbs or CNS. Wnt2 has previously been shown to be expressed in the heart pericardium and regions associated with foetal-maternal communication (McMahon and McMahon, 1989) and more recently in the lung mesenchyme of 11.5 and 13.5 dpc embryos (Bellusci et al., 1996). Reported here for the first time is Wnt2 expression in the regions of the embryo associated with early heart development. The expression of Wnt2 in precardiac mesoderm makes it a potentially useful marker for the initial stages of heart development and suggests a possible role for Wnt2 in this process. Using gene targeting, Wnt2 knockout mice have been generated that as adults lack any detectable expression of the wild-type Wnt2 transcript. No heart defects were observed in the Wnt2 mutant mice at any stage of development. Hearts of mutant homozygous mice began beating at the normal time (8.0-8.5 dpc) and appeared...
Table 2. Survival data on animals delivered by Caesarean section

<table>
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<tr>
<th>Wnt2+/+</th>
<th>Wnt2+/−</th>
<th>Wnt2−/−</th>
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<tbody>
<tr>
<td>Birth</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>1 dpp</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Difference</td>
<td>0</td>
<td>1(4%)</td>
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Table 3. Average body weights of Wnt2−/− and controls

<table>
<thead>
<tr>
<th>Age</th>
<th>Average body weights(g) +/- s.e.m.</th>
<th>Wnt2−/−</th>
<th>P values*</th>
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<tbody>
<tr>
<td>10.5 dpc</td>
<td>0.028±0.002</td>
<td>0.029±0.001</td>
<td>&gt;0.1</td>
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<tr>
<td>14.5 dpc</td>
<td>0.291±0.009</td>
<td>0.278±0.009</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>15.5 dpc</td>
<td>0.443±0.036</td>
<td>0.413±0.020</td>
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<tr>
<td>17.5 dpc</td>
<td>1.15±0.03</td>
<td>0.98±0.02</td>
<td>&lt;0.001</td>
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<td>C-section(18.5 dpc)</td>
<td>1.48±0.03</td>
<td>1.09±0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>28 day females</td>
<td>18.43±0.67</td>
<td>15.33±0.56</td>
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<tr>
<td>28 day males</td>
<td>21.89±0.78</td>
<td>18.79±0.83</td>
<td>&lt;0.01</td>
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*P values were calculated using the Mann-Whitney test.
†Both Wnt2+/− and Wnt2−/−
Defective placental structure and function may reduce the birth weight of Wnt2 mutant mice by impairing nutritional uptake or by affecting the placenta’s hormonal regulation of foetal growth retarding the maturation of the embryo. In humans there is evidence for a link between placental pathology and low birthweight babies in a clinical outcome of pregnancy termed intrauterine growth retardation (IUGR) (Redline, 1995). IUGR results in low birthweight for gestational age babies and is often associated with a number of clinical and environmental factors such as preeclampsia, hypertension, maternal vascular disorders, infection and smoking; although, in many cases, the aetiology of IUGR remains unknown. Pathology associated with IUGR includes intervillous fibrin, abnormal placental blood flow (both maternal and foetal), maternal floor infarction and a reduction in villous surface area (equivalent to reduced foetal vascularisation) (Redline, 1995). There is disagreement as to the relationship between some of these pathologies and IUGR, although a reduction in villous surface area clearly correlates with IUGR in at least one study (Boyd and Scott, 1985). While there are certain structural differences between the placenta of mice and humans, they contain essentially the same basic elements (Ramsey, 1975). The reduction in birthweight of Wnt2 mutant mice could be attributed to some of the placental defects seen in these mice (including apparent reduction in vascularisation and an increase in fibrin) which approximate the human pathologies of intrauterine growth retardation (IUGR) (Redline, 1995). VCAM1 (Gurtner et al., 1995), α4 Integrins (Yang et al., 1995), Mash-2 (Guillemot et al., 1994) and LIFR (Ware et al., 1995). Disruption of VCAM1 and the α4 Integrin genes results in defects in the fusion of the allantois to the chorionic plate resulting in death of the embryo due to lack of formation of the chorioallantoic placenta. In support of a role for Wnt2 in the consolidation of the foetal vessel network, but not in its initial establishment, such a fusion defect was not observed in the Wnt2 Null mice. Only disruption of the low affinity leukemia inhibitory factor receptor (LIFR) shows pathology similar to Wnt2 mutants. All LIFR mutant animals die perinatally at various times within the first 24 hours of delivery and there is some indication that they are smaller than their normal littersmates. It is difficult to correlate the perinatal lethality with the placental pathology since there are a number of other potentially lethal defects found in these embryos (Ware et al., 1995).

Perinatal death and running of Wnt2 mutant animals

Approximately half the Wnt2 homozygous mutants born die perinatally and many have a lower than average body weight. Since no other gross defects were apparent and there was a close correlation in the mutants between severity of placental defect and the degree of running and viability, it seems likely that placentation defects are the cause of the these phenotypic effects. The placental defects are apparent approximately 3 days prior to the time when the running of the embryos becomes significant, which supports this cause-effect relationship.
Wnt2 is required for placenta development
often associated with IUGR. If this is the case the Wnt2 mice generated in this study may be of value in understanding the relationship between placental defects and IUGR.

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Wnt2 is required for placenta development


