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Placental Development in a Mouse Model of Spinal Muscular Atrophy

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Abstract

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder, leading to fatal loss of motor neurons. It is caused by loss of function of the SMN gene, which is expressed throughout the body, and there is increasing evidence of dysfunction in non-neuronal tissues. Birthweight is one of most powerful prognostic factors for infants born with SMA, and intrauterine growth restriction is common. In the SMN^{-/-} mouse model of SMA, pups with the disease lived 25% longer when their mothers were fed a higher fat, “breeder” diet. The placenta is responsible for transport of nutrients from mother to fetus, and is a major determinant of fetal growth. Thus, the present study tested the hypothesis that placental development is impaired in SMN^{-/-} conceptuses. Detailed morphological characterization revealed no defects in SMN^{-/-} placental development, and expression of key transcription factors regulating mouse placental development was unaffected. The intrauterine growth restriction observed in SMA infants likely does not result from impaired placental development.

Keywords

Spinal Muscular Atrophy; Placenta; trophoblast differentiation

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Introduction

Spinal Muscular Atrophy (SMA) is an autosomal recessive, neurodegenerative disease that is the leading genetic cause of death in infants^[1]. SMA is caused by the loss of survival motor neuron (SMN), a ubiquitously expressed protein^[2]. SMA is mainly characterized by the loss of alpha-motor neurons, leading to atrophy in the lower limbs and trunk muscles. However, restoration of SMN expression only in motor neurons is not sufficient to rescue SMN mice, nor does knocking out SMN in only motor neurons and oligodendrocytes phenocopy the whole-body loss of SMN^[3,4]. Defects in heart, lung, pancreas^[1,5,6,7], and liver have been identified in humans and in mouse models of SMA¹. SMN performs a critical cellular function in the formation of snRNPs for all tissues within the body, however, it is currently unclear whether defects in snRNP biogenesis or an alternative axonal-specific defect account for the development of SMA.

In humans there are two SMN genes, SMN1 and SMN2. SMN1 and SMN2 are more than 99.9% identical; however, one of the non-polymorphic nucleotide differences dramatically alters the alternative splicing of the SMN2 gene. A C to T (+6) transition in SMN2 disrupts an important splice enhancer leading to aberrant splicing of the overwhelming majority of SMN2-derived transcripts. The alternatively spliced product, called SMN 7, is truncated and encodes an unstable, dysfunctional protein^[8,9]. Homozygous loss of SMN1 leads to individuals developing SMA. Disease severity is modified by SMN2 copy number, with a greater number of copies resulting in milder disease^[10]. Mice only have one SMN gene which is analogous to SMN1, therefore to model SMA in rodents; the human SMN2 transgene was inserted into the *Smn*-null background [11]rescuing embryonic lethality. This model, while viable, was still very severe, typically only living 4-6 days. To extend survival and address the functionality of the SMN 7 protein, the SMN 7 transgene was inserted into the SMN2-model mice (SMN2^{+/+}, SMN 7^{+/+}, mSmn^{-/-}), resulting in a slightly less severe model that exhibits many hallmarks of SMA, and lives approximately 14 days^[12].

There is evidence of growth restriction in at least some SMA infants^[13,14]. In a small study, birth weight was the only significant predictor of survival longer than 24 months in patients with SMA^[4], suggesting that intrauterine growth may be related to prognosis. Similarly, in the mouse model of severe SMA (*Smn*^{-/-} SMN2^{tg/tg}), a subset of pups are born underweight, and these die at, or shortly after birth¹¹. SMN 7 pups also have a smaller mean birth weight, albeit with range that overlaps that of controls^[12]. SMN 7 pups born to mothers fed a higher fat diet during gestation (9% g/fat vs 5.2 % g/fat) lived 25 percent longer than their counterparts born to mothers fed a standard diet^[15]. Because the placenta is responsible for the transfer of nutrients, including fatty acids, between mother and fetus and is a major determinant of fetal growth, we hypothesized that placental development may be impaired in SMN 7 conceptuses, leading to impaired fetal growth. To test this hypothesis, we investigated expression of key genes involved in placental development and lipid transport, and evaluated the morphology of placentae from SMN 7 versus wild type offspring.

Methods

Mice

SMN⁻⁷ SMA fetuses (hSMN2^{+/+}; SMN⁻⁷ ^{+/+}; mSmn^{-/-}), heterozygotes (hSMN2^{+/+}; SMN⁻⁷ ^{+/+}; mSmn^{+/-}) and wildtypes (hSMN2^{+/+}; SMN⁻⁷ ^{+/+}; mSmn^{+/+}), were generated by mating mSmn^{+/-}; hSMN2^{+/+}; SMN⁻⁷ ^{+/+} male and female mice. Dams were maintained on Picolab20 Mouse diet (Purina #5058). Daily plug check was performed and the day of plug detection was referred to as pregnancy d0.5. On d18.5 of gestation, pregnant females were sacrificed by using a CO₂ chamber and cervical dislocation. Fetuses from these mothers were genotyped using tail biopsies as previously described [16]. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the University of Missouri.

Placental Morphology

Placentae from one uterine horn from each mother were fixed at room temperature overnight in 4% paraformaldehyde and then embedded in paraffin. Five μ m mid-sagittal sections were stained with hematoxylin and eosin. Stained placentae from seven SMN⁻⁷ fetuses and five littermate controls (4 SMN^{+/+}; 1 SMN^{+/-}) were visualized under an Olympus I $\times$ 81 light microscope fitted with a color camera. A full mid-sagittal cross section of each placenta was captured by using a series of images taken with a 4 $\times$ objective and digitally “stitched” with MicroSuite software, as previously described [17]. Cross sectional areas from SMN⁻⁷ placentae were compared to those of controls using ImageJ software, free hand tool selection to outline the entire placenta, the labyrinth zone and the junctional zone. These areas were quantified using ROI manager function of ImageJ. In each placenta, five images within the labyrinth zone were captured by using a 40 $\times$ objective. Maternal and fetal blood spaces were manually selected in these images and areas quantified by using ImageJ software¹⁷.

Reverse transcription real-time PCR Analysis

Placentas from the contralateral horn were bisected, and one half was stored in TriReagent (Sigma) at -80°C. RNA was isolated from seven SMN⁻⁷ placentas and seven littermate controls (5 SMN^{+/+}; 2 SMN^{+/-}). The tissue was homogenized in TriReagent using an OMNI International GLH1 rotor, and total RNA extracted according to manufacturer's protocol with modification, as follows: after chloroform extraction, the aqueous phase was further purified by using the RNeasy mini kit (Qiagen), according to manufacturer's protocol. Two micrograms of total RNA were reverse transcribed using Invitrogen superscript III reverse transcriptase kit and random hexamers to generate first strand cDNA as outlined in manufacturer's protocol.

Forward and reverse primers and probes were synthesized by Integrated DNA Technologies (Table 1). Real-time PCR was performed in an ABI PRISM 7500 real time detection system using SABiosciences RT² Sybr Green/ROX qPCR master mix or Applied Biosystems Taqman Universal PCR master mix on settings 50°C for 2mins, 95°C for 10mins, and 40 cycles of 95°C for 15 secs, 60° C for 1min. All reactions were performed in triplicate. Dissociation curves were examined to ensure amplification of a single product. Reaction

efficiency was validated by using serial dilutions of template cDNA. Relative levels of mRNA were calculated using the C_t method, with normalization to 18s gene expression[18].

Statistical Analysis

Placental weights, cross- sectional areas and blood space areas were compared using Students' t-test with GraphPad Prism software. To compare mRNA levels, C_t were analyzed by Students' t-test.

Results

Placental Morphology

There were no significant differences in the weight of placentae among SMN genotypes, although there was some indication of a trend of increasing weight with increasing SMN1 copy number (linear regression, $p=0.058$) (figure 1a). There was no difference in the total cross-sectional area of the placenta between SMN 7 and control mice (figure 1b, figure 2a-b). The areas of the two major placental zones, the labyrinth zone, which is involved in the exchange of nutrients, gases and hormones between maternal and fetal circulations, and the junctional zone, which mediates the attachment of the trophoblast to the decidua, and is a source of proliferating trophoblast for the growth and development of the placenta were examined. There were no differences in size of either zone (figure 1c-d) or in the ratio of junctional:labyrinth zone in placentas of SMN 7 fetuses.

Placental cross sections were examined carefully at a higher magnification for signs of necrosis or inflammation, and no abnormalities were noted (figure 2). The parietal trophoblast giant cell layer, lining the interface of the junctional zone, spongiotrophoblast, glycogen cells, and maternal decidua were all morphologically normal (figure 2a-d). The structure of the labyrinth layer was also indistinguishable between control and SMN 7 placentas (figure 2e-f). Within the labyrinth, the cross-sectional areas of the maternal blood sinuses and fetal blood vessels were measured, and also were not different (figure 3)

Placental Gene Expression

To further screen for major abnormalities in placental development, we measured relative concentrations of mRNA for transcription factors characteristic of key trophoblast lineages by real-time PCR (Table 1). Markers of giant cell (*Hand1*), spongiotrophoblast (*Tpbpa*, *Ascl2*), and syncytiotrophoblast (*Esx1*) were present at similar levels in SMN 7 and control placentas (figure 4). Additionally, mRNA concentrations of *Stmn1*, stathmin1, which is highly expressed in trophoblast giant cells, were not different in SMN 7 and control placentas (figure 4), nor were key fatty acid transport and metabolism-related genes *Cd36* and *Srebp1c*.

Discussion

It was previously reported that SMN 7 pups from dams reared on a diet with higher fat content lived longer than pups from low fat-fed litters[15]. This observation, and knowledge

that the placenta is responsible for the transfer of nutrients, gases and waste between the mother and fetus and as such plays a crucial role in fetal health, prompted us to investigate whether placental development is impaired in SMA offspring.

The fetal portion of the placenta is compartmentalized into two layers: the junctional zone and labyrinth zone. Alterations in the relative areas of the junctional and labyrinth zones have been observed under several conditions, and serve as an indicator of disrupted placental development. For example, in placentas of embryos derived from nuclear transfer, the junctional zone is dramatically expanded[19] whereas in food-restricted mice, the size of the junctional zone relative to the labyrinth zone is decreased[17,20]. Thus, we quantified the relative areas of these zones in placentae from control and SMN^{-/-} 7 pups, but found no alterations of either.

Each zone is comprised of morphologically and functionally distinct cell types. The junctional zone consists of giant cells, glycogen cells and predominantly spongiotrophoblast cells[21]. Giant cells are polyploid cells, a subset of which invade the maternal decidua and maternal spiral arteries[21]. Both giant cells and spongiotrophoblast secrete hormones that regulate maternal metabolism, pregnancy recognition and other factors essential for fetal growth. Spongiotrophoblast cells are precursors to glycogen cells and both spiral artery and canal trophoblast giant cells[22]. Glycogen cells, as the name implies, store glycogen, which appears as vacuoles in paraffin embedded sections (figure 2c-d)[23]. Many glycogen cells migrate from the junctional zone into the decidua in the latter half of pregnancy, and they also cluster near maternal sinuses within the junctional zone[22]. The labyrinth zone, the area for maternal-fetal exchange, consists of maternal blood spaces lined by trophoblast giant cells, and is separated from fetal blood vessels by two layers of syncytiotrophoblast[24].

These lineages have been well studied in the mouse placenta, and genes characteristic of each have been defined. Thus, we quantified mRNA for selected markers to further screen for potential abnormalities in SMN^{-/-} 7 placentae. *Tpbpa* is expressed only in spongiotrophoblast cells in the mature placenta, though *Tpbpa*⁺ precursors give rise to giant cells and glycogen cells[22,25]. *Ascl2* (*Mash2*) and *Hand1* have opposing roles in placental trophoblast giant cell differentiation with *Ascl2* inhibiting, and *Hand1* promoting it²⁴. Though *Ascl2* knockouts display both spongiotrophoblast and labyrinthine defects, chimera experiments show that it is required only within spongiotrophoblast[26,27]. *Hand1*, on the other hand, is required for giant cell differentiation and it is expressed in giant cells of the mature placenta[28,29,30]. *Esx1* mRNA is limited to the labyrinthine layer of the mature placenta[31]. *Esx1* null mice have improperly organized labyrinthine layers leading to impaired fetal growth[31]. Each of these key placental lineage determinants was normally expressed in SMN^{-/-} 7 placentae, supporting the conclusion that morphogenesis of the SMN^{-/-} 7 placenta is not defective.

In addition to these lineage markers, we examined mRNA for *Stmn1* or stathmin, a protein expressed throughout the female reproductive tract, but highly expressed by human extravillous trophoblast cells during the first trimester of pregnancy and by their mouse analog, trophoblast giant cells[32,33,34]. Stathmin has been implicated in trophoblast

migration and differentiation in vitro[32]. Stathmin is significantly upregulated in motor neurons from the *Smn*^{-/-}*SMN2*^{+/-} mouse model of SMA[35]. Thus, we hypothesized that its expression may also be dysregulated in SMA placentae, but found no evidence of this.

Finally, because a higher fat maternal diet promoted *SMN*^{-/-} pup survival, we measured relative mRNA concentrations of two other placentally-expressed genes, *Cd36*, which promotes fatty acid translocation in multiple tissue types, and *Srebp1c*, which promotes fatty acid synthesis³⁵. These also were not differentially expressed in control and SMA placentae.

Overall, this study indicates that there are no major defects in placental structure in *SMN*^{-/-} mice, nor in the expression of the transcription factors controlling placental development. While this study cannot rule out impairments in nutrient transport activity in the *SMN*^{-/-} placenta, they suggest a fetal, rather than placental, cause of intrauterine growth restriction in this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Shababi M, Lorson CL, Rudnik-Schoneborn SS. Spinal muscular atrophy: a motor neuron disorder or a multi-organ disease? *J Anat.* 2014; 224:15–28. [PubMed: 23876144]
- Lefebvre S, Burglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell.* 1995; 80:155–165. [PubMed: 7813012]
- Gogliotti RG, Quinlan KA, Barlow CB, Heier CR, Heckman CJ, Didonato CJ. Motor neuron rescue in spinal muscular atrophy mice demonstrates that sensory-motor defects are a consequence, not a cause, of motor neuron dysfunction. *J Neurosci.* 2012; 32:3818–3829. [PubMed: 22423102]
- Park HB, Lee SM, Lee JS, Park MS, Park KI, Namgung R, Lee C. Survival analysis of spinal muscular atrophy type I. *Korean J Pediatr.* 2010; 53:965–970. [PubMed: 21218019]
- Bevan AK, Hutchinson KR, Foust KD, Braun L, McGovern VL, Schmelzer L, Ward JG, Petruska JC, Lucchesi PA, Burghes AH, Kaspar BK. Early heart failure in the *SMN*^{Δ7} model of spinal muscular atrophy and correction by postnatal scAAV9-*SMN* delivery. *Hum Mol Genet.* 2010; 19:3895–3905. [PubMed: 20639395]
- Schreml J, Riessland M, Paterno M, Garbes L, Rossbach K, Ackermann B, Kramer J, Somers E, Parson SH, Heller R, Berkessel A, Sterner-Kock A, Wirth B. Severe SMA mice show organ impairment that cannot be rescued by therapy with the HDACi JNJ-26481585. *Eur J Hum Genet.* 2013; 21:643–652. [PubMed: 23073311]
- Bowerman M, Michalski JP, Beauvais A, Murray LM, DeRepentigny Y, Kothary R. Defects in pancreatic development and glucose metabolism in *SMN*-depleted mice independent of canonical spinal muscular atrophy neuromuscular pathology. *Hum Mol Genet.* 2014; 23:3432–3444. [PubMed: 24497575]

8. Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc Natl Acad Sci U S A*. 1999; 96:6307–6311. [PubMed: 10339583]
9. Monani UR, Lorson CL, Parsons DW, Prior TW, Androphy EJ, Burghes AH, McPherson JD. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum Mol Genet*. 1999; 8:1177–1183. [PubMed: 10369862]
10. Feldkotter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am J Hum Genet*. 2002; 70:358–368. [PubMed: 11791208]
11. Monani UR, Sendtner M, Coovert DD, Parsons DW, Andreassi C, Le TT, Jablonka S, Schrank B, Rossoll W, Prior TW, Morris GE, Burghes AH. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in *Smn*(^{-/-}) mice and results in a mouse with spinal muscular atrophy. *Hum Mol Genet*. 2000; 9:333–339. [PubMed: 10655541]
12. Le TT, Pham LT, Butchbach ME, Zhang HL, Monani UR, Coovert DD, Gavrilina TO, Xing L, Bassell GJ, Burghes AH. SMN^{Delta7}, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. *Hum Mol Genet*. 2005; 14:845–857. [PubMed: 15703193]
13. Gonzalez De Dios J, Martinez Frias ML, Arroyo Carrera I, Fondevilla Sauci J, Sanchis Calvo A, Hernandez Ramon F, Martinez Guardia N, Garcia Gonzalez MM. [Role of signs of fetal hypokinesia in the diagnosis of spinal muscular atrophy of neonatal onset]. *An Esp Pediatr*. 2002; 56:233–240. [PubMed: 11864521]
14. Markowitz JA, Tinkle MB, Fischbeck KH. Spinal muscular atrophy in the neonate. *J Obstet Gynecol Neonatal Nurs*. 2004; 33:12–20.
15. Butchbach ME, Rose FF Jr, Rhoades S, Marston J, McCrone JT, Sinnott R, Lorson CL. Effect of diet on the survival and phenotype of a mouse model for spinal muscular atrophy. *Biochem Biophys Res Commun*. 2010; 391:835–840. [PubMed: 19945425]
16. Dale JM, Shen H, Barry DM, Garcia VB, Rose FF Jr, Lorson CL, Garcia ML. The spinal muscular atrophy mouse model, SMN^{Delta7}, displays altered axonal transport without global neurofilament alterations. *Acta Neuropathol*. 2011; 122:331–341. [PubMed: 21681521]
17. Schulz LC, Schlitt JM, Caesar G, Pennington KA. Leptin and the placental response to maternal food restriction during early pregnancy in mice. *Biol Reprod*. 2012; 87:120. [PubMed: 22993381]
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} Method. *Methods*. 2001; 25:402–408. [PubMed: 11846609]
19. Tanaka S, Oda M, Toyoshima Y, Wakayama T, Tanaka M, Yoshida N, Hattori N, Ohgane J, Yanagimachi R, Shiota K. Placentomegaly in cloned mouse concepti caused by expansion of the spongiotrophoblast layer. *Biol Reprod*. 2001; 65:1813–1821. [PubMed: 11717146]
20. Coan PM, Vaughan OR, Sekita Y, Finn SL, Burton GJ, Constancia M, Fowden AL. Adaptations in placental phenotype support fetal growth during undernutrition of pregnant mice. *J Physiol*. 2010; 588:527–538. [PubMed: 19948659]
21. Rai A, Cross JC. Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. *Dev Biol*. 2014; 387:131–141. [PubMed: 24485853]
22. Hu D, Cross JC. Ablation of Tpbpa-positive trophoblast precursors leads to defects in maternal spiral artery remodeling in the mouse placenta. *Dev Biol*. 2011; 358:231–239. [PubMed: 21839735]
23. Coan PM, Conroy N, Burton GJ, Ferguson-Smith AC. Origin and characteristics of glycogen cells in the developing murine placenta. *Dev Dyn*. 2006; 235:3280–3294. [PubMed: 17039549]
24. Anson-Cartwright L, Dawson K, Holmyard D, Fisher SJ, Lazzarini RA, Cross JC. The glial cells missing-1 protein is essential for branching morphogenesis in the chorioallantoic placenta. *Nat Genet*. 2000; 25:311–314. [PubMed: 10888880]
25. Carney EW, Prideaux V, Lye SJ, Rossant J. Progressive expression of trophoblast-specific genes during formation of mouse trophoblast giant cells in vitro. *Mol Reprod Dev*. 1993; 34:357–368. [PubMed: 8471259]
26. Guillemot F, Nagy A, Auerbach A, Rossant J, Joyner AL. Essential role of Mash-2 in extraembryonic development. *Nature*. 1994; 371:333–336.

27. Tanaka M, Gertsenstein M, Rossant J, Nagy A. Mash2 acts cell autonomously in mouse spongiotrophoblast development. *Dev Biol.* 1997; 190:55–65. [PubMed: 9331331]
28. Scott IC, Anson-Cartwright L, Riley P, Reda D, Cross JC. The HAND1 basic helix-loop-helix transcription factor regulates trophoblast differentiation via multiple mechanisms. *Mol Cell Biol.* 2000; 20:530–541. [PubMed: 10611232]
29. Riley P, Anson-Cartwright L, Cross JC. The Hand1 bHLH transcription factor is essential for placentation and cardiac morphogenesis. *Nat Genet.* 1998; 18:271–275. [PubMed: 9500551]
30. Hughes M, Dobric N, Scott IC, Su L, Starovic M, St-Pierre B, Egan SE, Kingdom JC, Cross JC. The Hand1, Stra13 and Gcm1 transcription factors override FGF signaling to promote terminal differentiation of trophoblast stem cells. *Dev Biol.* 2004; 271:26–37. [PubMed: 15196947]
31. Li Y, Behringer RR. Esx1 is an X-chromosome-imprinted regulator of placental development and fetal growth. *Nat Genet.* 1998; 20:309–311. [PubMed: 9806555]
32. Yoshie M, Kashima H, Bessho T, Takeichi M, Isaka K, Tamura K. Expression of stathmin, a microtubule regulatory protein, is associated with the migration and differentiation of cultured early trophoblasts. *Hum Reprod.* 2008; 23:2766–2774. [PubMed: 18718898]
33. Tamura K, Yoshie M, Nishi H, Osakabe Y, Isaka K, Hara T, Kogo H. Expression of stathmin in human uterus and decidualizing endometrial stromal cells. *Reproduction.* 2006; 132:625–636. [PubMed: 17008474]
34. Schulz LC, Widmaier EP. The effect of leptin on mouse trophoblast cell invasion. *Biol Reprod.* 2004; 71:1963–1967. [PubMed: 15306556]
35. Wen HL, Lin YT, Ting CH, Lin-Chao S, Li H, Hsieh-Li HM. Stathmin, a microtubule-destabilizing protein, is dysregulated in spinal muscular atrophy. *Hum Mol Genet.* 2010; 19:1766–1778. [PubMed: 20176735]
36. Schaiff WT, Knapp FF Jr, Barak Y, Biron-Shental T, Nelson DM, Sadovsky Y. Ligand-activated peroxisome proliferator activated receptor gamma alters placental morphology and placental fatty acid uptake in mice. *Endocrinology.* 2007; 148:3625–3634. [PubMed: 17463056]
37. Carter MG, Sharov AA, VanBuren V, Dudekula DB, Carmack CE, Nelson C, Ko MS. Transcript copy number estimation using a mouse whole-genome oligonucleotide microarray. *Genome Biol.* 2005; 6:R61. [PubMed: 15998450]
38. Schulz LC, Widmaier EP, Qiu J, Roberts RM. Effect of leptin on mouse trophoblast giant cells. *Biol Reprod.* 2009; 80:415–424. [PubMed: 19038858]
39. Sferruzzi-Perri AN, Macpherson AM, Roberts CT, Robertson SA. Csf2 null mutation alters placental gene expression and trophoblast glycogen cell and giant cell abundance in mice. *Biol Reprod.* 2009; 81:207–221. [PubMed: 19228596]

Abbreviations

SMA	Spinal Muscular Atrophy
SMN	spinal motor neuron

Highlights

- Spinal Muscular Atrophy is the second leading genetic cause of death in infants
- SMA infants can be growth restricted and their survival is influenced by maternal diet
- Our goal was to determine whether placental development is impaired in SMN^{-/-} mice
- No significant differences were observed in placental gene expression or morphology
- Data suggest SMA does not impair placental trophoblast differentiation

Figure 1.

Size of the placenta and its major zones in a mouse model of SMA. (a) placental wet weights in mice expressing human SMN2 and SMN 7, and zero (*Smn*^{-/-}, n=8), one (*Smn*^{+/-}, n=14) or two (*Smn*^{+/+}, n=7) copies of mouse *Smn*. (b) Total cross sectional area of the placenta and areas of the (c) junctional and (d) labyrinth zones were determined by image analysis in control (*hSMN2*^{+/+}; *SMN 7*^{+/+}; *mSmn*^{+/+} or *mSmn*^{+/-}, n) and *SMN 7* (*hSMN2*^{+/+}; *SMN 7*^{+/+}; ^{-/-}) mice. Bars represent SEM.

Figure 2. Placental morphology in control (a,c,e) and SMN⁻⁷ (b,d,f) mice. (a,b) Full mid-sagittal cross section of placenta, created from multiple images. (c,d) Representative images of the junctional zone, photographed with a 40× objective lens. (e,f) Representative images of the labyrinth zone, photographed with a 40× objective lens.

Figure 3.

Average cross sectional areas of maternal (a) and fetal (b) blood spaces in five randomly selected fields in each placenta (n=1) from control (n=5) and SMN Δ 7 (n=7) mice. Bars represent SEM.

Figure 4. Relative mRNA levels were quantified by real-time RT-PCR in control and SMN Δ 7 placentas (n=7). Columns represent fold difference versus control placentas and bars represent the range of fold differences calculated from standard error of the dCT. No significant differences were detected.

Table 1

Primer and Probe Sequences for real-time RT-PCR

Gene	Trophoblast lineage	Source	Forward	Reverse	Probe
<i>Asc2</i>	Spongiotrophoblast Glycogen cells	Primer Express	5'-GTG AAGGTGCAA ACG TCC ACT T-3'	5'-TCTGGTGCGGCA CAGGAA-3'	5'-56-FAM/CGCACC CGGTTCCCTC GCGA-3'
<i>Exx1</i>	Syneytiotrophoblast	[36]	5'-CCCATGCATCCT CAA ATG ATG-3'	5'-GCCTAAATG GTG GAGGCATTG-3'	N/A
<i>Hand1</i>	Giant cells	[37]	5'-GAGATGTATACC TGAGAGCAACAGG CATGATAG GTA G-3'	5'-CTTCTCCTTCATT TCCTTCCTTTTCCTT C-3'	N/A
<i>Simn1</i>	Ubiquitous; upregulated in giant cells	[38]	5'-TGGCCAGTGTC ACITTTACT TCCC-3'	5'-CCCTTGAGCCCC TAA AAC ATC-3'	5'-56-FAM/CTAGAA GCCGATGTAGGAC CGT-3'/6-TAM
<i>Tpbpa</i>	Spongiotrophoblast	[39]	5'-GCCAGTTGT TGA TGACCCCTGA-3'	5'-CCCCATCGCC ACT CTCTGTGT-3'	N/A
<i>18s</i>	Control	[38]	5'-TTCGGAACTGAG GCCATG AT-3'	5'-TTTCGCTCTGGT CCGTCTTG-3'	N/A