PAPP-A2 deficiency does not exacerbate the phenotype of a mouse model of intrauterine growth restriction

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Abstract

Background: Pregnancy-associated plasma protein-A2 (PAPP-A2) is consistently upregulated in the placenta of pregnancies complicated by preeclampsia and fetal growth restriction. The causes and significance of this upregulation remain unknown, but it has been hypothesized that it is a compensatory response to improve placental growth and development. We predicted that, if the upregulation of PAPP-A2 in pregnancy complications reflects a compensatory response, then deletion of Pappa2 in mice would exacerbate the effects of a gene deletion previously reported to impair placental development: deficiency of matrix metalloproteinase-9 (MMP9).

Methods: We crossed mice carrying deletions in Pappa2 and Mmp9 to produce pregnancies deficient in one, both, or neither of these genes. We measured pregnancy rates, number of conceptuses, fetal and placental growth, and the histological structure of the placenta.

Results: We found no evidence of reduced fertility, increased pregnancy loss, or increased fetal demise in Mmp9−/− females. In pregnancies segregating for Mmp9, Mmp9−/− fetuses were lighter than their siblings with a functional Mmp9 allele. However, deletion of Pappa2 did not exacerbate or reveal any effects of Mmp9 deficiency. We observed some effects of Pappa2 deletion on placental structure that were independent of Mmp9 deficiency, but no effects on fetal growth. At G16, male fetuses were heavier than female fetuses and had heavier placentae with larger junctional zones and smaller labyrinths.

Conclusions: Effects of Mmp9 deficiency were not exacerbated by the deletion of Pappa2. Our results do not provide evidence that upregulation of placental PAPP-A2 represents a mechanism to compensate for impaired fetal growth.

Keywords: Placenta, Pregnancy, Preeclampsia, Intrauterine growth restriction, Pregnancy associated plasma protein, Matrix metalloproteinase, Insulin-like growth factor

Background

Intrauterine growth restriction and preeclampsia threaten the health and wellbeing of both the fetus and the mother, affecting 5–7% of pregnancies and constituting leading causes of perinatal and maternal mortality [1]. These conditions are thought to be caused, at least in part, by abnormal placental development and function [2]. There have been enormous efforts to identify the molecular mechanisms responsible for placental dysfunction in preeclampsia and intrauterine growth restriction, with numerous studies examining placental gene expression at delivery. Pregnancy-associated plasma protein-A2 (PAPP-A2) is one of the genes most consistently found to be upregulated in preeclampsia [3–7] and is also associated with fetal growth restriction [8]. Furthermore, elevated levels of PAPP-A2 in the maternal circulation in the first trimester have also been associated with preeclampsia [9, 10]. PAPP-A2 is a protease of insulin-like growth factor binding protein 5 (IGFBP-5) [11] and is thought to regulate insulin-like growth factor (IGF) availability, although it may also function through other pathways [12]. IGFs play key roles in placental development [13], and their availability is regulated by six IGF binding proteins (IGFBPs). IGFs are released primarily through cleavage of the IGFBPs by proteases [14].
PAPP-A2 deficiency would therefore be expected to reduce IGF availability, and indeed loss-of-function mutations in humans reduce stature [15] while Pappa2 deletion in mice reduces body size [16–18] and increases IGFBP-5 levels [19].

Despite the high expression of PAPP-A2 in mouse placenta [20], Pappa2 deletion has no effect on pregnancy outcomes, apart from slightly reduced birthweight, which may be due to deletion in the fetus itself [16, 21]. We therefore hypothesized that the upregulation of PAPP-A2 in human pregnancy complications represents a compensatory response to increase IGF signaling to promote placental growth and development [10, 22, 23]. To test this hypothesis, we deleted Pappa2 in a mouse model of preeclampsia and intrauterine growth restriction: deficiency of matrix metalloproteinase-9 (MMP9) [24]. We selected this model since Mmp9 deletion impairs early placental development [24], and thus reflects “canonical” preeclampsia, rather than preeclampsia of other etiologies [25]. Deficiencies in early placental development would be expected to be ameliorated by increased IGF availability and, therefore, by increased PAPP-A2 expression. We predicted that, if the elevated expression of PAPP-A2 in preeclampsia in humans reflects a compensatory response, then deletion of Pappa2 in mice would exacerbate effects of Mnp9 deletion, i.e., mice null for both Pappa2 and Mmp9 would show a more severe phenotype than mice null for Mmp9 only. We focused on the number and size of fetuses, as well as placental histology since these were traits expected to be affected by Mmp9 deficiency [24] and potentially ameliorated by PAPP-A2 and increased IGF availability.

**Methods**

All work was carried out in accordance with the guidelines of the Canadian Council on Animal Care and approved by the SFU University Animal Care Committee (protocol 1188B). Pappa2 deletion mice with a C57BL/6 background were generated as previously described [16, 19]. Females homozygous for Pappa2 deletion (Pappa2−/−) were crossed with a male homozygous for Mmp9 deletion (Mmp9−/−) obtained from the Jackson Laboratory (stock number 007084) to produce offspring heterozygous at both genes. The first generation (F1) offspring were crossed to produce an F2 population that included mice with all nine possible genotypes (three Mmp9 genotypes x three Pappa2 genotypes). Females and males from the F2 population were selected based on Mmp9 and Pappa2 genotype for breeding experiments, and were mated as described in Table 1. Rather than using only homozygous mice, we performed a variety of crosses to make use of as many mice as possible (Table 1). Furthermore, mating type 1 with Mmp9−/− males was previously reported to show a reduction in litter size and placental abnormalities [24]. Beginning at approximately 8 weeks of age, females were placed with a male for one night, checked for vaginal plugs, and removed from the male whether or not a plug was observed. If no vaginal plug was observed and/or if female weight had not increased by ~ 1 g 1 week after mating, females were paired again. F2 females were collected at day 16 of gestation (G16; where the day after mating = day 0). To obtain mice for a further cohort, some females heterozygous at both genes (Mmp9+/−; Pappa2+/−) were paired with males homozygous for both deletions (Mmp9−/−; Pappa2−/−) and not collected during pregnancy. We produced a backcross (BC) population, rather than crossing heterozygotes, to increase the number of mice homozygous for Mmp9 and/or Pappa2 deletion. Females and males from the BC population were mated in the same manner as the F2 population, except that females were collected at day 18 of gestation (G18). Pregnancies were sampled at G18 in case effects were apparent only after G16. 63 F2 females were mated, yielding 43 G16 pregnancies (although one was mistakenly not collected during pregnancy) whereas 24 BC females were mated, yielding 20 G18 pregnancies.

At collection, females were blood sampled by cardiac puncture and the entire uterus was placed in 4% formaldehyde solution in phosphate buffered saline for 3 days before it was dissected to count and weigh individual fetuses and placentae, and to count putative fetal resorptions (green or green/brown masses). Maternal serum vascular endothelial growth factor (VEGF) was measured by enzyme-linked immunosorbent assay (R&D Systems, MMV00). Fixed placentae were stored in 70% ethanol until embedded in paraffin. A subset of placentae were selected for sectioning, attempting to include one male and one female placenta for each female, and excluding heterozygous genotypes. Because previous work reported that both embryonic and maternal Mmp9 deficiency affect placental development [24], we selected Mmp9−/− placenta from both Mmp9−/− and Mmp9+/− dams, as well as Mmp9+/− placenta from Mmp9+/− dams. For each placenta, multiple sections (6 μm) were obtained ~440 μm apart, up to a maximum of 10 sections per placenta. Sections were stained with haematoxylin and eosin and the areas of the labyrinth, junctional zone and

| Table 1 | Mmp9 crosses performed in experiments |
|---------|--------------------------------------|
| Mating type | Female Mmp9 genotype | Male Mmp9 genotype |
| 0 | −/− | −/− |
| 1 | −/− | +/− |
| 2 | +/− | −/− |
| 3 | +/− | +/− |
| 4 | +/+ | +/+ |

Each type of Mmp9 cross included either no functional Pappa2 alleles (Pappa2+/− x Pappa2−/−) or at least one functional Pappa2 (achieved with various combinations of female and male genotype)
decidua were measured using ImageJ 1.48v. Damaged sections, and sections close to the edge of the placenta (i.e., where the labyrinth was mostly surrounded by junctional zone) were excluded, yielding 454 sections from 62 placentae (average: 7.3 sections per placenta). To obtain a single value for each of the labyrinth, junctional zone and decidua for each placenta, we analysed the areas from all 454 sections using a general linear model (proc GLM, SAS, Version 9.4) including terms for placenta identity and section location (i.e., close to the centre vs. further from the centre; sections further from the centre had smaller areas). From this analysis, we obtained the least squares mean for each placenta for each of the labyrinth, junctional zone and decidua.

To obtain tissue for genotyping, mice were ear-clipped at weaning or a small section of fetal tail was collected. Pappa2 genotype [19] and fetal sex [26] were determined as previously described. Mmp9 genotype was determined by PCR as recommended by the Jackson Laboratory (primers used: 5'-CTGAATGAACTGCA GGACGA-3'; 5'-ATACCTTCTCGCAGGAGCA-3'; 5'-GTGGGACCATCATAACATCACA-3'; 5'-CTCG CGGCAAGTCTTCAGAGTA-3').

All statistical analyses were performed using general linear models (proc GLM) or repeated measures analyses (proc MIXED) in SAS, Version 9.4 (SAS Institute Inc., Cary, NC). Repeated measures analyses (with dam as a random factor) were used for placental and fetal traits where there were multiple offspring per dam, since the dam was the unit of replication.

Results

The genotype ratios and postnatal growth of the F2 and BC populations are presented in the Additional file 1. Combining F2 and BC females, we found no evidence of reduced fertility or increased pregnancy loss in Mmp9−/− females. The proportion of females that became pregnant did not differ between Mmp9−/− females and other females, whether all females were analysed together (Fisher’s Exact Test \( P = 0.47 \)) or separately based on whether at least one wild-type Pappa2 allele was present (Fisher’s Exact Test \( P = 1.00 \)) or not (Fisher’s Exact Test \( P = 0.27 \)) (Table 2). Similarly, the proportion of females that had at least one failed mating (i.e., a vaginal plug was detected following mating, but pregnancy did not develop) did not differ between Mmp9−/− females and other females. This was true whether all females were analysed together (Fisher’s Exact Test \( P = 0.47 \)) or separately based on whether at least one wild-type Pappa2 allele was present (Fisher’s Exact Test \( P = 0.33 \)) or not (Fisher’s Exact Test \( P = 1.00 \)) (Table 2). The number of times a female was paired with a male before becoming pregnant did not differ between Mmp9−/− and other females (\( F_{1,59} = 2.22; P = 0.14 \)), or by the interaction between these two factors (\( F_{1,59} = 0.10; P = 0.76 \)) (Table 2). Since the number of times a female was paired with a male varied only from 1 to 6, we also analysed these data using a non-parametric Wilcoxon test. Again there was no difference between Mmp9−/− and other females (\( P = 0.71 \)), pooling matings with and without at least one wild-type Pappa2 allele present.

We also found no evidence of reduced fecundity or increased fetal loss in Mmp9−/− females. Including females collected at either G16 or G18, the number of fetuses did not differ between Mmp9−/− and other females (\( F_{1,58} = 0.28; P = 0.60 \)), and was not affected by whether at least one wild-type Pappa2 allele was present (\( F_{1,58} = 0.00; P = 0.99 \)) or the interaction between these two factors (\( F_{1,58} = 0.17; P = 0.68 \) (Fig. 1)). The number of putative embryo resorptions ranged from 0 to 4, and tended to be slightly lower in Mmp9−/− females (mean = 0.6) than in other females (mean = 0.9; Wilcoxon test \( P = 0.25 \)). The proportion of females that had at least one resorption did not differ between Mmp9 genotypes (11/30 Mmp9−/− females vs. 16/32 other females; Fisher’s Exact Test \( P = 0.32 \)).

At G16, the average fetal mass and average placental mass did not differ between Mmp9−/− and other females,

**Table 2** Effects of Mmp9 and Pappa2 deletion on fertility including F2 and BC females, i.e., females collected at G16 or G18

|                         | No wild-type Pappa2 alleles | At least one wild-type Pappa2 allele |
|-------------------------|-----------------------------|-------------------------------------|
| Mmp9 mating type        |                             |                                     |
| 0–1                     | 2.3 ± 0.4                   | 2.7 ± 0.3                           |
| 2–4                     | 2.3 ± 0.3                   | 2.9 ± 0.3                           |
| # matings for pregnancy |                             |                                     |
| \# females that became pregnant | 11                          | 19                                   |
|                        | 16                          | 17                                   |
| \# females that did not become pregnant | 2                           | 7                                    |
|                        | 9                           | 6                                    |
| \# females with failed matings | 9                           | 18                                   |
|                        | 18                          | 19                                   |
| \# females with failed matings | 4                           | 8                                    |
|                        | 7                           | 4                                    |

\( a \) Least-squares means ± standard error from a general linear model including Mmp9 mating type, whether mating had any wild-type Pappa2 alleles, and the interaction between these two terms

\( b \) A failed mating was defined as when a vaginal plug was detected following mating, but pregnancy did not develop; this analysis includes females that subsequently became pregnant, and those that never became pregnant.
and was not affected by whether at least one wild-type Pappa2 allele was present or the interaction between these two factors (controlling for the number of fetuses, which was negatively related to fetal and placental mass; Table 3). There was no excess of very small or runted pups in Mmp9<sup>−/−</sup> pregnancies (Fig. 2). There was a tendency for Mmp9<sup>−/−</sup> females to have heavier placentae, but this was marginally non-significant (P = 0.07, Table 3). Considering only pregnancies with both Mmp9<sup>+/−</sup> and Mmp9<sup>−/−</sup> fetuses, Mmp9<sup>−/−</sup> fetuses and their placentae were significantly lighter than their Mmp9<sup>+/−</sup> siblings, but there was no effect of whether a wild-type Pappa2 allele was present in the pregnancy, and no interaction between Mmp9 and Pappa2 (Table 4; Fig. 3). Male fetuses were heavier than female fetuses and had heavier placentae. The number of Mmp9<sup>−/−</sup> to Mmp9<sup>+/+</sup> conceptuses did not differ from the expected 1:1 ratio (76 Mmp9<sup>−/−</sup> vs. 73 Mmp9<sup>+/−</sup>; χ<sup>2</sup> = 0.06; P = 0.81).

We also studied fetuses at G18, in case growth restriction was more apparent later in pregnancy. As at G16, the average fetal mass and average placental mass did not differ between Mmp9<sup>−/−</sup> and other females, and was not affected by whether at least one wild-type Pappa2 allele was present or the interaction between these two factors, controlling for the number of fetuses (Table 3; Fig. 2). Average fetal mass tended to be lighter in Mmp9<sup>−/−</sup> females but this was marginally non-significant (P = 0.08, Table 3). Considering only pregnancies segregating at Mmp9, Mmp9<sup>−/−</sup> fetuses were lighter than their Mmp9<sup>+/−</sup> and Mmp9<sup>+/+</sup> siblings, but there was no effect of whether a wild-type Pappa2 allele was present in the pregnancy, and no interaction

![Fig. 1 Effect of Mmp9 and Pappa2 deletion on the number of fetuses, including females collected at either G16 or G18](image)

Table 3 Effects of Mmp9 and Pappa2 deletion on average fetal mass and average placental mass

| Term in model | Mmp9 | Pappa2 | Mmp9*Pappa2 interaction | Number of fetuses |
|---------------|------|--------|-------------------------|-------------------|
| G16           |      |        |                         |                   |
| Average fetal mass | 0.89 | 0.35   | 0.44                   | 6.51              |
| Average placental mass | 3.50 | 0.07   | 0.82                   | 10.76             |
| G18           |      |        |                         |                   |
| Average fetal mass | 3.41 | 0.08   | 1.13                   | 2.08              |
| Average placental mass | 0.61 | 0.45   | 1.00                   | 1.11              |

Statistics are from general linear models including effects of Mmp9 deletion (mating types 0 and 1 compared with others), Pappa2 deletion (whether the cross included at least one functional Pappa2 allele or not), the interaction between these two factors, and the number of fetuses as a covariate.
Fig. 2 Effect of Mmp9 and Pappa2 deletion on fetal weight at G16 (upper panels) and G18 (lower panels)

| Term in model     | Mmp9   | Pappa2 | Mmp9*Pappa2 interaction | Number of fetuses | Sex of fetus |
|-------------------|--------|--------|-------------------------|-------------------|--------------|
| G16               |        |        |                         |                   |              |
| Fetal mass        | F_1,16 | P      | F_1,18                  | F_1,16            | P            |
|                   | 9.69   | 0.007  | 0.07                    | 0.80              | 0.40         |
| Placental mass    | F_1,16 | P      | F_1,18                  | F_1,18            | P            |
|                   | 12.63  | 0.003  | 0.06                    | 0.80              | 0.01         |
| G18               |        |        |                         |                   |              |
| Fetal mass        | F_1,10 | P      | F_1,11                  | F_1,10            | P            |
|                   | 9.53   | 0.012  | 0.59                    | 0.46              | 0.03         |
| Placental mass    | F_1,10 | P      | F_1,11                  | F_1,11            | P            |
|                   | 0.81   | 0.39   | 1.13                    | 0.31              | 1.26         |

These analyses included multiple conceptuses per dam, and so statistics are from repeated measures analyses (with dam as a random factor), including effects of Mmp9 genotype of conceptus (Mmp9\(^{−/−}\) vs. Mmp9\(^{+/−}\) and Mmp9\(^{+/+}\)), Pappa2 deletion (whether the cross included at least one functional Pappa2 allele or not), the interaction between these two factors, fetal sex, and the number of fetuses as a covariate.
between Mmp9 and Pappa2 (Table 4; Fig. 3). There was no effect of Mmp9 genotype on placental weight. As at G16, male fetuses had heavier placentae than female fetuses. The genotype ratios did not differ from the expected Mendelian ratios in litters segregating for 2 genotypes (16 Mmp9−/−: 9 Mmp9+/−; \( \chi^2_1 = 1.96; P = 0.16 \)) or three genotypes (26 Mmp9−/−: 40 Mmp9+/−: 12 Mmp9+/+; \( \chi^2_1 = 5.08; P = 0.08 \)). Though non-significant, the trend was for an excess of Mmp9−/− conceptuses.

At G16, we observed little effect of Mmp9 deficiency on the areas of the labyrinth, junctional zone or decidua, either in absolute terms or in terms of each component as a percentage of the total area (Table 5; Figs. 4 and 5). There was generally no interaction between Mmp9 deficiency and whether at least one wild-type Pappa2 allele was present (Table 5; Fig. 4), although Mmp9−/− placentae from Mmp9−/− dams with no Pappa2 had slightly smaller decidua area, when measured as a percentage of the total area (Table 5; Fig. 4). Pregnancies with no Pappa2 had smaller deciduas in absolute terms, and had larger labyrinths and smaller deciduas as a percentage of the total area, compared to pregnancies where at least one wild-type Pappa2 allele was present (Table 5; Fig. 4). Placentae of male fetuses had larger junctional zone

![Fig. 3](image-url) Effect of Mmp9 and Pappa2 deletion on fetal weight at G16 (left) and G18 (right) in pregnancies segregating at Mmp9. Open symbols are Mmp9−/− fetuses and solid symbols are their Mmp9+/− and Mmp9+/+ siblings. Error bars are pooled per Mmp9 genotype (i.e., pooling Pappa2 genotype)

### Table 5 Effects of Mmp9 and Pappa2 deletion on the areas of the labyrinth, junctional zone and decidua, either in absolute terms, or as a percentage of total area

| Term in model | Mmp9\(^a\) | Pappa2 | Mmp9\*Pappa2 interaction | Sex of fetus |
|---------------|------------|--------|--------------------------|-------------|
| F\(_{2,28}\)  | P          | F\(_{1,28}\) | P                              | F\(_{1,20}\) | P |
| Absolute      |            |         |                           |             |
| Labyrinth     | 0.20       | 0.82    | 0.00                      | 0.98        | 1.64 | 0.21 | 0.10 | 0.75 |
| Junctional Zone| 0.38       | 0.69    | 1.93                      | 0.18        | 0.69 | 0.51 | 14.76 | 0.001\(^b\) |
| Decidua       | 3.17       | 0.06    | 7.89                      | 0.01        | 2.62 | 0.09 | 2.90 | 0.10 |
| Percentage of total |           |         |                           |             |
| Labyrinth     | 1.15       | 0.33    | 6.89                      | 0.01        | 1.80 | 0.18 | 15.01 | 0.001\(^b\) |
| Junctional Zone| 1.41       | 0.26    | 0.79                      | 0.38        | 0.04 | 0.96 | 11.63 | 0.003\(^b\) |
| Decidua       | 4.10       | 0.03    | 6.97                      | 0.01        | 3.96 | 0.03 | 0.00 | 0.98 |

These analyses included multiple conceptuses per dam, and so statistics are from repeated measures analyses (with dam as a random factor), including effects of Mmp9 group, Pappa2 deletion (whether the cross included at least one functional Pappa2 allele or not), the interaction between these two factors, and fetal sex

\(^a\)In these analyses, there were three Mmp9 groups: Mmp9−/− placenta from Mmp9−/− dams, Mmp9+/− placenta from Mmp9+/− dams, and Mmp9+/+ placenta from Mmp9+/+ dams

\(^b\)Area of the junctional zone, both absolute and as a percentage of the total area, was larger in males than females, while the area of the labyrinth as a percentage of the total was smaller in males
**Fig. 4** Effect of *Mmp9* and *Pappa2* deletion on the areas of the labyrinth, junctional zone and decidua in absolute terms (upper panels) and as a percentage of the total area (lower panels). Open symbols are pregnancies without a functional *Pappa2* allele and solid symbols are pregnancies with at least one functional *Pappa2* allele. Error bars are from repeated measures analyses (with dam as a random factor), including effects of *Mmp9* group, *Pappa2* deletion, the interaction between these two factors, and fetal sex.

**Fig. 5** Representative images of G16 placental sections from *Mmp9*^−/−^ placenta from *Mmp9*^−/−^ dams and *Mmp9*^+/+^ placenta from *Mmp9*^+/+^ dams, with and without *Pappa2*, showing the outlined labyrinth (L), junctional zone (J) and decidua (D). All placentae are from female fetuses.
areas in absolute terms and, as a percentage of the total area, had larger junctional zones and smaller labyrinths.

The previous report of Mmp9 deletion mice [24] described reduced serum VEGF levels in Mmp9−/− females. We analysed VEGF levels at G16 in a subset of pregnancies of Mmp9−/− and Mmp9+/− females, all with at least one wild-type Pappa2 allele. VEGF levels did not differ between Mmp9−/− and Mmp9+/− females (F1,10 = 0.12; P = 0.73), but were positively related with the number of conceptuses (F1,10 = 13.70; P = 0.004; Fig. 6).

Discussion

In humans, placental PAPP-A2 is upregulated in preeclampsia [3–7] and fetal growth restriction [8]. However, deletion of Pappa2 in mice has little effect on pregnancy outcome [16, 21], suggesting that the upregulation of PAPP-A2 in human pregnancy complications may represent a compensatory response [10, 22, 23]. If PAPP-A2 is important in compensating for placental insufficiency, it would be expected that its absence would exacerbate the effects of placental dysfunction. Previously, Mmp9 deficiency has been reported to cause placental abnormalities resulting in growth restriction [24]. We observed that Mmp9−/− fetuses were lighter than Mmp9+/− siblings, but this difference was not exacerbated by deletion of Pappa2. Therefore, our results do not provide evidence that PAPP-A2 contributes to placental mechanisms that compensate for poor fetal growth.

Surprisingly, we found no effect of Mmp9 deletion, with or without deletion of Pappa2, on fertility, fecundity, pregnancy loss, fetal loss or placental structure. While the publication describing Mmp9 deletion as a model of preeclampsia and intrauterine growth restriction reported “as much as a 50% reduction in litter size” [24], no data were presented, and the original report of Mmp9 deletion described a much more modest reduction in litter size (1.6 pups) [27]. In our experiments, Mmp9−/− females were compared with control siblings, and no experimental females were daughters of Mmp9−/− females. It is therefore possible that by avoiding maternal effects of Mmp9 deletion, the severity of the deletion was reduced; whether previous reports [24, 27] used our breeding scheme is not clear.

The previous report of the effects of Mmp9 deficiency on pregnancy also reported reduced levels of VEGF in the maternal circulation [24]. VEGF influences placental angiogenesis, and maternal circulating VEGF levels are reduced in preeclamptic human pregnancies [28]. While there was no effect of Mmp9 deficiency on circulating maternal VEGF in the present study, we found a positive association between VEGF levels and the number of conceptuses. The previous report of reduced VEGF in Mmp9−/− females [24] may therefore have been due to reduced numbers of conceptuses, rather than to placental pathology.

In addition to some modest effects of Mmp9 and Pappa2 deletion, we observed more robust differences between the sexes in fetal mass, placental mass, and placental structure at G16, as well as placental mass at G18. These differences may reflect sex-specific strategies for fetal growth and placental function [29] with potential long term effects on offspring health [30].

Conclusions

Previous work reported that deletion of Mmp9 reduced pregnancy rates and implantation success following embryo transfer, decreased litter size, and increased rates of fetal demise and growth restriction [24]. In contrast, we found only modest effects of Mmp9 deletion on fetal growth, and found no effects on the number of fetuses, the number of putative embryo resorptions, or female fertility (number of matings required to achieve pregnancy, the proportion of females that became pregnant, or the number of females with failed matings). The difference in results between our study and previous work may have been due to our experimental design, which compared Mmp9−/− females with control siblings and thus avoided confounding maternal effects. The effects of Mmp9 deficiency were not exacerbated by the deletion of Pappa2, and therefore our results do not support the hypothesis that PAPP-A2 upregulation in human pregnancy complications represents a compensatory response to ameliorate placental growth and development. Our results provide insight into the role of PAPP-A2 dysregulation in devastating pregnancy complications, which may inform the use of this protein as an early biomarker of placental health [9, 10]. Our work also serves as a caution regarding the use of Mmp9 deficiency as a model of these complications.
Additional files

**Additional file 1:** Genotype ratios and postnatal growth of F2 and BC populations. (DOCX 326 kb)

**Additional file 2:** Datasets analysed in this study. (XLSX 69 kb)

**Abbreviations**

BC: Backcross; F1: First generation; F2: Second generation; G16, G18: day 16 or 18 of gestation, where the day after mating = day 0; IGF: Insulin-like growth factor; IGFBP: Insulin-like growth factor binding protein; MMP9: Matrix metalloproteinase-9; PAPP-A2: Pregnancy-associated plasma protein-A2; VEGF: Vascular endothelial growth factor

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**Availability of data and materials**

The dataset supporting the conclusions of this article is included as an additional file (Additional file 2).

**Authors’ contributions**

JKC conceived of the study, contributed to laboratory work, carried out statistical analyses, and wrote the manuscript. KIL performed the laboratory work and helped draft the manuscript. NB contributed to laboratory work, statistical analyses, and wrote the manuscript. JKC, SMP, and JMC contributed to manuscript preparation. All authors approve and are accountable for the final version.

**Ethics approval**

All work was carried out in accordance with the guidelines of the Canadian Council on Animal Care and approved by the SFU University Animal Care Committee (protocol 11888).

**Competing interests**

The authors declare that they have no competing interests.

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