## Heart defects in *Ly6e* mutant mice are likely a secondary consequence of impaired placental development

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Mouse knockout experiments have provided evidence for a relationship between the developing heart and the placenta. Germ-line deletions for either *p38*α (*Mapk14*) or *Peroxisome-proliferator activator receptor gamma* (*Ppar*γ) cause severe cardiac and placental defects, but when the placental deficiencies are selectively rescued by aggregation with wildtype tetraploid embryos, the heart defects in *Mapk14*$^{-/-}$ and *Ppar*γ$^{-/-}$ embryos are corrected (Adams *et al.*, 2000; Barak *et al.*, 1999). These experiments demonstrate that heart defects can be a secondary consequence of impaired placentation, although the precise nature of this relationship remains ill defined. Both *Mapk14* and *Ppar*γ mutations do however affect the formation of the placental labyrinth; the site where the maternal and fetal circulations become torturously intertwined to facilitate two-way exchange, suggesting that vascularization of the placenta may be implicated.

In a previous study we reported the expression of *Ly6e* in the transporting trophoblast cells of the labyrinth (Hughes *et al.*, 2013). *Ly6e* encodes a small molecular weight, GPI-linked glycoprotein of the Ly-6 family of cell surface proteins. Genetic deletion of *Ly6e* is embryonic lethal by mid-gestation, a phenotype originally attributed to heart abnormalities (Zammit *et al.*, 2002). However, *Ly6e* is not appreciably expressed in heart, suggesting the resultant dilated cardiomyopathy and ventricular trabeculation defects may be secondary to placental defects. We were fortunate to obtain the *Ly6e* mutant mouse strain to investigate placental morphogenesis in the absence of *Ly6e*.

Our analysis demonstrated that *Ly6e*$^{-/-}$ placentae are abnormal, both in the gross organisation of the fetal vasculature and in the ultrastructural organisation of the interhaemal membrane, the cellular barrier separating the fetal and maternal blood compartments. *In situ* hybridisation using genetic markers for a number of placental trophoblast subtypes indicated that trophoblast differentiation is not affected in *Ly6e*$^{-/-}$ placenta *per se*, but a reduction in villous branching is clearly evident. Interestingly, a loss of *Ly6e* in trophoblast does appear to affect the specification of fetal endothelial cells, situated on the opposite side of the interhemal membrane. Electron microscopy of *Ly6e*$^{-/-}$ interhaemal membranes revealed areas of disrupted syncytiotrophoblast fusion and an overall increase in barrier thickness. Knockdown of *Ly6e* expression in differentiating trophoblast stem cells *in vitro* confirmed the syncytiotrophoblast fusion defect. Together our observations suggest profound deficiencies in placental function in the absence of *Ly6e*. Furthermore, these placental phenotypes precede the appearance of heart abnormalities, and are therefore the likely cause of both the heart defects and mid-gestational lethality in these mice.

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