Orphan G protein-coupled receptor GPR37L1 and the cardiovascular system: variability across methods and models

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G protein-coupled receptors (GPCRs) have been blockbuster pharmaceutical targets for the treatment of various aspects of cardiovascular disease and currently comprise the largest category of FDA-approved drugs. Thus, the discovery that genetic deletion of an orphan GPCR (no identified ligand) called GPR37L1 led to an ~62 mmHg increase in murine blood pressure (Min *et al.*, 2010) was seen as strong evidence that GPR37L1 might be a druggable target for the treatment of the underlying causes of essential hypertension.

To thoroughly assess the potential mechanism(s) underlying such a profound blood pressure change, we obtained the original mixed background GPR37L1 null mice (Min *et al.*, 2010) and also generated our own pure C57BL/6J mice using the EUCOMM conditional-ready system (Coleman *et al.*, 2015). Our studies of the original GPR37L1 null mice failed to recapitulate the hypertensive phenotype observed; indeed we saw no cardiovascular phenotype at all in these mice when we compared them to their own wild type controls. In contrast, baseline characterisation of blood pressure haemodynamics (isoflurane anaesthesia followed by micromanometry or conscious radiotelemetry measurements) and post-mortem heart morphometry in our own GPR37L1 knock-out (KO) mice revealed a modest but reproducible elevated blood pressure phenotype in female GPR37L1 KO mice only (+11 mmHg micromanometry, +9 mmHg telemetry; Coleman *et al.*, 2018). The response to short term cardiovascular challenge with AngII (2 mg/kg/d for 7 days) was similarly sexually dimorphic, with male GPR37L1 KO mice advancing to heart failure, while female GPR37L1 KO mice were protected from cardiac fibrosis (Coleman *et al.*, 2018). In a separate cohort of untreated mice that were aged to 52 weeks, we observed no blood pressure difference between KO and wild type for either sex, but both GPR37L1 KO sexes developed significant cardiac hypertrophy, again indicating that GPR37L1 plays some kind of role in cardiovascular homeostasis.

Because our GPR37L1-lacZ reporter mice showed GPR37L1 expression was exclusively limited to the brain, particularly glial cells, we hypothesized that GPR37L1 must be mediating its cardiovascular effects *via* control of sympathetic tone. We addressed this in two ways: (1) third order mesenteric artery wire myography, and (2) blood pressure radiotelemetry recording with spectral analysis during behavioural and pharmacological interventions. While our myography data suggested that GPR37L1 resistance vessels were less sensitive to endothelium-dependent relaxation, consistent with our previous *in vivo* studies, we were surprised to see that there were few differences in the cardiovascular characterisation of both GPR37L1 KO sexes in our new radiotelemetry cohort. We found no genotype-specific changes in 24 h or spectral analyses of pressure or heart rate, nor was there an effect of pharmacological intervention with pentolinium or enalaprilat. However, female GPR37L1 KO mice did have a counterintuitively blunted reaction to aversive stress tests (dirty cage swap or restraint), which may instead report on reduced emotional reactivity rather than sympathetic drive, *per se*.

In summary, we have comprehensively phenotyped both male and female GPR37L1 KO mice. For every cohort of mice investigated, we observed at least one cardiovascular difference between wild type and GPR37L1 KO mice, but these endpoints were not consistent between studies. We attribute this variability to the marginal blood pressure phenotype that we are measuring (maximum of 11 mmHg difference; gender- and age-specific) and differences in blood pressure recording protocols. On this basis, we conclude that GPR37L1 is not a robust or druggable target for the treatment of essential hypertension.

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