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P462 -Using ultrasound scanning to investigate and treat a mouse model of obstructive uropathy

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Obstructive uropathy accounts for up to 20% of paediatric end stage renal failure, with irreparable damage occurring to the kidneys before surgical correction of obstruction is possible. In cases of Posterior Urethral Valves (PUV), problems due to a thickened bladder wall persist throughout life. Animal models are essential tools; both for investigating developmental disorders and for developing future treatments. Most uropathy models rely on surgery to generate obstruction, which can only be performed at later stages of development.

The T30 Homozygote (T30H) mouse has a balanced, heritable chromosomal translocation specifically compromising smooth muscle development in the bladder. No overt defects are present in other organs. This lack of smooth muscle means that T30H mice are unable to void the bladder in utero, and is associated with hydronephrosis and reduced nephron numbers, mimicking the kidney symptoms of children with obstructions perfectly, despite the translocation having the opposite effect normally seen in the bladder. The fact that the bladder is non-emptying also means that it is the perfect target for injectable gene therapy, as the contents are retained throughout gestation.

The onset of the phenotype can be identified using ultrasound scanning. T30H mice are indistinguishable from wild type littermates at E14, but at E16 there is a drastic expansion in bladder size, with the bladder taking up much of the abdominal cavity. In these animals hydronephrosis can be identified in both kidneys.

The translocation is between chromosomes 2 and 11, and on chromosome 11 the translocation point is upstream of the gene myocardin, a master regulator of smooth muscle expression. Myocardin undergoes alternative splicing specific to the bladder, making it particularly sensitive to changes in myocardin's regulatory mechanisms, resulting the T30H phenotype. In T30H mice there are reduced smooth muscle markers present on staining, and the expression ratio of myocardin splice isoforms is different to wild types using RT-PCR and qPCR.

Using primary cell culture, we have knocked down myocardin expression using a lentiviral vector. This causes both reduced cell growth and reduced expression of smooth muscle cell markers. We have hypothesised that an increase in the level of myocardin expression will increase the growth of smooth muscle cells, and may rescue the T30H phenotype. We have developed a methodology using ultrasound guided injection to deliver AAV9 mediated overexpression of myocardin to the bladder of T30H mice in utero at E16.

The T30H mouse perfectly recapitulates the kidney phenotype in patients with urinary tract obstruction. We have identified myocardin as a key gene involved in correct bladder smooth muscle development, and used it in a model of targeted gene therapy in utero. In the future this approach could be applied to diseases like PUV, where the bladder is non-emptying, but there must be a decrease in the level of muscle growth for normal function.