Identifying Testosterone Producing Hydroxysteroid Dehydrogenase Enzymes in *Hsd17b3*-Deficient Mice

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Testosterone is essential for the regulation of androgen-dependent functions including male sexual development and spermatogenesis. In the adult testis, androgens are synthesised by highly specialised cells, known as the Leydig cells. Adult Leydig cells synthesise testosterone via the canonical androgen biosynthesis pathway, where the HSD17B3 enzyme catalyses the conversion of the androgen precursor androstenedione into testosterone. Consequently, loss of function mutations in the human *HSD17B3* gene results in a disorder of sexual development. 46,XY *HSD17B3*-deficient individuals retain internal Wolffian structures, however the external genitalia is undermasculinised, appearing as female or ambiguous.

Two independent research groups have generated *Hsd17b3*-deficient mouse models. Surprisingly, and in contrast to human cases of *HSD17B3*-deficiency, male *Hsd17b3* knockout mice are masculinised from birth and are fertile in adulthood. Although *Hsd17b3* knockout mice exhibit high androstenedione/testosterone ratios (indicative of HSD17B3 dysfunction), intratesticular testosterone remains normal. This data suggests that mice have compensatory mechanisms/alternative enzymes which enable the continued production of testosterone in the absence of HSD17B3. We aimed to identify alternative hydroxysteroid dehydrogenase (HSD) enzymes that may be responsible for continued testosterone biosynthesis in *Hsd17b3* knockout mice.

We have identified mouse HSD enzymes that can convert the precursor androstenedione into testosterone and validated this conversion *in vitro*. We have demonstrated that a key amino acid in a particular HSD allows it to synthesise testosterone, in contrast to the human enzyme which has a different amino acid and is unable to produce testosterone. To model human androgen production in mice, we developed a humanised transgenic mouse line expressing the HSD that is altered to express the human amino acid and is thus unable to produce testosterone. To determine if this mutated HSD enzyme is unable to compensate for the lack of *Hsd17b3* we have cross bred this humanised HSD mouse line with *Hsd17b3* knockout mice. The humanised mutation in mice has been validated by genomic sequencing and the phenotype has been characterised. Intratesticular and circulating steroid analysis is being performed and will indicate if this HSD plays an important functional role in testosterone biosynthesis in *Hsd17b3* knockout mice.

In conclusion, we are generating mouse models that can be used to better understand human disorders of androgen biosynthesis and can be exploited to identify novel therapies for androgen deficiency.