Characterisation of a Novel Cystic Kidney Disease Model

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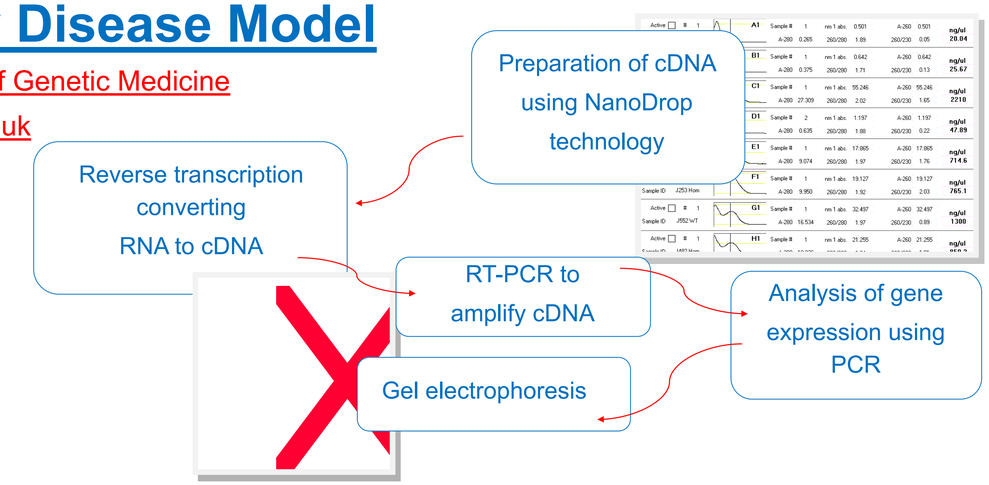
Joubert Syndrome is a genetic disorder characterised by three predominant clinical features;

- Molar tooth sign; due to hypoplasia (underdevelopment) of the cerebellar vermis, large superior cerebellar peduncles and a deep
- interpeduncular fossa
- Hypotonia (low muscle tone)
- Abnormal development

Nephronophthisis is an autosomal recessive cystic kidney disease which presents in juvenile Joubert Syndrome patients

This project aimed to:

- Characterise a novel cystic kidney disease model
- Analyse genetic changes which occur during the onset of nephronophthisis



A workflow diagram representing the methodology followed.

Analysis of Mouse Cell Lines Using B-Geo PCR

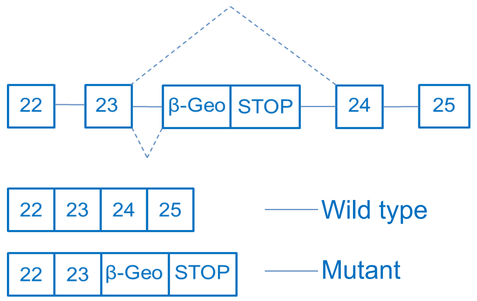


Figure 1: A diagram of the CEP290 290 mutation. Insertion of the β-geo reporter gene occurs such that the resulting mutant has impaired CEP290 protein functioning.

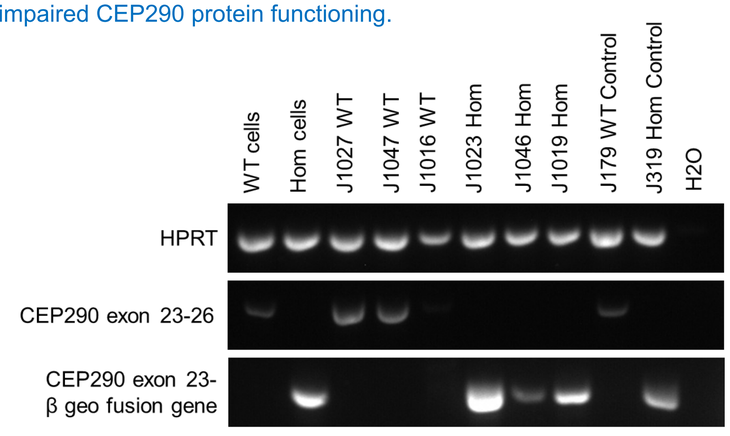


Figure 2: Gel electrophoresis of RT-PCR products enabling identification of mutant cells. The fusion gene is only present in mutant cells. The downstream exons fail to be expressed leading to impaired protein functioning. HPRT is a house keeping protein used as a comparative control.

Characterisation of Kidney Samples and Cell Lines

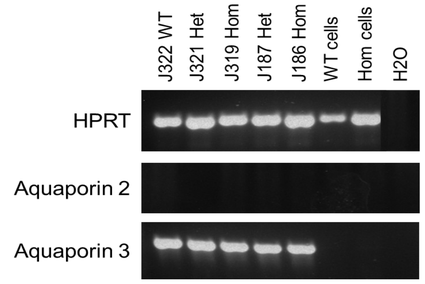


Figure 3: Gel electrophoresis of RT-PCR products primed against aquaporin 2 and 3. The aquaporins are water transporter molecules which have been previously implicated in cystic kidney disease.

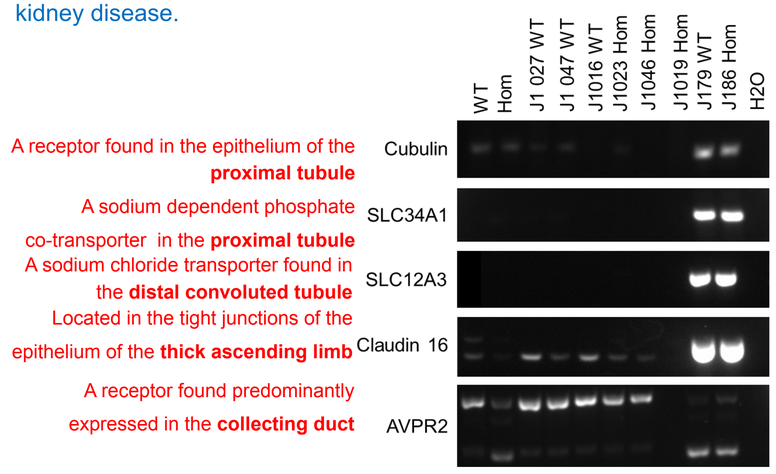


Figure 4: Gel electrophoresis of RT-PCR products following priming with tubule specific markers to allow characterisation of cell lines. Particular note should be paid to the amplification of cubulin, indicative of a proximal tubule cell line.

Analysis of Claudin Expression in Mouse Kidney Samples

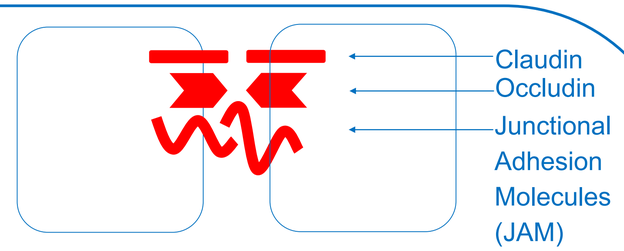


Figure 5: A diagram showing the constituents of a tight junction found paracellularly between epithelial cells.

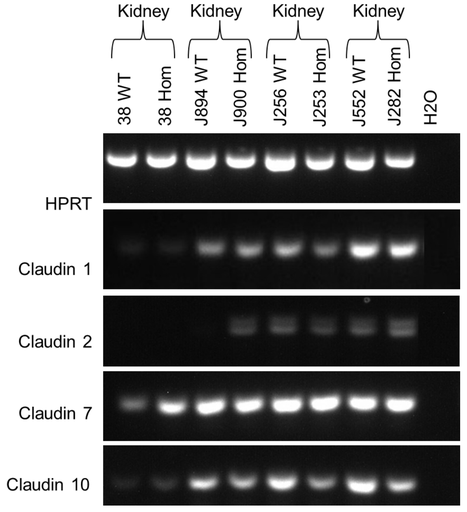


Figure 6: Gel electrophoresis of RT-PCR products following priming of kidney samples with claudin specific primers. This allowed assessment of tight junction genes given the levels of aquaporin expression.

Conclusions

- Claudin 10 expression was markedly reduced in the homozygote kidney samples, particularly in the P32 and 1 year kidney, compared to the corresponding wild-type
- This observation supports the findings of previous microarray data from another cystic kidney disease model implicating Claudin expression in the onset of nephronophthisis
- Such genetic changes could prove to be of therapeutic importance and hence further research would seem beneficial