Genes and Joubert

Identifying genes involved in Joubert syndrome

By Jessica Downing | 140079391 | j.downing2@newcastle.ac.uk | Biomedical Genetics

Research carried out at: The Institute of Genetic Medicine, International Centre for Life, Newcastle upon Tyne, Newcastle upon Tyne

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Introduction

Joubert syndrome (JS) is a rare genetic disorder which affects children. There are three main signs of Joubert:

- Eye sight – retinal degeneration
- Brain - "molar tooth sign"
- Kidneys – Nephronophthisis

Patients with JS also show a huge range of severity, which makes diagnosis and treatment difficult.

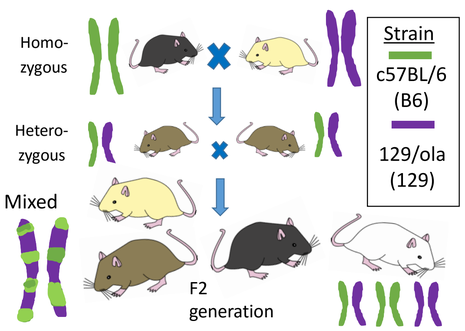
There are already 23 genes which have been identified as important factors in Joubert S, including the gene CEP290.

Aims

- Identify genes involved in Joubert syndrome
- Determine whether different genetic backgrounds affect severity of symptoms
- Use mouse models with the CEP290 mutation that represent JS patients
- Observe areas on the genome that effect severity of kidney disease and retinal degeneration

(Cep290) Mouse Model

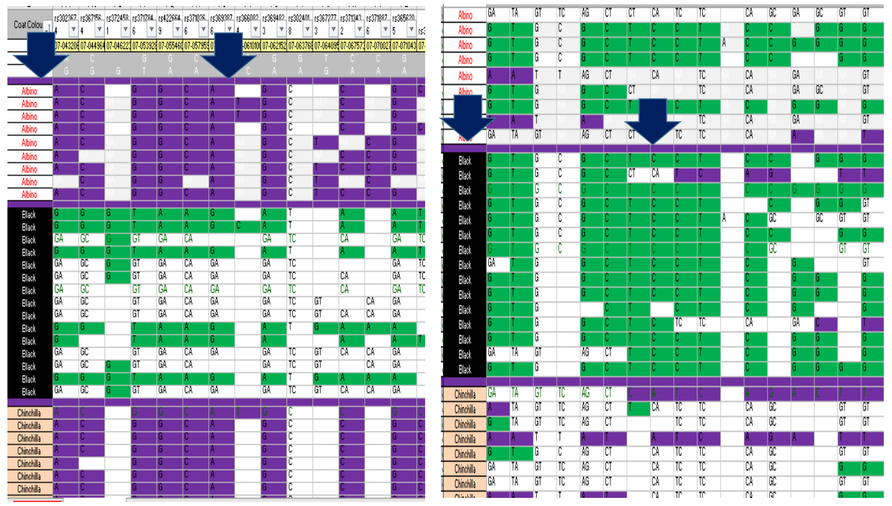
Mouse models were created by causing a deletion of the Cep290 gene, a known gene to cause JS when mutated in JS humans. The mice then model symptoms of human patients, along with mixing two strains to observe different genetic backgrounds.



SNP array - Coat Colour Control

- There are 89 mice and 4 controls. DNA was extracted and used in a SNP (single nucleotide polymorphism) array. This was done using the MassARRAY ® system. The results were then transferred onto Excel format. From Excel the array will show the genetic background of each individual mice at each specific locus.
- The prediction was that mice with a homozygous B6 background will show a higher severity of symptoms.
- In Excel the cells which showed heterozygous for that SNP have a white background, whereas the homozygous B6 were filled in green and homozygous 129 are purple.

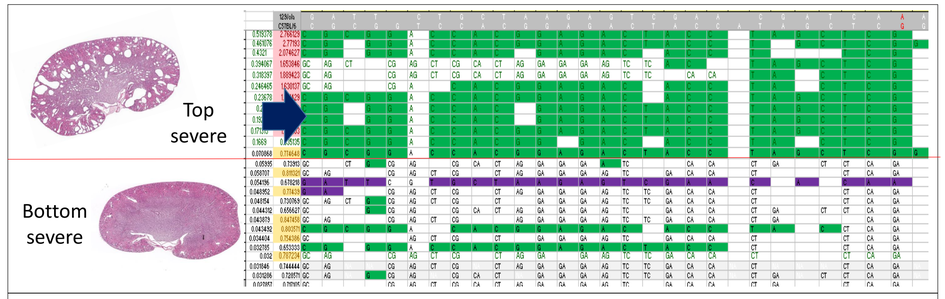
1(a) 1(b)



As a control test the mice were grouped into coat colour because this is a phenotype known to be directly related to the genotype. Above shows the mice split into Albino, black and chinchilla. 1(a) shows a large purple region shared by all albino mice. This part of chromosome 7 contains the gene Oca2 which is linked to albinism. 1(b) shows a block of green (B6 hom.) shared by the black mice. In this region of chromosome 2 there is the 'a' nonagouti gene which causes a black coat.

SNP array – Kidneys

- The mice all have the Cep290 gene deletion and therefore present with kidney disease. However the severity differs between the mice, this is likely due to the different genetic backgrounds.
- Areas of homozygosity of one background (B6 or 129) being present in only the top severe kidneys will suggest a gene in that region which when present creates a more diseased phenotype.
- Many of the kidneys present cysts yet the size of them vary . The size of the cysts were correlated with genetic background, as determined by SNP array analysis to identify particular regions of the genome associated with disease severity.

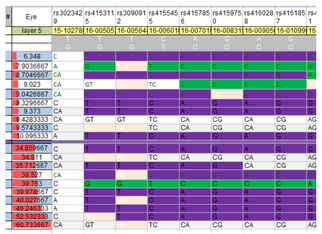


Above shows the SNP data ordered by cystic index. This is a zoomed out view of the dataset representing all mice with the top 16 being the most effected. There is a large block of green present , showing a cluster of the B6 genotype at those loci observed in the most severe mice. This is a region of interest where further identification of genes in that region can be carried out for possible modifiers.

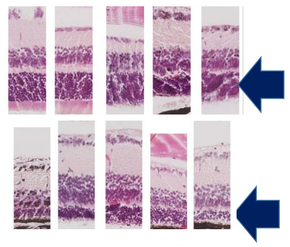
SNP array – Eye

Many JS patients have retinal degeneration ranging from being completely blind or only having minor sight issues.

- The mice eyes were removed and prepared for H and E staining. The eyes were then photographed and rotated at a set size (1.75inX0.5in).
- The eyes have 6 distinguishable layers which were then measured individually.
- One noticeable layer was the outer nuclear layer which varied dramatically in size.
- However when reviewing the SNP data of the most severe (smallest size) to least, no clear relationships between severity and genotype could be seen



Right shows images of retinal layers from the top 5 severe and bottom 5 mice, with the outer nuclear layer highlighted (arrow). The left shows a representative region of the genome showing no clear correlation with severity.



Summary

- Joubert syndrome patients vary in the severity of their symptoms. Research will aid diagnosis of patients by being able to predict how severe their symptoms may be, subsequently aiding treatment options and suggesting novel targets for therapeutic development.
- The experimental approach was validated by examining coat colour genetics.
- Results from the Kidney analysis present an interesting region containing genetic modifiers that make JS more severe.
- More analysis is needed on the eyes using bioinformatic statistical techniques, to evaluate patterns more difficult to see from viewing the data alone. Increasing the number of informative SNPs (blank cells) should facilitate this.

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References:

- Mice and kidney pictures: Dr Simon Ramsbottom
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