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SCIENTIFIC BUSINESS ABSTRACTS

identified potential association (exomewide corrected $P < 0.05$) with variants in several other candidates including RYR1, ZFPM1, CAMTA2, DLX6 and PCM1.

Conclusion: The NOTCH1 locus is the most frequent site of genetic variants predisposing to non-syndromic TOF, followed by FLT4. Together, variants in these genes are found in almost 7% of TOF patients.

(12) Genome editing of haemopoietic stem cells for treatment of thalassaemia

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Biography: James Davies is a clinician scientist with a specialist interest in haemopoietic stem cell transplantation, genome editing, genomics and bioinformatics. He studied medicine at Oxford University before going on to complete specialist training in haematology and intensive care medicine in London. He is an honorary consultant haematologist with the allogeneic transplant service in Oxford at present. He was awarded Wellcome Trust Clinical Research Training Fellowship to do a DPhil with Prof Doug Higgs and Prof Jim Hughes at the Weatherall Institute of Molecular Medicine. This focussed on developing next generation sequencing-based methods for interrogating the physical structure that genes form with their distal regulatory elements. This work lead to important insights into how genes are controlled and he was awarded the RDM Graduate Prize for this project. He has subsequently developed an interest in the clinical application of genome editing technology. In 2017, he was awarded an MRC Clinician Scientist fellowship to leverage his expertise in gene regulation, bioinformatics and development of high throughput sequencing assays to develop novel safe approaches for treating inherited disorders of haemopoiesis, such as thalassaemia.

Aim: Thalassaemia is commonly due to mutations at the beta globin (HBB) locus, and this causes transfusion dependent anaemia in severe cases. A key pathophysiological factor is the imbalance of alpha and beta globin production. This results in accumulation of excess alpha globin chains, which are toxic and cause cell death. Patients who co-inherit partial deletions of the alpha globin genes with beta thalassaemia usually have a mild phenotype and are transfusion independent. We aim to develop genome editing strategies of haemopoietic stem cells to exploit this for use as part of an autologous transplant to treat thalassaemia.

Methods: CRISPR-Cas9 was used to edit the most important enhancer of the alpha globin gene to elicit a controlled reduction in alpha globin expression. In silico methods were used to define the key sequences to delete to abrogate transcription factor binding. This allowed us to develop a strategy to disrupt single transcription factor binding sites using Cas9 ribonucleoprotein.

Results: Our in silico approaches allowed us to define three key transcription factor binding sites within the enhancer. We were able to achieve indel efficiencies in excess of 75% as measured by next generation sequencing. This resulted in a much more controlled reduction in alpha globin expression than was achieved by deletion of the whole enhancer.

Discussion: In silico prediction allows the identification of the sites within enhancers that allow genome editing to be used to reduce gene expression in a highly controlled manner.