

Expression of the human alpha-globin cluster in the absence of the major regulatory element MCS-R2

The alpha globin genes are located in the subtelomeric region of the short arm of chromosome 16. Expression of each allele is controlled by 4 distal *cis*-acting enhancers (MCS-R1-4) located 48, 40, 33 and 10kb upstream of the duplicated structural alpha genes . Experimental and clinical studies have shown that R1 and R2 are the most important enhancers of alpha-globin production. Here we present an individual with alpha-thalassemia who inherited one copy of chromosome 16 from which both alpha genes have been deleted (--^{SEA}). This individual's other copy of chromosome 16 harbours a small deletion of R2. Thus all alpha globin expression in this individual originates from one copy of the alpha cluster in which R1 drives expression of the two alpha-globin genes in *cis*. Remarkably, this patient maintains a relatively normal degree of oxygenation despite a severe degree of alpha thalassaemia.

This female patient was born in Surinam and her mother and father were of Indian and Indonesian origins respectively. She most recently presented, aged 26 yrs, with tiredness and shortness of breath on exertion. She had no previously diagnosed medical conditions, but had received two blood transfusions as a child, both prompted by concurrent infections. Her growth and development were normal. She had suffered one miscarriage at 13 weeks gestation aged 25 yrs. There was nothing of note in the family history and the patient is currently employed as a clerical worker. Cardiovascular examination was normal with no hepatosplenomegaly. Her CBC was consistent with HbH disease, with a Hb of 73g/L, Hct 25%, MCV 45fl, MCH 13.2pg with a blood film showing marked microcytosis anisopoikilocytosis, target cells and 30.7% HbH inclusions on Brilliant Cresyl Blue staining. HPLC showed evidence of a fast-moving band, HbA 88.4% and HbA₂ 1.3%, HbE 5.1%. MLPA analysis showed that the father and proband both carried heterozygous deletions of R2, and the mother and proband both carried the --^{SEA} deletion on one allele. Due to the severity of the anaemia and the striking red cell indices it was highly likely that she had intact regulatory elements in *cis* with the --^{SEA} deletion on one allele, and on the other, a regulatory network missing R2 lying upstream of the two intact alpha-globin genes

To investigate this further, CD34+ cells were extracted from the patient's peripheral blood and differentiated using a liquid culture system (Trakarnsanga *et al.* Nature Comms. 2017). The alpha/non-alpha-globin synthetic ratio of mRNA as measured by qPCR at peak globin-production was markedly reduced compared to WT cells and those of a patient carrying the --^{SEA} deletion on one allele alone: WT 1.26, SEA 0.65, Patient 0.11.

The upstream enhancers are associated with regions of open chromatin that can be detected by chromatin accessibility assays. To see whether the deletion of R2 had caused new regulatory elements to appear or whether accessibility of the existing elements was altered, ATAC-seq was performed on Day 13 of differentiation. This showed a reduction in the peak at R2 in keeping with its deletion on one allele, but showed no change in the peak height of R1 compared to that in the individual with the --^{SEA} deletion alone or WT. To assess chromatin interactions across the domain Capture-C, a sensitive assay to detect interactions between selected regions of chromatin, was performed (Davies *et al.* Nature Methods 2016). Compared to WT, when capturing from the alpha-globin promoters

interactions with R1, R3 and R4 were observed with no interactions with R2 detected, in keeping with the hypothesised distribution of deletions. No interactions *in trans* between the intact R2 and alpha-globin genes were detected.

Previous cases of deletions of the upstream alpha-globin regulatory element have either involved deletions of the regulatory elements on one allele only, or involved both alleles but with a full complement of alpha-genes. Our findings show that whilst a severe HbH phenotype was observed, it is possible to maintain transfusion independence with only R1, R3, R4 and two alpha-globin genes. Considering the ATAC-seq and Capture-C findings, as well as comparison with the homologous locus in mouse it is likely that the bulk of regulation in this patient occurs via R1. The observed phenotype is consistent with redundancy within the regulatory elements of the alpha gene locus, which may enable continued expression of key cell-specific genes in the event of adverse genotypic events.