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Use of next generation sequencing to identify AML with germline predisposition; an essential diagnostic test

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INTRODUCTION

Acute myeloid leukaemia (AML) with biallelic mutation of *CEBPA* is a distinct subtype of AML with a favourable prognosis. In approximately 10% of cases, the mutation of one *CEBPA* allele is germline enabling a classification of "AML with germline *CEBPA* mutation". Patients with a germline mutation typically present at a young age and are prone to multiple relapses. This prospect brings stem cell transplant (SCT) into the management plan and thus the need for timely genetic screening of related, potential donors.

Here we report on a case of AML with germline *CEBPA* mutation detected at 1st relapse, how it reflects advances in molecular diagnostics and impacts on patient management.

CASE

Presentation
The patient initially presented aged 5 with pancytopenia and circulating blasts. He had no past medical history and no known family history of haematological malignancy. The diagnosis of AML was confirmed by bone marrow aspirate (BMA) with a typical immunophenotype (CD34+, CD117+, CD13+, CD33+, CD117+, CD7+). A normal karyotype was observed (46, XY) with no mutation within *FLT3* or *NPM1*.

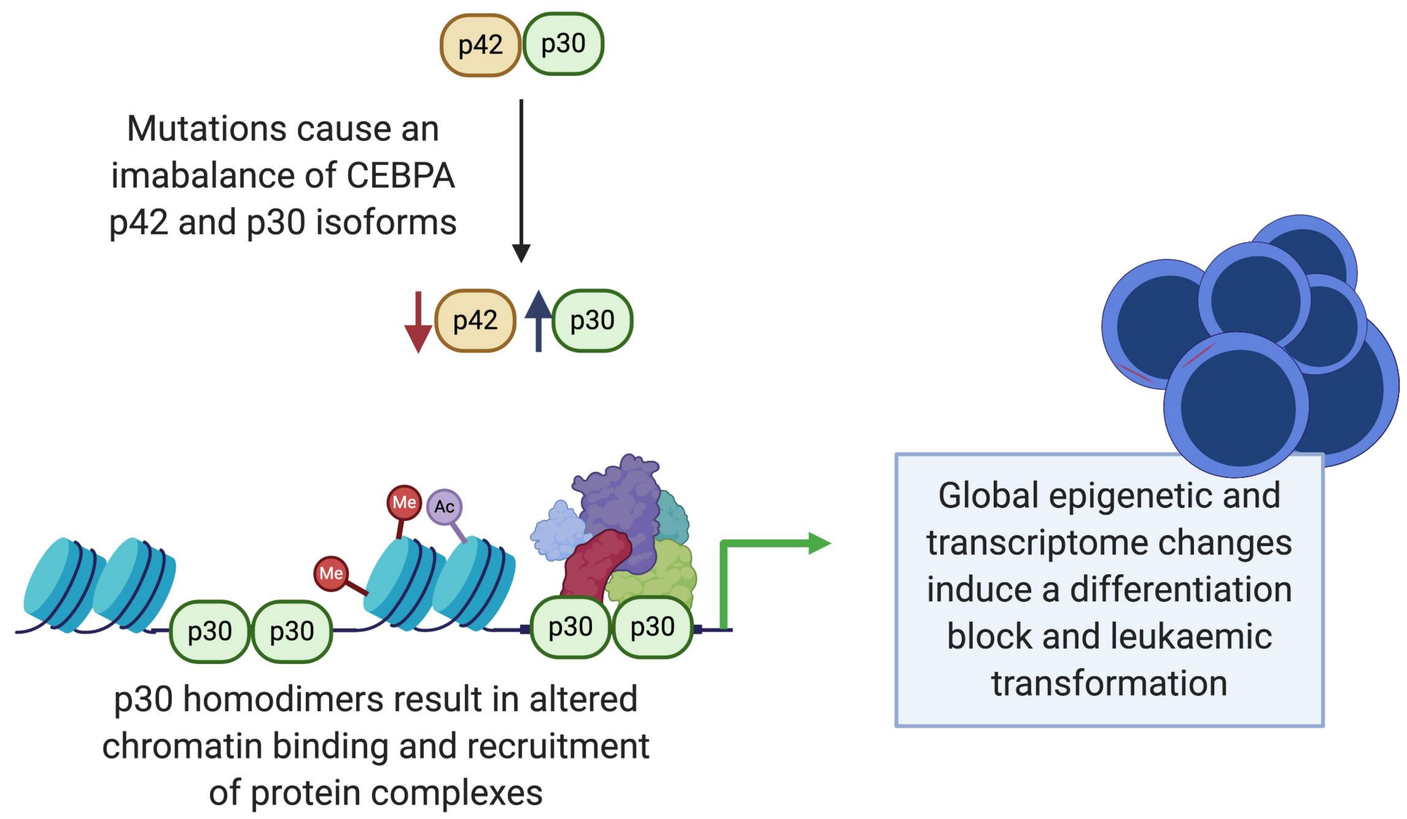
Initial treatment
He was recruited to the MRC AML15 trial and treated with a non-transplant protocol (2 cycles of FLAG-Ida induction and 3 cycles of high dose cytarabine consolidation). He achieved CR1 and completed treatment without significant complications. There was no evidence of long-term therapy related toxicity on routine follow up.

Relapse
Twelve years later, aged 17, he presented with a persisting upper respiratory tract infection. A full blood count demonstrated a neutropenia ($1.59x10^9$/L) with circulating blasts (<5%ANC). Further investigations demonstrated AML. The immunophenotype was identical to that at diagnosis. The karyotype was also normal (46, XY). No mutation within *FLT3* or *NPM1* was observed. As the initial working diagnosis was therapy-related AML he was commenced on Vyxeos chemotherapy with a plan for allogeneic SCT at CR1.

Next generation sequencing
To further characterise his disease, a next generation sequencing (NGS) panel was performed (Oncomine Myeloid Assay, Thermofisher). Two pathogenic variants within *CEBPA* were identified: c.190dup [p.(Ile64Asnfs*44)] and c.931_932insGAC [p.(Thr310_Gln311insArg)]. The disease was thus classified as AML with biallelic *CEBPA* mutation. Of note, the variant allele frequency (VAF) of the c.190dup variant was 52% whereas the VAF of the c.931_932insGAC variant was 33% raising the possibility the c.190dup variant represented a germline mutation. NGS and conventional sequencing of the relevant *CEBPA* region in DNA from a subsequent remission sample and saliva sample demonstrated the c.190dup but not the c.931_932insGAC variant.

These results indicate the presence of a germline *CEBPA* mutation with subsequent acquisition of a second somatic *CEBPA* mutation, consistent with the molecular pathogenesis of this disorder.

CEBPA IN AML



Normal function of CEBPA in haematopoiesis
CEBPA has an important role in regulating haemopoietic stem and progenitor function and myeloid lineage differentiation. CEBPA exerts multiple effects on epigenetic and transcriptome regulators. *CEBPA* mRNA can result in the expression of a full-length protein, a p42 and a p30 isoform. The p42:p30 ratio is critical in determining cell fate and is kept in strict homeostatic balance.

CEBPA in AML
CEBPA mutations are present in approximately 15% of cases of AML and have been a focus of interest due to an association with a favorable prognosis. There are three possible mutant patterns: 1. AML with single *CEBPA* mutation (~50% of cases), 2. AML with biallelic *CEBPA* mutation (as in the case presented), 3. AML with biallelic *CEBPA* mutation due to loss of heterozygosity.

The exact mechanisms of leukaemia pathogenesis with mutated *CEBPA* is not fully known. However, mutations of *CEBPA* lead to an imbalance between the p42 and p30 isoforms with a premature stop on p42 translation. Increased p30 levels are associated with immature cell state and inhibition of termination differentiation in myeloid cells. P30 binds chromatin directly and interacts with different co-factors of gene regulation than when in a heterodimer state with p42. This includes global epigenetic regulators such as *KMT2A*. Overall, *CEBPA* mutations have a genome wide impact on epigenetic and transcriptional regulation which confers a pro-leukaemogenic cell state.

CONCLUSIONS

CEBPA variants are often complex insertion/deletions. These results demonstrate successful incorporation of an amplicon based NGS strategy for the identification of germline and somatic *CEBPA* variants in AML with a normal karyotype and the importance of germline assessment in patients with AML with biallelic *CEBPA* mutation.

In retrospect the clinical presentation is typical of AML with germline *CEBPA* mutation. Beyond implications for prognosis, family screening and family planning the presence of a germline mutation is particularly important to establish in the context of a planned related donor SCT. New pathways must therefore be developed to allow timely access to Clinical Genetics.

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CEBPA in AML figure created with BioRender.com

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