Familial Cancer (2009) 8:581–584
DOI 10.1007/s10689-009-9289-x

Molecular study of *CEPBA* in familial hematological malignancies

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Published online: 3 September 2009

Abstract Familial aggregation in patients with several haematological malignancies has been described, but the genetic basis for this familial clustering is not known. Few genes predisposing to familial haematological malignancies have been identified, among which *RUNX1* and *CEBPA* have been described as predisposing genes to acute myeloid leukemia (AML). Recent studies on *RUNX1* suggest that germline mutations in this gene predispose to a larger panel of familial haematological malignancies than AML. In order to strengthen this hypothesis, we have screened *CEBPA* for germline mutations in several families presenting aggregation of hematological malignancies (including chronic or acute, lymphoid or myeloid leukemias, Hodgkin's or non Hodgkin's lymphomas, and myeloproliferative or myelodysplastic syndromes) with or without solid tumours. Although no deleterious mutations were found, we report two novel and rare variants of uncertain significance. In addition, we confirm that the in frame insertion c.1175_1180dup (p.P194_H195dup) is a germline polymorphism.

Keywords *CEBPA* · Germline mutation · Haematological familial malignancies

The CCAAT/enhancer-binding protein alpha (C/EBPα or CEBPA) is the founding member of a family of related leucine zipper transcription factors that play important

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roles in myeloid differentiation [1]. C/EBPα expression is predominant in myelomonocytic cells and is specifically upregulated during granulocyte differentiation [2]. This protein is encoded by the *CEBPA* intronless gene that spans 2,783 bp long and maps to human chromosome 19q13.1 (Genebank accession number NM_004364).

On the basis of the observation that C/EBPα-deficient mice lack mature granulocytes [3], it has been speculated that *CEBPA* mutations might contribute to the differentiation block specific to acute myeloid leukemia (AML). According to this hypothesis, somatic *CEBPA* mutations, have been reported in various studies, and are found in 5–14% of AML patients [4]. These mutations consist in N-terminal frameshift abnormalities, leading to increased translation of a 30 kDa isoform with dominant-negative properties, or in frame insertions/deletions disrupting the bZIP domain (bipartite DNA-binding domain and "leucine zipper" dimerization region) located in the C-terminus.

Recently, Fuchs et al. [5] reported the presence of somatic *CEBPA* mutations in patients with hematological malignancies other than AML. Namely, they found *CEBPA* mutations in 4.2% of patients with myelodysplastic syndrome (MDS), in 3.6% of patients with non Hodgkin's lymphoma (NHL) and in 5.1% of patients with multiple myeloma (MM).

Moreover, germline *CEBPA* mutations have been identified in patients affected by AML from five different families [6–10]. Up to 1.1% of all the AML patients included in Pabst et al. study carried a germline *CEBPA* mutation [8] suggesting that germline *CEBPA* mutations are a more frequent event than commonly anticipated.

Another gene has been described as a candidate to AML development, the *AML1* gene (*RUNX1*). A recent study suggests that germline mutations predisposing to AML might also predispose to ALL (Acute lymphoblastic leukemia) [11]. Similarly we hypothesize that germline *CEBPA* mutations might be predisposing to familial hematological malignancies other than AML.

In consequence, and for the first time to our knowledge, we screened *CEBPA* for germline mutations, in large collection of patients presenting familial aggregations of hematological malignancies and solid tumours. Our aim was to determine the incidence of germline *CEBPA* mutations in familial hematological malignancies and better understand the role of *CEBPA* as a general inhibitor of cell proliferation and tumour suppressor.

We sequenced the entire *CEBPA* coding region in 119 patients belonging to 99 independent families recruited through a national cooperative network supported by the French National Cancer Institute (INCa) committed to familial hematological malignancies (call for proposal 2005). The sample is constituted of 95 patients belonging to 75 familial forms of hematological malignancies (at least two cases of hematological malignancies with or without solid tumours in first, second or third degree relatives); 23 patients from 23 families with aggregation of tumours including one case of hematological malignancy in first, second or third degree relatives; and 1 patient who had a multiple primitive tumour with hematological malignancy but without family history. Fourteen families from a total of 99 included AML (11 familial aggregations of hematological malignancies and 3 familial aggregations of hematological malignancy and other solid tumours).

Mutation screening was performed on genomic DNA extracted from peripheral blood cells after obtaining informed consent from patients in accordance with the Declaration of Helsinki. Peripheral blood samples were obtained during complete remission (CR). Genomic DNA was extracted using standard procedures.

All primers and PCR conditions are available under request. Amplified PCR products were purified (Qiagen Inc, Valencia, CA, USA) and both strands were sequenced using BigDye Terminator chemistry implemented on an ABI Prism 3130 sequencer (Applied Biosystems). Sequences were analyzed and compared to reference sequences using the software package Seqscape (v2.5, Applied Biosystems).

Table 1 summarizes the results of *CEBPA* screening. Sequences alignment and comparison allowed us to identify two novel DNA variations: c.724G>A and c.111G>A (Genebank accession number NM_004364), respectively in

Table 1 Summary of results of *CEBPA* mutation screening in a series of 119 patients

Nucleotide change[a]	Predicted protein change	Occurence	Familial context	Reference
c.111G>A	p.Ala37Ala	1	FHM with solid tumours	Our study
c.573C>T	p.His191His	3	FHM with or without solid tumours	[2, 5]
c.584_589dupCACCCG	p.Pro194_His195dup	20	FHM with or without solid tumours, 1 HM and solid tumours	[2, 5]
c.690G>T	p.Thr230Thr	19	FHM with or without solid tumours, 1 HM and solid tumours PMT with 1 HM	[2, 5]
c.724G>A	p.Gly242Ser	1	FHM with solid tumours	Our study

FHM familial hematological malignancies, *HM* hematological malignancy, *PMT* primitive multiple tumour

[a] Numbering according to Genebank accession number NM_004364

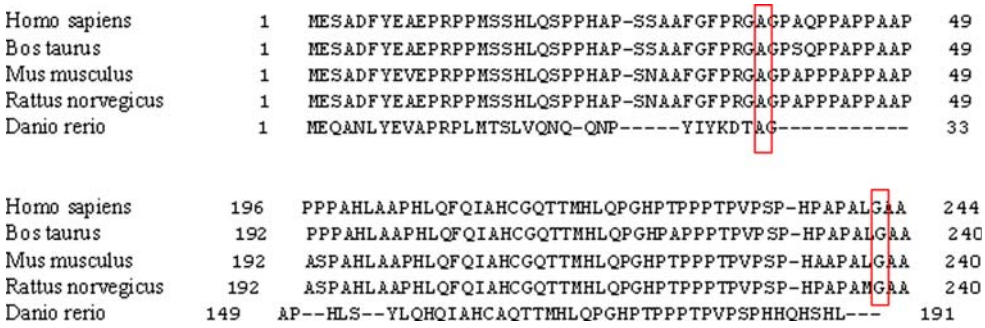

Fig. 1 Sequence alignments of C/EBPα protein between different species. In *black box* are shown the 37 and 242 amino acid positions implicated in the two nucleotide changes

two (distinct) patients, namely 0,8% for each. Up to now, these two variants had not yet been reported. The first one is a missense variant (c.724G>A, p.Gly242Ser) and the second one is a silent nucleotide change (c.111G>A, p.Ala37Ala). Interestingly, these two nucleotide changes were not detected in 163 screened controls. The two patients carrying these variants are issued from families affected with hematological malignancies with solid tumours. The c.724G>A substitution in a patient with CLL diagnosed at 37 years and the c.111G>A transition was identified in a patient affected by NHL diagnosed at 45 years.

In addition two already described silent polymorphisms were identified: the (c.690G>T) and the (c.573C>T) in 19 (16%) and 3 (2.5%) of 119 patients, respectively. They were previously described as polymorphisms by various studies in same proportions [2, 5].

Moreover, we detected a 6 bp in-frame insertion in the second transactivation domain (TAD2) (c.543_589dupCACCCG, p.Pro194_His195dup) in 20 patients (17%). This polymorphism was first reported as a mutation [2, 12]. Also, we found that, the c.584-589dup is observed in 10% of the tested controls confirming the last reported data [5, 13–15] indicating that this duplication is a common population polymorphism.

The significance of the two novel alterations reported here is more uncertain and difficult to define. Considering that they were never found before in more of 1,400 AML patients described in the literature and that they were not found in 163 controls (this study), and that they were identified in patients with hematological malignancies other than AML, we could not exclude that they were germline deleterious mutations. Moreover, Fuchs et al. detected novel *CEBPA* mutations in MDS (myelodysplastic syndrome), MM (multiple myeloma) and NHL (Non Hodgkin lymphoma) patients suggesting a possible specific mutation profile according to the hematological pathology [5]. Finally, these two amino acids are conserved among human, mouse, bull, rat and zebrafish (Fig. 1).

We underline that the c.724G>A substitution occurred in a patient that developed CLL at a young age, that may reveal implication of predisposing *CEBPA* genetic alteration. It would be interesting to test *CEBPA* in patient's relatives and other familial aggregations of haematological malignancies.

On the contrary, we could then hypothesize that these substitutions are rare polymorphisms of *CEBPA* gene rather than pathologic mutations, because they did not affect a functional domain of C/EBPα.

Extreme caution should be taken when classifying genetic alterations as either causative mutations or polymorphisms. As reported by Fröhling et al. at least three steps are necessary to identify pathogenic mutations. First, identified sequence variations should be absent in normal control samples. Second, analysis of germline DNA and somatic DNA should be analyzed consecutively. And finally, the functional consequences are experimentally tested [16].

Although the 2 new mutations that were found in this study occurred in the coding region of the CEBPA gene and none of them observed in the normal control group, further functional studies may be of value to distinguish "driver" mutations underlying oncogenesis or clinicance relevance from biologically neutral "passenger" alterations.

We report the first study of *CEBPA* gene in a familial hematological malignancies context, to our knowledge. Further investigations are needed to differentiate polymorphisms from mutations before clinical correlation and prognostic impact of CEBPA mutations could be ascertained in any clinical studies.

Acknowledgments This work was supported by la Société Française d'Hématologie, le Groupe Génétique et Cancer and l'Institut National du Cancer INCa. We would like to thank Dr. Valérie Delague for helpful discussions and all technicians of laboratory for their expert technical assistance.

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