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Impact of the Functional *CD5* Polymorphism A471V on the Response of Chronic Lymphocytic Leukaemia to Conventional Chemotherapy Regimens

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Summary

The CD5 lymphocyte receptor -a *bona fide* immunohistochemical marker of chronic lymphocytic leukaemia (CLL) cells- is a negative regulator of activation signals from the antigen-specific B-cell receptor (BCR). Given that signalling components of the BCR are important contributors to the variable clinical behaviour of CLL, the relevance of functional variants of CD5 on CLL prognosis was explored. The results show that germline-encoded *CD5* variants influence the survival to conventional chemotherapies from CLL patients with unmutated *IGVH* genes. This result supports the notion that CD5 is not only a phenotypic marker but a relevant player in CLL cell biology.

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Competing interests
The authors have no competing interests.

Author contributions
All authors were involved in the design and approval of the final manuscript. FL supervised the work and wrote the manuscript. JD analyzed the data and wrote the manuscript. TB, LB, EC-M and EB performed the genetic analyses. XP, DC and EC provided the patient's samples and clinical data.

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Keywords

chronic lymphocytic leukaemia; *CD5* polymorphism; B-cell receptor signalling; B1a lymphocytes; progression-free survival

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Introduction

Chronic lymphocytic leukaemia (CLL) is considered the result of abnormal clonal expansion of mature antigen-experienced $CD5^+$ B1a cells (Chiorazzi *et al*, 2005). Such leukemic cells are resistant to apoptosis and present with a highly heterogeneous clinical course depending on several associated biological parameters such as the presence or absence of chromosomal aberrations, somatic mutations within the immunoglobulin variable heavy chain genes (*IGHV*), and surface CD38 or intracytoplasmic ZAP-70 expression. The fact that key signalling components of the B-cell receptor (BCR) are relevant contributors to the variable clinical behaviour of CLL (Stevenson *et al*, 2011) made us to explore the influence of functionally relevant germline variants of *CD5*, a *bona fide* phenotypical marker of CLL cells, on CLL prognosis. The rationale behind this is that CD5, a lymphocyte surface receptor normally expressed in all T cells and the B1a cell subset (Soldevila *et al*, 2011), is considered a negative modulator of intracellular signalling mediated by the antigen-specific receptor present on both T (TCR) and B1a (BCR) cells (Tarakhovsky *et al*, 1995; Bikah G et al, 1996), to which physically associates (Beyers *et al*, 1992; Lankester *et al*, 1994). Moreover, in both normal and leukemic B cells, CD5 signalling is also relevant for production of the B1a cell autocrine growth factor IL-10 (Gary-Gouy *et al*, 2002), as well as for acquisition of a common gene transcription signature (Gary-Gouy *et al*, 2007).

Although no human CD5 deficiencies have been reported so far, recent evidence show the existence of common nonsynonymous *CD5* single nucleotide polymorphisms (SNPs) of functional relevance to CD5-mediated signal transduction (Carnero-Montoro *et al*, 2012). Two of them, rs2241002 (C>T) and rs2229177 (C>T), coding for amino acid changes at the extracellular (P224>L) and cytoplasmic (A471>V) regions of CD5, respectively, conform haplotypes that have been subjected to positive selection in recent human evolution. The functionality of those SNPs is demonstrated by the fact that homozygous carriers of the ancestral P224-A471 (CC) haplotype are less efficient than the more recently derived P224-V471 (CT) haplotype in providing inhibitory signals to TCR-mediated activation responses and are associated with more clinically aggressive forms of systemic lupus erythematosus (Cenit *et al*, 2014). In light of this evidence, we decide to analyse the putative influence of the *CD5* SNPs rs2241002 and rs2229177 on clinical outcome of a series of CLL patients from our Hospital.

Materials and Methods

Patient selection

CLL cases with DNA available and information on morphologic and immunologic diagnosis of CLL according to the WHO classification, age, sex, Binet stage, date and type of frontline

CLL-specific therapy, date of progression after frontline therapy and follow-up was retrospectively screened.

Analysis of *IGHV* mutation status, CD38 and ZAP-70 expression, and chromosomal abnormalities were performed using conventional methods (Crespo *et al*, 2003). The latter were classified according to Dohner's hierarchical model and subdivided in unfavourable (17p and 11q deletion) or favourable (others). Standard criteria were used for both initiation of therapy and response evaluation.

Evaluation of P224L and A471V polymorphisms

DNA samples were genotyped for the analyzed SNPs rs2241002 (C>T, Pro224>Leu) and rs2229177 (C>T, Ala471>Val) using predesigned TaqMan assays (Applied Biosystems, Foster City, California, USA) with IDs: C_3237272_10 and C_25472293_20. In 505 patients, the SNP results were directly retrieved from whole genome/exome sequencing data (Puente *et al*, 2015).

Statistical analysis

Baseline characteristics and prognostic factors were compared by the Chi-square or Fisher exact tests for discrete variables, and the Mann-Whitney or Kruskal-Wallis tests for continuous variables. The primary endpoints for comparison were progression-free survival (PFS) from first line therapy and overall survival (OS) from diagnosis. Kaplan-Meier curves were plotted and the effect of the different variables assessed using the log-rank test. Variables analyzed were FISH abnormalities (unfavourable vs. favourable), CD38 and ZAP-70 expression (positive *vs.* negative), *IGHV* status (mutated *vs.* unmutated), B2M (<2 *vs.* ≥2 times the upper limit of normal) and V471A polymorphism. Unadjusted two-sided $P<0.05$ were considered significant. Statistical analyses were performed using R, version 3.2.2.

Results

The characteristics of the subject study population are shown in Table 1. In total, 935 patients from our CLL database had DNA samples available for study. Median age was 63 years (range, 18-98 years) at CLL diagnosis. Binet stage was A in 83% of patients, B in 12% of patients, and C in 5% of patients. Results of FISH, CD38 expression, ZAP70 expression and *IGHV* mutation status were available in 81%, 77%, 83% and 79% of patients. Median follow-up was 34 months (range, 1-158) from frontline therapy and 93 months (range, 1-471) from diagnosis. Four hundred and seventeen (44%) patients required therapy at least once. The most commonly used chemotherapeutic agents were fludarabine, alone or in combination (37%), chlorambucil (28%), COP/CHOP chemotherapy (17%) and cladribine (6%). Only 10% of these patients received rituximab as part of their frontline therapy. The A471V genotypes were available in 903 patients, and undetermined in the remaining 32 patients. Genotypes CC, CT and TT were observed in 202 (22%), 432 (48%) and 269 (30%) patients, respectively. Regarding P224L, genotypes CC, CT and TT were documented in 575 (63%), 316 (34%) and 29 (3%) patients, respectively (undetermined in 15 patients). There

were no significant differences across all different A471V genotypes in terms of age, sex, prognostic factors, therapy administered or follow-up (Table S1).

Median PFS from frontline therapy was 46 months (95% confidence interval: 38-52 months) for those patients who required therapy. Known adverse prognostic factors, such as high ZAP-70 expression ($P < 0.001$), unmutated *IGHV* status ($P < 0.001$), adverse FISH aberrations ($P = 0.002$) and high CD38 expression ($P = 0.007$) had a significant impact on PFS when the entire cohort was analysed. The A471V polymorphism had a trend towards a significant impact on PFS ($P = 0.052$), while P224L had no significant effect on this outcome.

In light of these results, only patients who required therapy but were homozygous for the most prevalent P224L genotype (P224P, CC) were selected for evaluation of the impact of the A471V polymorphism. Baseline characteristics of this selected population of patients are displayed in Table 1. Age, sex, prognostic factors, therapy and follow-up were well balanced across all three genotypes. High ZAP-70 expression ($P = 0.001$), high CD38 expression ($P = 0.008$), and unmutated *IGHV* status ($P < 0.001$) were equally significant in terms of PFS in this selected population (Fig. S1), as was the A471V genotype. Indeed, patients carrying a CC or CT A471V genotype had a significantly prolonged median PFS compared to TT carriers (51 vs. 41 months, $P = 0.024$) (Fig. 1A). Furthermore, this prognostic impact was particularly evident in patients with mutated *IGHV* (110 vs. 40 months, $P = 0.028$), and not in patients with unmutated *IGHV* (36 vs. 47 months, $P = 1.0$) (Fig. 1B).

Finally, we also analysed overall survival but neither A471V, P224L nor the combined effect had any significant impact on any of these outcomes, although there was a trend towards a significantly longer overall survival for patients carrying a CC or CT genotype for A471V but only in patients with P224P (CC) genotype and mutated *IGHV* genes ($P = 0.073$) (Fig. S2).

Conclusions

The present results indicate that CLL patients either homo- or heterozygous for the ancestral A471 (C) allele of *CD5* have a significantly prolonged PFS, but exclusively in cases with mutated *IGHV* genes. This indicates that the influence of the *CD5* A471V SNP in PFS is only apparent in less aggressive CLL cases such as those presenting mutated *IGHV* genes. These results are reminiscent of those from a previous single report on the prognostic impact of the *CD5* A471V SNP in patients with CLL requiring therapy (Sellick *et al*, 2008). In that study, the more recently derived V471 (T) allele was associated with a shorter PFS independently of *IGHV* mutational status. In our study, this effect was particularly evident when we restricted the analysis to patients with the P224P (CC) genotype and mutated *IGHV* genes, and non-existent in patients with unmutated *IGHV* genes. In this regard, it is important to highlight that nearly all unmutated *IGHV* CLL cases contain mutations in driver genes, most of them conferring a bad prognosis and faster progression (Puente *et al*, 2015). By contrast, most mutated *IGHV* cases have mutations in better prognosis drivers such as *MYD88* or del(13q14), resulting in a less aggressive disease. These differences might allow the observation of the protecting effect of *CD5* polymorphisms in mutated

IGHV cases, but not on unmutated *IGHV* ones. How the *CD5* A471V variants can influence the natural behaviour of CLL cells is an unresolved question. However, based on the lower signal transducing capability of the A471 variant (Carnero-Montoro *et al*, 2012; Cenit *et al*, 2014), this may result in either 1) a lower capability for negatively modulating the antigen-receptor (BCR) mediated signalling (Soldevila *et al*, 2011), or 2) a lower anti-apoptotic signalling (Tibaldi *et al*, 2011). Whatever the case, the present results support the notion that CD5 is not only a phenotypic marker but a relevant player in CLL cell biology as demonstrated by the influence of germline-defined functional *CD5* variants on CLL outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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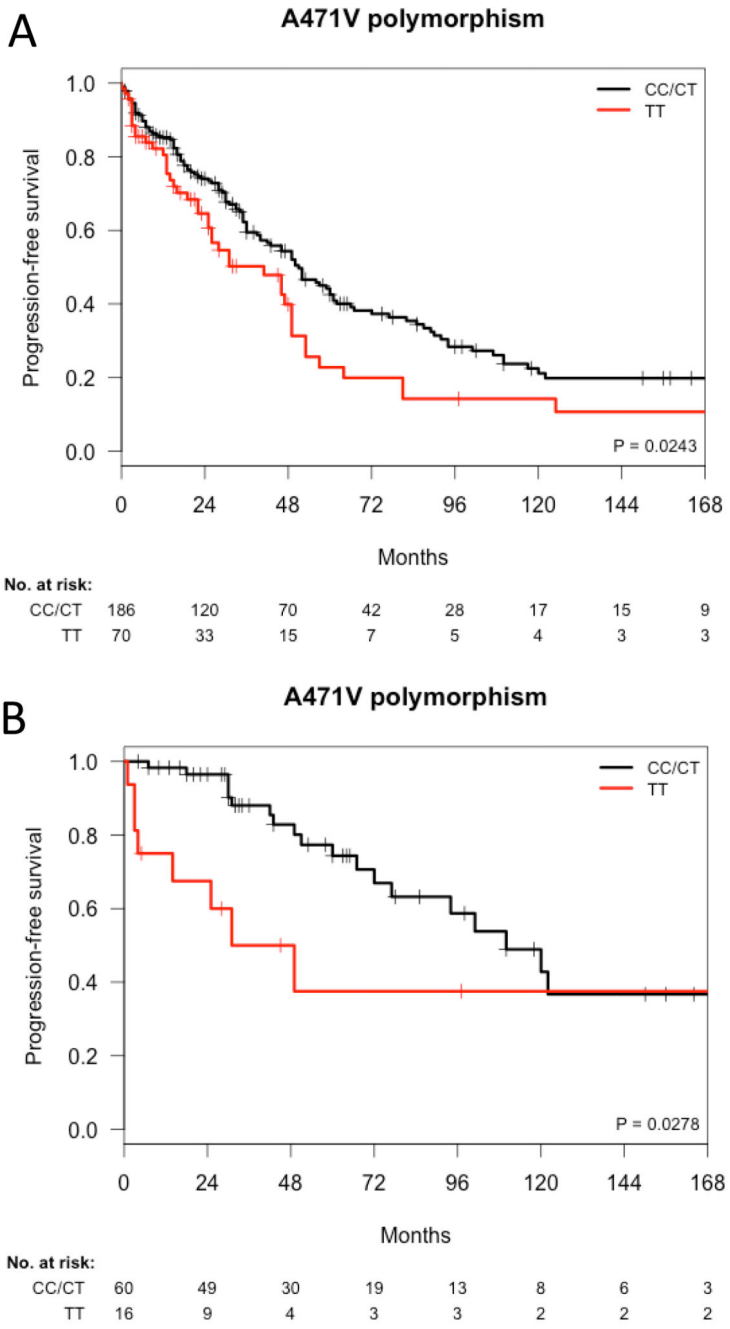

Fig. 1.

(A) Progression-free survival according to the A471V polymorphism in patients who required CLL-specific therapy and had a homozygous P224P (CC) genotype (P = 0.024).

(B) Progression-free survival according to the A471V polymorphism in patients who required CLL-specific therapy, and had a homozygous P224P (CC) genotype and mutated *IGHV* (P = 0.028).

Table 1

Baseline characteristics according to the A471V polymorphism of patients who required CLL-specific therapy and had a homozygous P224P (CC) genotype

A471V genotype	**CC (*n* = 156)**	**CT (*n* = 249)**	**TT (*n* = 141)**	***P* value**
Age (y), median (range)	65 (32-93)	64 (28-98)	63 (18-94)	.855
Sex, % male/female	58/42	53/47	60/40	.280
Binet stage B or C, *n* (%)	26 (17)	40 (16)	14 (10)	.180
Serum beta2-microglobulin (mg/l), median (range)	1.9 (1.0-33)	2.1 (1.0-17)	2.2 (1.5-11)	.129
Positive CD38 expression, *n* (%)	33 (27)	59 (31)	27 (24)	.445
Positive ZAP70 expression, *n* (%)	34 (25)	49 (24)	36 (30)	.429
Unmutated *IGHV* gene, *n* (%)	47 (37)	66 (33)	45 (40)	.464
FISH aberrations (Döhner's hierarchical model):				.502
Favorable (13q-,+12, none)	105 (81)	180 (86)	96 (83)	
Unfavorable (11q-, 17p-)	24 (19)	29 (14)	19 (17)	
Frontline therapy:				.412
Chlorambucil, *n* (%)	21 (32)	29 (28)	14 (22)	
CHOP/COP, *n* (%)	11 (17)	14 (14)	6 (10)	
Fludarabine mono/combo, *n* (%)	22 (33)	36 (35)	21 (33)	
Cladribine monotherapy, *n* (%)	5 (8)	4 (4)	6 (10)	
RCHOP, *n* (%)	1 (1)	0 (0)	2 (3)	
RFC/RFCM, *n* (%)	2 (3)	10 (10)	5 (8)	
Others, n (%)	4 (6)	9 (9)	9 (14)	
Follow-up from frontline therapy (mo), median (range)	49 (4-150)	34 (3-158)	28 (4-143)	.056
Follow-up from B-CLL diagnosis (mo), median (range)	101 (3-314)	87 (4-463)	86 (4-277)	.313

Abbreviations: FISH, fluorescent in-situ hybridization; B-CLL, B-cell chronic lymphocytic leukaemia; IGHV, immunoglobulin heavy chain variable region; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; COP, cyclophosphamide, vincristine and prednisone; RCHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; RFCM, rituximab, fludarabine, cyclophosphamide and mitoxantrone; RFC, rituximab, fludarabine and cyclophosphamide.