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Case report

# The YPEL5–PPP1CB fusion transcript is detected in different hematological malignancies and in normal samples

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ABSTRACT

Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in Western adults. It was suggested that transcripts from a reciprocal trans-splicing event between YPEL5 and PPP1CB were present exclusively in CLL patients (more than 90%). Here we show that the YPEL5–PPP1CB fusion is not specific for CLL but is also detected in other hematological malignancies such as chronic myeloid leukemia, monoclonal B cell lymphocytosis or acute leukemia and also in normal samples. As such, it is unlikely that the YPEL5–PPP1CB fusion is a good drug target in CLL or a suitable target to monitor disease.

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## 1. Introduction

Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in adults in the Western society and is characterized by accumulation of clonal mature B cells [1–3]. In most patients, CLL is preceded by a monoclonal B cell lymphocytosis (MBL), characterized by less than 5000 monoclonal B cells/μL in the peripheral blood [4]. Although most CLL patients have an indolent clinical course, some cases present with a more aggressive evolution, leading to high morbidity and mortality [2]. Although several frequent mutations have been found in CLL [3], there is no common translocation or mutation known.

Recently, a reciprocal fusion between YPEL5 (yippee-like 5) and PPP1CB (serine/threonine protein phosphatase-1 beta catalytic subunit) was described to be exclusively expressed in more than 90% of primary CLL samples [5] and not in control samples or samples from patients with other hematological malignancies. The two chimeric RNA transcripts appeared be the result of trans-splicing as no sign of genomic reorganization could be detected. The discovery of a common, CLL-specific molecular defect is interesting as it provides a unique target for drug discovery and molecular monitoring.

Here, we show that the YPEL5–PPP1CB chimeric transcript is not specific for CLL as it is also detected in primary patient samples of the CLL-precursor MBL, acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML) and normal samples. As such, it should not be used as a diagnostic marker and the relevance for this fusion transcript in CLL as a drug target and molecular marker is questionable.

## 2. Materials and methods

### 2.1. Samples

Diagnostic or follow-up blood and bone marrow samples from patients with CLL, MBL, AML, CML and ALL were collected from February 2012 to September 2013 in the course of normal treatment. Diagnosis was based on the World Health Organization 2008 criteria [2]. Permission to use left-over samples was obtained from the ethical committee of Ghent University Hospital and all samples were treated according to the Declaration of Helsinki. White blood cells were isolated by erythrocyte lysis. RNA was isolated using the RNeasy kit (Qiagen) according to the manufacturer's instructions.

### 2.2. cDNA synthesis

cDNA was made using SuperScript II or SuperScript III (Life Technologies) according to the manufacturer's instructions. Briefly, 1 μg RNA was reverse transcribed in a total volume of 40 (SuperScript II) or 50 μL (SuperScript III).

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### 2.3. qPCR

For the NuPCR (Illumina; product discontinued), primers and MNAzyme were designed with the DesignStudio. YPEL5-PPP1CB and PPP1CB–YPEL5 PCRs (both with FAM-labeled probe) were performed in a two multiplex PCRs, each in combination with an ABL1 PCR (HEX-labeled probe). The primers used are: 5′-TAAACCTGGAGACTCGGGTGG-3′ and 5′-CTTTCCTGGACGACATCCTCGTA-3′ for YPEL5-PPP1CB, 5′-AGTCTGTGCCGACAAGATGGC-3′ and 5′-TCCATCTGTCAAGACGACGTA-GAATAAAA-3′ for PPP1CB-YPEL5 and 5′-CAGAGGTCCATCTCGCTGAGA-TA-3′ and 5′-CTCGGAGGAGACGTAGAGCTT-3′ for ABL1. The sequence of the MNAzymes were not provided by the manufacturer. The NuPCR was performed according to the manufacturer's instructions. Briefly, 5 μL cDNA was used in a 20 μL total reaction volume. Cycling was done on a CFX96 cycler (Bio-Rad) following this protocol: 95 °C 2 min, 40 cycles of 95 °C for 15 s, 50 °C for 30 s and 72 °C for 30 s.

For the EvaGreen qPCR, we used the published SYBR Green assay primers [5] for YPEL5–PPP1CB and 5′-TGACAGGGGA-CACCTACACA-3′ and 5′-ATACTCCAAATGCCCAGACG-3′ for ABL1 in combination with the SsoFast EvaGreen Supermix (Bio-Rad). For the PCR, 5 μL cDNA was used in a 20 μL total reaction volume using 300 nM primers. Cycling was done on a CFX96 cycler (Bio-Rad) with the following PCR protocol: 95 °C for 30 s and 50 cycles of 95 °C for 5 s and 60 °C for 20 s. Following the PCR, a melting curve from 65 °C to 95 °C was done (0.5 °C/5 s).

For both PCR strategies, expression of YPEL5–PPP1CB was normalized to the expression of ABL1. All PCRs included proper negative controls, which never showed amplification. Samples were analyzed for tumor-specific markers (e.g. BCR–ABL1 expression in CML samples) to confirm their identity as non-CLL samples.

## 3. Results and discussion

The apparent frequent and exclusive occurrence of the trans-splicing of YPEL5 and PPP1CB in CLL [5] could be a good target for directed therapy and molecular monitoring of this disease. To maximize the sensitivity and specificity in the detection of this fusion transcript, we used the recently introduced NuPCR technique [6]. The combination of two specific primers and a specific MNAzyme ensures a high specificity of the PCR in combination with a sensitive detection method (Fig. 1).

Initially, we tested four samples of CLL patients for the expression of both YPEL5–PPP1CB and PPP1CB–YPEL5 transcripts. In all samples, we observed low expression of the YPEL5–PPP1CB transcript. In contrast, the PPP1CB–YPEL5 transcript was not detected in any sample (data not shown). Therefore, we focused on the YPEL5–PPP1CB transcript in our further analyses. The panel of primary diagnostic CLL samples was expanded and in total, we detected variable expression of the YPEL5–PPP1CB transcript in 17/18 CLL samples (94%) (Fig. 2A – black bars), confirming the recurrent expression of the YPEL5–PPP1CB transcript in CLL [5].

To test the specificity of the expression of YPEL5–PPP1CB, we tested diagnostic samples of patients with AML ($n=3$), ALL ($n=4$), MBL ($n=7$), CML ($n=3$), different hematological cell lines ($n=10$; data not shown) and samples from healthy individuals ($n=3$) with the NuPCR for YPEL5–PPP1CB. All patient samples and three cell lines (ME-1, Kasumi-1, and MonoMac-6) tested positive, albeit at different levels (Fig. 3A – black bars), showing that the presence of YPEL5–PPP1CB is not specific for CLL. The expression levels in the different sample types were highly variable, but correspond to the levels observed in primary CLL samples. To investigate the discrepancy between the NuPCR and the results obtained by Velusamy et al. [5], we repeated the analysis on the same samples with the primers from the published SYBR Green assay [5] (Fig. 1; further referred to as EvaGreen method). After the PCR, one melting peak was obtained and sequence analysis confirmed the identity of the YPEL5–PPP1CB fusion (Supplementary Fig. S1). The patient samples showed similar expression with the EvaGreen method as obtained by the NuPCR (Figs. 2A and 3A – white bars). This confirmed the wide expression in non-CLL samples, disproving the statement that the YPEL5–PPP1CB is specific for CLL. Globally, the expression level was comparable regardless of the PCR technique used (Figs. 2B and 3B).

A report on high-throughput mRNA sequencing in CLL patients [7] also described a low-level expression of the YPEL5–PPP1CB transcript, both in normal and cancer samples. Although the expression level was below the threshold set by the authors, this finding is concordant with our results.

The discrepancy between our results and the results from Velusamy et al. [5] is potentially explained by the recent finding that incubation-induced gene dysregulation affects the expression level and splicing of numerous genes in leukemic samples [8]. As the expression level of YPEL5–PPP1CB is very low, even small differences in splicing efficiency, as a result of different sample processing times, could make the difference between being able to detect the transcript or not.

To conclude, we find that the YPEL5–PPP1CB transcript is not exclusively present in CLL samples and its usability as a molecular marker and the relevance for CLL therapeutics should be scrutinized thoroughly before taking steps in the development of target-specific drugs.

## Authors' contributions

KV: designed the study, performed experiments, drafted the manuscript, approved the final version.

JP, BD: designed the study, revised the manuscript, approved the final version.

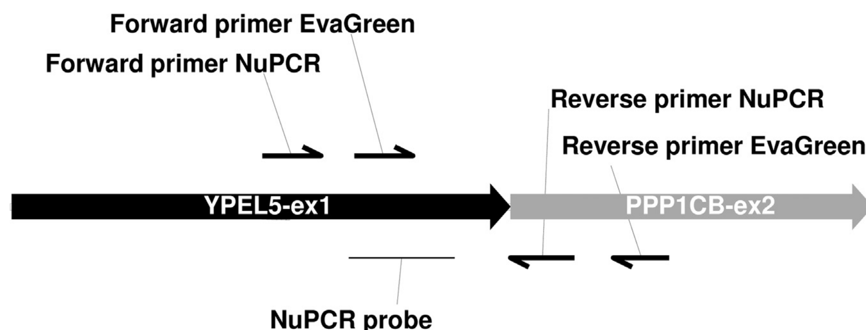


**Fig. 1.** Location of primers and probes. The chimeric transcript starts with the first exon of YPEL5 (black) and is followed by the second exon of PPP1CB (gray). The primers used in the analyses are shown as arrows.

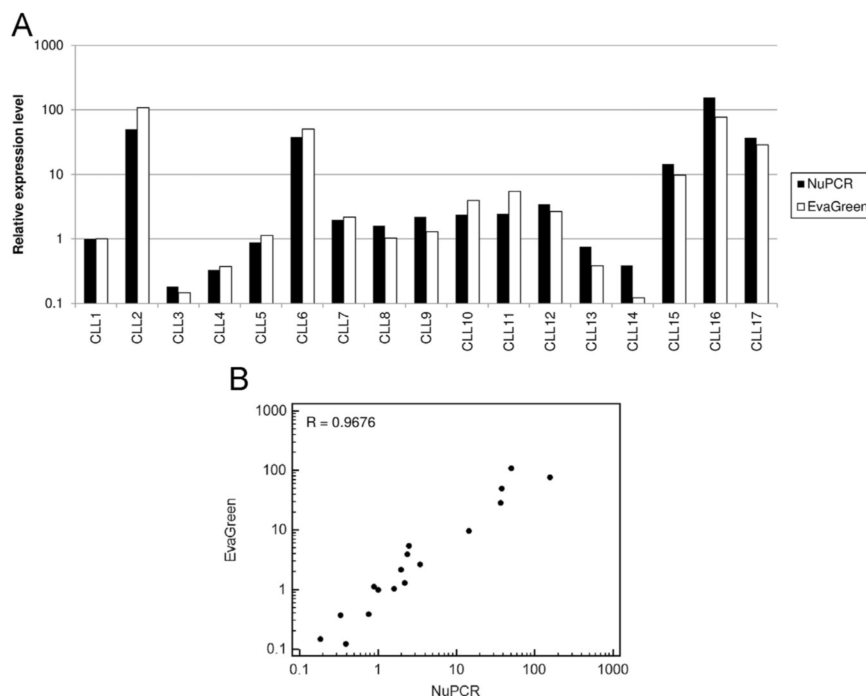


**Fig. 2.** Expression of YPEL5–PPP1CB in diagnostic samples of CLL patients. (A) Diagnostic CLL samples ($n$=17) were analyzed for YPEL5–PPP1CB expression using two different PCR strategies (NuPCR (black) and EvaGreen (white)). The relative expression is highly variable but shows overall a good comparison between the two techniques used. Only positive samples with quantifiable results are shown. The expression in the sample CLL1 was set to 1 and all other samples are shown relative to this sample. (B) Scatter plot comparing the results from the NuPCR with the EvaGreen PCR. A correlation coefficient of 0.9676 was obtained.

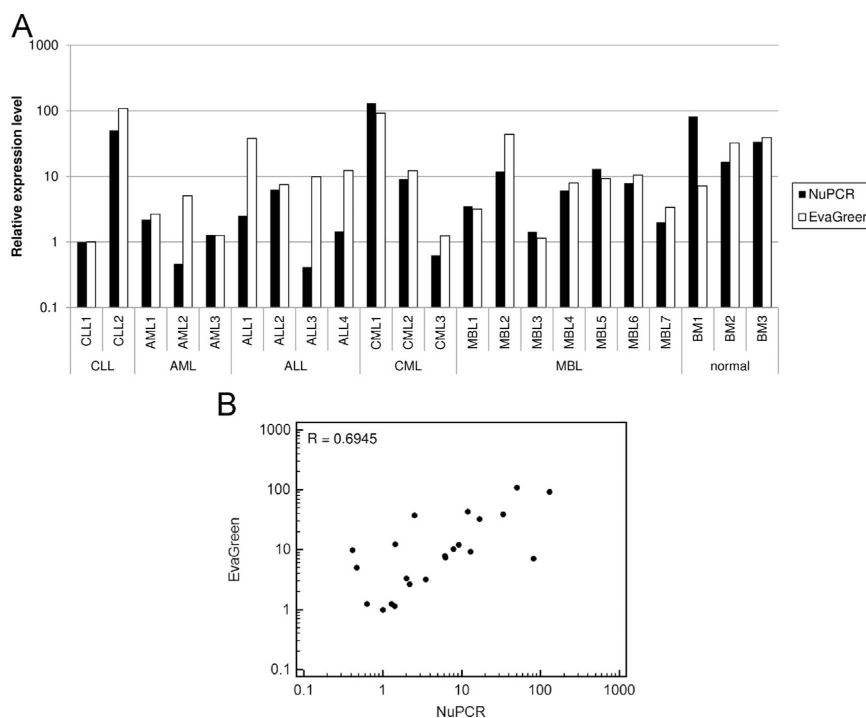


**Fig. 3.** Expression of YPEL5–PPP1CB in diagnostic and remission samples of patients with different hematological malignancies. (A) Diagnostic AML ($n$=3), ALL ($n$=4), CML ($n$=3), and MBL ($n$=7) samples, and normal bone marrow samples ($n$=3) were analyzed for YPEL5–PPP1CB expression using two different PCR strategies (NuPCR (black) and EvaGreen (white)). CLL1 and CLL2 samples are reproduced from Fig. 2 for comparison. All samples show expression levels in the same range as the CLL samples. The expression in the sample CLL1 was set to 1 and all other samples are shown relative to this sample. (B) Scatter plot comparing the results from the NuPCR with the EvaGreen PCR. A correlation coefficient of 0.6945 was obtained.

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## Appendix A. Suplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.lrr.2015.07.001.

## References

[1] N. Chiorazzi, K.R. Rai, M. Ferrarini, Chronic lymphocytic leukemia, N. Engl. J. Med. 352 (8) (2005) 804–815.
[2] S.H. Swerdlow, et al., WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, World Health Organization, Lyon, France, 2008.
[3] G. Gaidano, R. Foa, R. Dalla-Favera, Molecular pathogenesis of chronic lymphocytic leukemia, J. Clin. Invest. 122 (10) (2012) 3432–3438.
[4] G.E. Marti, et al., Diagnostic criteria for monoclonal B-cell lymphocytosis, Br. J. Haematol. 130 (3) (2005) 325–332.
[5] T. Velusamy, et al., Recurrent reciprocal RNA chimera involving YPEL5 and PPP1CB in chronic lymphocytic leukemia, Proc. Natl. Acad. Sci. USA 110 (2013) 3035–3040.
[6] E. Mokany, et al., MNAzymes, a versatile new class of nucleic acid enzymes that can function as biosensors and molecular switches, J. Am. Chem. Soc. 132 (3) (2010) 1051–1059.
[7] P.G. Ferreira, et al., Transcriptome characterization by RNA sequencing identifies a major molecular and clinical subdivision in chronic lymphocytic leukemia, Genome Res. 24 (2) (2014) 212–226.
[8] H. Dvinge, et al., Sample processing obscures cancer-specific alterations in leukemic transcriptomes, Proc. Natl. Acad. Sci. USA 111 (47) (2014) 16802–16807.