Locked Nucleic Acid-Enhanced Detection of 1100delC CHEK2 Germ-Line Mutation in Spanish Patients with Hematologic Malignancies, Maria Collado,1 Olfert Landt,2 Eva Barragán,1 Ulrich Less,2 José Cervera,3 Miguel A. Sanz,3 and Pascual Bolufer1*

We studied 107 patients with acute leukemia (AL). Two patients had AL (one type B, common; and one biphenotypic), and 105 had acute myeloid leukemia: 1 with French-American-British subtype Mo, 11 with subtype M1, 13 with subtype M2, 65 with subtype M3, 4 with subtype M4, 2 with subtype M4Eo, 5 with subtype M5, 2 with subtype M6, 1 who was not classified, and 1 with subtype M1 at relapse. There were 52 males and 55 females, and the median age was 46 years (range, 1–78 years). We also studied a group of 26 patients with TRL/ MDS (15 males and 11 females) with a median age of 64 years (range, 7–87 years) at the time of diagnosis of the primary tumor (Table 1). The control group consisted of 176 healthy volunteers (69 males and 107 females) who had a median age of 36.5 years (range, 16–75 years).

DNA was extracted from 500 µL of whole blood anticoagulated with EDTA by use of MagNA Pure LC DNA Isolation Kits-Large Volume (Roche Diagnostics) with the MagNA Pure LC System (Roche).
One established method for detecting 1100delC CHEK2 starts with a long-range PCR amplifying an ~10-kb fragment, because there are at least six to eight genomic copies of similar genes containing identical exons 10–14 (Dr. Mieke Schutte, Medical Oncology, Rotterdam, The Netherlands, personal communication) \(^{(11)}\). For the analysis of variants, we strongly prefer mutation-specific probes because the high melting temperature is valid proof of sequence identity and presence of the mutation. However, direct analysis of the gene using the primers CHEK2F (gi 6911603, positions 104228–104254) and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the primers CHEK2F and CHEK2R; the amplification product was then diluted with a conventional PCR using the pr
for 2 s, 64 °C for 5 s, and 72 °C for 10 s. The final melting was performed by increasing the temperature from 40 °C to 90 °C at 0.2 °C/s and continuously reading the fluorescence (channels F2/F1). In every assay, an 1100delC- positive heterozygous control (kindly provided by Dr. Mieke Schutte) was run to ensure its detection. The control sample was verified by DNA sequencing.

We compared different DNA and LNA-modified hybridization probes specific for the wild-type and the mutated gene. Wild-type-specific DNA detected the mutation in PCR products without the preamplification step, whereas the mutation-specific DNA probes failed to produce a signal.

In contrast, the LNA-modified probes showed a substantially decreased melting temperature for the mismatched target. Both wild-type- and mutation-specific probes enabled detection of the deletion without the preamplification step. With the mutation-specific LNA-modified hybridization probe, we obtained melting temperatures of 54 °C for the deleted allele and 46 °C for the wild-type allele (Fig. 1).

None of the 309 samples analyzed among the three groups (AL, TRL/MDS, and controls) carried the 1100delC CHEK2 mutation, making the differences in the age ranges between the test and control populations irrelevant. Despite these negative results, in all samples tested, the reliability of the method was supported by the positive heterozygous control for 1100delC systematically used in every assay.

The results are in complete agreement with previous reports for Spanish patients with familial breast cancer (13) in which the authors were unable to detect the 1100delC CHEK2 mutant variant in any of 856 samples analyzed for both cases and controls. A very low incidence of 0.3% was described for this mutation in a population from New York (14). However, this mutation has been found at a frequency of 1.4% in a Finnish population (6) and 1.1% in control individuals from the United Kingdom, The Netherlands, and North America (1).

The lack of detection of this germ-line mutation among the 107 patients with AL is in agreement with most reports on hematologic malignancies, which show a scarcity of somatic CHEK2 mutations (8–10), but not the CHEK2*1100delC germ-line mutation.

The association of this mutation with cancer risk is dependent mainly on its incidence in the general population, which varies greatly among the groups studied. Thus, in the studies carried out in populations from The Netherlands or Finland, where the incidence of the 1100delC CHEK2 mutant in controls was highest, the presence of the 1100delC CHEK2 allele was associated with a high risk for breast cancer (1, 6). However, the absence of this mutation in the present study and in another study involving a Spanish population (13) and the very low incidence found in a study performed in New York (14) make the presence of this mutation clinically irrelevant.

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References


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