## *NRIP3*: a novel translocation partner of *MLL* detected in a pediatric acute myeloid leukemia with complex chromosome 11 rearrangements

*MLL*-rearranged acute myeloid leukemia (AML) is associated with an adverse outcome in most treatment protocols. The *MLL*-gene exhibits an 8-kb breakpoint cluster region, which behaves as a hotspot for chromosomal translocations. So far more than 50 different fusion partners of the *MLL*-gene have been identified.[1] In pediatric AML, the main translocations are: t(9;11), t(11;19); t(6;11) and t(10;11) accounting for almost 15-20% of the cases(2). Here, we report on a novel translocation partner of *MLL* on chromosome 11 in a pediatric AML case.

A 5-month old boy presented with AML FAB-M5 and a white blood cell count of $9.8\times10^9$/L. CSF analysis showed CNS involvement with a cell count of $1.4\times10^6$ cells/mm$^3$ and with 93% blasts. Immunophenotyping of bone marrow and peripheral blood further confirmed a monoclonal population in at least 50% of which showed the following aberrant phenotype: CD34$^-$, CD117$^+$, CD13$^+$, CD33$^+$, CD15s$^+$, CD14$^-$, CD4$^+$, CD45$^+$ and MPO$^+$. The child responded well to chemotherapy according to the DCOG-AML 97 protocol. The patient is in continuous complete remission seven years after diagnosis.

RBA and QFQ-banded karyotyping and fluorescence *in situ* hybridization (FISH) showed a double inversion on chromosome 11 in combination with a rearrangement involving chromosome 3 (Figure 1).

At the time of diagnosis the karyotype was *46,XY,der(3)t(3;11)(p21;q23)ins(3;11)(q23;p12p15),der(11)del(11)(p12p15)inv(11)(p1?2q1?1)inv(11)(q1?1q23)t(3;11)(p21;q23)*. FISH using the *MLL* Dual Color, Break Apart Rearrangement Probe (Vysis/Abbott, Des Plaines, IL, USA) confirmed the *MLL* gene rearrangement showing the 3' probe on the short arm of the der(3) and the 5' probe high on the long arm of the der(11).

Long distance inverse (LDI)-PCR was performed as previously described.[1] In this case this technique revealed that 5'*MLL* (intron 9) was fused to *NRIP3* (intron 1) located on chromosome 11p15. Moreover, 3'*MLL* was fused to sequences from chromosome 3q21.3 (*FLJ40473*). Subsequently, the *MLL-NRIP3* fusion gene was identified with RT-PCR (Figure 2), using an *MLL* exon 8 specific forward primer (5'-CGTCGAGGAAAAGAGTGA-3') combined with an *NRIP3* exon 3 specific reverse primer (5'-CAGGCCAAAGAGATGAGAT-3').

Since these results were not in concordance with the observed karyotype, additional FISH analysis was performed. A paint for chromosome 11 showed that there were chromosome 11 sequences present on the short arm of the der(3), and on a small region on the der(3)(q). The pericentric inversion of chromosome 11 was confirmed using probes on both sides of the centromeric region (Figure 2). The 11p telomeric probe was present on the top of the der(11)(p). The paracentric inversion of 11q was confirmed using probes on 11q13 and 11q21. The 11q telomeric probe was present on the top of the der(3)(p) confirming the t(3;11)(p21;q23). The break apart probes for *NUP98* (11p15.4)(3) were unexpectedly detected on the top of the der(3)(p), near the 3'*MLL* localization. Subsequent hybridization using a more centromeric probe on 11p15.4 (RP11-21N2), which is 5 Mb telomeric to *NRIP3*, showed the signal on the der(11)(q) near the probe for 5'*MLL* based on inverted DAPI banding pattern. A probe covering part of the *FLJ40473* region showed in addition to the normal location on 3q21, a weaker signal on the top of the der(3)(p) near the location of 3'*MLL* and *NUP98* (Figure 2). We were not able to determine the origin of the small insertion of chromosome 11 sequences on the der(3)(q). However, FISH results clearly demonstrated that there were many more chromosome 11 and 3 rearrangements present than expected on the basis of the conventional karyotyping.

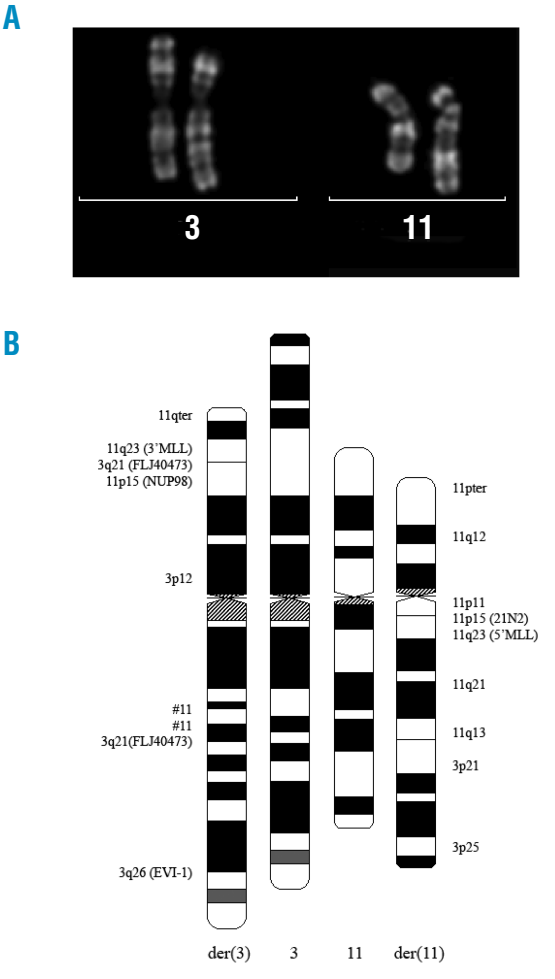


**Figure 1.** Karyogram of chromosomes 3 and 11. (A) Partial RBA-banded karyogram showing chromosomes 3 and 11. The der(3) and the der(11) are shown on the right. (B) Schematic representation of the normal chromosome 3 and 11 (middle) and the der(3) (left) and the der(11) (right). The band designations provided were all observed using specific FISH probes. The designation #11 is where the whole paint for chromosome 11 showed hybridization to the der(3). Used probes were (from pter to qter) for chromosome 3: RP11-438J1 (3p25), RP11-969E9 (3p21), RP11-451E6 (3p12.3), RP11-79F5 (3p12.1), RP11-456K4 (3q21), RP11-82C9 (3q26); for chromosome 11: RP11-120E20/348A20 (NUP98, 11p15.4), RP11-21N2 (11p15.4), RP11-102E22 (11p11.2), pLC11a (centromere 11), RP11-114D10 (11q12.2), 4179 (11q13), cos3.16 (11q21), MLL break apart (11q23.3), RP11-133I16 (11q23.3), 11qtel.

In this case with complex rearrangements of chromosome 3 and 11 a novel translocation partner of the *MLL*-gene was detected. We have shown that the translocation partner was found on chromosome 11 with LDI-PCR. This technique revealed the *NRIP3* gene on 11p15 as a novel translocation partner of *MLL* in pediatric AML, while the 3' part of *MLL* was translocated to chromosome 3. The latter is thought not to be of importance since the reciprocal *MLL* translocations are often not expressed. Furthermore, it has been suggested that the *MLL* translocation partners are not randomly selected but that they are part of a protein network serving common functional processes. For example, interactions have already been described between AF4 and AF9 and ENL and AF4/AF10, which play a functional role in leukemogenesis.[4,5] So far, the function of *NRIP3* is not known, although it is one of the genes to be frequently hypermethylated in non-small cell lung cancer, hence it may potentially play a role in the pathogenesis of other cancers.[6] As this is the first case in which *NRIP3* is involved as a translocation partner for *MLL*, no conclusions can be drawn with respect to the clinical relevance and prognostic value. However, our patient has been in continuous complete remission for more than seven years.

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## No evidence for association between *TGFB1* promoter SNPs and the risk of childhood pre-B acute lymphoblastic leukemia among French Canadians

TGFB1 is a potent growth inhibitor to a wide variety of cell types including hematopoietic cells and deregulation of the TGFB1 signaling pathway has been implicated in the development of several cancers.[1] Functional regulatory polymorphisms of the *TGFB1* gene have been directly associated with inter-individual variability in TGFB1 plasma levels and modified risk of breast,[2] lung,[3] colorectal,[4] and prostate[5] cancers. No studies, to date, have examined the association between genetic polymorphisms in *TGFB1* and childhood leukemia. In this study we examined the role of *TGFB1* promoter SNPs (pSNPs), -1886G>A, -1571A>G, -1550DEL/AGG, and -509C>T, as genetic modulators of childhood pre-B ALL susceptibility among the French Canadian population.

In a case-control study, we investigated genotypic, haplotypic, as well as multi-SNP combination associations with childhood pre-B ALL. The study population and inclusion criteria were described previously.[6] Childhood pre-B ALL cases (n=321) consisted of 189 boys and 132 girls with a median age of 4.7 years. Parental DNA was available for 203 of these children. Healthy controls (n=329) were recruited at the Sainte-Justine Hospital. Study individuals were all French Canadian from the province of Quebec, Canada. Candidate pSNPs were previously identified[7] and were selected based on their frequency in the European population. A PCR-based allele-specific oligonucleotide hybridization approach was used to genotype samples, described as previously[8].

Hardy-Weinberg equilibrium was tested using the $\chi^2$ goodness of fit test and PedCheck (Version 1.1) was used to identify genotype incompatibilities using the familial data.[9] Pearson's $\chi^2$ test or Fisher's exact test, as appropriate, was used to compare allele/genotype/haplotype carriership in patients and controls. Crude odds ratios (ORs) were measured using logistic regression in STATA (Release 9.2) and are given with 95% confidence intervals (CIs). Haplotype frequencies were estimated using the FAMHAP Software (Version 16) using parental data when available.[10] A likelihood ratio test implemented in FAMHAP was used to examine global haplotype associations with disease status. Multimarker combinations were tested for association with disease using the method proposed by Becker and Knapp implemented in FAMHAP.[11] Multiple testing was controlled for using the false discovery rate[12] with a type I error rate of 10%.

Frequencies of pSNPs were in agreement with those previously reported in other populations of European descent and all distributions were in Hardy-Weinberg equilibrium. *TGFB1* pSNP distributions did not differ significantly between cases and controls (Table 1).