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 χ^2 goodness of fit test and PedCheck (Version 1.1) was used to identify genotype incompatibilities using the familial data.[9] Pearson's χ^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).

Suggestive evidence of a reduced risk of childhood pre-B ALL was demonstrated when carriers of at least one variant -1886A allele were compared to homozygous individuals for the ancestral -1886G allele (OR=0.58, 95% CI: 0.33-1.01, p=0.042). However these results did not sustain multiple testing corrections. A total of 12 promoter haplotypes (pHaps) were inferred but only haplotypes TGH*1, *2 and *3 had frequencies ≥0.05 and represented 98% of the observed haplotypes in the population tested (Table 2). The remaining 2% of the chromosomes carried nine minor haplotypes that were grouped under TGH*. We found no significant difference in the overall distribution of the 12 *TGFB1*-derived pHaps between cases and controls (Global c^2= 10.85, 11 degrees of freedom, p=0.46) and found no evidence of association between individual haplotypes and the risk of pre-B ALL. Investigating multi-SNP combinations, the best result was obtained for marker combination -1886A/-1550AGG/-509C, which was associated with a decreased risk of pre-B ALL (OR= 0.86) however the global test statistic failed to reach significance (p=0.15).

Though the expected variability of *TGFB1* expression levels due to promoter SNPs could indeed contribute to leukemogenesis, our data did not support a role for *TGFB1* promoter variants -1886G>A, -1571A>G, -1550DEL/AGG or -509C>T in the etiology of childhood pre-B ALL, at least among the French Canadian population. Having screened 2kb upstream of the transcription start site in 40 individuals,[7] a strength of this study was our ability to capture and survey most common genetic variation within the region under investigation with reasonable statistical power. Despite the relatively small size of this dataset, we had 80% power at the 5% level to detect a minimum OR of 1.5 with a minor allele frequency ≥15%. However it remains possible that other variants within the *TGFB1* gene and surrounding regulatory sequences that were not assessed within the scope of this study could modify disease susceptibility. Additional analyses in independent datasets are required to further support the lack of association between these *TGFB1* variants and childhood pre-B ALL since our study did have limited statistical power to detect associations involving weak effects or rarer variants.

Table 1. Allele and genotype frequencies of pSNPs in *TGFB1* in childhood pre-B ALL patients and controls.

DNA variants, genotypes and alleles	N. (%) ALL patients	Controls	OR (95% CI)	p
-1886G>A				
Alleles				
-1886G	215 (34.0)	201 (31.3)	1 (referent)	–
-1886A	417 (66.0)	441 (68.7)	0.88 (0.70-1.12)	0.30
Genotypes				
GG	40 (12.7)	25 (7.8)	1 (referent)	–
GA	135 (42.7)	151 (47.0)	0.56 (0.32-0.97)	0.038
AA	141 (44.6)	145 (45.2)	0.61 (0.35-1.05)	0.076
GA+AA vs. GG	276 (87.3)	296 (92.2)	0.58 (0.33-1.01)	0.042
-1571A>G				
Alleles				
-1517A	254 (41.1)	253 (39.4)	1 (referent)	–
-1517G	364 (58.9)	389 (60.6)	0.93 (0.74-1.17)	0.54
Genotypes				
AA	52 (16.8)	45 (14.0)	1 (referent)	–
AG	150 (48.5)	163 (50.8)	0.80 (0.50-1.26)	0.33
GG	107 (34.6)	113 (35.2)	0.82 (0.51-1.32)	0.42
-1550DEL/AGG				
Alleles				
-1550AGG	417 (67.0)	448 (69.1)	1 (referent)	–
-1550DEL/AGG	205 (33.0)	200 (30.9)	1.10 (0.87-1.39)	0.42
Genotypes				
AGG AGG	142 (45.7)	150 (46.3)	1 (referent)	–
AGG DEL/AGG	133 (42.8)	148 (45.7)	0.95 (0.68-1.32)	0.76
DEL/AGG DEL/AGG	36 (11.6)	26 (8.0)	1.46 (0.84-2.55)	0.18
-509C>T				
Alleles				
-508C	418 (66.6)	446 (68.8)	1 (referent)	–
-508T	210 (33.4)	202 (31.2)	1.11 (0.88-1.40)	0.39
Genotypes				
CC	141 (44.9)	148 (45.7)	1 (referent)	–
CT	136 (43.3)	150 (46.3)	0.95 (0.69-1.32)	0.77
TT	37 (11.8)	26 (8.0)	1.49 (0.86-2.59)	0.15

Percentages indicate number of individuals with a given genotype/total number of individuals in the datase or the number of chromosomes with given allele/total number of chromosomes in the dataset. OR indicates crude odds ratio; —, not applicable.

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Table 2. Distribution of *TGFB1* promoter haplotypes in pre-B ALL patients and controls.

Haplotype	DNA variant -1886G>A	-1571A>G	-1550DEL/AGG	-509C>T	N. (%) ALL patients	Controls	OR (95% CI)	p	Global X^2 (df)	Global p
TGH*1	A	G	AGG	C	359 (56.45)	386 (59.75)	0.87 (0.69-1.10)	0.23	10.85 (11)	0.46
TGH*2	G	A	DEL/AGG	T	205 (32.24)	196 (30.34)	1.09 (0.86-1.39)	0.46		
TGH*3	A	A	AGG	C	50 (7.86)	53 (8.21)	0.95 (0.62-1.46)	0.82		
TGH*	*	*	*	*	22 (3.45)	11 (1.70)	–	–		

The risk of ALL was evaluated for each haplotype compared with all other possible haplotypes combined. Percentages indicate number of chromosomes with given haplotype/total number of chromosomes. Haplotypes with relative frequencies <5% are grouped under TGH and are represented as * combinations of the four DNA variants. A likelihood ratio test was performed in FAMHAP to compare global haplotype differences between cases and controls and is reported here as a Global X^2 test with number of haplotype parameters different from zero-1 degrees of freedom. OR indicates crude odds ratio; df, degrees of freedom; and —, not applicable.*

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Physiological *PTEN* expression in peripheral T-cell lymphoma not otherwise specified

Peripheral T-cell lymphomas not otherwise specified (PTCL/NOS) is the commonest subtype of PTCL.[1] This is a complex entity, characterized by great morphologic, immunophenotypic and clinical variability, whose molecular pathology is still largely unknown.[2]

Recently, gene expression profiling (GEP) studies allowed the identification of PTCL/NOS-associated signatures, leading to the better understanding of its histogenesis, pathogenesis and prognostication.[2] Interestingly, proliferation pathways were found to be commonly altered in PTCL,[2,3] highly proliferative cases being characterized by poorer prognosis.[4,5]

The tumor suppressor PTEN (phosphatase tensin homolog), is a critical regulator for multiple cellular processes including proliferation. In addition to its well-defined role in signaling in the cytoplasm, its nuclear localization seems to contribute to its physiological tumor suppressor activity. Interestingly, somatic mutations of PTEN, including null or missense mutations, and truncations, occur in multiple sporadic tumors. Furthermore, deletions of 10q22-25 have been reported in ~5-10% of B-NHLs, raising the possibility of PTEN involvement in the pathogenesis of lymphoid malignancies.[6] Importantly, the great majority of such mutations abolish (81%) or greatly decrease (10%) PTEN phosphatase activity, by determining the absence of the encoded product or the presence of a mutated, non-functional protein, finally leading to tumorigenesis.

Interestingly, experimental evidence showed that mice with loss of heterozygosity of the wildtype *mPTEN* allele develop T-cell lymphomas.[7] Furthermore, down-regulation of this molecule was found to be essential for the formation of the typical nuclear lobules in adult T-cell leukemia/lymphoma[8] and its alterations may be important in the pathogenesis and progression of mycosis fungoides[9] and T-cell acute lymphoid leukemia.[10] Notably, partial or complete loss of PTEN was detected in 66.7% of anaplastic large cell lymphomas and in 12.5% of the few other mature T-/NK-cell lymphomas so far studied.[11] Nevertheless, the number of PTCLs/NOS was indeed limited and no definitive conclusion can be drawn concerning the possible involvement of PTEN in the pathogenesis of such tumors. The present study was designed to evaluate the possible occurrence of PTEN aberration in PTCL/NOS. We analyzed GEP data of 28 PTCLs/NOS, and 20 samples of normal T cells. Technical details have been previously reported (*http://www.ncbi.nlm.nih.gov/projects/geo/*).[3,12]

Genotypes were determined by PCR amplification and direct sequencing. In particular, PCR products of all nine exons and exon-intron junctions of PTEN were directly sequenced in 72 PTCLs/NOS. Primers and relative conditions of amplification are detailed in Table 1.

Finally, we studied PTEN expression by immunohistochemistry (IHC) on tissue micro-arrays (TMAs) containing 34 PTCL/NOS cases.[4] PTEN cytoplasmatic expression was tested by a mouse monoclonal antibody, PTEN/MMAC1 Ab-4 (clone 17.A, Thermo Scientific), at a 1:20 dilution, while PTEN nuclear expression was tested by a specific mouse monoclonal antibody, PTEN Ab-6 (clone 28H6, Thermo Scientific), which was applied at a 1:10 dilution. The sections underwent antigen retrieval in citrate buffer (pH=6.0) in a micro-waver at 900W (3 cycles lasting 5' each) and revealed by the EnVision and APAAP techniques, respectively.

First, GEP showed no significant differences in *PTEN* expression when PTCL/NOS cases were compared to normal T-lymphocytes (Figure 1).

Secondly, direct sequencing did not reveal any significant abnormality. Similarly, PTEN locus was found to be intact in the course of a high-density karyotyping study carried out by the Affymetrix 250k SNPs-array in a series of PTCL/NOS (*Hartmann et al., manuscript in preparation*).

We then studied the protein expression and localization in a series of 34 PTCL/NOS cases. Importantly, IHC documented physiological nuclear and cytoplasmic expression in 31/31 and 23/26 evaluable cases, respec-