Molecular characterization and clinical impact of t(11;15)(q23;q14-15) *MLL-CASC5* rearrangement

The identification of novel translocation partner genes involved in *MLL* rearrangements increases the chances of identifying novel possibilities for a targeted, molecular therapy. Concerted efforts by many research groups in collaboration with the Diagnostic Center of Acute Leukemia (DCAL) at Frankfurt University have made it possible to establish the *MLL* recombinome database.[1] From these studies it became clear that just a few translocation partner genes account for over 90% of leukemia patients, and that the vast majority of *MLL* partner genes is relatively infrequent. Nevertheless, these rare but recurrent *MLL* rearrangements are also of importance, and, therefore, collaborative efforts are required to analyze their function(s)

Table 1. Clinical and laboratory features of three new *MLL-CASC5* rearrangement.

Gender/Age (Country)	Case 1 M/50 (Korea)	Case 2 M/15 (Czech Republic)	Case 3 F/22 (Austria)
Past history	AGC, total gastrectomy, (January, 2008)	Glomerulonephritis (January, 2007)	Medulloblastoma (October, 2000)
Previous toxic exposure i.e. topoisomerase (duration)	Tegafur-uracil (20 months)	None	HIT91 trial, radiation
Hematologic diagnosis	t-MDS	AML-M4	t-MDS
CBC finding at diagnosis	WBC 7,300/uL; Hb 10.1 g/dL; PLT 182,000/uL	WBC 39,000/uL; Hb 8.4 g/dL; PLT 43,000/uL	WBC 1,400/uL; Hb 9.2 g/dL; PLT 54,000/uL
BM interpretation	Normocellular with trilineage dysplasia (nuclear budding, multinuclearity, megaloblastic changes, dysgranulation) blast count (5.4%)	Blast count (24%)	Hypocellular bone marrow, slightly dysplastic myelopoiesis and erythropoiesis, minor population of undifferentiated precursor cells
Chromosome study	46,XY,del(3)(q21),t(11;15)(q23;q15)[12]/47,sl,+21[6]/48,sdl1,+21[2]	46,XY,der(3),t(11;15)(q23;q?14)	46-48,XX,der(3)t(3;?)(q11;?),t(11;15)(q23;q21), del(14)(q11q23?)[4],+21[4], +1-2mar[cp21]
MLL FISH analysis (break apart %)	Positive (83%)	Positive (92%)	Positive (67%)
Genomic breakpoints confirmed by LDI-PCR	MLL (Intron 10) – CASC5 (Exon 11)	MLL (Intron 9) – CASC5 (Exon 11)	MLL (Intron 9) – CASC5 (Exon 11)
RAS mutation study	K-RAS (no mutation), N-RAS (not available)	K-RAS (No mutation), N-RAS (G12C mutation)	Not conducted
Microarray studies (Human Genome Build 37.2)	Done	Done	Not conducted
Regions of loss (size)*	chr3:100,678,757-107,018,905 (6,340 kb) chr3:107,970,337-145,133,136 (37,163 kb) chr3:147,878,002-167,019,831 (19,142 kb)	chr3:83,272,290-100,008,560 (16,736 kb) chr3:102,523,816-106,076,839 (3,553 kb) chr3:106,748,975-131793628 (25,045 kb) chr3:133,454,250-139,471,363 (6,017 kb) chr3:140,292,285-141,501,836 (1,210 kb)	
Regions of gain (size)†	chr3:167,026,939-170,408,789 (3382 kb) chr3:182,415,039-186,599,696 (4185 kb)	chr3:131,795,746-132,103,139 (307 kb) chr3:132,744,510-133,453,323 (709 kb) chr3:139,483,424-140,284,421 (801 kb) chr3:141,502,230-147,025,026 (5,523 kb) chr3:155,324,552-155,920,227 (596 kb) chr3:168,509,556-172,476,033 (3,966 kb)	
Treatment	Vidaza	HAM, AI/2CDA, FLAG	DNX-FLAG, SCT
Clinical course	Progression to AML	No remission	Relapse (remission on Day 28, SCT)
Survival length (clinical outcome)	8 months (dead)	8 months (dead)	22 months (dead after relapse)

*M: male; F: female; t-MDS: therapy-related myelodysplastic syndrome; AML: acute myeloid leukemia; WBC: white blood cell; Hb, hemoglobin; Plt: platelet; AGC: advanced gastric cancer; RT-PCR: reverse transcriptase PCR; LDI-PCR: long distance inverse PCR; HAM: high-dose cytarabine and mitoxantrone; AI/2CDA: Ara-C Idarubicin/Cladribine; FLAG: fludarabine, high dose ara-C, and G-CSF. *The common deleted region of these 2 cases included following genes: MIR548A3, ALCAM, CBLB, LOC100302640, LOC344595, HHLA2, MYH15, KIAA1524, DZIP3, RETNLB, TRAT1, GUCA1C, MORC1, FLJ22763, C3orf66, DPPA2, DPPA4, FLJ25363, PVRL3, CD96, ZBED2, PLCXD2, PHLDB2, ABHD10, TAGLN3, TMPRSS7, C3orf52, MIR567, GCET2, SLC9A10, CD200, BTLA, ATG3, SLC35A5, CCDC80, CD200R1L, CD200R1, GTPBP8, C3orf17, BOC, WDR52, SPICE1, SIDT1, KIAA2018, NAA50, ATP6V1A, GRAMD1C, ZDHHC23, KIAA1407, QTRTD1, DRD3, ZNF80, TIGIT, MIR568, ZBTB20, GAP43, LSAMP, LOC285194, IGSF11, C3orf30, UPK1B, B4GALT4, ARHGAP31, TMEM39A, POGLUT1, C3orf1, CD80, ADPRH, PLA1A, POPDC2, COX17, C3orf15, NR1I2, GSK3B, GPR156, LRRC58, FSTL1, MIR198, NDUFB4, HGD, RABL3, GTF2E1, STXBP5L, POLQ, ARGFX, FBXO40, HCLS1, GOLGB1, IQCB1, EAF2, SLC15A2, ILDR1, CD86, CASR, CSTA, CCDC58, FAM162A, WDR5B, KPNA1, PARP9, DTX3L, PARP15, PARP14, HSPBAP1, DIRC2, LOC100129550, SEMA5B, PDIA5, SEC22A, ADCY5, PTPLB, MYLK, CCDC14, ROPN1, KALRN, UMPS, ITGB5, MUC13, HEG1, SLC12A8, ZNF148, SNX4, OSBPL11, MIR548I1, LOC100125556, ALG1L, ROPN1B, SLC41A3, ALDH1L1, KLF15, CCDC37, ZXDC, UROC1, CHST13, C3orf22, TXNRD3IT1, TXNRD3, C3orf46, CHCHD6, PLXNA1, TPRA1, MCM2, PODXL2, ABTB1, MGLL, KBTBD12, SEC61A1, RUVBL1, EEFSEC, MIR1280, DNAJB8, GATA2, LOC90246, C3orf27, RPN1, RAB7A, LOC653712, ACAD9, KIAA1257, CCDC48, GP9, RAB43, ISY1, CNBP, COPG, C3orf37, H1FX, C3orf47, RPL32P3, SNORA7B, C3orf25, MBD4, IFT122, RHO, H1FOO, PLXND1, TMCC1, TRH, ALG1L2, LOC729375, COL6A4P2, COL6A5, COL6A6, PIK3R4, ATP2C1, ASTE1, NEK11, NUDT16P1, NUDT16, MRPL3, SNORA58, CPNE4, SRPRB, RAB6B, C3orf36, SLCO2A1, RYK, AMOTL2, ANAPC13, CEP63, KY, EPHB1, PPP2R3A, MSL2, PCCB, STAG1, TMEM22, NCK1, IL20RB, SOX14, CLDN18, DZIP1L, A4GNT, DBR1, ARMC8, TXNDC6, MRAS, ESYT3, CEP70, FAIM, PIK3CB, FOXL2, C3orf72, PRR23A, PRR23B, PRR23C, BPESC1, PISRT1, MRPS22, COPB2, RBP2, RBP1, NMNAT3, TRIM42, SLC25A36, SPSB4, ACPL2, ZBTB38, RASA2, RNF7, and GRK7. †The common duplicated region included EGFEM1P, MIR551B, MECOM, TERC, ARPM1, MYNN, LRRC34, LRRIQ4, LRRC31, SAMD7, LOC100128164, SEC62, GPR160, PHC3, PRKCI, SKIL, CLDN11, and SLC7A14.*

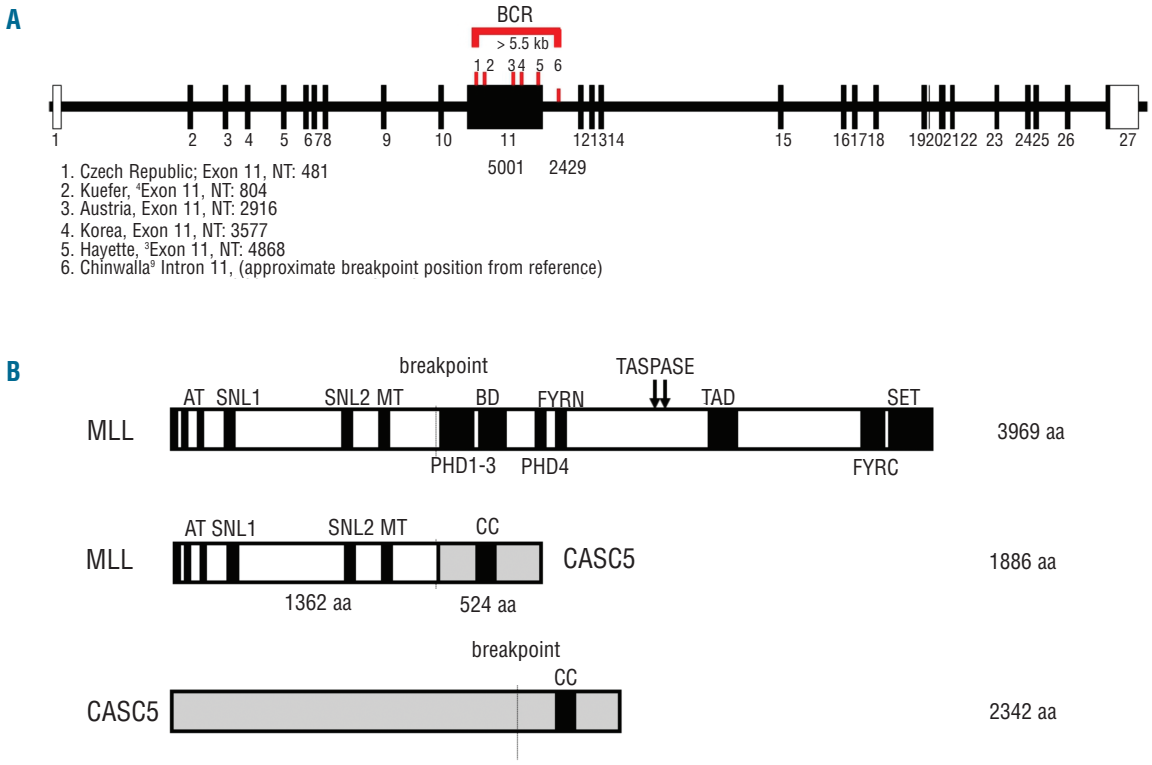

Figure 1. (A) Breakpoint cluster region (BCR) of the *CASC5* gene. (B) Size and location of functional domains of the MLL *wt*, CASC5 *wt*, and of the MLL-CASC5 (predicted) fusion protein. AT: AT hook, SNL: subnuclear localization; MT: methyltransferase; BD: binding domain; TAD: transcriptional activation domain; PHD: plant homeo domain; SET: Su(var)3e9; Enhancer-of-zeste: Trithorax; CC: coiled coil.

and impact on clinical outcome. In this regard, we recently reported *MLL-TET1* rearrangements, which belong to a category of rare *MLL* rearrangements.[2]

Here we present comprehensive analyses of patients with *MLL-CASC5* rearrangements resulting from t(11;15)(q23;q14-15) chromosomal translocations of which only 13 cases have been reported so far.[3-9] The *CASC5* (alias *AF15q14*) gene is located at chromosome 15q14, consists of 27 exons, and spans a genomic region of 70.32 kb. The encoded CASC5 protein is known to be associated with cell growth suppression and/or maturation enhancement and thus its disruption could be a key factor for leukemogenesis.[4] To date, 3 cases have been molecularly confirmed as *MLL-CASC5* positive leukemia (*MLL-CASC5*$^+$).[3,4] However, little is known about the common features of *MLL-CASC5*$^+$ leukemia both in terms of detailed molecular data and clinical aspects. In order to gain further insights into this rare MLL fusion, we have collected t(11;15)(q23;q14-15)/*MLL-CASC5*$^+$ leukemia cases and describe their clinical features (Table 1). By analyzing the molecular and clinical characteristics of these patients along with previously reported t(11;15)(q23;q14-15) cases, we aimed to unravel the distinct features of this cytogenetically described leukemia subtype.

Using the long distance inverse-polymerase chain reaction (LDI-PCR) method, 3 new *MLL-CASC5*$^+$ cases were identified, which have also been included in the recently published MLL recombinome database.[1] Basic demographic data, past medical history, and clinical course (if available), as well as cytogenetic, FISH, and LDI-PCR data for the 3 *MLL-CASC5* cases were collected and specimens for further analyses were obtained after informed consent. Altogether, now a total of 16 cases with a t(11;15)(q23;q14-15) were analyzed including 10 acute myeloid leukemia (AML) cases (2 M1, 4 M2, 3 M4, and 1 NOS (leukemia not otherwise specified)), 4 acute lymphoblastic leukemia (ALL) cases (1 L1, 2 L2, and 1 unspecified), and 2 cases of myelodysplastic syndrome (MDS). Mean age of the patients was 20.6 years (range 1-54); there were 11 males and 5 females. Although karyotypes were available in all cases, molecular data, including confirmation of *MLL-CASC5*, and clinical datasets were rather limited.

It is worthy to note that, as previously observed by others,[3] abnormalities of chromosome 3 were seen in 10 out of 16 cases (62.5%) with a t(11;15)(q23;q14-15). In an attempt to detect additional copy number alterations, we conducted SNP-microarray analysis on 2 of our 3 cases with chromosome 3 abnormalities (cases 1 and 2) using Affymetrix CytoScan 750K (Affymetrix, Santa Clara, CA, USA). Human Genome Build 37.2 was used for the analyses of copy number variations. These analyses reconfirmed the presence of chromosome 3 abnormalities (case 1: arr 3q12.2q27.3(100,678,757-186,599,696)cth; case 2: arr 3p12.2q26.31(83,272,290-172,476,033)cth). A common deleted or duplicated region on chromosome 3q was identified in both analyzed cases (Table 1). It will be interesting to determine whether the interstitial deletion and 3q gain observed in these 2 cases represent recurrent features in other t(11;15) cases, and whether any commonly deleted or duplicated genes at chromosome 3 cooperate in the leukemogenesis of this rare *MLL*$^+$ leukemia. Another notable finding from LDI-PCR analyses of the fusion breakpoints was that our 3 cases showed genomic break-

points located within exon 11 of the CASC5 gene, suggesting a common breakpoint cluster region (Figure 1). The structures of MLL, CASC5, and the putative fusion protein are presented in Figure 1. Although CASC5 is a known component of the MIS12 complex, which may be fundamental for kinetochore formation and proper chromosome segregation during mitosis (acts in coordination with CENPK to recruit the NDC80 complex to the outer kinetochore),[10,11] there is not yet enough knowledge on the mechanism of leukemogenesis of MLL-CASC5 fusion protein. According to a recent study by Grossman *et al.*,[12] not only additional cytogenetic aberrations (42.4%) but also RAS pathway mutations including NRAS (22%) and KRAS (20.3%) are frequently found in *MLL*-rearranged AML (RAS mutation results in our patients are provided in Table 1). Therefore, further studies on the MLL-CASC5 fusion protein itself, and their cooperative mutations such as RAS signaling pathway mutations or chromosome 3q abnormalities, would be required in the near future.

Regarding clinical outcome, out of 8 patients for whom clinical data were available, only 3 are in complete remission, whereas 5 patients died with a mean survival period of 10.4 months. Based on these data it appears that the clinical course and prognosis of *MLL-CASC5*$^+$ leukemia are generally poor and that such patients have relatively short periods of survival. Since *MLL* fusion genes, particularly in treatment-related or secondary hematopoietic malignancies, are indicative of a rather poor clinical outcome, monitoring for therapy response or an impending relapse using the genomic MLL fusion sequences as patient-specific molecular targets bone marrow transplantation may be indicated.[13,14] However, due to the small number of *MLL-CASC5*$^+$ leukemia cases, no final conclusion regarding its prognostic relevance can be drawn.

Taken together, to the best of our knowledge, this is the most comprehensive analysis of *MLL-CASC5*$^+$ leukemia to date. Although only a handful of cases have now been confirmed on the molecular level, it is efforts such as the use of LDI-PCR or other new genomic analyses that allowed the advancement of the classification and characterization of MLL-associated leukemias.[1,15] Further studies on *MLL-CASC5*$^+$ patients with regards to the molecular and clinical features will increase our understanding of this specific subtype of MLL-rearranged leukemias.

John Jeongseok Yang,[1] Tae Sung Park,[2] Seung-Tae Lee,[3]* Ja-Young Seo,[3] Seung Hwan Oh,[4] Eun Hae Cho,[5] Sabine Strehl,[6] Nora Mühlegger,[6] Michael N. Dworzak[6,7] Jan Zuna,[8] Dagmar Pospisilova,[9] Claus Meyer,[10] Rolf Marschalek,[10] Hee-Jin Kim,[3] Sun-Hee Kim[3]*

** TSP, STL, and SHK equally contributed to this work.*

[1]Department of Medicine, Graduate School Kyung Hee University, Seoul, Korea; [2]Department of Laboratory Medicine, School of Medicine, Kyung Hee University, Seoul, Korea; [3]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [4]Department of Laboratory Medicine, Inje University College of Medicine, Busan, Korea; [5]Green Cross Genome, Yongin, Korea; [6]CCRI, Children's Cancer Research Institute, St. Anna Kinderkrebsforschung e.V., Vienna, Austria; [7]St. Anna Children's Hospital, Department of Pediatrics, Medical University of Vienna, Vienna, Austria; [8]CLIP (Childhood Leukaemia Investigation Prague) – Department of Paediatric Haematology and Oncology, 2nd Faculty of Medicine, Charles University Prague and University Hospital Motol, Prague, Czech Republic; [9]Department of Paediatrics, Faculty Hospital of Palacky University, Olomouc, Czech Republic; and [10]Institute of Pharmaceutical Biology, ZAFES, Diagnostic Center of Acute Leukemia, Goethe-University of Frankfurt, Germany

Correspondence: 153jesus@hanmail.net
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