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Original Contribution

# The frequency of *NOTCH1* variants in T-acute lymphoblastic leukemia/lymphoma and chronic lymphocytic leukemia/small lymphocytic lymphoma among Jordanian patients

Nezeen Z. Abualhaj[a], Zain Dardas[a], Belal Azab[a,b], Dema Ali[b], Maher A. Sughayer[c], Tariq N. Aladily[a], Mamoun Ahram[d,*]

[a] Department of Pathology, Microbiology and Forensic Medicine, School of Medicine, The University of Jordan, Amman, Jordan
[b] Cell Therapy Center, The University of Jordan, Amman, Jordan
[c] Department of Pathology, King Hussein Cancer Center, Amman, Jordan
[d] Department of Physiology and Biochemistry, School of Medicine, The University of Jordan, Amman, Jordan

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ABSTRACT

The transmembrane receptor NOTCH1 is thought to be associated with the development and progression of T-acute lymphoblastic leukemia (T-ALL)/T-lymphoblastic lymphoma (T-LBL) and chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). The current study aimed to characterize NOTCH1 expression and elucidate the variants in the functional PEST domain of the receptor in T-ALL/LBL and CLL/SLL. The nuclear expression of NOTCH1 protein was detected in 25% and 5% of cases of T-ALL/LBL and CLL/SLL, respectively, whereas cytoplasmic expression was detected in 33.3% and 15% cases, respectively. The frequency of variants in T-ALL/LBL was 33%, whereas 40% of CLL/SLL cases possessed variants. Four novel variants were identified; three of which were non-synonymous and one common variant c.7280_7280delG between T-ALL/LBL and CLL/SLL cases. The previously described variant, c.7541_7542delCT, was detected in 3 cases of CLL/SLL. These results provide support for the contribution of NOTCH1 in the etiology of these types of cancers.

## 1. Introduction

NOTCH1 receptor plays an important role in the regulation of critical cellular processes such as proliferation, stem cell maintenance, and differentiation during embryonic and adult development [1]. It is a transmembrane protein that can also act as a ligand-activated transcription factor [2]. NOTCH1 signaling pathway is initiated by Jagged or Delta ligand. The ligand binds to the receptor on neighboring cells and induces a cascade of proteolytic cleavages. This results in the release and nuclear translocation of the notch intracellular domain (NICD) initiating a short-term transcriptional activation cascade [3]. Aberrations in this signaling pathway have been reported in various diseases especially hematological and solid malignancies [2].

In adult hematopoiesis, NOTCH1 has an indispensable function in T-cell development and differentiation [4,5]. The constitutive activation of *NOTCH1* gene was first observed in the t(7;9)(q34;q34.3) translocation found in < 1% of human T-acute lymphoblastic leukemia (T-ALL) [6]. A previous study has revealed that > 50% of human T-ALL cases harbor activating *NOTCH1* variants [7]. These variants involve mostly the heterodimerization domain (HD) and/or the C-terminal PEST domain. Variants within the HD region result in constitutive activation of the signaling pathway due to ligand-independent cleavage of NOTCH1 [8,9]. On the other hand, PEST variants increase the half-life and the stability of NOTCH1 [10].

Likewise, NOTCH1 activating variants are recurrently associated with chronic lymphocytic leukemia (CLL) [11]. In contrast to T-ALL, NOTCH1 signaling activation mechanism in CLL is ligand dependent [12]. The *NOTCH1* variants occur in ~10% of CLL at diagnosis and their frequency increases with disease progression [13]. Due to the oncogenic effect of NOTCH1, there is interest in therapeutic targeting of the protein in cancers with antagonists such as inhibitory antibodies and gamma-secretase inhibitors (GSI) [14]. Ideally, such trials focus on treatment of patients whose tumors show evidence of constant NOTCH1 activation.

Although there are several reports of *NOTCH1* variants, most of them have come from Western countries [14-17] and few from East Asia [18,19]. There are limited data from the Middle East and none from Jordan. In this study we aimed to investigate variants within the

* Corresponding author at: Department of Physiology and Biochemistry, School of Medicine, The University of Jordan, Amman 11942, Jordan.
*E-mail address:* m.ahram@ju.edu.jo (M. Ahram).

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PEST domain of *NOTCH1* in T-ALL/lymphoblastic lymphoma (LBL) and CLL/small lymphocytic lymphoma (SLL) cancers, and to compare their frequency with the reported studies. Moreover, we characterized *NOTCH1* expression in lymph node (LN) and bone marrow (BM).

## 2. Materials and methods

### 2.1. Samples

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks of cases diagnosed in 2014 and 2015 were retrospectively supplied from Jordan University Hospital and King Hussein Cancer Center. A total of eighty two (n = 82) FFPE tissue specimens were tested in this study. Thirty seven of them were diagnosed as T-ALL/LBL (12 LN and 25 BM), whereas forty five samples were diagnosed as CLL/SLL (20 LN and 25 BM). The study was approved by the Scientific Research Committee at the School of Medicine, The University of Jordan, and the Institutional Review Board at King Hussein Cancer Center. Tissue sections were used for both immunohistochemistry and DNA extraction.

### 2.2. Immunohistochemistry

Monoclonal anti-NOTCH1 antibody (clone A6, Quartett Immunodiagnostika, Biotechnologie, + Kosmetik Vertriebs GmbH, Germany) was used at a dilution of 1:20. Pancreatic tissue was stained in each run as a positive control. All tissue specimens were cut into 4-μm thin sections. The sections were rehydrated in xylene and alcohol of different concentrations. Antigen retrieval was performed by placing the tissue sections in a citrate buffer (pH 6.0) and heated at 95 °C for 15 min in a microwave. Incubation with the primary antibody was performed for 60 min followed by the secondary horseradish peroxidase-conjugated anti-mouse antibody (Leica, United Kingdom) for 30 min. Staining was detected using the Novolink Detection kit (Leica, United Kingdom). Nuclear staining within the cells was considered as a positive result, whereas cytoplasmic staining was noted as a negative result. All cases were reviewed by at least two hematopathologists.

### 2.3. DNA extraction and amplification

DNA was extracted from the FFPE tissue samples using DNA extraction kit (Zymo Research, USA) following manufacturer's protocol. Four pairs of primers were optimized for DNA amplification (Table 1). The amplicons cover 80% of PEST domain. Amplification of a portion of the actin gene was used as a control to ensure absence of PCR inhibitors in the reaction. Reactions lacking DNA template were used as a negative control to rule out the presence of contaminating DNA residues.

### 2.4. Sequence analysis

The PCR products were purified and sequenced using the forward primer by Sanger sequencing (Genewiz Inc., South Plainfield, NJ, USA). Sequence analysis was performed using ChromasPro software (Technelysium Pty Ltd., Tewantin, Australia). Sequences were compared with the reference sequences for genomic DNA (GenBank accession number: NG_007458) using Magic-Basic Local Alignment Search Tool (Magic-BLAST version 1.1.0) of the National Center for Biotechnology Information (NCBI). Possible deleterious effects of each variant on protein structure/function were predicted using the *in silico* tools SIFT algorithm (http://sift.bii.a-star.edu.sg/), PROVEAN (http://provean.jcvi.org/genome_submit_2.php), Polymorphism Phenotyping v2 (Polyphen2; http://genetics.bwh.harvard.edu/pph2/), and MutationTaster tool (www.mutationtaster.org). The frequency of variants was assessed using the Genome Aggregation Database (gnomAD).

## 3. Results

### 3.1. Expression of active NOTCH1 in T-ALL and CLL/SLL

Upon investigating expression of NOTCH1, 3/12 (25%) LN cases and none of BM samples of T-ALL showed diffuse nuclear staining for NICD (Fig. 1A). On the other hand, cytoplasmic positivity was noted in 4 LN cases and 3 BM cases (Fig. 1B). In contrast, nuclear staining in CLL/SLL cases was less frequent. Only 1/20 (5%) was positive in LN cases (Fig. 1C), in addition to 3 cases having cytoplasmic staining in the LNs (Fig. 1D). None of the BM samples of CLL/SLL cases was positive. Focal positivity was noted in stromal cells in the BM, which were consistent morphologically with histiocytes. A positive control of pancreatic tissue was routinely performed (Fig. 1E).

### 3.2. NOTCH1 variants in T-ALL

The frequency of *NOTCH1* variants was analyzed in 12 T-ALL samples that were successfully amplified. Sequencing revealed that 4/12 (33%) of the samples possessed somatic variants. In total, three novel variants were identified: A novel one bp deletion (c.7280_7280delG) was found in three samples (3163, 5015, and 2412), and two novel missense variants c.7322C > A and c.7093T > G in samples 5015 and 7075 respectively (Table 2; Fig. 1S). The c.7280_7280delG variant would result in a frameshift (p.Gly2427AlafsTer8) leading to a premature termination codon (PTC) at amino acid residue 2434. However, *in silico* prediction tools predicted the c.7322C > A variant as benign, whereas c.7093T > G variant was predicted as pathogenic (Table 2). Both variants were highly conserved in vertebrates and not reported in gnomAD.

### 3.3. NOTCH1 variants in CLL/SLL

Twenty LN cases of CLL/SLL were successfully amplified and analyzed for the frequency of *NOTCH1* variants. Three somatic variants were identified in 8/20 (40%) of cases. Interestingly, the novel deletion (c.7280_7280delG), which was shared among three T-ALL samples, was also found in 4 CLL samples (776, 1536, 4624, and 5103). Furthermore, a previously reported 2-bp deletion c.7541_7542delCT was identified in three samples (1917, 4055, and 5103) (Fig. 2S). This deletion causes a frameshift (p.Pro2514ArgfsTer4) that leads to a PTC at amino acid residue 2517. In two samples (2334 and 7349) a novel missense variant was found (c.7048C > A) (Fig. 3S). The *in silico* predictions for this variant were contradictory. While it was predicted as tolerated, benign, and neutral by SIFT, PolyPhen2, and Provean, respectively, it was predicted as disease causing by Mutation Taster (Table 2).

**Table 1**
Primer used to amplify the DNA region of the PEST domain.

| Primer number | Forward primer | Reverse primer | Product size/bp |
|---|---|---|---|
| NOTCH1-1 | GGGCCCTGAATTTCACTGT | AGGCCCTGGTAGCTCATCAT | 229 |
| NOTCH1-2 | GCTGCACAGTAGCCTTGCT | CTGAGCTCACGCCAAGGT | 224 |
| NOTCH1-3 | ACATCCAGCAGCAGCAAAG | GTGGGACCAGCGAGGATG | 222 |
| NOTCH1-4 | CACTATTCTGCCCCAGGAGA | CAGTCGGAGACGTTGGAATG | 234 |

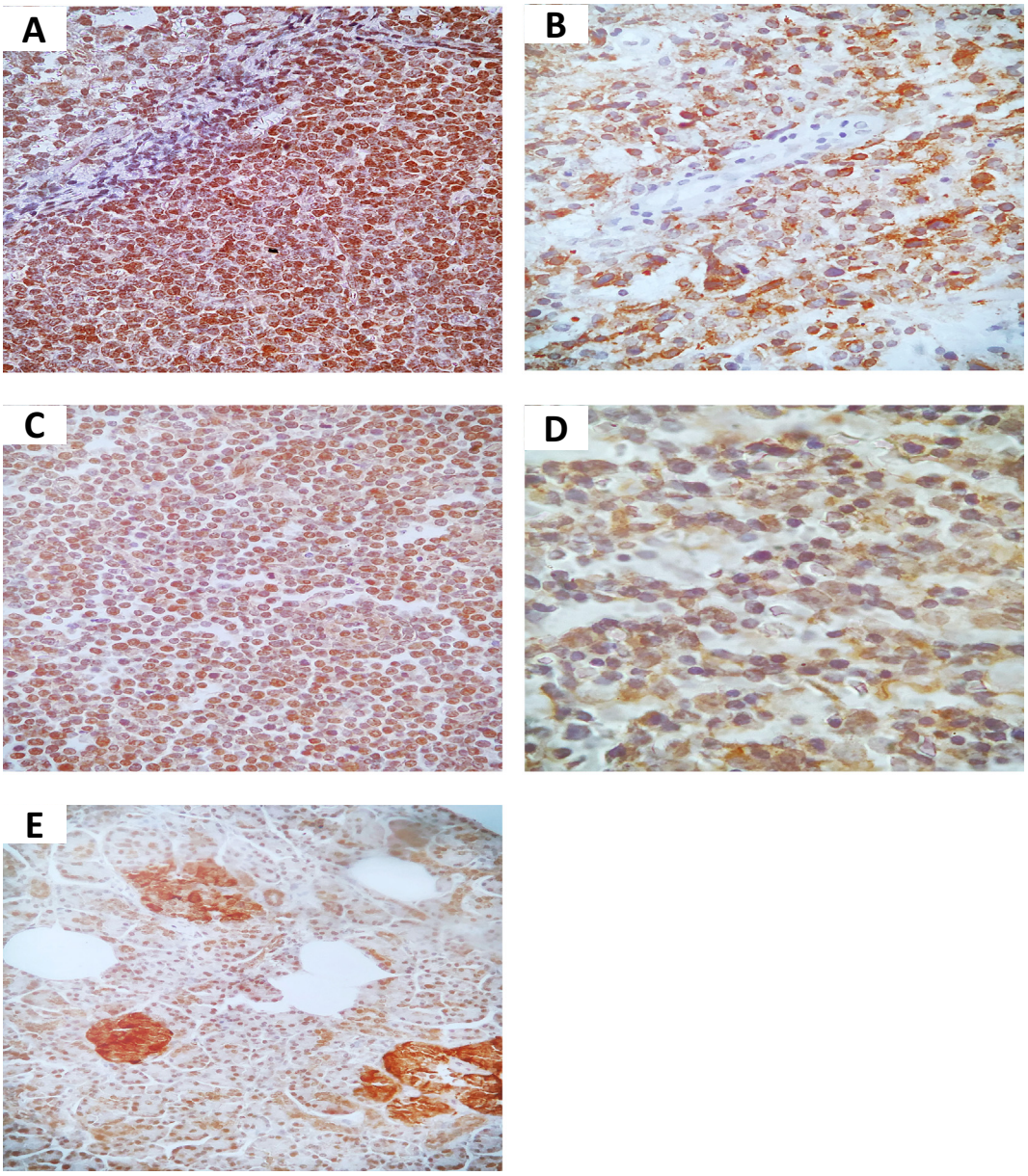


**Fig. 1.** Immunohistochemical staining of NOTCH1. (A) A case of T-ALL positive for nuclear staining. (B) Another case of T-ALL with positive cytoplasmic staining only. (C) A case of SLL showing nuclear localization. (D) A case of SLL with only positive cytoplasmic expression. (E) A positive control showing nuclear and cytoplasmic staining in pancreatic tissue.

## 4. Discussion

T-ALL/T-LBL are malignancies of T-lymphoblasts [20,21]. T-ALL accounts for about 15% of childhood ALL and 25% of adult ALL cases [22]. It is more common among older adolescents, with male predominance. ALL primarily affects the thymus then infiltrates into other tissues, especially organs that play a role in fetal hematopoiesis, which are often manifested by hepatosplenomegaly or lymphadenopathy [23]. In contrast, T-LBL is implicated in mass lesions, especially lymph nodes, and accounts for 2% of non-Hodgkin lymphoma [20-22]. Similarly, CLL and SLL are considered different manifestations of the same entity [24]. The hematological abnormalities and the lymph node infiltration are used to differentiate CLL from SLL [25]. In addition, CLL is the most common leukemia in Western countries [26] and appears particularly in older people [27]. The molecular etiology of T-ALL/LBL and CLL/SLL has yet to be determined.

*NOTCH1* has varied roles in cancer acting as either an oncogene or a tumor suppressor gene depending on the cellular context. Gain-of-function mutations of *NOTCH1* are common in T-ALL/LBL [8,28] and have also been described in subsets of CLL [12,15,29]. In this study we investigated for the first time the frequency of *NOTCH1* PEST domain variants in T-ALL/T-LBL and CLL/SLL cancers among Jordanian patients. We also analyzed the expression of *NOTCH1* in LN and BM FFPE tissues.

Interestingly, our analyses revealed a higher frequency of PEST domain variants in T-ALL/LBL (33% of cases) than the previously reported frequencies of a 4–27% range [8,16-19]. Similarly, the frequency of variants within the PEST domain in CLL/SLL cases was more frequent in this study where it occurred in 40% of cases in comparison to the previously reported 4–12% frequency [12,29-33].

Collectively, we have identified five variants (c.7280_7280delG, c.7541_7542delCT, c.7322C > A, c.7093T > G, c.7048C > A) in which four of them (80%) were novel. The c.7280_7280delG was the most common variant among all cases. It was detected in 25% of T-ALL/LBL

**Table 2**
NOTCH1 PEST domain variants.

| Disease | Sample ID | IHC | Primer | Variant coordinate hg19 | Variation | | | Clinvar | Maximum MAF (gnomad) | *In silico* prediction tool | | | | Reported/ novel | Morphological immunophenotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | HGVS c.DNA | HGVS A.A | Consequence | | | SIFT | PolyPhen2 | Provean | Mutation Taster | | |
| T-ALL | 3163 | +ve | NOTCH1-4 | chr9:139390911 | c.7280_7280delG | p.Gly2427AlafsTer8 | Frameshift | NA | NA | NA | NA | NA | DC | Novel | $CD3^+$, $TdT^+$, $CD79a^-$ |
| | 5015 | +ve | NOTCH1-4 | chr9:139390911 | c.7280_7280delG | p.Gly2427AlafsTer8 | Frameshift | NA | NA | NA | NA | NA | DC | Novel | $CD3^+$, $CD5^+$, $TdT^+$, |
| | | | | chr9:139390869 | c.7322C > A | p. Ala2441Glu | Missense | NA | NA | T | B | NE | P | Novel | $CD45^-$,$CD2^-$, $CD8^-$, $CD34^-$, $MPO^-$ $CD79a^-$ |
| | 2412[a] | +ve | NOTCH1-4 | chr9:139390911 | c.7280_7280delG | p.Gly2427AlafsTer8 | Frameshift | NA | NA | NA | NA | NA | DC | Novel | $CD3^+$, $CD5^+$, $BCL2^+$, $TdT^+$, $CD99^+$, $CD20^-$, $CD10^-$, BCL-$6^-$, $CD34^-$, $CD1a^-$, PAX-$5^-$ |
| | 7075 | −ve | NOTCH1-2 | chr9:139391098 | c.7093T > G | p.Tyr2365Asp | Missense | NA | NA | D | PD | DE | DC | Novel | $CD3^+$, $CD5^+$, $TdT^+$, $CD45^-$, $CD2^-$, $CD8^-$, $CD34^-$, $MPO^-$, CD79a, $LCA^+$, $CD5^+$, $CD99^+$, $TdT^+$, Pancytokeratin$^-$, CK8/$18^-$, synaptophysin$^-$, chromogranin$^-$, $CD10^-$ |
| CLL | 776 | −ve | NOTCH1-4 | chr9:139390911 | c.7280_7280delG | p.Gly2427AlafsTer8 | Frameshift | NA | NA | NA | NA | NA | DC | Novel | $LCA^+$, $CD5^+$, $CD20^+$, $CD23^+$, $CD43^+$, $CD3^+$, Cyclin $D1^-$, $CD15^-$, $CD30^-$, $CD138^-$, $CK^-$ |
| | 1536 | −ve | NOTCH1-4 | chr9:139390911 | c.7280_7280delG | p.Gly2427AlafsTer8 | Frameshift | NA | NA | NA | NA | NA | DC | Novel | $LCA^+$, $CD5^+$, $CD20^+$, $CD23^+$, $BCL2^+$,$CD3^+$ in T cells, Cyclin $D1^-$, $CD10^-$ |
| | 4624 | −ve | NOTCH1-4 | chr9:139390911 | c.7280_7280delG | p.Gly2427AlafsTer8 | Frameshift | NA | NA | NA | NA | NA | DC | Novel | $CD5^+$, $CD20^+$, $CD23^+$, $CD3^+$ in T cells |
| | 5103 | −ve | NOTCH1-4 | chr9:139390911 | c.7280_7280delG | p.Gly2427AlafsTer8 | Frameshift | NA | NA | NA | NA | NA | DC | Novel | $CD5^+$, $CD20^+$, $CD23^+$ |
| | | | NOTCH1-5 | chr9:139390649 | c.7541_7542delCT | p.Pro2514ArgfsTer4 | Frameshift | NA | NA | NA | NA | NA | DC | Reported | |
| | 1917[b] | −ve | NOTCH1-5 | chr9:139390649 | c.7541_7542delCT | p.Pro2514ArgfsTer4 | Frameshift | NA | NA | NA | NA | NA | DC | Reported | $CD5^+$, $CD20^+$, $CD23^+$, $CD3^-$, Cyclin $D1^-$ |
| | 4055 | −ve | NOTCH1-5 | chr9:139390649 | c.7541_7542delCT | p.Pro2514ArgfsTer4 | Frameshift | NA | NA | NA | NA | NA | DC | Reported | $CD20^+$, $CD23^+$, $CD3^+$, $CD5^+$, Cyclin $D1^-$ |
| | 2334 | −ve | NOTCH1-2 | chr9:139391143 | c.7048C > A | p.Leu2350Met | Missense | NA | NA | T | B | NE | DC | Novel | $CD20^+$, $CD23^+$, $CD5^+$, $CD10^-$, Cyclin $D1^-$, $CD3^+$ in T cells |
| | 7349 | −ve | NOTCH1-2 | chr9:139391143 | c.7048C > A | p.Leu2350Met | Missense | NA | NA | T | B | NE | DC | Novel | $CD5^+$, $CD20^+$, $CD23^+$ |

T-ALL: T-acute lymphoblastic leukemia, CLL: chronic lymphocytic leukemia, IHC: immunohistochemistry, NA: not available, MAF: minor allele frequency, T: tolerated, D: damaging, B: benign, PD: probably damaging, NE: neutral, DE: deleterious, DC: disease causing, P: polymorphism.

[a] A negative result for BCR/ABL1 gene translocation and no abnormal clone has been identified by karyoptyping.

[b] A negative result for the presence of a clone with deletion 13q and deletion 19p.

cases and 20% of CLL/SLL cases. This novel deletion introduces a frameshift mutation (p.Gly2427AlafsTer8) that truncates 122 of the *NOTCH1* amino acids. The other deletion, c.7541_7542delCT, was found in 15% of CLL/SLL cases. It also leads to a frameshift (p.Pro2514ArgfsTer4) causing a PTC 39 amino acids upstream of the normal stop codon. This variant was previously reported to account for 80–94% of *NOTCH1* variants in CLL [33-35]. Consequently, both deletions generate a NOTCH1 protein lacking the C-terminal PEST domain, where phosphorylation of NOTCH1 inactivates NOTCH1 signaling. In fact, a truncated NOTCH1 is a more stable protein that is constitutively active [33,36].

The other three novel variants were missense variants (c.7322C > A, c.7093T > G, c.7048C > A). All of them are highly conserved in vertebrates not reported in the population databases, and expected to cause a significant change in protein structure and/or function. Nevertheless, further work is warranted to reach a solid conclusion regarding the pathogenicity of these variants.

Few studies have investigated the expression of *NOTCH1* in T-ALL/LBL and CLL/SLL. For instance, Kluk et al. found that 57% of T-ALL/T-LBL cases and 89% of CLL cases were positive for nuclear NICD staining [14]. Another study assessed the expression of *NOTCH1* in CLL patients and found that 79.3% of cases have positive nuclear staining [31]. Moreover, Arruga et al. showed that 27% of CLL cases displayed diffused NICD staining, whereas cytoplasmic staining was mostly localized in tumors with wild-type *NOTCH1* [36]. In this study, positive expression of *NOTCH1*, indicated by nuclear staining, was observed in 25% of T-ALL/LBL-LN tissues and 5% of CLL/SLL-LN tissues. However, 33.3% of CLL/SLL-LNs, 15% of CLL/SLL-LNs, and 12% of T-ALL/LBL-BM samples displayed cytoplasmic expression. None of the BM samples showed nuclear staining. Clearly, our results indicate lower positive staining than other studies. This could be due to the type of antibodies used for staining where some of the previous studies used polyclonal antibodies for detecting NICD, whereas we utilized a monoclonal antibody that recognizes a cleaved peptide of Notch1. The nuclear expression was not significantly associated with the *NOTCH1* variants. This is similar to other reports that have also not found a significant association [14,31].

In summary, a high frequency of *NOTCH1* variants was observed in our cohort. This study has identified four novel variants that add new insights into the genetic heterogeneity of T-ALL/LBL and CLL/SLL. All variants require further investigation of their pathogenic contribution in disease transformation, progression, and response to therapy. In addition, a large series of T-ALL/LBL and CLL/SLL cases, as well as their relationship with other clinical and biological features, are warranted to elucidate the relevance of the *NOTCH1* variants in the Jordanian population.

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## Conflict of interest

All authors declare no conflict of interest of any type.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.anndiagpath.2019.01.004.

## References

[1] Borggrefe T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. Cell Mol Life Sci 2009;66:1631–46. https://doi.org/10.1007/s00018-009-8668-7.

[2] Gianfelici V. Activation of the NOTCH1 pathway in chronic lymphocytic leukemia. Haematologica 2012;97:328–30. https://doi.org/10.3324/haematol.2012.061721.

[3] Liu N, Zhang J, Ji C. The emerging roles of Notch signaling in leukemia and stem cells. Biomark Res 2013;1:23. https://doi.org/10.1186/2050-7771-1-23.

[4] Deftos ML, Bevan MJ. Notch signaling in T cell development. Curr Opin Immunol 2000;12:166–72.

[5] Bigas A, Espinosa L. Hematopoietic stem cells: to be or Notch to be. Blood 2012;119:3226–35. https://doi.org/10.1182/blood-2011-10-355826.

[6] Palomero T, Barnes KC, Real PJ, Glade Bender JL, Sulis ML, Murty VV, et al. CUTLL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to γ-secretase inhibitors. Leukemia 2006;20:1279–87. https://doi.org/10.1038/sj.leu.2404258.

[7] Li X, von Boehmer H. Notch signaling in T-cell development and T-ALL. ISRN Hematol 2011;2011:921706. https://doi.org/10.5402/2011/921706.

[8] Weng AP, Ferrando AA, Lee W, Morris JP, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306:269–71. https://doi.org/10.1126/science.1102160.

[9] Malecki MJ, Sanchez-Irizarry C, Mitchell JL, Histen G, Xu ML, Aster JC, et al. Leukemia-associated mutations within the NOTCH1 heterodimerization domain fall into at least two distinct mechanistic classes. Mol Cell Biol 2006;26:4642–51. https://doi.org/10.1128/MCB.01655-05.

[10] Thompson BJ, Jankovic V, Gao J, Buonamici S, Vest A, Lee JM, et al. Control of hematopoietic stem cell quiescence by the E3 ubiquitin ligase Fbw7. J Exp Med 2008;205:1395–408. https://doi.org/10.1084/jem.20080277.

[11] Rosati E, Sabatini R, Rampino G, Tabilio A, Di Ianni M, Fettucciari K, et al. Constitutively activated Notch signaling is involved in survival and apoptosis resistance of B-CLL cells. Blood 2009;113:856–65. https://doi.org/10.1182/blood-2008-02-139725.

[12] Di Ianni M, Baldoni S, Rosati E, Ciurnelli R, Cavalli L, Martelli MF, et al. A new genetic lesion in B-CLL: a *NOTCH1* PEST domain mutation. Br J Haematol 2009;146:689–91. https://doi.org/10.1111/j.1365-2141.2009.07816.x.

[13] Rossi D, Rasi S, Fabbri G, Spina V, Fangazio M, Forconi F, et al. Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. Blood 2012;119:521–9. https://doi.org/10.1182/blood-2011-09-379966.

[14] Kluk MJ, Ashworth T, Wang H, Knoechel B, Mason EF, Morgan EA, et al. Gauging NOTCH1 activation in cancer using immunohistochemistry. PLoS One 2013;8:e67306https://doi.org/10.1371/journal.pone.0067306.

[15] Villamor N, Conde L, Martínez-Trillos A, Cazorla M, Navarro A, Beà S, et al. NOTCH1 mutations identify a genetic subgroup of chronic lymphocytic leukemia patients with high risk of transformation and poor outcome. Leukemia 2013;27:1100–6. https://doi.org/10.1038/leu.2012.357.

[16] Breit S, Stanulla M, Flohr T, Schrappe M, Ludwig W-D, Tolle G, et al. Activating NOTCH1 mutations predict favorable early treatment response and long-term outcome in childhood precursor T-cell lymphoblastic leukemia. Blood 2006;108:1151–7. https://doi.org/10.1182/blood-2005-12-4956.

[17] Mansour MR, Linch DC, Foroni L, Goldstone AH, Gale RE. High incidence of Notch-1 mutations in adult patients with T-cell acute lymphoblastic leukemia. Leukemia 2006;20:537–9. https://doi.org/10.1038/sj.leu.2404101.

[18] Bhanushali AA, Babu S, Thangapandi VR, Pillai R, Chheda P, Das BR. Mutations in the HD and PEST domain of Notch-1 receptor in T-cell acute lymphoblastic leukemia: report of novel mutations from Indian population. Oncol Res 2010;19:99–104.

[19] Zhu Y-M, Zhao W-L, Fu J-F, Shi J-Y, Pan Q, Hu J, et al. NOTCH1 mutations in T-cell acute lymphoblastic leukemia: prognostic significance and implication in multifactorial leukemogenesis. Clin Cancer Res 2006;12:3043–9. https://doi.org/10.1158/1078-0432.CCR-05-2832.

[20] Hoelzer D, Gökbuget N. T-cell lymphoblastic lymphoma and T-cell acute lymphoblastic leukemia: a separate entity? Clin Lymphoma Myeloma 2009;9:S214–21. https://doi.org/10.3816/CLM.2009.s.015.

[21] Ribera J-M. T-cell acute lymphoblastic leukemia and lymphoblastic lymphoma. XXXVII. Ankara, Turkey: Ulusal Hematoloji Kongresi; 19–22 October 2011https://pdfs.semanticscholar.org/a263/19a95600d2f8170653f6645257183a551c57.pdf.

[22] Zerbini MCN, Soares FA, Morais JC, Vassallo J, Deolinda Rodrigues Pereira Velloso E, Chaufaille MLLF, et al. World Health Organization classification of tumors of hematopoietic and lymphoid tissues. 2008-major changes from the 3rd edition. 4th ed.57. Revista da Associação Médica Brasileira; 2001. p. 66–73. (1992. Impresso).

[23] Harmening D. Clinical hematology and fundamentals of hemostasis. 5th ed. F.A. Davis Co: Philadelphia PA; 2009.

[24] Thomas LC, Maida MJ, Martinez-Outschoorn U, Filicko-O'Hara J, Morris GJ. Chronic lymphocytic leukemia/small lymphocytic lymphoma with pancytopenia and chylothorax. Semin Oncol 2011;38:165–70. https://doi.org/10.1053/j.seminoncol.2010.11.013.

[25] Gibson SE, Swerdlow SH, Ferry JA, Surti U, Dal Cin P, Harris NL, et al. Reassessment of small lymphocytic lymphoma in the era of monoclonal B-cell lymphocytosis. Haematologica 2011;96:1144–52. https://doi.org/10.3324/haematol.2011.042333.

[26] Parikh SA, Rabe KG, Kay NE, Call TG, Ding W, Schwager SM, et al. Chronic lymphocytic leukemia in young (≤55 years) patients: a comprehensive analysis of prognostic factors and outcomes. Haematologica 2014;99:140–7. https://doi.org/10.3324/haematol.2013.086066.

[27] Armitage JO. Non-Hodgkin lymphomas. Lippincott Williams & Wilkins; 2009.

[28] Ferrando AA. The role of NOTCH1 signaling in T-ALL. Hematol Am Soc Hematol Educ Progr 2009:353–61. https://doi.org/10.1182/asheducation-2009.1.353.

[29] Fabbri G, Rasi S, Rossi D, Trifonov V, Khiabanian H, Ma J, et al. Analysis of the chronic lymphocytic leukemia coding genome: role of *NOTCH1* mutational activation. J Exp Med 2011;208:1389–401. https://doi.org/10.1084/jem.20110921.

[30] Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, et al. *SF3B1* and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med 2011;365:2497–506. https://doi.org/10.1056/NEJMoa1109016.
[31] Onaindia A, Gómez S, Piris-Villaespesa M, Martínez-Laperche C, Cereceda L, Montes-Moreno S, et al. Chronic lymphocytic leukemia cells in lymph nodes show frequent NOTCH1 activation. Haematologica 2015;100:e200–3. https://doi.org/10.3324/haematol.2014.117705.
[32] Puente XS, Pinyol M, Quesada V, Conde L, Ordóñez GR, Villamor N, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 2011;475:101–5. https://doi.org/10.1038/nature10113.
[33] Willander K, Dutta RK, Ungerbäck J, Gunnarsson R, Juliusson G, Fredrikson M, et al. NOTCH1 mutations influence survival in chronic lymphocytic leukemia patients. BMC Cancer 2013;13:274. https://doi.org/10.1186/1471-2407-13-274.
[34] Zou Y, Fan L, Xia Y, Miao Y, Wu W, Cao L, et al. NOTCH1 mutation and its prognostic significance in Chinese chronic lymphocytic leukemia: a retrospective study of 317 cases. Cancer Med 2018;7:1689–96. https://doi.org/10.1002/cam4.1396.
[35] Burns A, Alsolami R, Becq J, Stamatopoulos B, Timbs A, Bruce D, et al. Whole-genome sequencing of chronic lymphocytic leukaemia reveals distinct differences in the mutational landscape between IgHVmut and IgHVunmut subgroups. Leukemia 2018;32:332–42. https://doi.org/10.1038/leu.2017.177.
[36] Arruga F, Gizdic B, Serra S, Vaisitti T, Ciardullo C, Coscia M, et al. Functional impact of NOTCH1 mutations in chronic lymphocytic leukemia. Leukemia 2014;28:1060–70. https://doi.org/10.1038/leu.2013.319.