Clinical and Experimental Medicine (2019) 19:261–267
https://doi.org/10.1007/s10238-019-00551-0

CASE REPORT

1q23.1 homozygous deletion and downregulation of Fc receptor-like family genes confer poor prognosis in chronic lymphocytic leukemia

Giulia Daniele[1] · Alberto L'Abbate[1] · Antonella Turchiano[1] · Orazio Palumbo[2] · Massimo Carella[2] · Crocifissa Lo Cunsolo[3] · Paolo Iuzzolino[3] · Angelo Lonoce[1] · María Hernández-Sánchez[4] · Carla Minoia[5] · Patrizia Leone[6] · Jesus Maria Hernandez-Rivas[4] · Clelia Tiziana Storlazzi[1]

Received: 23 October 2018 / Accepted: 11 March 2019 / Published online: 15 March 2019

Abstract

The identification of chromosome 1 translocations and deletions is a rare and poorly investigated event in chronic lymphocytic leukemia (CLL). Nevertheless, the identification of novel additional molecular alterations is of great interest, opening to new prognostic and therapeutic strategies for such heterogeneous hematological disease. We here describe a patient affected by CLL with a mutated *IGHV* status, showing a balanced t(1;3)(q23.1;q21.3) translocation and a der(18)t(1;18)(q24.2;p11.32), accompanying the recurrent 13q14 heterozygous deletion in all analyzed cells at onset. By combining whole-genome sequencing, SNP array, RNA sequencing, and FISH analyses, we defined a 1q23.1 biallelic minimally deleted region flanking translocations breakpoints at both derivative chromosome 1 homologues. The deletion resulted in the downregulation of the Fc receptor-like family genes *FCRL1*, *FCRL2*, and *FCRL3* and in the lack of expression of *FCRL5*, observed by RT-qPCR. The mutational status of *TP53*, *NOTCH1, SF3B1*, *MYD88*, *FBXW7*, and *XPO1* was investigated by targeted next-generation sequencing, detecting a frameshift deletion within *NOTCH1* (c.7544_7545delCT). We hypothesize a loss of tumor suppressor function for *FCRL* genes, cooperating with *NOTCH1* mutation and 13q14 genomic loss in our patient, both conferring a negative prognosis, independently from the known biological prognostic factors of CLL.

Keywords CLL · 1q23.1 deletion · *FCRL* · *IGHV* · *NOTCH1* · Translocation

Background

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western countries (Europe and North America), mainly affecting elderly people [1]. Deletions of 11q22-q23, 13q14, and 17p13, as well as trisomy of chromosome 12, were recognized as the most frequent aberrations in this disease, and their detection guides the therapeutic approach [2]. Conversely, translocations are generally rare in CLL and mainly involve chromosomes harboring immunoglobulin gene loci [3].

Considering the high heterogeneity of CLL natural course, the identification of new additional molecular alterations is of great interest, opening to new prognostic and therapeutic strategies. The impact of chromosomal translocations [3] and deletions involving chromosome 1 [4] has

Giulia Daniele and Alberto L'Abbate equally contributed to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10238-019-00551-0) contains supplementary material, which is available to authorized users.

✉ Clelia Tiziana Storlazzi
cleliatiziana.storlazzi@uniba.it

[1] Department of Biology, University of Bari "Aldo Moro", Via G. Orabona No. 4, 70126 Bari, Italy

[2] Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy

[3] UO Anatomia Patologica, Ospedale S. Martino, Belluno, Italy

[4] Servicio de Hematología, Hospital Universitario de Salamanca, IBSAL, IBMCC, Centro de Investigación del Cáncer, Universidad de Salamanca, CSIC, Salamanca, Spain

[5] Haematology Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy

[6] Department of Biomedical Sciences and Human Oncology, Internal Medicine Unit G. Baccelli, University of Bari Aldo Moro Medical School, Bari, Italy

been poorly investigated so far. Here, we characterized two novel chromosomal translocations [t(1;3)(q23.1;q21.3) and t(1;18)(q24.2;p11.32)] accompanied by rearrangement of both chromosomes 1 in a CLL patient, possibly correlated with the development and the prognosis of the disease. To gather a complete view of these chromosomal imbalances, we integrated next-generation sequencing (NGS) [whole-genome sequencing (WGS) and RNA sequencing (RNA-Seq)], SNP array, PCR techniques, and fluorescence in situ hybridization (FISH).

Case presentation

A 76-year-old male patient was admitted to the Belluno Central Hospital (Italy) for absolute lymphocytosis (lymphocytes 10.2×10^3 µL) in October 2014. Flow cytometry immunophenotyping of the peripheral blood (PB) was consistent with the diagnosis of CLL/SLL (chronic lymphocytic leukemia/small lymphocytic lymphoma) and was negative for the main biological prognostic markers (ZAP-70, CD38, CD49d, CD69, CD305/LAIR1). The patient was asymptomatic. Evaluated as Binet stage B, the patient underwent a close follow-up. A disease progression was documented (lymphocyte doubling time, thrombocytopenia, some episodes of mild night sweats) in May 2015. Cytogenetic analysis on metaphase spreads obtained by PB cultured in the presence of ChromoLymphoB proliferation reagent (Euroclone, Pero, Italy) defined the karyotype as follows: 46,XY,t(1;3)(q?11;q?11),del(13)(q13q14),-18, +mar [20] (Online Resource 1) [5]. Subsequent multi-color-FISH (M-FISH) analysis, performed by using the commercially available 24XCyte multi-color probe kit (MetaSystems, Milan, Italy) according with the manufacturer's instructions, detected two copies of a der(1)t(1;3)(q23;q21) and one copy of a der(3)t(1;3)(q23;q21) (Fig. 1a), as well as a der(18) resulting from a t(1;18)(?;p11.3) translocation, undetected by classical cytogenetic analysis (Fig. 1b). A not deleted status for *TP53* was observed by FISH, while a mutated status of the immunoglobulin heavy variable (*IGHV*) was disclosed (4.5% of identity compared to the *IGHV* germline sequence counterpart) [6]. Since February 2016, the patient was then treated with 6 courses of rituximab–bendamustine allowing a complete response (IWCLL 2008 criteria), but he presented an early relapse with a disease-free survival of 10 months. The karyotype at this stage was not available, due to the lack of metaphases in the PB. Due to the low tumor burden at relapse, the patient underwent a close follow-up (Online Resource 2).

Structural variations (SVs) and copy number (CN) information on PB genomic DNA of the patient were identified by combining WGS and SNP array data. WGS library preparation was performed according to manufacturer's instructions using the TruSeqDNA v2 protocol (Illumina, San Diego, CA, USA) and sequenced on the Illumina X Ten platform (Illumina, San Diego, CA, USA), in a paired-end 2 × 150-cycle run, with a mean coverage of 30 × per sample. Raw reads were aligned to the human reference genome (GRCh37/hg19) using BWA-MEM (v.0.7.12) (http://bio-bwa.sourceforge.net/) with default configuration. PCR duplicates were removed using Picard tools (v.1.119, https://broadinstitute.github.io/picard/). A total of 16,745 SVs (Online Resource 3) were identified using DELLY software (v.0.7.1) and Integrative Genomics Viewer (IGV) analysis. The occurrence of an interstitial deletion at 13q14 (chr13:50,052,441-51,667,198, Online Resource 4a), which led to the loss of *TRIM13, miR-3613, KCNRG, DLEU2, miR-16-1, miR-15a,* and *DLEU1,* as frequently observed in CLL [7], was also confirmed. Moreover, WGS analysis showed that chromosome 1 at nt 157,121,603 was fused to chromosome 3 at nt 129,066,511 on both der(1) chromosomes (Fig. 1a). On der(3), chromosome 3 at nt 128,646,792 was joined to chromosome 1 at nt 158,308,307 (Fig. 1a). The fusion of chromosome 1 (at nt 168,080,587) to chromosome 18 (at nt 2,825,548) was also detected on der(18), revealing a breakpoint at 1q24.2 (Fig. 1b).

SNP array data were obtained by using the Affymetrix CytoScan HD platform (Affymetrix, Santa Clara, CA, USA), following the manufacturer's protocol, and analyzed by the Chromosome Analysis Suite 3.1 software. The resulting data (Online Resource 5), combined with IGV visual inspection of WGS results, showed copy number switches on chromosome 1 at nt 157,121,603, at nt 158,308,307, and at nt 168,080,587 (Online Resource 4b), corresponding to a homozygous and a heterozygous deletion at 1q23.1 (chr1:157,121,603-158,308,307) and 1q23.1-q24.2 (chr1:158,308,307-168,080,587), respectively. Similarly, sequence coverage switches were also observed on chromosomes 3 (chr3:128,646,792-129,066,511) and 18 (chr18:1-2,825,548) (Online Resource 4c-d).

FISH assays [8] on PB metaphase chromosomes and nuclei, performed with bacterial artificial chromosome (BAC) probes (Fig. 1, Online Resource 6) and PCR (Online Resource 7a), followed by Sanger sequencing, confirmed the translocations breakpoints and the size of all deletions flanking the t(1;3)(q23.1;q21.3) and t(1;18)(q24.2;p11.32) (Online Resource 6, Online Resource 8, and data not shown). Therefore, the karyotype has been refined as follows: 46,XY,t(1;3)(q23.1;q21.3), der(1)t(1;3)(q23.1;q21.3),del(13)(q13q14),der(18)t(1;18)(q21.2;p11.32).

The availability of good quality RNA material prompted us to investigate the presence of possible fusion transcripts. RNA-Seq library was prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA) and sequenced on the Illumina HiSeq 2500 platform, in a paired-end 2 × 101-cycle run (60 Mreads/sample). Fusion

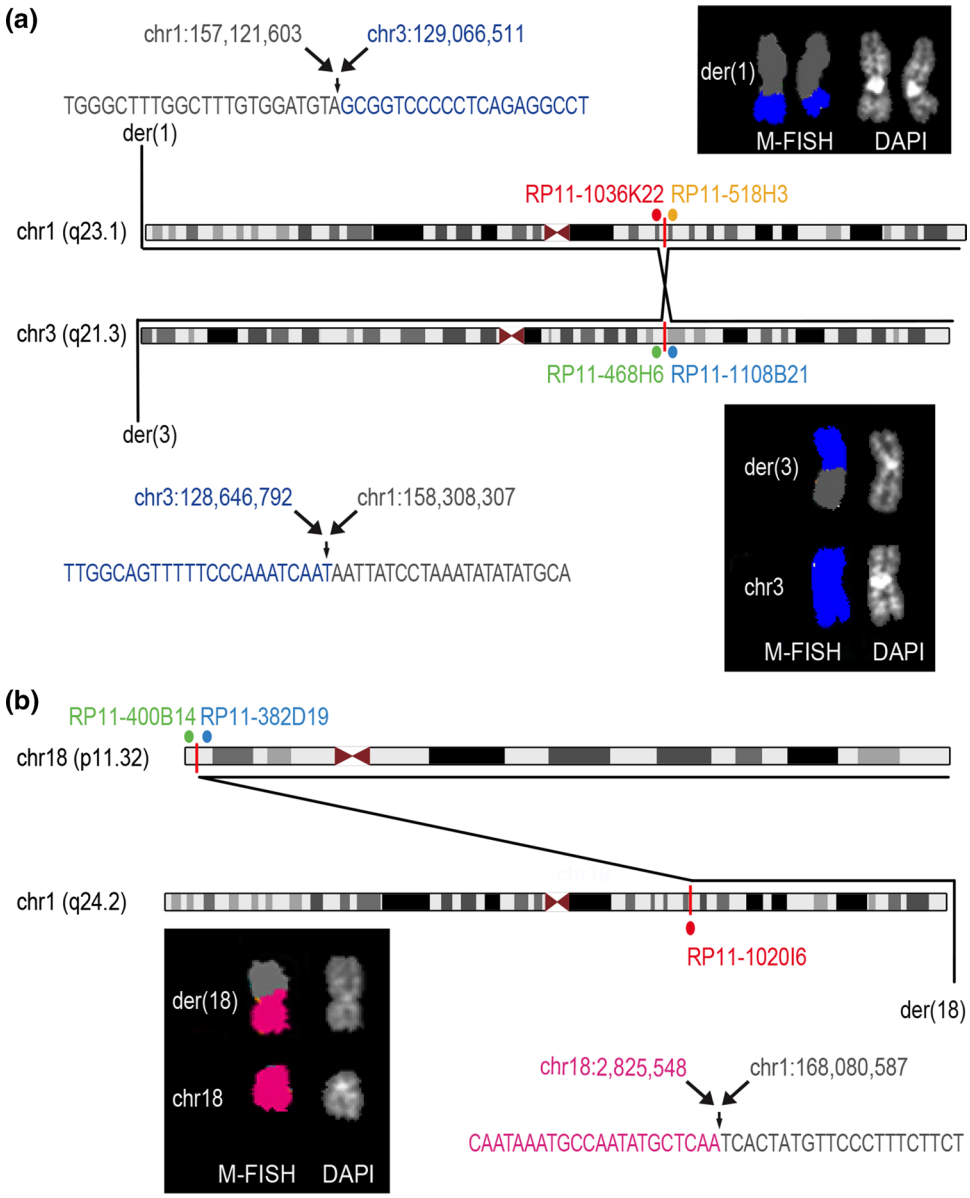

Fig. 1 Chromosome 1 translocations breakpoints, accompanied by deletions, mapped at nucleotide level. Ideograms of chromosomes 1, 3, and 18 showing breakpoints (red bars) of t(1;3)(q23.1;q21.3) (**a**) and of t(1;18)(q24.2;p11.32) (**b**) translocations, respectively. Fusion junction sequences for each derivative chromosome are indicated together with nucleotide map positions (black arrows). Partial M-FISH karyotypes of pseudo-colored and corresponding DAPI staining images of derivative chromosome are also shown on the left and right sides of the black boxes, respectively. Consistently colored FISH probes used to confirm translocations and deletions breakpoints are also indicated. (Corresponding FISH pseudo-colored images are shown in Online Resource 8.) (color figure online)

transcripts calls were performed using ChimeraScan software with default parameters. Ten chimeric transcripts were selected according to the number of supporting reads, as well as to the biological role of partner genes (highlighted in Online Resource 9), but their validation by RT-PCR, with primers designed according to ChimeraScan results (Online Resource 7b), provided negative results.

We then focused our attention on the genes mapping in the 1q23.1 homozygously deleted region (*FCRL1*, *FCRL2*, *FCRL3*, *FCRL4*, *FCRL5*, *CD1A*, *CD1C*, *CD1D*, *CD5L*, *ETV3*, and *KIRREL*), taking into account their known leukemogenic role, to identify those ones with a potential behavior as tumor suppressor genes (TSGs). Their expression level was evaluated by RT-qPCR with primer pairs designed on exons shared by multiple transcripts isoforms (Online Resource 7c). The experiments were performed using the Roche LightCycler 96 System with SYBR Green (FastStart Universal SYBR Green Master, Roche, Monza, Italy), according to manufacturer's instruction. The relative quantification analysis was performed using the LightCycler 96 software (Online Resource 10). The geometric mean of *B2 M* and *HPRT1* housekeeping genes was used as reference and a pooled sample, obtained by mixing RNAs of CLL Sel+ CD19+ cells from three cases without structural variations involving chromosome 1 (ctrl#1, ctrl#2, and ctrl#3), as calibrator. For two of these control cases (ctrl#2, ctrl#3), information about *IGHV* mutational status (unmutated and mutated, respectively) and clinical outcome (both favorable) was available.

Among the homozygously deleted genes, in our patient we detected the downregulation of three out of five genes belonging to the *Fc receptor-like (FcRL)* family (*FCRL1*, *FCRL2*, and *FCRL3*), as well as the upregulation of three members of the *CD1* family (*CD1A*, *CD1C*, and *CD1D*) and of *ETV3*, if compared with the pooled sample of the CLL cases used as controls. On the contrary, *FCRL4*, *FCRL5*, *CD5L*, and *KIRREL* showed an undetectable expression level (Fig. 2). Particularly, we confirmed the lack of *FCRL4* expression in the patient as well as in controls [9]. As

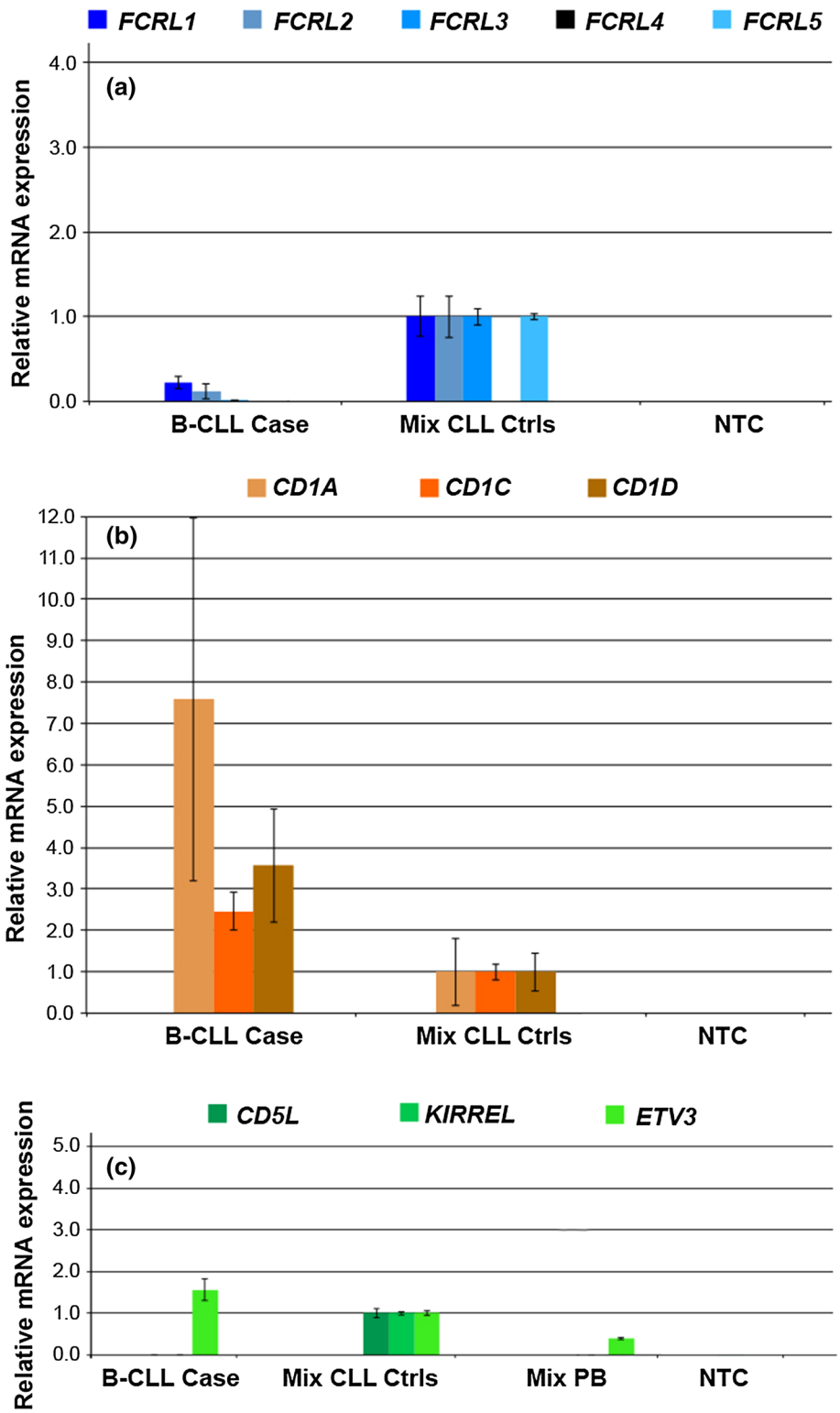

Fig. 2 Expression levels of genes mapping to the 1q23.1 homozygously deleted region. RT-qPCR results showing *FcRL* family (**a**), *CD1* family (**b**), and *CD5L*, *KIRREL*, and *ETV3* (**c**) expression in the present B-CLL case, and in appropriate normal tissues in comparison with a pool of three B-CLL controls

observed by ChimeraScan analysis, none of these genes were involved in the genesis of chimeras (Online Resource 9).

Additionally, to define the molecular profile of the patient for prognostic evaluation purposes [10], we investigated the mutational status of genes with prognostic significance in CLL [*TP53* (ENST00000269305, exons 4-10, 8 amplicons), *NOTCH1* (ENST00000277541, exons 33-34 corresponding to the C-terminal PEST domain, 7 amplicons), *SF3B1* (ENST00000335508, exons 10-16 of *SF3B1*, 7 amplicons), *MYD88* (ENST00000396334, exons 4 and 5, 2 amplicons), *FBXW7* (ENST00000281708, exons 8-12, 5 amplicons), and *XPO1* (ENST00000281708, exons 8-12, 5 amplicons)]. A targeted sequencing was performed using the GS FLX and Junior Sequencer Instrument software version 2.7 (Roche Applied Science) with the 454 Titanium Amplicon chemistry (Roche Applied Science, Penzberg, Germany). The oligonucleotide design was performed as part of the IRON-II network, and the workflow has been previously reported [11]. The median number of reads generated per gene was 1098 (coverage range 434–2091-fold) (Online Resource 11). To detect variants, filters were set to display those ones occurring in more than 2% of bidirectional reads per amplicon [12]. The analysis identified a frameshift *NOTCH1* deletion (c.7544_7545delCT/p.P2515 fs*4) with a mutational burden of 46.5%, affecting the C-terminal PEST domain of the encoded protein (Online Resource 12), probably cooperating with 1q23.1 loss and 13q14 deletion. All the other tested genes (*TP53*, *SF3B1*, *MYD88*, *FBXW7*, and *XPO1*) showed a wild-type allelic status.

Discussion and conclusions

We here report a *IGHV*-mutated CLL case with 13q14 deletion, *NOTCH1* mutation and a novel 1q23.1 homozygous deletion with concomitant downregulation of the *FcRL* family genes *FCRL1*, *FCRL2*, and *FCRL3*. This homozygous deletion (1,124 Kb in size), flanking the breakpoint of a t(1;3)(q23.1;q21.3) translocation, involved two copies of a der(1)t(1;3) chromosome. The two der(1) copies replaced both normal chromosome 1 counterparts. Such aberrations were observed in all the analyzed metaphases of the patient's sample at onset, suggesting they occurred as early mutational events in the tumor cells of the patient.

FcRL genes encode for FCRL glycoproteins containing an immuno-receptor tyrosine-based activating/inhibitory motif (ITAM/ITIM), able to confer a dual role in regulating B cell responses and function [13]. They were previously investigated on their possible impact upon diagnosis, prognosis and treatment of B cell malignancies. *FCRL1, FCRL2, FCRL3, FCRL4,* and *FCRL5* gene upregulation was found in CLL samples also showing a mutated *IGHV* status [14, 15]. Particularly, *FCRL2* expression has been considered a potential prognostic marker if associated with the *IGHV* mutational status. Indeed, a low expression level of *FCRL2* was correlated with the *IGHV* unmutated status, CD38 and ZAP70 surface expression, and unfavorable prognosis [14, 15]. Our CLL case, displaying a mutated *IGHV* status, a negative CD38 and ZAP70 expression and a complex karyotype, showed the downregulation of *FCRL1*, *FCRL2*, and *FCRL3*, as well as the lack of *FCRL5* expression and clinically presented a poor outcome. Due to the lack of concordance between *FCRL2* expression level and *IGHV* mutational status [14], our patient makes an exception to what reported so far in the literature.

FCRL proteins disclosed an inhibitory effect on BCR signaling [16, 17] that, if otherwise activated, may stimulate the aberrant proliferation of malignant B cells [18]. Therefore, we speculate that the pathogenesis of CLL in the case under study could be strongly connected with the lack of *FCRL* genes, due to the absence of a negative regulation on the BCR signaling.

Additionally, the mutational analysis displayed a deletion in the PEST domain of *NOTCH1*. It caused a loss of the C-terminus of the protein that, in turn, could lead to enhance NOTCH1 stability and signaling [19]. As reported [20], NOTCH1 signaling is constitutively activated in CLL cells and its activation increases the resistance to spontaneous apoptosis, as well as NF-kB activity. NF-kB pathway activation, causing aberrant cell proliferation, may also depend on BCR signaling [21]. Intriguingly, also 13q deletion (when detected in more than 80% of cells, as in the present case) is associated with a specific gene expression pattern resulting in BCR, NF-kB, and antiapoptotic signaling stimulation [22]. Additionally, *DLEU7*, mapping to 13q14 and coding for a potent inhibitor of NF-kB signaling, when deleted, may cause the NF-kB pathway activation [7].

Hence, *FCRLs* deregulation, mutated *NOTCH1*, and 13q14 loss might cooperate by stimulating B cells proliferation through the activation of all these pathways.

We also found the upregulation of *CD1* family genes (*CD1A*, *C*, and *D*). These genes encode for transmembrane glycoproteins known to present antigens to T-cells, playing an important role in the regulation of immunity against infection. Our result is apparently in contrast with the literature indicating *CD1C* and *CD1D* as downregulated in CLL [23]. Such decrease in the expression level of these genes leads to a disturbance of microbial immunity and tumor prevention in CLL patients. Furthermore, high levels of CD1D expression were correlated with a poor prognosis, due to an uncontrolled CLL cell proliferation caused by natural killer T-cells control failure [24].

Similarly, we found the upregulation of *ETV3* in comparison with the pool of the three CLL control cases. Since a preliminary finding of a potential role of *ETV3* as a TSG in lymphoma was reported as a consequence of its loss of

heterozygosity [25], further analysis in a larger cohort of CLL patients is therefore mandatory to clarify its role in CLL leukemogenesis [25].

In conclusion, we here describe a *IGHV*-mutated case with a 1q23.1 homozygous deletion as a novel cytogenetic abnormality in CLL, associated with the downregulation of *FCRL* embedded genes. Independently from known prognostic factors (*IGHV*, 17p13, and *TP53* mutations) and in agreement with previous results [14, 15] concerning the low expression of *FCRL2*, this patient showed a poor disease-free survival. A possible cooperation with CLL recurrent abnormalities, such as the 13q14 deletion and *NOTCH1* mutation, could be hypothesized. The lack of oncogenic fusion transcripts in our case further corroborates the hypothesis that such 1q23.1 loss should be considered as a driver event toward CLL. Further investigations in a larger cohort would be helpful to verify the recurrence of this cytogenetic abnormality, as well as the impact of this specific gene loss upon the expression profile. Moreover, since the biological function of these molecules is still poorly investigated in leukemia, further functional experiments are needed.

Authors' contribution GD and AT performed and analyzed FISH, M-FISH, RT-PCR, and RT-qPCR experiments. AL'A analyzed WGS and RNA-Seq data. OP and MC performed and analyzed SNP array data. CLC and PI performed cytogenetic examinations and flow cytometry immunophenotyping. AL and PL performed and analyzed RT-PCR experiments. MHS and JMHR performed and interpreted mutational analysis. CM and JMHR analyzed and explained the patient data regarding the hematological disease. CTS conceptual organized and supervised the experiments. GD, AL'A, AT, MHS, and CTS were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the AIRC (Associazione Italiana per la Ricerca sul Cancro; AIRC IG No. 15413 for CTS).

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

References

1. Fischer K, Hallek M. Optimizing frontline therapy of CLL based on clinical and biological factors. Hematol. Am. Soc. Hematol Edu. Program. 2017;2017(1):338–45. https://doi.org/10.1182/asheducation-2017.1.338.
2. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N. Engl. J. Med. 2000;343(26):1910–6. https://doi.org/10.1056/NEJM200012283432602.
3. Michaux L, Wlodarska I, Rack K, Stul M, Criel A, Maerevoet M, et al. Translocation t(1;6)(p35.3;p25.2): a new recurrent aberration in "unmutated" B-CLL. Leukemia. 2005;19(1):77–82. https://doi.org/10.1038/sj.leu.2403543.
4. Pfeifer D, Pantic M, Skatulla I, Rawluk J, Kreutz C, Martens UM, et al. Genome-wide analysis of DNA copy number changes and LOH in CLL using high-density SNP arrays. Blood. 2007;109(3):1202–10. https://doi.org/10.1182/blood-2006-07-034256.
5. ISCN 2016. An international system for human cytogenomic nomenclature (2016) McGowan-Jordan J, Simons A, Schmid M (eds), Karger, Basel.
6. Ghia P, Stamatopoulos K, Belessi C, Moreno C, Stilgenbauer S, Stevenson F, et al. ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia. 2007;21(1):1–3. https://doi.org/10.1038/sj.leu.2404457.
7. Palamarchuk A, Efanov A, Nazaryan N, Santanam U, Alder H, Rassenti L, et al. 13q14 deletions in CLL involve cooperating tumor suppressors. Blood. 2010;115(19):3916–22. https://doi.org/10.1182/blood-2009-10-249367.
8. L'Abbate A, Lo Cunsolo C, Macri E, Iuzzolino P, Mecucci C, Doglioni C, et al. FOXP1 and TP63 involvement in the progression of myelodysplastic syndrome with 5q- and additional cytogenetic abnormalities. BMC Cancer. 2014;14:396. https://doi.org/10.1186/1471-2407-14-396.
9. Jourdan M, Robert N, Cren M, Thibaut C, Duperray C, Kassambara A, et al. Characterization of human FCRL4-positive B cells. PLoS ONE. 2017;12(6):e0179793. https://doi.org/10.1371/journal.pone.0179793.
10. Jeromin S, Weissmann S, Haferlach C, Dicker F, Bayer K, Grossmann V, et al. SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. Leukemia. 2014;28(1):108–17. https://doi.org/10.1038/leu.2013.263.
11. Quijada-Alamo M, Hernandez-Sanchez M, Robledo C, Hernandez-Sanchez JM, Benito R, Montano A, et al. Next-generation sequencing and FISH studies reveal the appearance of gene mutations and chromosomal abnormalities in hematopoietic progenitors in chronic lymphocytic leukemia. J. Hematol. Oncol. 2017;10(1):83. https://doi.org/10.1186/s13045-017-0450-y.
12. Grossmann V, Roller A, Klein HU, Weissmann S, Kern W, Haferlach C, et al. Robustness of amplicon deep sequencing underlines its utility in clinical applications. J. Mol. Diagn. 2013;15(4):473–84. https://doi.org/10.1016/j.jmoldx.2013.03.003.
13. Capone M, Bryant JM, Sutkowski N, Haque A. Fc Receptor-like proteins in pathophysiology of B-cell disorder. J. Clin. Cell Immunol. 2016. https://doi.org/10.4172/2155-9899.1000427.
14. Li FJ, Ding S, Pan J, Shakhmatov MA, Kashentseva E, Wu J, et al. FCRL2 expression predicts IGHV mutation status and clinical progression in chronic lymphocytic leukemia. Blood. 2008;112(1):179–87. https://doi.org/10.1182/blood-2008-01-131359.
15. Nuckel H, Collins CH, Frey UH, Sellmann L, Durig J, Siffert W, et al. FCRL2 mRNA expression is inversely associated with clinical progression in chronic lymphocytic leukemia. Eur. J. Haematol. 2009;83(6):541–9. https://doi.org/10.1111/j.1600-0609.2009.01328.x.
16. Kochi Y, Myouzen K, Yamada R, Suzuki A, Kurosaki T, Nakamura Y, et al. FCRL3, an autoimmune susceptibility gene, has inhibitory potential on B-cell receptor-mediated signaling. J. Immunol. 2009;183(9):5502–10. https://doi.org/10.4049/jimmunol.0901982.
17. Shabani M, Bayat AA, Jeddi-Tehrani M, Rabbani H, Hojjat-Farsangi M, Ulivieri C, et al. Ligation of human Fc receptor like-2 by monoclonal antibodies down-regulates B-cell receptor-mediated signalling. Immunology. 2014;143(3):341–53. https://doi.org/10.1111/imm.12311.
18. Burger JA, Chiorazzi N. B cell receptor signaling in chronic lymphocytic leukemia. Trends Immunol. 2013;34(12):592–601. https://doi.org/10.1016/j.it.2013.07.002.

19. 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(6):689–91. https://doi.org/10.1111/j.1365-2141.2009.07816.x.
20. 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(4):856–65. https://doi.org/10.1182/blood-2008-02-139725.
21. Hertlein E, Byrd JC. Signalling to drug resistance in CLL. Best Pract. Res. Clin. Haematol. 2010;23(1):121–31. https://doi.org/10.1016/j.beha.2010.01.007.
22. Hernandez-Sanchez M, Rodriguez-Vicente AE, Hernandez JA, Lumbreras E, Sarasquete ME, Martin AA, et al. MiRNA expression profile of chronic lymphocytic leukemia patients with 13q deletion. Leuk. Res. 2016;46:30–6. https://doi.org/10.1016/j.leukres.2016.04.008.
23. Zheng Z, Venkatapathy S, Rao G, Harrington CA. Expression profiling of B cell chronic lymphocytic leukemia suggests deficient CD1-mediated immunity, polarized cytokine response, altered adhesion and increased intracellular protein transport and processing of leukemic cells. Leukemia. 2002;16(12):2429–37. https://doi.org/10.1038/sj.leu.2402711.
24. Gorini F, Azzimonti L, Delfanti G, Scarfo L, Scielzo C, Bertilaccio MT, et al. Invariant NKT cells contribute to chronic lymphocytic leukemia surveillance and prognosis. Blood. 2017;129(26):3440–51. https://doi.org/10.1182/blood-2016-11-751065.
25. Green MR, Jardine P, Wood P, Wellwood J, Lea RA, Marlton P, et al. A new method to detect loss of heterozygosity using cohort heterozygosity comparisons. BMC Cancer. 2010;10:195. https://doi.org/10.1186/1471-2407-10-195.