RESEARCH ARTICLE

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Potential Candidate Genes for Therapeutic Targeting in Chronic Myeloid Leukemia: A Pilot Study

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Abstract

Background: Chronic myeloid leukemia (CML) is a prevalent hematological malignancy known for the presence of the Philadelphia chromosome and activation of the BCR-Abl kinase activity. Although tyrosine kinase inhibitors are widely used as the standard treatment, resistance remains a concern among certain patients. This study aimed to investigate the gene expression profile of a group of CML patients in comparison to a control group in order to identify novel candidate genes associated with the disease. **Methods:** Whole transcriptome sequencing was performed, and gene expression levels were validated using quantitative real-time PCR. Additionally, single nucleotide and insertion/deletion variants were analyzed in the selected candidate genes among 10 CML patients and 4 healthy control subjects. **Results:** Analysis revealed a set of differentially expressed genes, whose up- or downregulation was further confirmed by qRT-PCR. Among the upregulated genes in the patient group were ribosomal protein like (RPL) members, specifically RPL9, RPL34, RPL36A, and RPL39, while downregulation was observed in CCDC170, LDB1, and SBF1 compared to the healthy subjects. Furthermore, gene variant studies identified novel genetic changes in these candidate genes, suggesting potential clinical significance in CML. **Conclusions:** This study highlights RPL9, RPL34, RPL36A, RPL39, CCDC170, LDB1, and SBF1 as potential targets in CML. Additionally, it underscores the importance of investigating these genes and their variants in larger cohort studies to assess their clinical significance in CML patients.

Keywords: CCDC170- CML- LDB1- RPL- SBF1

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Introduction

Chronic myeloid leukemia (CML), previously referred to as chronic granulocytic leukemia, is one of the earliest recognized malignancies dating back to 1845. The association between chromosomal abnormalities and cancer was first discovered in CML, with the identification of the Philadelphia chromosome resulting from translocation between chromosomes 9 and 22 (Minciacchi et al., 2021). The disease is characterized by three different phases based on the differentiation of myeloid cells: the chronic phase, accelerated phase, and blast crisis. The blast crisis, observed in 70% of cases, can manifest as either myeloid or lymphoid blast crisis (Arber et al., 2016). Notably, CML stem cells have been identified, and this subset of cells is believed to be responsible for therapy resistance, self-renewal, and survival, leading to relapse after remission in certain patients. Blast crisis stem cells have been reported among these cell populations. Different subpopulations of leukemia stem cells exhibit distinct genetic signatures that can be identified through transcriptomic sequencing and analysis (Giustacchini et al., 2017).

The BCR-ABL kinase, resulting from the formation of the Philadelphia chromosome, plays a central role in leukemogenesis by activating numerous downstream effectors (Cilloni and Saglio, 2012). Although treatment with tyrosine kinase inhibitors has shown positive clinical outcomes and remission in many patients, a significant proportion of treated patients develop resistance to these inhibitors (Zhou et al., 2018). Additionally, chromosomal abnormalities beyond the Philadelphia chromosome have been reported in CML patients, potentially influencing treatment responses. Therefore, exploration and investigation of these abnormalities in leukemia patients during the treatment course is warranted (Clark et al., 2021).

Recent reports have highlighted the value of whole-transcriptome sequencing and bioinformatics data in comparing the genetic profiles of different CML subgroups. This approach can aid in identifying new intervention pathways and enhancing our understanding

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of the disease biology (Youn et al., 2021). Furthermore, the use of sequencing platforms has shown promise in analyzing and predicting patients' response to tyrosine kinase inhibitors, as well as identifying novel markers in patients resistant to treatment (Youn et al., 2021).

This study aimed to investigate the whole transcriptome of treated chronic myeloid leukemia (CML) patients compared to normal control subjects. The main objectives were to explore differential gene expression in CML patients and identify potential candidate genes for further validation. The study also aimed to analyze gene variants in the identified candidate genes.

Materials and Methods

This cross-sectional study received approval from the institutional review board of Imam Abdulrahman Bin Faisal University (IRB-2017-03-147). The study sample included 10 confirmed CML patients and 4 healthy controls. All participants provided informed consent, and patient recruitment occurred between 2018 and 2021 at King Fahd University Hospital in Dammam, Saudi Arabia. Exclusion criteria encompassed individuals with hematological conditions other than CML, as well as those with solid tumors or other chronic diseases.

RNA Sequencing and Data Analysis

Blood samples were collected from both patients and controls using PAXgene blood RNA tubes (Preanalytix, Switzerland). The tubes were allowed to settle at room temperature for 30 minutes before being frozen at -80 °C until RNA extraction. RNA was extracted following the protocol of the PAXgene Blood RNA Kit (Catalogue #762164, Preanalytix, Switzerland). The Agilent 2100 platform (Agilent RNA 6000 Nano kit #5067-1511, Agilent Technologies, Germany) was used to assess the quantity and integrity of the extracted RNA prior to creating RNA libraries for sequencing.

All samples underwent triplicate analysis after passing the quality control check, and a cDNA library was created and amplified following the protocol described in our previous publication, with ribosomal RNA removal (El-Masry et al., 2022). RNA sequencing was done using DNBSEQ platform. Gene expression quantification was performed by aligning the sequencing data to the reference genome using HISAT2 (Kim et al., 2015). Gene expression quantification was achieved using the RNA sequencing by Expectation-Maximization (RSEM) algorithm (Li and Dewey, 2011). DESeq2 was used for differential gene expression analysis (Love et al., 2014), and the differentially expressed genes were clustered using R package pheatmap . Gene expression levels were reported as fragment per kilo base million (FPKM), with higher values indicate corresponding to higher expression levels of specific genes.

Quantitative Polymerase Chain Reaction (qPCR)

To validate the gene expression quantification from RNA sequencing, quantitative polymerase chain reaction (qPCR) was performed. The primers listed in Table 1 were used to amplify the candidate genes, and the analysis was conducted in quadruplicate sets. The cycling program followed the instructions provided in the datasheet of the one-step SYBR Green PCR master mix (Molecular-On, cat. # OCQ-M-001-100, Auckland, New Zealand). The amplification profile included an initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of denaturation at 95 °C for 10 seconds and annealing and extension at 60 °C for 30 seconds. A melting analysis was conducted as well.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene, and relative gene expression was calculated for all samples relative to the gene expression level in control #1 using the $2^{-\Delta\Delta CT}$ method. The ΔCT value was calculated by subtracting the CT value of the reference gene from the CT value of the target gene for each sample. The ΔΔCT value was then calculated by subtracting the ΔCT of the control samples from that of the patient samples. The fold change in gene expression was estimated in each sample using the $2^{-\Delta\Delta CT}$ value. All patient and control samples were normalized to the value calculated for control #1, allowing the estimation of the relative gene expression of the target genes in all samples.

Gene Variant Analysis

To ensure data quality, low quality locus information was filtered out, and single nucleotide variants (SNV) and insertion/deletion (INDEL) changes were analyzed using the Genome Analysis Toolkit (GATK) (McKenna et al., 2010). GATK offers a powerful computational engine for mutation detection and genotyping purposes.

Statistical Analysis

In the bioinformatics report, the significance of differential gene expression was assessed using the false discovery rate (Q value) to minimize false positive results. An unpaired t-test was performed to compare gene expression levels between patients and controls, with statistical significance set at $p \leq 0.01$ to ensure robust statistical findings. GraphPad Prism (version 7) was utilized for statistical analysis.

Results

Patients' Presentation and Treatment

The study sample consisted of 3 females and 7 males with a mean age of 55 years, all diagnosed with CML and undergoing treatment. The healthy control group comprised four individuals (mean age = 39 years), including 1 female, to reflect the transcriptomic profile of both genders. CML diagnosis was established based on complete blood counts, revealing a significant increase in white blood cells accompanied by mild to severe splenomegaly. General fatigue, weakness, and abdominal pain were common symptoms observed at the time of hospital admission. Diagnosis confirmation involved the detection of the Philadelphia chromosome through karyotyping and molecular analysis of BCR-Abl (p210). Figure 1 illustrates the average BCR-Abl levels in patients over the years. Patients #2, 3, 7, and 8 exhibited the highest BCR-Abl levels, potentially indicating a greater disease

Table 1. Quantitative PCR Primers List

Gene	Forward primer	Reverse primer
RPL9	GCTCACTTCCCCATCAACGT	CTGGTCTCATCCGAACCCTG
RPL34	GCCTATGGTGGTTCCATGTG	TGTGCTTGTGCCTTCAACAC
RPL36A	AAGCGGCGTTATGACAGGAA	GCTCAACGCACTCAAGCCTT
RPL39	AATCGTCCCATTCCCCAGTG	CCCAGCTTGGTTCTTCTCCA
CCDC170	TACACATGAGCCTCCTCCGG	CCTGATGGTAAGATGCGCGT
LDB1	TGCGGATAAAGACGTGGCAC	CAGCCCACACCGAGTGATGT
SBF1	CATCCGCTTCCATAAGGCAG	GCTCTGACACAAAGCCAGCA

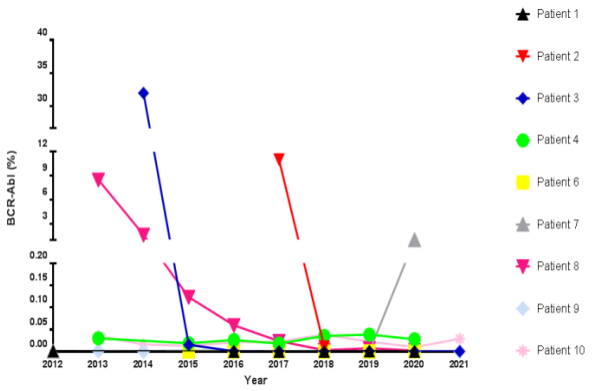

Figure 1. BCR-Abl (%) Average Levels in CML Patients. The data shows that the highest levels of BCR-Abl were detected in patients 2, 3, 7 and 8. The highest value was recorded at 32% for patient #3 in 2015. The last BCR-Abl level of patient #7 recorded in 2020 confirmed an active disease stage. All other patients were in Major Molecular Remission.

burden in these individuals. All patients initiated treatment with a daily dose of 200 mg imatinib until achieving BCR-Abl reduction to <0.1%, indicative of major molecular remission (MMR) according to international standards. One patient (Patient #8) remained on imatinib for 12 years due to BCR-Abl levels >10%. Subsequently, treatment was switched to nilotinib for two years before reverting back to daily imatinib (400 mg). MMR was eventually attained in this patient. Another individual (Patient #7) was initially treated with nilotinib and subsequently transitioned to imatinib, but remains in the active disease stage, although BCR-Abl levels decreased from >10% to below 2%.

Differential Gene Expression

In the study sample, a total of 17,469 genes were identified, with an average mapping ratio of 89.5% to the reference genome. Among these genes, 78 were found to be differentially expressed in CML patients compared to the healthy controls. Four closely related genes, namely ribosomal proteins L9, L34, L36A, and L39 (RPL9, RPL34, RPL36A, and RPL39), showed a fold change greater than 2 and were selected for further confirmation of their relative gene expression levels using quantitative PCR. Three downregulated genes, CCDC170, LDB1, and SBF1, were also quantified by quantitative PCR (Table 2, 3).

Table 2. Potential Candidate, Differentially Expressed, Upregulated Genes in CML Patients

Gene symbol	log2fold change	fold change	Q value
RPL34	1.456	2.744	0.0003
RPL36A	1.360	2.568	0.0142
RPL39	1.328	2.510	6.64E-05
RPL9	1.328	2.510	0.0004

Quantitative Gene Expression

The results of quantitative real-time PCR confirmed the upregulation of RPL9, RPL34, RPL36A, and RPL39 in CML patients compared to the control group. The average relative fold change in RPL9 expression was 22.48 times higher in CML patients versus 6.42 times in controls. For RPL34, RPL36A, and RPL39, the average fold change values were 20.7, 14, and 18.77 in CML patients compared to 6.12, 4.5, and 6.37 in controls, respectively. These findings align with the RNA sequencing results, which also demonstrated upregulation of these genes in the patient group. In contrast, the level of CCDC170 was lower in the patient group compared to the control group, while the levels of LDB1 and SBF1 exhibited similar expression levels in both groups.

Gene Variants

Analysis of single nucleotide variants (SNVs) and insertion/deletion changes using the Genome Variation Server database revealed a significant number of variants in the candidate genes of the study sample. For simplicity, only the novel variants are reported here. SNVs found in the upregulated genes, including *RPL9, RPL34,* and *RPL39*, are presented in Table 4A. These variants, potentially acting as modifiers, could have an impact on gene phenotype, although their clinical significance remains to be elucidated. SNVs identified in the downregulated genes are summarized in Table 4B, with a notable finding of a nonsense mutation in SBF1 that leads to the gain of a stop codon, potentially exerting a high impact on gene phenotype. INDEL changes are presented in Tables 5A (upregulated genes) and 5B (downregulated genes). The results revealed a diverse array of changes

Table 3. Potential Candidate, Differentially Expressed, Downregulated Genes in CML Patients

Gene symbol	log2fold change	fold change	Q value
LDB1	-0.2667	0.8312	0.0167
SBF1	-0.3269	0.7972	0.0278
CCDC170	-0.9294	0.5251	0.0321

Table 4A. Novel Single Nucleotide Variants in the Upregulated Genes

Gene name	Reference Nucleotide	Change Nucleotide	Location	Position	Chromosome
RPL9	C	A	Downstream variant	39453904	4
	C	G	Intron variant	39455515	
	T	C	Intron variant	39456079	
RPL34	G	A	Upstream variant	108618695	4
	G	A	Upstream variant	108619698	
	T	C	5 prime UTR variant	108621665	
	A	G	Intron variant	108623616	
	A	G	Downstream variant	108626984	
	A	G	Downstream variant	108626985	
	A	G	Intron variant	108627771	
	A	G	Intron variant	108627794	
	A	G	Intron variant	108627807	
	A	G	Intron variant	108628949	
	A	G	Intron variant	108628959	
	A	G	Intron variant	108628985	
RPL39	T	C	Intron variant	119789072	X

in *RPL9, RPL34, RPL39, CCDC170, LDB1,* and *SBF1.* Notably, three frameshift mutations were identified, one in CCDC170 and two in SBF1. These frameshift changes have the potential for a high impact on gene product function (Figure 2,3).

Discussion

The exploration of patients' genomic profiles provides valuable insights into disease pathophysiology and individual variations in resilience. In this study, we aimed to understand the transcriptomic profile of a cohort of CML patients who had been diagnosed with CML and treated with tyrosine kinase inhibitors (TKIs) such as Imatinib and/or nilotinib. Although we did not have access to baseline expression levels at the time of diagnosis, the differential gene expression patterns observed in this study indicated that the treatment did not completely normalize

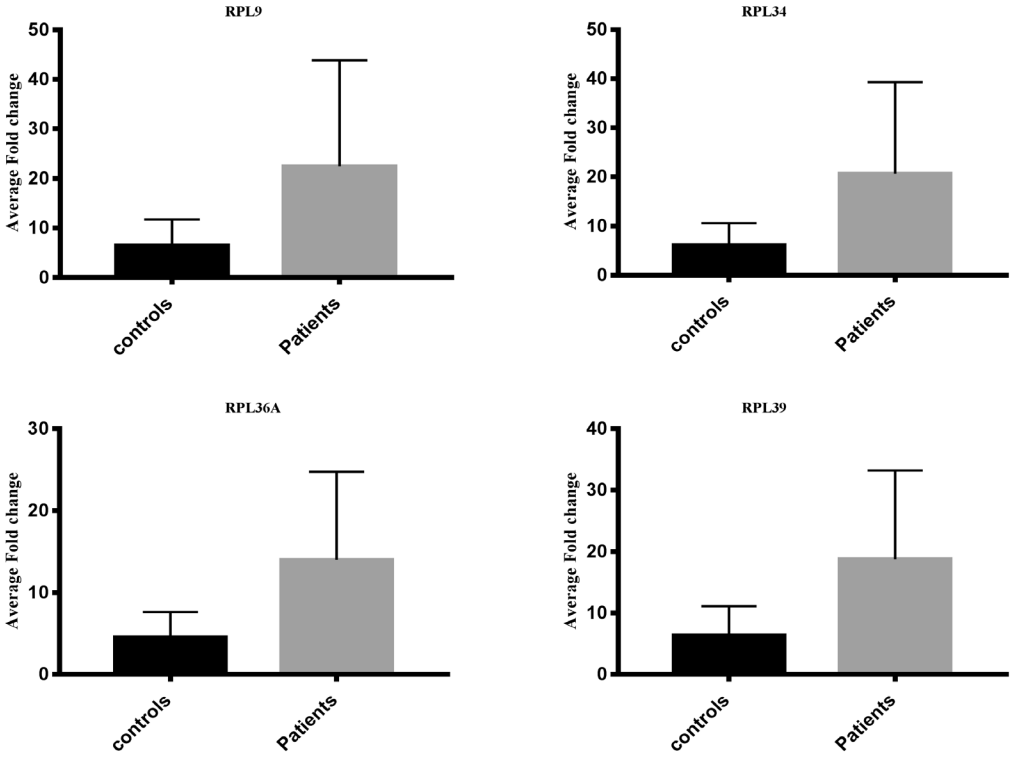

Figure 2. The Upregulated RPL Gene Family Memebers in CML Patients. qRT-PCR results confirmed that the average relative gene expression of RPL9, 34, 36A and 39 was apparently higher than that in the control group by several fold changes. The difference was not statistically significant.

Table 4B. Novel Single Nucleotide Variants in the Downregulated Genes

Gene name	Reference Nucleotide	Change Nucleotide	Location	Position	Chromosome
CCDC170	G	T	5 prime UTR variant	151494126	6
	A	T	Intron variant	151494746	
	A	G	Intron variant	151502145	
	A	G	Intron variant	151502165	
	A	G	Intron variant	151505863	
	G	T	Intron variant	151509489	
	G	A	Intron variant	151513504	
	A	G	Intron variant	151521009	
	A	G	Intron variant	151521472	
	A	G	Intron variant	151533421	
	T	C	Intron variant	151552677	
	T	C	Intron variant	151566475	
	G	T	Intron variant	151586636	
	A	G	Intron variant	151589112	
	G	C	Synonymous variant	151596493	
	G	T	Intron variant	151614597	
	A	G	3 prime UTR variant	151618855	
	A	G	3 prime UTR variant	151618868	
	G	T	3 prime UTR variant	151618937	
	G	T	3 prime UTR variant	151619560	
	G	T	3 prime UTR variant	151620324	
	G	T	3 prime UTR variant	151621112	
	G	C	Downstream gene variant	151622997	
LDB1	G	A	Intron variant	102111335	10
	T	C	Intron variant	102111739	
	C	A	Intron variant	102111878	
	C	A	Intron variant	102112839	
	A	G	Upstream gene variant	102121651	
SBF1	A	G	Downstream gene variant	50443003	22
	A	G	Downstream gene variant	50443054	
	A	G	Intron variant	50451876	
	G	A	Intron variant	50452897	
	G	A	Intron variant	50454777	
	C	A	Splice region variant + intron variant	50455417	
	C	A	Intron variant	50456133	
	A	G	Intron variant	50457930	
	C	A	Stop gained (Nonsense)	50466026	
	C	A	Intron variant	50469333	

the gene expression profiles to the control levels.

Our findings revealed differential upregulation of ribosomal proteins L9, L34, L36A, and L39 within the ribosomal protein-like family in the CML cohort, as confirmed by the results of quantitative real-time PCR (qRT-PCR). On the other hand, CCDC170, LDB1, and SBF1 were downregulated. The qRT-PCR results confirmed the downregulation of CCDC170, while LDB1 and SBF1 exhibited similar expression levels in both the patient and control groups. It is important to note that there is limited evidence in the literature regarding the role of these genes specifically in CML.

However, there is existing evidence linking the ribosomal protein-like family to human cancer. For instance, RPL9 has been associated with a lower likelihood of recurrence in patients with non-muscle invasive bladder cancer after treatment (Piao et al., 2022). Conversely, another study reported the tumorigenic potential of RPL9 in human colorectal cancer (Baik et al., 2016). Additionally, high expression of RPL9 in glioma cells has been correlated with enhanced cell migration through the positive regulation of vimentin,

Table 5A: Novel insertion/deletion variants in the upregulated genes

Gene name	Reference Nucleotide	Change Nucleotide	Location	Position	Chromosome
RPL9	A	AT	Intron variant	39455793	4
	AT	A	Intron variant	39456087	
RPL34	AG	A	Intron variant	108623182	4
	C	CA	Intron variant	108623332	
	G	GT	Splice region variant	108625367	
	CG	C	Downstream variant	108626965	
	C	CA	Intron variant	108627424	
RPL39	G	GA	Intron variant	119786740	X
	C	CA	Intron variant	119789101	
	TC	T	Upstream variant	119792179	

Table 5B. Novel Insertion/Deletion Variants in the Downregulated Genes

Gene name	Reference Nucleotide	Change Nucleotide	Location	Position	Chromosome
CCDC170	GA	G	Intron variant	151504837	6
	CAAA	C	Intron variant	151509015	
	AATG	A	Intron variant	151515338	
	G	GAA	Intron variant	151516066	
	CAA	C	Intron variant	151516624	
	GT	G	Intron variant	151516871	
	TG	T	Frame shift variant	151548460	
	AT	A	3 prime UTR variant	151618226	
	TAG	T	3 prime UTR variant	151619126	
	TGC	T	3 prime UTR variant	151619553	
	C	CA	3 prime UTR variant	151619609	
	C	CAA	3 prime UTR variant	151619656	
	G	GA	3 prime UTR variant	151620129	
	C	CAA	3 prime UTR variant	151620189	
	A	AAAG	3 prime UTR variant	151620204	
	TA	T	3 prime UTR variant	151620947	
LDB1	CA	C	Downstream variant	102105064	10
	AT	A	Intron variant	102112875	
	TC	T	Intron variant	102113488	
	AAT	A	Upstream variant	102120518	
	C	CTT	Upstream variant	102122462	
SBF1	CTT	CT,CTTT,C	Downstream variant	50444323	22
	CGGGGTG	C,CGGGGTGGGGGTG	Downstream variant	50444524	
	GT	G,GTT	Downstream variant	50444618	
	G	GA	Downstream variant	50444724	
	GC	G	Intron variant	50449127	
	C	CA	Intron variant	50449172	
	GA	G	Intron variant	50449523	
	CG	C	Intron variant	50455596	
	CA	C	Intron variant	50458820	
	AG	A	Intron variant	50458958	
	CT	C	Intron variant	50463183	
	AT	A	Frame shift variant	50464643	
	GC	G	Frame shift variant	50465075	
	AAC	A	Intron variant	50467076	

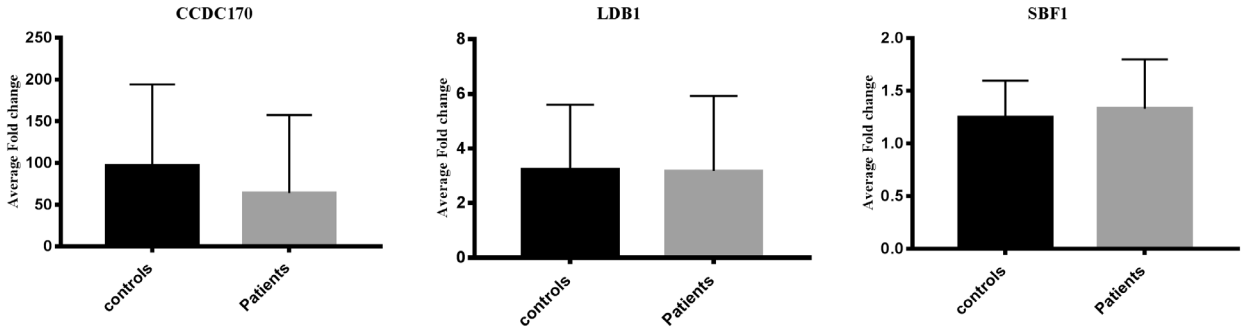

Figure 3. The Downregulated Genes, *CCDC170, LDB1* and *SBF1* in CML Patients. qRT-PCR results showed that *CCDC170* expression level in CML patients was lower than that in the control group. *LDB1* and *SBF1* gene expression was similar in CML and control groups. The difference was not statistically significant.

a marker of epithelial-mesenchymal cell transition and metastasis (Tian et al., 2018). Given these findings, further exploration of the role of RPL9 in CML is warranted. The observed overexpression of RPL9 in the CML cohort in our study could potentially serve as a prognostic marker for monitoring disease progression and predicting overall survival in CML patients.

In line with the observed tumorigenic role of RPL9, similar findings have been for reported for RPL34 in esophageal cancer, where its tumorigenic influence was suppressed by a novel antisense RNA in esophageal cancer cells (Gong et al., 2019). Another study (Liu et al., 2015) reported that RPL34 knockdown in gastric cancer by small interfering RNA exerted anti-cancer effects, which confirms its malignancy-driving influence. Likewise, a tumorigenic role of RPL34 was reported in small cell lung carcinoma, contributing to proliferation in these cancers (Yang et al., 2016; Wei et al., 2016). A similar proliferation-promoting effect of RPL34 was also found in pancreatic cancer (). Interestingly, a recent study reported RPL34 as a tumor suppressor in cervical cancer (Zhu et al., 2021), indicating the potential for cancer-specific roles of RPL34 and underscoring the importance of investigating its contribution in leukemogenesis

Moving on to RPL36A, recent research has reported its involvement in radiotherapy resistance in one oral cavity cancer, as knockdown of RPL36A resulted in increased sensitivity to radiotherapy (Chen et al., 2021). Additionally, RPL36A has been identified as an oncogene in endometrial cancer and has been associated with disease progression in glioblastoma multiform (López-Ozuna et al., 2021; Alshabi et al., 2019). Similarly, overexpression of RPL39, another member of the ribosomal protein-like family, has been linked to poor prognosis in breast cancer and has demonstrated roles in breast carcinogenesis and metastasis to the lung (Dave et al., 2017; Dave et al., 2014). A similar tumorigenic potential of RPL39 was also reported in pancreatic cancer (Li et al., 2014). Likewise, the carcinogenic effect of RPL39 was recently reviewed in a recent study, which investigated its role in cell migration and invasion (Jie et al., 2021). In another recent study (Tong et al., 2022), the association between RPL39 and glioma progression was also reported. Considering the limited existing knowledge about the roles of RPL36A and RPL39 in CML, further investigation of their potential as therapeutic targets in the disease is warranted.

Understanding their specific contributions in this context could provide meaningful insights into CML pathogenesis and potentially offer new avenues for targeted treatment approaches. Moreover, the findings of this study revealed the downregulation of CCDC170, LDB1, and SBF1 in the CML cohort, suggesting their potential tumor-suppressive activity in CML.

In line with this, it was recently reported that overexpression of CCDC170 in breast cancer induces apoptosis and improves patients outcomes (Wang et al., 2020). A single nucleotide variant in CCDC170 gene increased the risk of breast cancer. Additionally, the same study reported that CCDC170 regulated cell polarity and migration (Jiang et al., 2017). In addition, gene variants of CCDC170 was reported to be associated with susceptibility to breast cancer (Wang et al., 2014). The downregulation of CCDC170 in CML patients in our study aligns with its tumor-suppressor activity in breast cancer.

Regarding LDB1, its relationship with oncogenicity in acute lymphoblastic leukemia has been reported, as it is required for the function of the Lmo2 oncogene (Li et al., 2020). High expression of LDB1 has also been correlated with reduced survival rates in colorectal cancer and with enhanced proliferation and invasiveness in head and neck cancer (García et al., 2016; Simonik et al., 2016). Alterations in the LDB1/Lmo2 complex have been implicated in acute leukemogenesis (Layer et al., 2020). Hence, the possible role of LDB1 in CML should be further explored, considering its downregulation in the CML cohort in our study.

Another downregulated gene in the CML cohort was SBF1. The antiproliferative effect of this gene has been reported in NIH3T3 cells (Firestein and Cleary, 2001). Additionally, SBF1 has shown a statistical association with treatment sensitivity in head and neck squamous cell carcinoma, suggesting its potential use as a positive prognostic marker in this type of cancer (Zhu et al., 2014). The clinical significance of SBF1 frameshift variants has also been reported in neuropathy (Gang et al., 2020), warranting exploration in other diseases such as CML. However, there is limited information available regarding the link between SBF1 and CML or other human cancers.

Further investigation is needed to elucidate their potential roles in CML pathogenesis.

In conclusion, the findings from previous studies and our own study provide support for the involvement of RPL family, *CCDC170, LDB1*, and *SBF1* genes in human cancers. These genes may have both pro- and anti-oncogenic effects, and their impact could vary depending on the specific type of cancer. The differential regulation of these genes observed in our study of CML patients, along with their reported effects in other human cancers, highlights the need for further investigation into these genes and their variants in the context of CML. However, it is important to note that the present study had a small sample size and the patients were already receiving treatment, which limited our access to baseline information at the time of initial diagnosis. Therefore, it is necessary to explore the role of these candidate genes in the initiation, progression, and monitoring of CML in larger cohorts, while carefully considering their contribution to the disease.Acknowledgements: The authors would like to express their sincere gratitude to the Deanship of Scientific Research at Imam Abdulrahman Bin Faisal University for funding this work.

Author Contribution Statement

Khaldoon M. Alsamman: data analysis and approval of the final draft. Ali M. Alamri: Clinical practice points and patients history. Chittibabu Vatte: Methodology and RNA extraction. Amani Y. Owaidah: Writing up and approval of the final manuscript. Fatimah Alhassan and Ruba Mubaraki: Methodology and quantitative PCR. Omar S. El-Masry: Study design and conceptualization and drafting the manuscript.

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Ethics Committee

All participants provided informed consent prior to enrollment, and the study was reviewed and approved by the Institutional Review Board at Imam Abdulrahaman Bin Faisal University (IRB# IRB# 2017-03-147).

Conflict of Interest

Each author declares that they have no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that could pose a conflict of interest in relation to the submitted article.

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