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# Isolation and analysis of candidate myeloid tumor suppressor genes from a commonly deleted segment of 7q22

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**Abstract**

Monosomy 7 and deletions of 7q are recurring leukemia-associated cytogenetic abnormalities that correlate with adverse outcomes in children and adults. We describe a 2.52-Mb genomic DNA contig that spans a commonly deleted segment of chromosome band 7q22 identified in myeloid malignancies. This interval currently includes 14 genes, 19 predicted genes, and 5 predicted pseudogenes. We have extensively characterized the *FBXL13, NAPE-PLD*, and *SVH* genes as candidate myeloid tumor suppressors. *FBXL13* encodes a novel F-box protein, *SVH*is a member of a gene family that contains Armadillo-like repeats, and *NAPE-PLD* encodes a phospholipase D-type phosphodiesterase. Analysis of a panel of leukemia specimens with monosomy 7 did not reveal mutations in these or in the candidate genes *LRRC17, PRO1598*,and *SRPK2*. This fully sequenced and annotated contig provides a resource for candidate myeloid tumor suppressor gene discovery.


*Keywords:* Chromosome 7; Acute myeloid leukemia; Myelodysplastic syndrome; Monosomy 7; F-box proteins

Monosomy 7 and del(7q) are among the most common cytogenetic alterations found in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [1–3]. These abnormalities occur in ~10% of de novo MDS and AML, in ~50% of patients with therapy-related leukemias [1], and in myeloid malignancies that arise in the context of constitutional predispositions such as Fanconi anemia, neurofibromatosis type 1, and severe congenital neutropenia. Importantly, monosomy 7 and del(7q) are strongly associated with adverse clinical features and with resistance to existing therapies.

A commonly deleted segment (CDS) of chromosome band 7q22 was defined by cytogenetic investigation of 81 patients with a malignant myeloid disorder characterized by a del(7q) [3]. Primary bone marrow cells from 15 of these cases were analyzed by fluorescence in situ hybridization (FISH) to identify the smallest commonly deleted interval. These studies delineated a region within 7q22 that was estimated to span 2–3 Mb of genomic DNA. The proximal boundary of the CDS was defined by 2 patients, whereas the distal boundary was defined by 3 patients [3]. We previously assembled a partial contig of this interval and excluded five candidate tumor suppressor genes (TSGs) by analyzing leukemia samples with monosomy 7 or a del(7q) for mutations [4]. A homolog of *Drosophila trithorax* called *MLL5* has also been cloned from the CDS; however, molecular analysis did not reveal "second hit" mutations in primary leukemias [5]. Here we describe a fully annotated genomic contig spanning the entire 7q22 CDS and the identification and analysis of additional candidate myeloid TSGs from this interval.

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## Results

Fig. 1 presents a tiling path comprising 28 overlapping bacterial and P1 artificial chromosome (BAC and PAC) clones spanning the 7q22 CDS, which is flanked distally by *D7S1841* and proximally by *D7S1503*. Selected polymorphic loci are shown within the contig, which includes 2.52 Mb of nonredundant DNA sequence. Additional information about the BAC and PAC clones that span the CDS, including names, insert sizes, and GenBank accession numbers, is available at http://itsa.ucsf.edu/~kmslab/data/data.html.

Genome databases were queried to identify known and predicted genes within the CDS. Candidate sequences were evaluated further by designing oligonucleotide primers to amplify and clone a segment of each predicted open reading frame from human bone marrow cDNA. These amplified fragments were used to probe Northern blots prepared from hematopoietic tissues and leukemia cell lines to characterize candidate genes and to ascertain the size(s) of the transcript(s). We have identified 14 genes from the 7q22 CDS that are expressed in normal bone marrow, including 11 known genes (*LRRC17 (P37NB), PMPCB, ZRF1, PSMC2, RELN, ORC5L, SRPK2, PRO1598, PRES, LHFPL3*,and *MLL5*) and 1 pseudogene (*S100A14*) [4,5]. Three novel candidate tumor suppressor genes that showed strong cross-species conservation and/or contained putative functional domains were characterized in detail. These studies uncovered the *FBXL13, NAPE-PLD*, and *SVH* genes, one of which (*SVH*) was entered into the National Center for Biotechnology Information (NCBI) database by others [6].

*FBXL13* encodes a novel F-box protein that is most similar to the F-box with leucine-rich repeats protein 2 (FBL2). F-box proteins with leucine-rich repeats mediate substrate-specific binding to ubiquitin ligase complexes, which target proteins to the proteasome for degradation [7]. The *VHL* TSG encodes an adapter component of a mammalian ubiquitin ligase complex that degrades hypoxia-inducible factor 1α [8], and hCdc4/Fbw7 is an F-box protein that has been implicated as a TSG [9,10]. Northern blot analysis demonstrated *FBXL13* RNA message sizes of ~1.6 and ~2.3 kb, respectively (Fig. 2A). The first transcript (GenBank Accession No. AY359238) is 1568 bp in length, has 13 exons, and contains an F-box protein domain and 5 leucine-rich repeats with the (cystine-containing) subfamily. The second *FBXL13*isoform (GenBank Accession No. AY359239) is 2274 bp, has 19 exons, and contains an F-box protein domain with 12 leucine-rich repeats (Fig. 2A). The *FBXL13* locus spans 261 kb. Most of the gene lies within BAC clones CTA-318M5 and RP11-645N11 (GenBank Accession Nos. AC005250 and AC073127) (Fig. 1), but it partially extends into the flanking BAC RP4-802G15 (GenBank Accession No. AC006477).

We also identified a partial sequence that showed homology with the hypothetical *Caenorhabditis elegans*

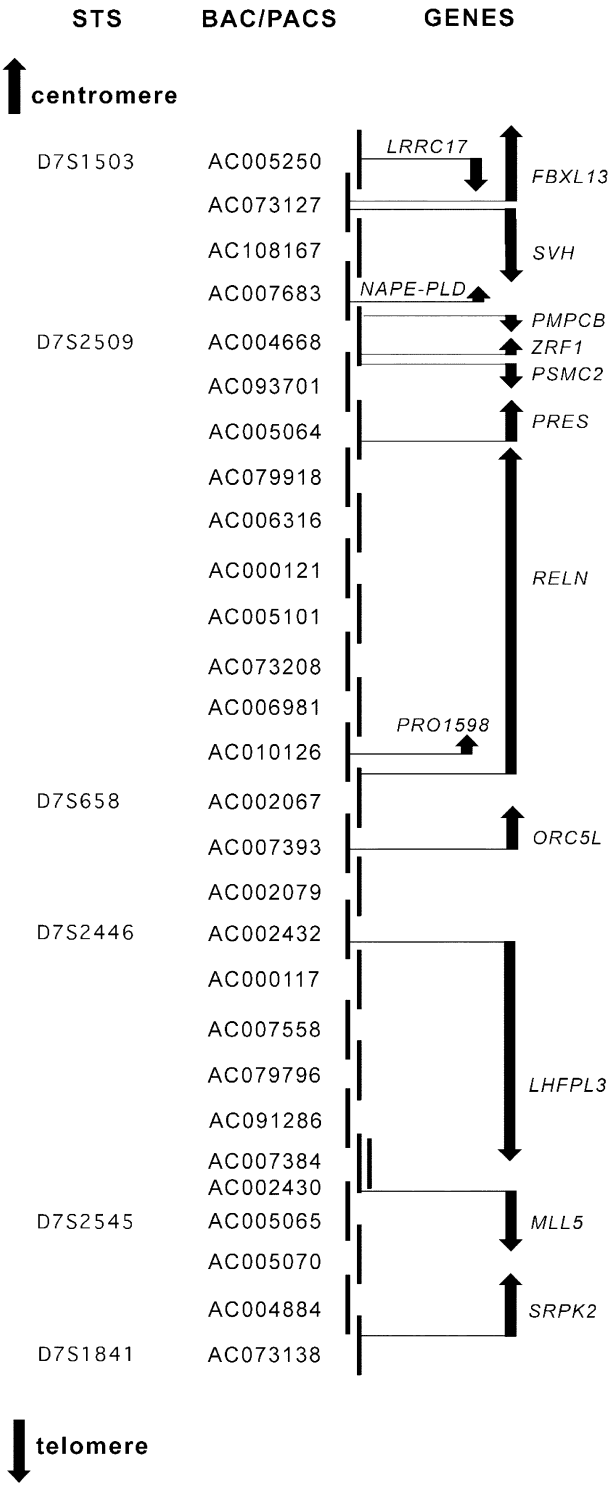


Fig. 1. Genomic organization of the 7q22 CDS. Left: The interval is flanked by the centromeric microsatellite marker *D7S1503* and by the telomeric marker *D7S1841*. Additional loci within the region are shown. Center: The CDS includes 28 overlapping BAC and PAC clones. GenBank accession numbers are shown. Right: The locations and approximate sizes of the 14 known genes are presented, with the 5′–3′ orientation of each gene indicated by the direction of the arrows. Leukemia samples have been screened for mutations in all of these genes except *PRES* and *LHFPL3*, which are in progress.