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Supplemental Data

Heterozygous Germline Mutations in the *CBL* Tumor-Suppressor Gene Cause a Noonan Syndrome-like Phenotype

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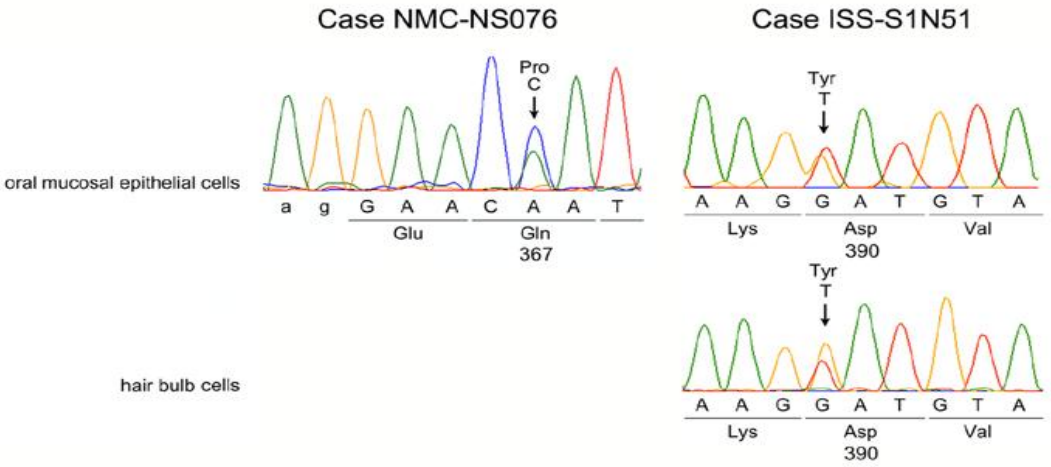
Table S1. Primer pairs and annealing temperatures used to amplify the *CBL* coding sequence, and sizes of PCR products.

Exon	Primer Sequence (5′→3′)		Ann. Temp. (°C)	Product Length (bp)
	Forward	Reverse		
1	TTCACGCCCTGCTTCTCTCC	TTCCTCCGTCCGCTCGTTCC	62[a]	355
2	CAATGGGGTTATGGATCTGC	CTATGTGTTACCCATTCAGGC	57	461
3	CTTGTATGGTGAATTTGGTGC	ATTACTTTTCTCAGAGTTCCC	55	334
4	TTGATTATGGCGATGCCTGG	TTTCTCTTCACCGAAGTAGC	56	281
5	CTCTGAGTTGGTTGTACATCTGAC	CAGAACCTTGGCTATTGCGAAAC	60	290
6	TTGCCTTCCACCGTAATACC	TCCCAGACTCTAACAGATGG	57	236
7	ATGGAGAAACTCCCAGATTCC	AGCTTGTGTCCAGTGATATGG	58	221
8	GTATAGGAAACAAGTCTTCAC	TCCAAGGTTATTACATAGCTG	54	270
9	AGCCTTTACTGATACAAGGG	TAGAAGACAACTCACAATGG	54	404
10	TTAGGAGAGTTGAAAGATGCC	TGTGGAAGCAGGGTGAAAGC	60	286
11	GCTTCTGCTCTTGGAACTGC	ACAGACATGAGCCACTGTGC	65[a]	566
12	AATAAGAGCAGAGGCTCAGC	AATCATTTCTACATGGTGCAG	54	289
13	GGTGACATGTATTTTGCTCTG	GGTGAAGGGTGTCAATTACC	56	300
14	CATACACCTATAACTTGCCAC	GATCAAGCTATCTCAATTGCC	56	322
15	TCTGTTTACATGGTGTTTGGC	AAACATACAGGCCACACTGC	60	395
16	ACATGTACCCAGTTTACAGG	TAACTCCCAACTCACTGGTC	56	452

[a]Exon 1 and exon 11 PCR products were amplified using FastStart Taq DNA Polymerase (Roche Diagnostics) in a buffer containing 1X GC rich solution (Roche Diagnostics).

Figure S1. Germline origin of *CBL* mutations causing a Noonan syndrome-like phenotype. (A) Sequence electropherograms of the *de novo* c.1100A>C (Gln367Pro) and c.1168G>T (Asp390Tyr) missense changes documenting the heterozygous state in hair bulb cells and/or oral mucosal epithelial cells from sporadic cases NMC-NS076 and ISS-S1N51. (B) Partial amino acid sequence alignment of CBL orthologs and paralogs showing conservation of Gln^{367}, Lys^{382}, Asp^{390} and Arg^{420} (arrows).

A



B

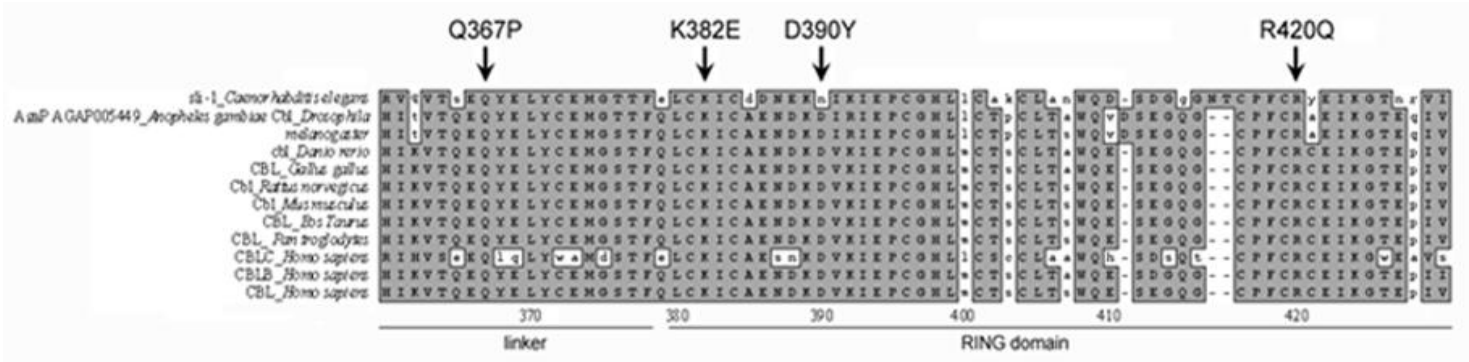


Figure S2. Location of mutated CBL amino acid residues in the three-dimensional structure of the protein complexed with the E2 ubiquitin-conjugating protein UBE2L3. Cα ribbon trace of the CBL tyrosine kinase binding domain (cyan), linker (orange), and RING finger domain (red) of CBL are shown together with the interacting portion of UBE2L3 (gray).[40] Mutated residues identified in this study are indicated with their side chains as blue thick lines. Residues of the linker (magenta) or RING domain (yellow) mutated in myeloid malignancies are also shown with their side chains. Visualization and analysis of the molecular structure was performed using the program UCSF Chimera.[54]