Supplementary Online Material

Babushok et al. Single Nucleotide Polymorphism Array Analysis of Bone Marrow Failure Patients Reveals Characteristic Patterns of Genetic Changes

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Supplemental Methods: CN-LOH Prediction

For detection of CN-LOH, we used the CN-LOH prediction algorithm available as part of the CNV Workshop[1] (Available for download at http://cnv.sourceforge.net). Following CN-LOH prediction, we used initial thresholds of a minimum of 100 homozygous SNPs and 1MB size to identify a high-confidence set of CN-LOH regions. In order to focus on CN-LOH regions most likely to be pathogenic, we analyzed CN-LOH regions exceeding the 99th percentile of CN-LOH size of normal controls. For the Quad610 Beadchip, 99th percentile of CN-LOH size in the control population was 5.3 MB (median 1.3MB). For Omni1-Quad Beadchip, 99th percentile was 8.5 MB (median 1.3 MB). All genomic positions were based on National Center for Biotechnology Information Build 37 of the human genome (hg19) from the University of California-Santa Cruz genome browser.

Supplemental Text: Case Descriptions

Supplemental Text 1A

Patient 1 was a 27 year old female with a ten-year history of severe aplastic anemia, with a small PNH clone. Metaphase cytogenetics showed inversion 9p13q21.1, a normal population variant. SNP-A performed for routine follow-up at the time of remission was unremarkable. However, SNP-A obtained two years later in the setting of a relapse revealed a new acquired CN-LOH clone at 5q (Figure 1B). The 5q CN-LOH clone persisted at 6 month follow-up (not shown).

Patient 3 was a 47 year old male with a new diagnosis of aplastic anemia, with a small PNH clone. Metaphase cytogenetics were normal. SNP-A at the time of diagnosis revealed multiple acquired CN-LOH clones involving both 6p and 6q. SNP-A performed after one course immunosuppressive therapy showed a loss of the 6q CN-LOH and an expansion of the 6p CN-LOH clone (Figure 1C).

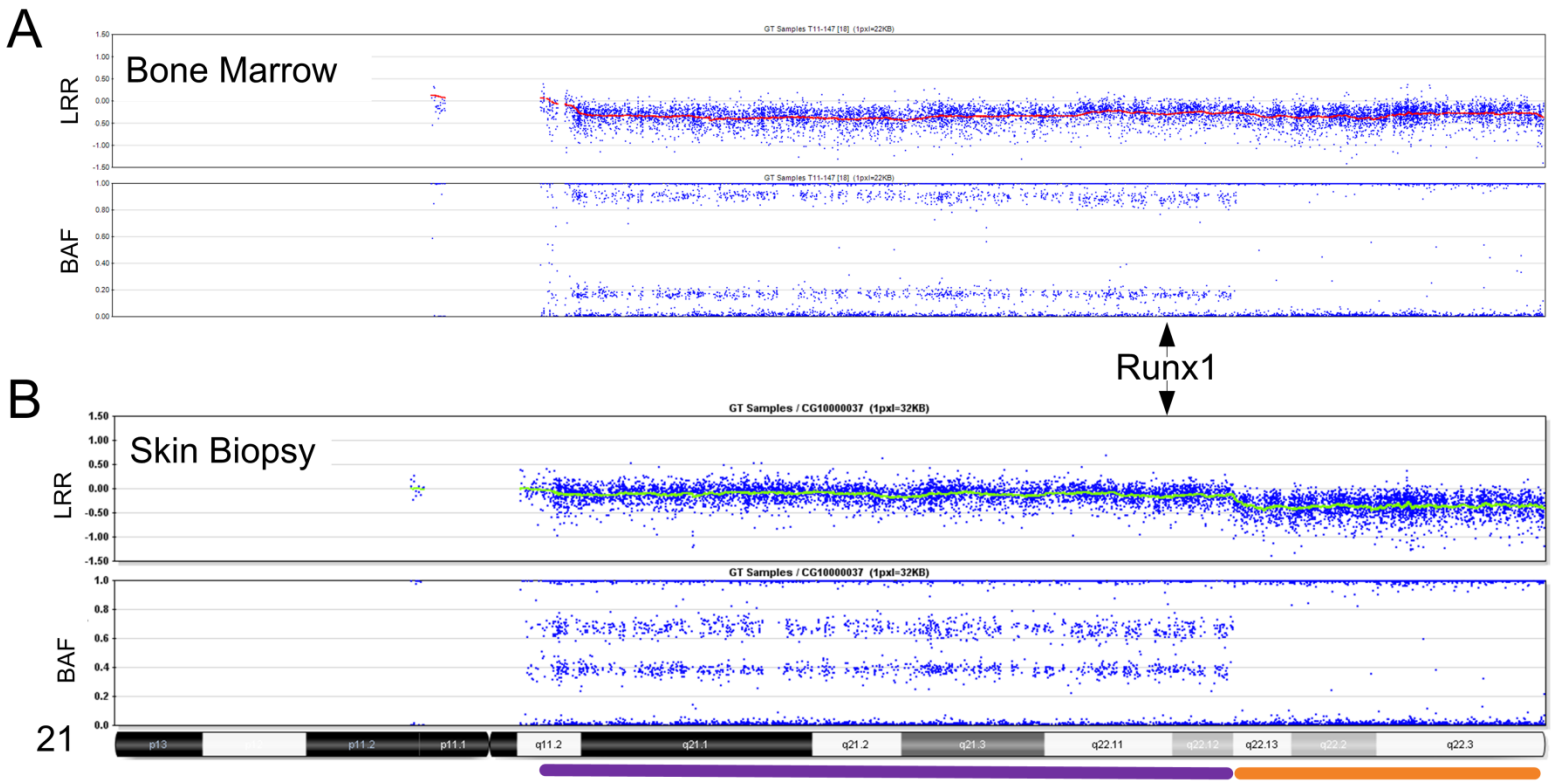
Supplemental Text 1B

Patient 14 was a 6 year old Caucasian female, referred for a life-long history of "Shwachman-like" bone marrow failure syndrome, with negative *SBDS* gene testing. The patient initially presented in infancy with failure to thrive, a history of pancreatic insufficiency that improved with pancreatic enzyme supplementation, and neutropenia. Additional symptoms included a hypogammaglobulinemia, a seizure disorder and skin changes. SBDS gene testing was negative, *ELA1* and *HAX1* testing was negative. Telomere length testing did not meet diagnostic criteria for Dyskeratosis Congenita. She was managed with G-CSF and IVIG infusions. Bone marrow biopsy at the time of referral showed a hypocellular marrow with 25% cellularity, M:E ratio of 2:1, and trilineage hematopoiesis without overt dysplasia or increase in blasts. Metaphase cytogenetics were normal. SNP array performed on the bone marrow aspirate revealed a clonal gain of chromosome 13 in 5-10% of cells. SNP array findings were confirmed by FISH with probes for the *RB1* and *FOXO1* loci on chromosome 13q14. Trisomy 13 has been previously described in the context of myelodysplasia, myeloproliferative disorders, and acute myeloid leukemias[2-4].

Supplemental Text 1C

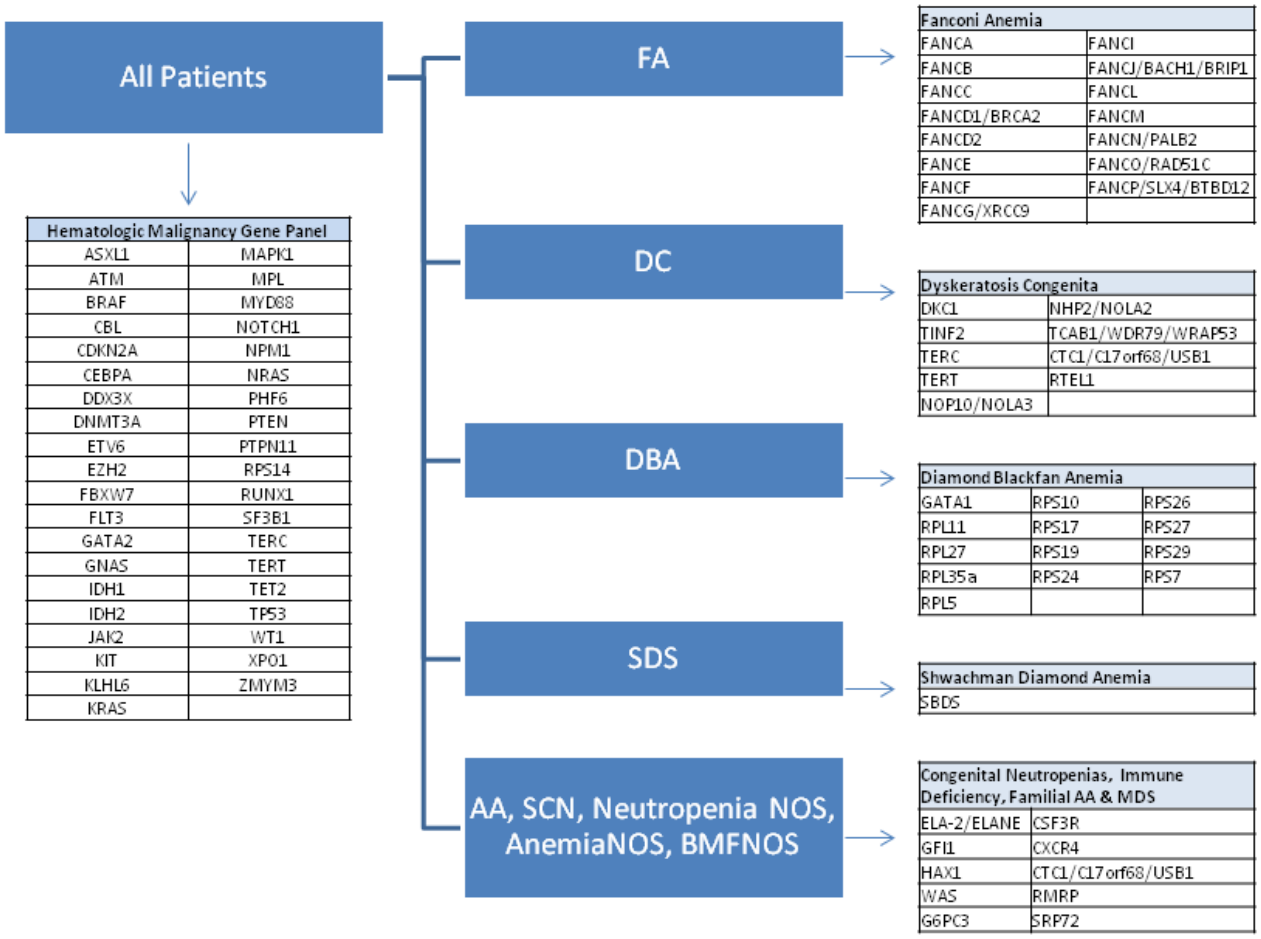
Patient 13 was a 1 year old Southeast Asian male, referred for congenital thrombocytopenia. The patient had intrauterine growth retardation and multiple congenital anomalies at birth including the middle interhemispheric variant of holoprocencephaly, bilateral ptosis, pyloric stenosis, and kyphosis of thoracic spine. Karyotype performed on skin biopsy at birth revealed a constitutional chromosomal abnormality of 46,XY,r(21)[17]/45,XY,-21[3]; SNP array performed on the same specimen showed that a 10.21 Mb deletion of 21q22.13q22.3 in the cell line containing the ring chromosome. A bone marrow biopsy performed at 1 year of age to evaluate persistent thrombocytopenia showed a cellular marrow with trilineage hematopoiesis without dyspoiesis. Bone marrow cytogenetic analysis showed an abnormal karyotype 46,XY,r(21)[1]/45,XY,-21[19]. SNP array analysis on the bone marrow aspirate

revealed an expansion of the monosomy 21 clone (85%) with ring 21 comprising only 15%; this was in contrast to the constitutional SNP array sample showing 85% with ring 21 and 15% with monosomy 21. The expansion of the monosomy 21 clone resulted in haploinsufficiency for *RUNX1*; heterozygous loss of *RUNX1* was previously shown to cause congenital thrombocytopenia [5-6]. Monosomy 21 has been seen in isolated cases of myeloid malignancies[7-8] and lymphomas[7, 9-10]; and was reported in a case of constitutional mosaic ring 21[11].



Supplemental Figure 1: Expansion of Monosomy 21 Clone Causing *RUNX1* Haploinsufficiency in a Patient with Congenital Thrombocytopenia and Congenital Anomalies.

Supplemental Figure 1: Expansion of Monosomy 21 Clone Causing *RUNX1* Haploinsufficiency in a Patient with Congenital Thrombocytopenia and Congenital Anomalies. Panel A shows SNP-A genotyping of bone marrow aspirate DNA from a 1-year old male with congenital thrombocytopenia and multiple anomalies. Genotyping results are depicted as two scatter plots: the top plot shows Log R Ratio (LRR, a measure of normalized total signal intensity for both alleles) on the Y-axis, and the chromosomal location on the X-axis. The bottom plot of each pair shows B-allele Frequency (BAF, a relative frequency of the minor allele) on the Y-axis, and the chromosomal location on the X-axis. Panel A shows a dominant clone of monosomy 21, seen as a prominent negative deflection of LRR from a normal diploid value of 0 in the region indicated by the purple line. There is a smaller, roughly 15% clone of ring chromosome 21 containing a terminal deletion (region highlighted by orange line). Panel B shows a paired SNP array obtained on DNA extracted from a skin biopsy from the same patient. Skin biopsy SNP-A reveals constitutional mosaicism for ring chromosome 21 estimated at roughly 85% and containing a terminal deletion of chromosome 21 (indicated by the orange line); there is a smaller monosomy 21 clone (15%) seen as a slight negative deflection of LRR in the region indicated by purple line. In conjunction, results of Panels A and B indicate an expansion of the monosomy 21 clone in the bone marrow compared to the skin in this patient with congenital somatic mosaicism for ring chromosome 21 and monosomy 21. Expansion of monosomy 21 leads to effective haploinsufficiency for *RUNX1*, which has been previously linked to congenital thrombocytopenia.



Hematologic Malignancy Gene Panel	
ASXL1	MAPK1
ATM	MPL
BRAF	MYD88
CBL	NOTCH1
CDKN2A	NPM1
CEBPA	NRAS
DDX3X	PHF6
DNMT3A	PTEN
ETV6	PTPN11
EZH2	RPS14
FBXW7	RUNX1
FLT3	SF3B1
GATA2	TERC
GNAS	TERT
IDH1	TET2
IDH2	TP53
JAK2	WT1
KIT	XPO1
KLHL6	ZMYM3
KRAS	

Fanconi Anemia	
FANCA	FANCI
FANCB	FANCJ/BACH1/BRIP1
FANCC	FANCL
FANCD1/BRCA2	FANCM
FANCD2	FANCN/PALB2
FANCE	FANCO/RAD51C
FANCF	FANCP/SLX4/BTBD12
FANCG/XRCC9	

Dyskeratosis Congenita	
DKC1	NHP2/NOLA2
TINF2	TCAB1/WDR79/WRAP53
TERC	CTC1/C17orf68/USB1
TERT	RTEL1
NOP10/NOLA3	

Diamond Blackfan Anemia		
GATA1	RPS10	RPS26
RPL11	RPS17	RPS27
RPL27	RPS19	RPS29
RPL35a	RPS24	RPS7
RPL5		

Shwachman Diamond Anemia
SBDS

Congenital Neutropenias, Immune Deficiency, Familial AA & MDS	
ELA-2/ELANE	CSF3R
GFI1	CXCR4
HAX1	CTC1/C17orf68/USB1
WAS	RMRP
G6PC3	SRP72

Supplemental Figure 2: Flow Diagram for Candidate Gene Analysis. SNP-A for all patients were visually analyzed in GenomeStudio Software for presence of abnormalities candidate genes listed on the left of the slide ("Hematologic Malignancy Gene Panel"). Additionally, patients from selected diagnostic groups (shown in blue rectangles) were screened for structural abnormalities in additional candidate genes as shown.

Supplemental References

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