Supplemental Figures

Improving diagnostic precision, care and syndrome definitions using comprehensive next generation sequencing for the inherited bone marrow failure syndromes

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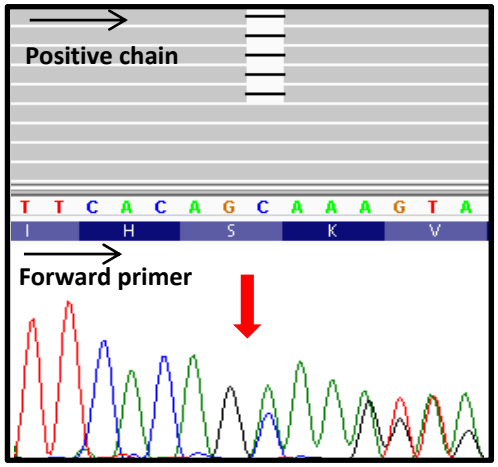
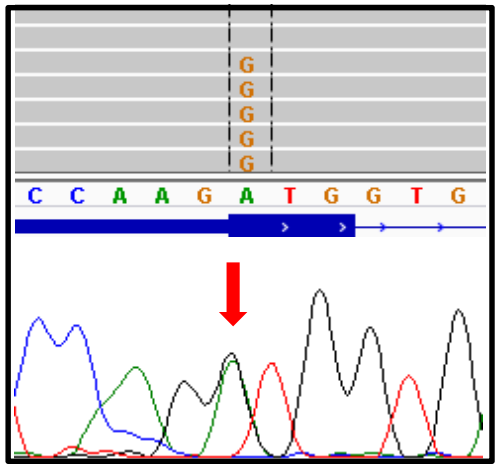
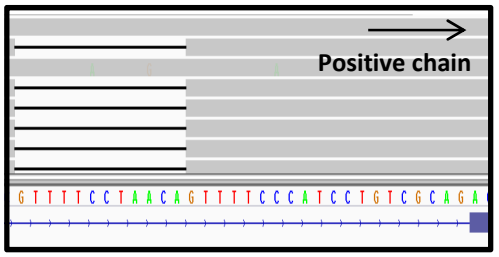
Figures 1-58 show the mutation areas from the next generation sequencing BAM files and information about Sanger sequencing validation. Please note that in the BAM files only a proportion of the reads are shown (about 7-8 of an average of 680 reads/patient).



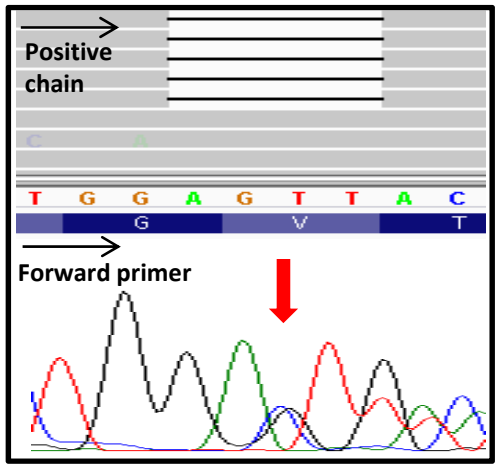
1. Patient 1 in in Supplemental Table 3. *RPS26* c.243delC



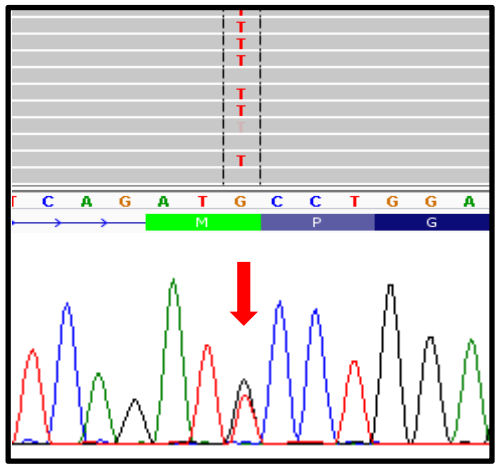
2. Patient 2 in Supplemental Table 3. *RPS26* c.1A>G



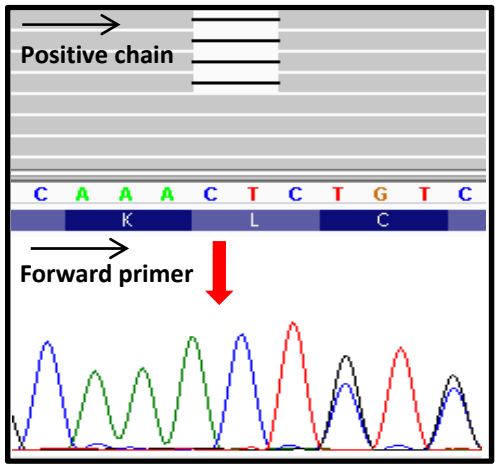
3. Patient 3 in Supplemental Table 3. *RPS26* c.4-32_21delGTTTTCCTAACA (Mutation was Validated in a clinical lab)



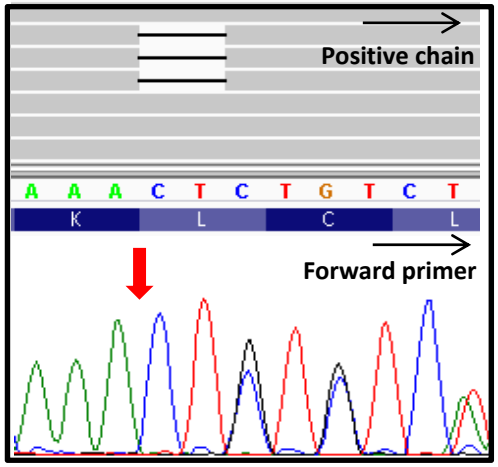
4. Patient 4 in Supplemental Table 3. *RPS19* c.10_13delAGTT



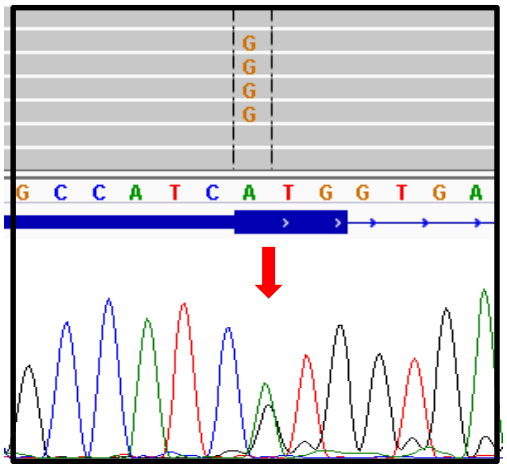
5. Patient 5 in Supplemental Table 3. *RPS19* c.3G>T



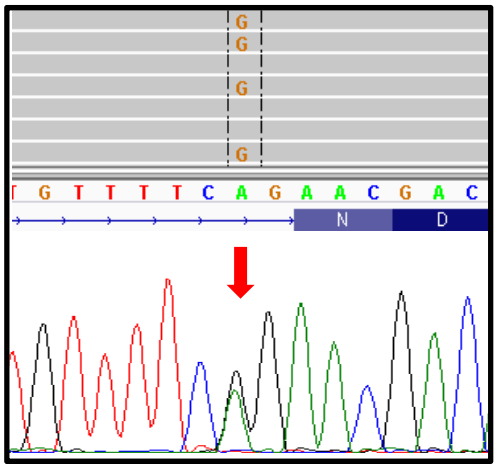
6. Patient 1 in Supplemental Table 3. *RPL11* c.60_61delCT



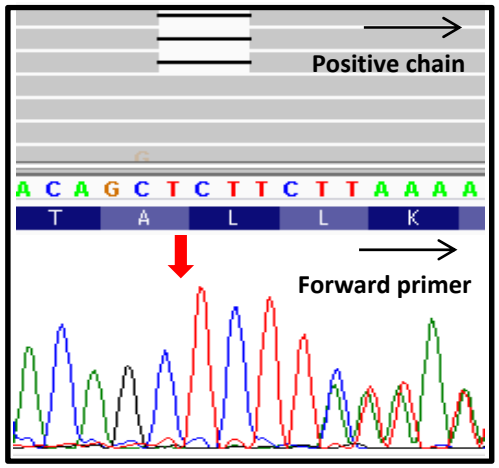
7. Patient 7 in Supplemental Table 3.
RPL11 c.60_61delCT



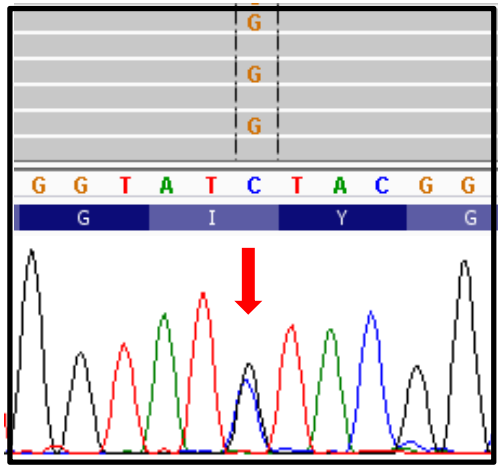
8. Patient 8 in Supplemental Table 3.
RPS24 c.1A>G



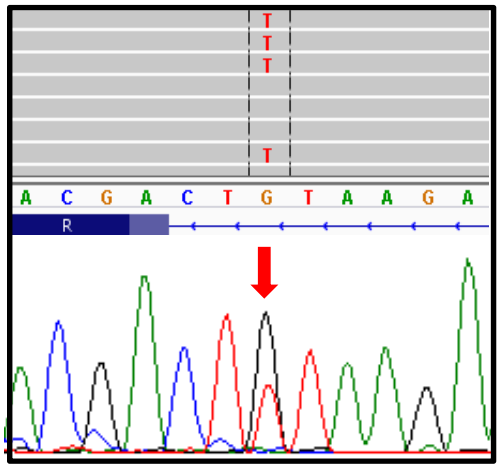
9. Patient 9 in Supplemental Table 3.
RPS24 c.4-2A>G



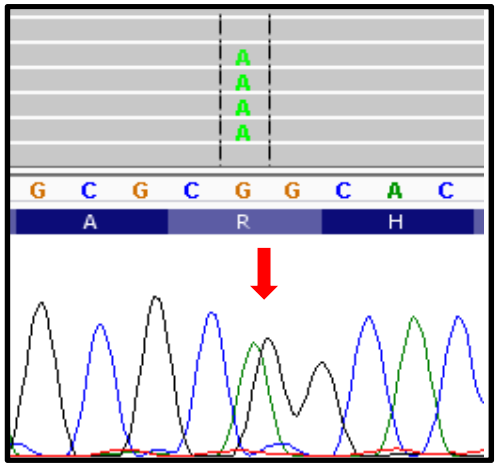
10. Patient 10 in Supplemental Table 3.
RPL35A c.78_80delTCT



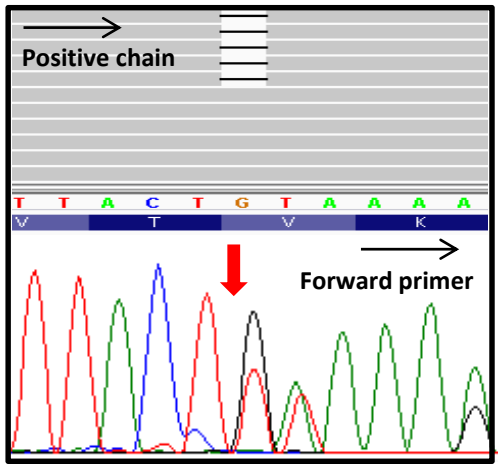
11. Patient 11 in Supplemental Table 3.
RPL11 c.372C>G



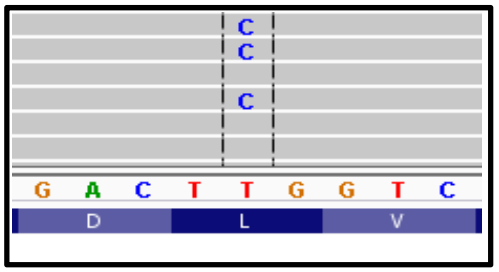
12. Patient 12 in Supplemental Table 3.
RPS29 c.63-3C>A



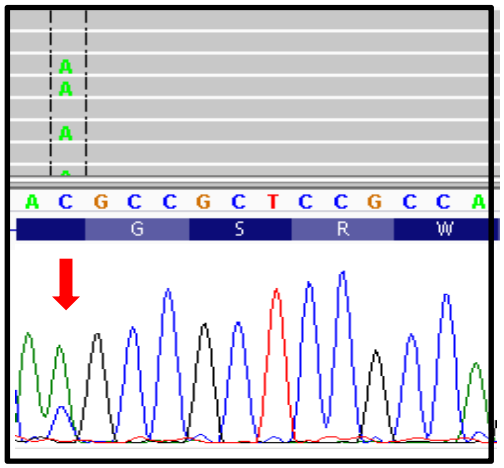
13. Patient 13 in Supplemental Table 3. *RPS19* c.185G>A



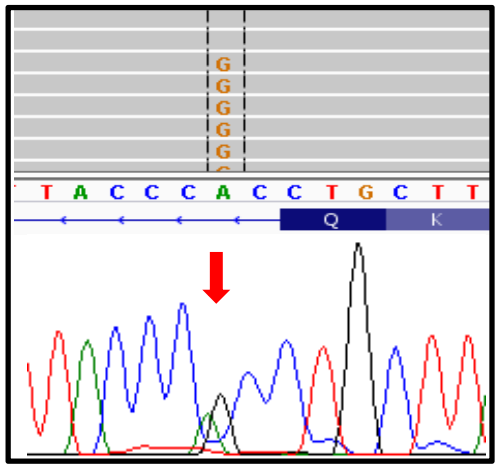
14. Patient 14 in Supplemental Table 3. *RPS19* c.16delG



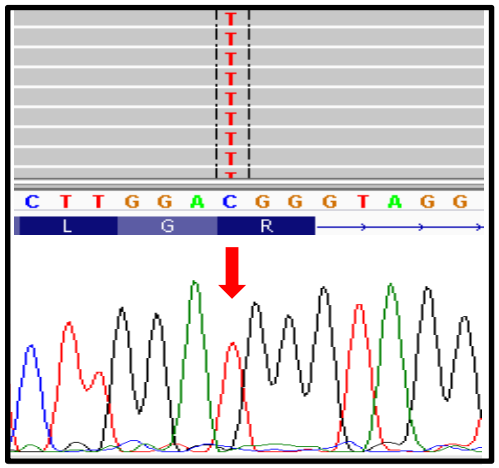
15. Patient 15 in Supplemental Table 3. *RPS7* c.398T>C. (Mutation was Validated in a clinical lab)



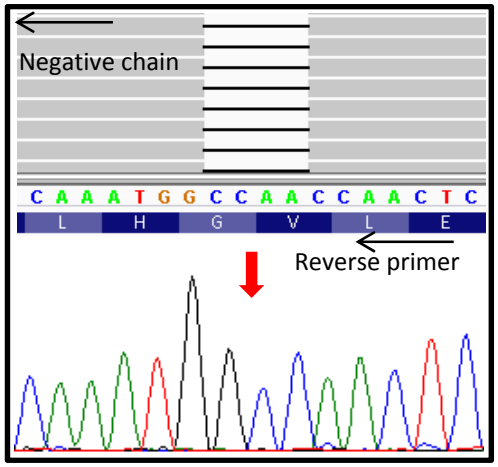
16a. Patient 16 in Supplemental Table 3. *SBDS* c.127G>T



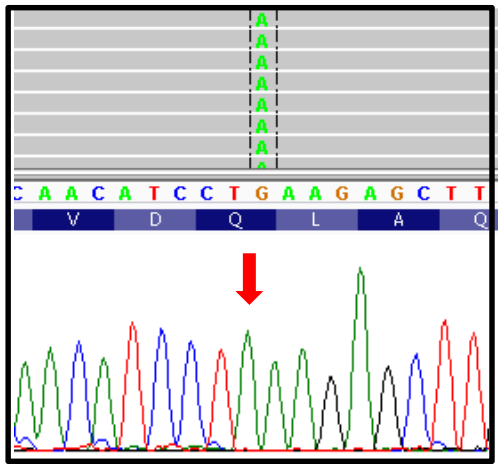
16b. Patient 16 in Supplemental Table 3. *SBDS* c.258+2T>C



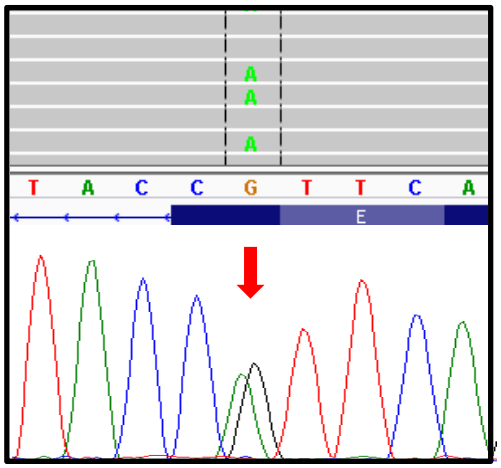
17. Patient 17 in Supplemental Table 3. *FANCE* c.1111C>T (hom)



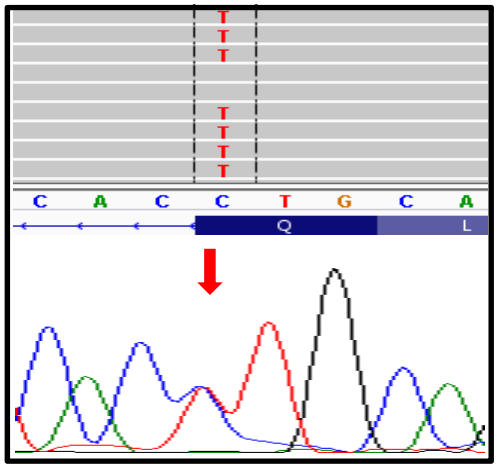
18. Patient 18 in Supplemental Table 3. *FANCA* c.1115_1118delTTGG (hom)



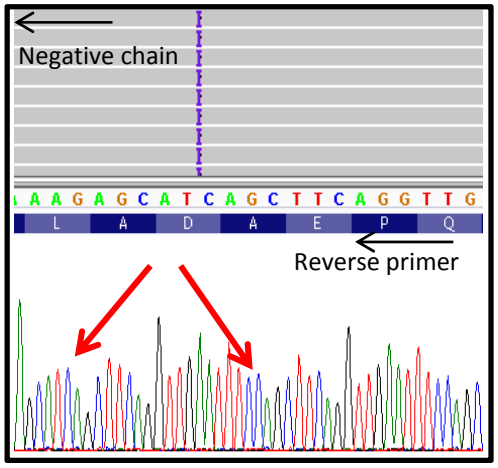
19. Patient 19 in Supplemental Table 3. *FANCA* c.1645C>T (hom)



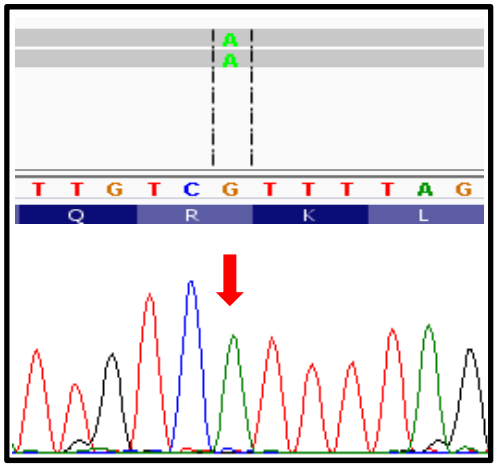
20a. Patient 20 in Supplemental Table 3. *FANCA* c.2851C>T



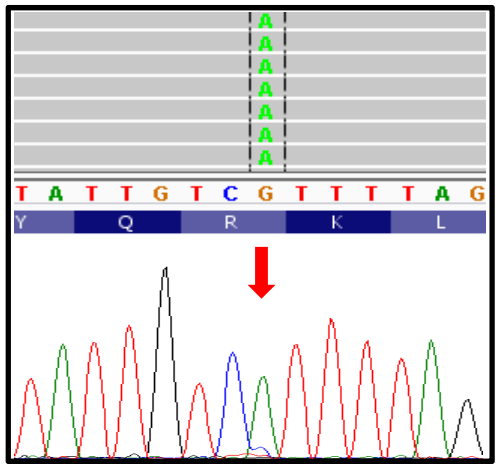
20b. Patient 20 in Supplemental Table 3. *FANCA* c.1470G>A



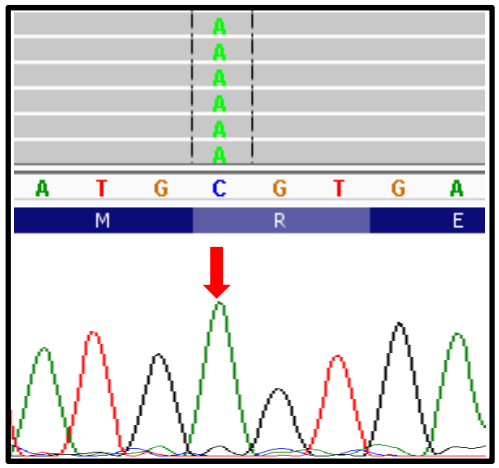
21. Patient 21 in Supplemental Table 3. *FANCA* c.2830_2831InsGAAATTCAACCT GAAGCTG (hom)



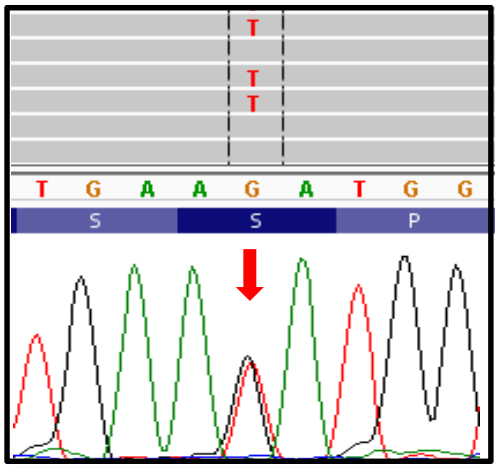
22. Patient 22 in Supplemental Table 3. *FANCJ/BRIP1* c.2392C>T (hom). See Results Section for more information about this homozygous mutation with 2 reads .



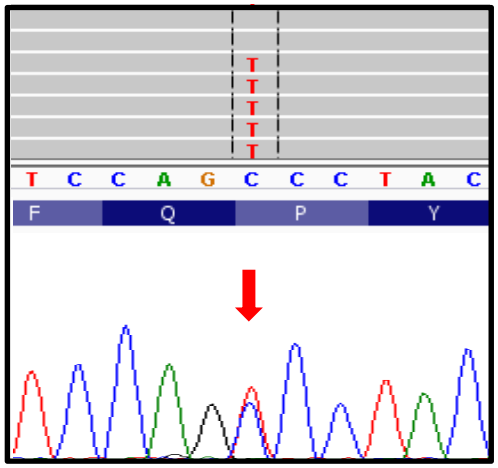
23. Patient 23 in Supplemental Table 3. *FANCJ/BRIP1* c.2392C>T (hom)



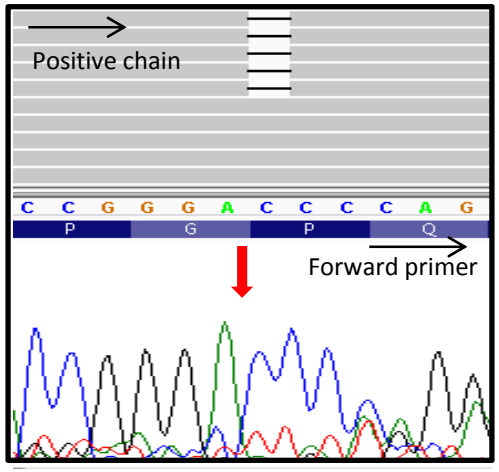
24. Patient 24 in Supplemental Table 3. *ERCC4* c.2065C>A (hom)



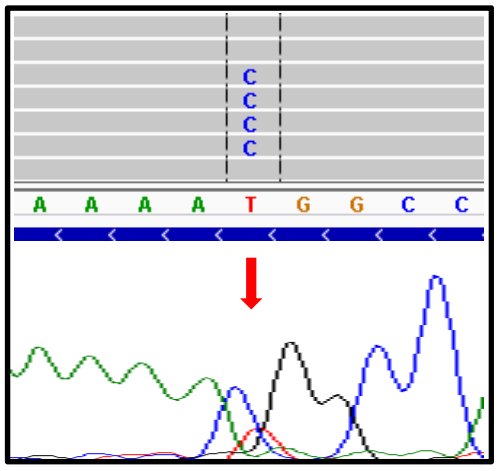
25. Patient 25 in Supplemental Table 3. *TINF2* c.734C>A



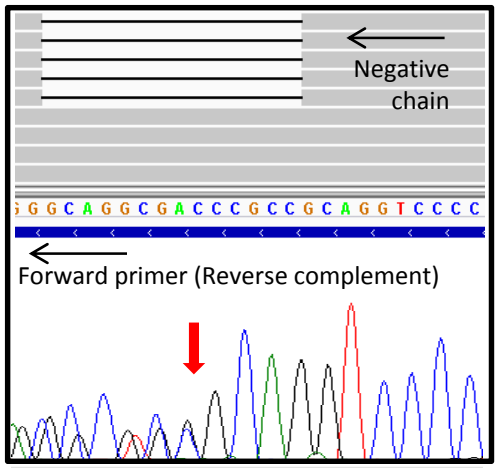
26a. Patient 26 in Supplemental Table 3. RTEL1 c.49C>T



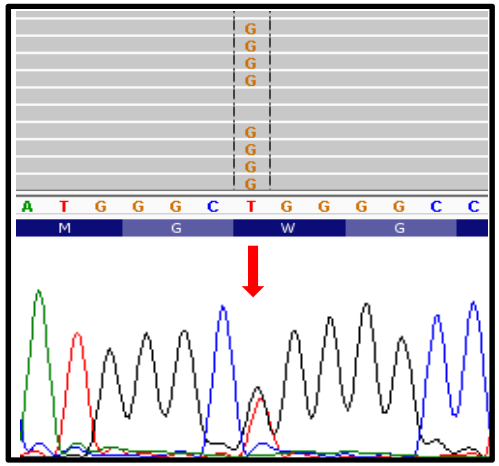
26b. Patient 26 in Supplemental Table 3. *RTEL1* c.3442delC



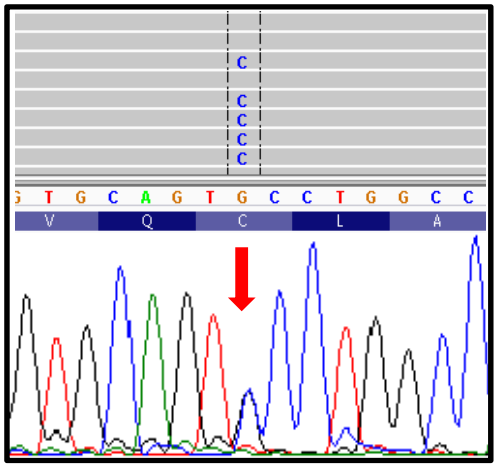
27a. Patient 27 in Supplemental Table 3. *TERC* c.37A>G



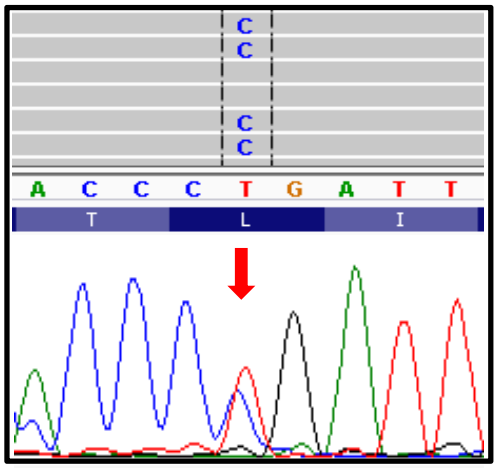
27b. Patient 27 in Supplemental Table 3.
TERC c.216_229del



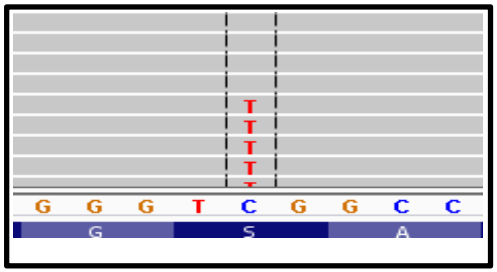
28. Patient 28 in Supplemental Table 3.
ELANE c. 466T>G



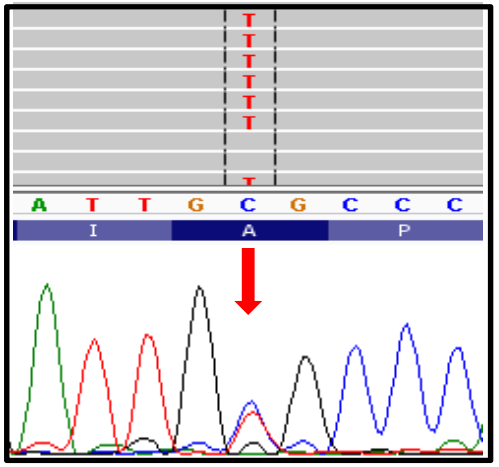
29. Patient 29 in Supplemental Table 3.
ELANE c.452G>C



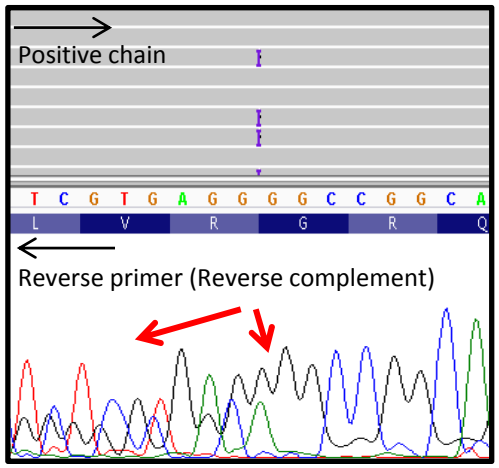
30. Patient 30 in Supplemental Table 3.
ELANE c.176T>C



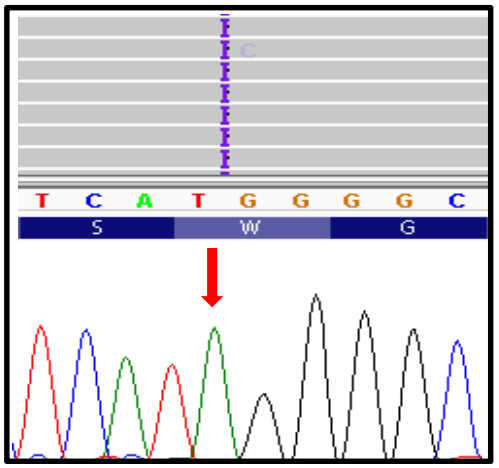
31. Patient 31 in Supplemental Table 3.
ELANE c.377C>T (Mutation was Validated in a clinical lab)



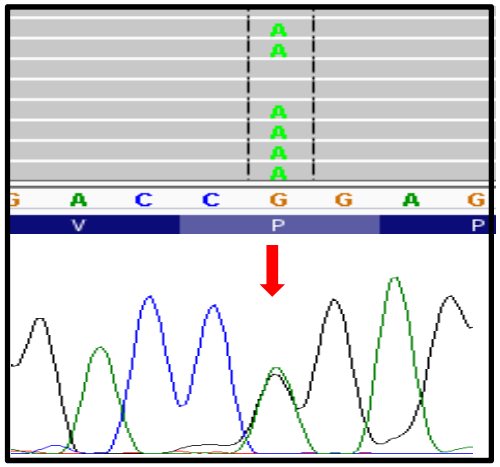
32. Patient 32 in Supplemental Table 3.
ELANE c.182C>T



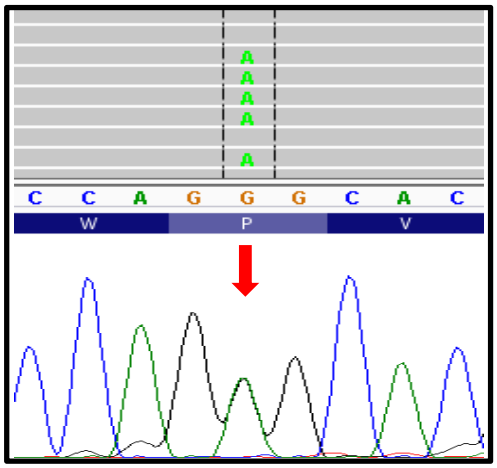
33. Patient 33 in Supplemental Table 3.
ELANE c.574_581dupGGCCGGCA



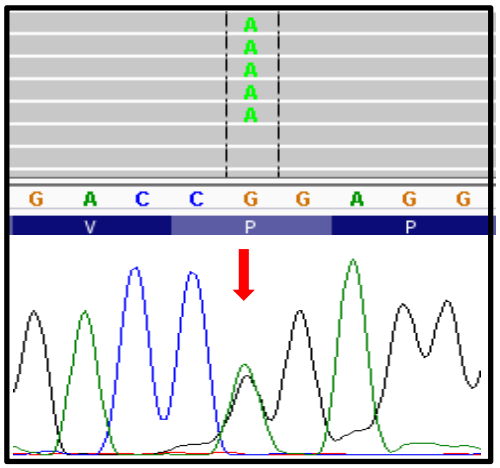
34. Patient 34 in Supplemental Table 3.
HAX1 c.131insA (hom)



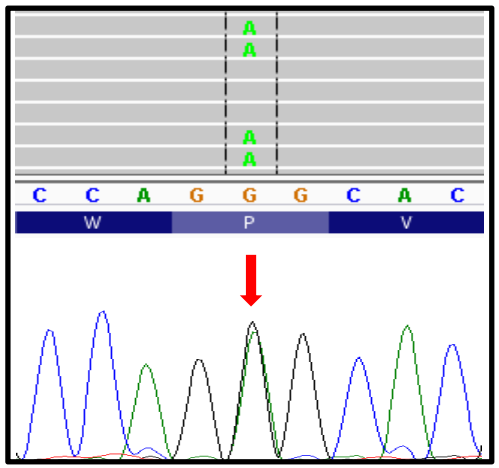
35a. Patient 35 in Supplemental Table 3.
CDAN1 c.2015C>T



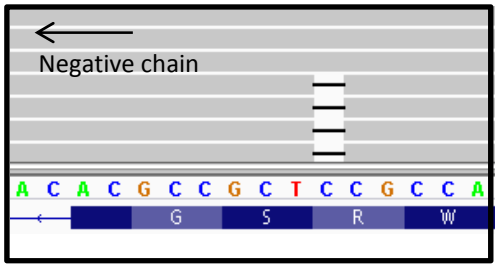
35b. Patient 35 in Supplemental Table 3.
CDAN1 c.2081C>T



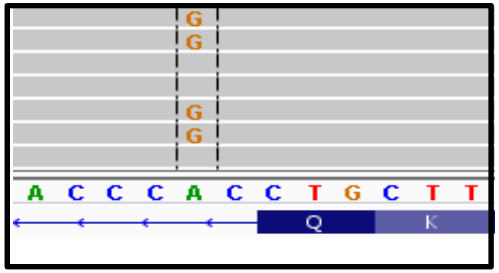
36a. Patient 36 in Supplemental Table 3.
CDAN1 c.2015C>T



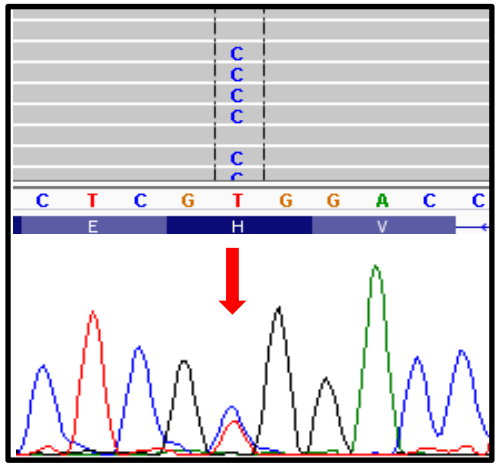
36b. Patient 36 in Supplemental Table 3.
CDAN1 c.2081C>T



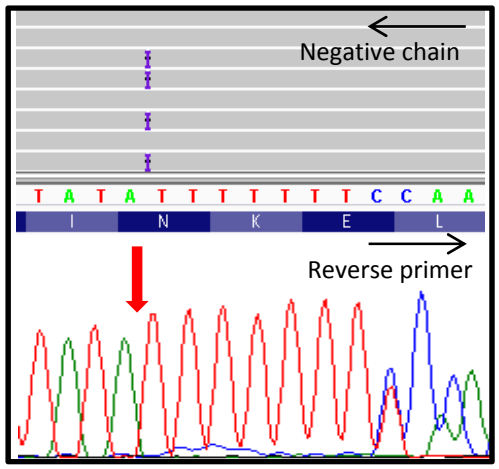
37a. Patient 37 in Supplemental Table 3. *SBDS* c.120delG (Mutation was Validated in a clinical lab)



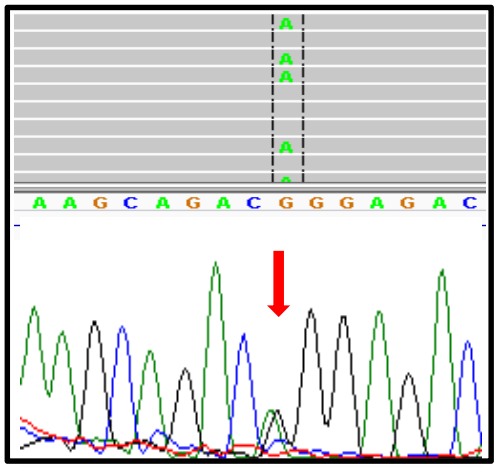
37b. Patient 37 in Supplemental Table 3. *SBDS* c.258+2T>C (Mutation was Validated in a clinical lab)



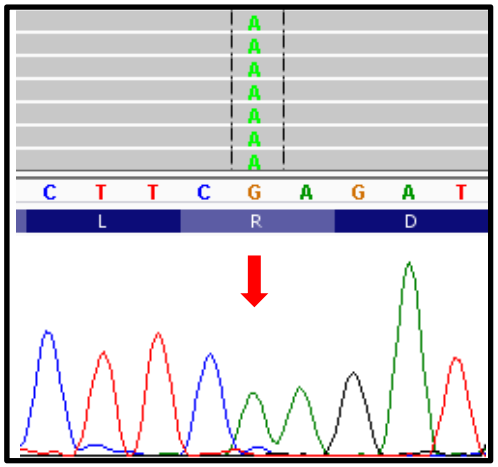
38. Patient 38 in Supplemental Table 3. *MYH9* c.4562A>G



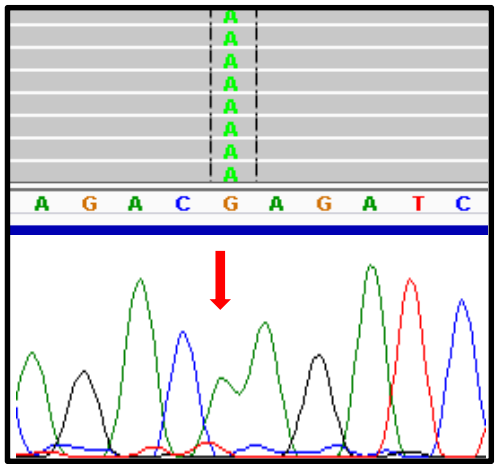
39. Patient 39 in Supplemental Table 3. *ANKRD26* c.4976dupA



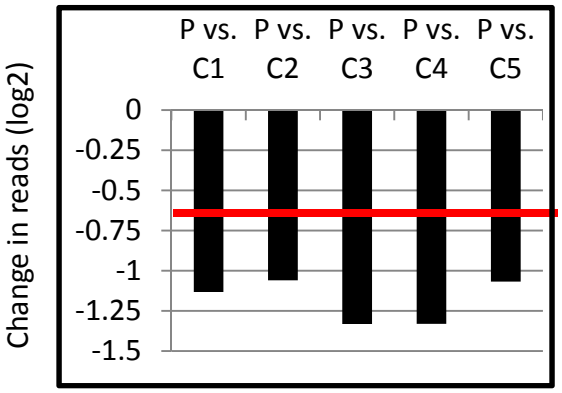
40. Patient 40 in Supplemental Table 3. *TERT* c.2383-15C>T. The NGS gene panel detected the same mutation in the affected mother of this patient (Supplemental Fig 3, Patient 42)



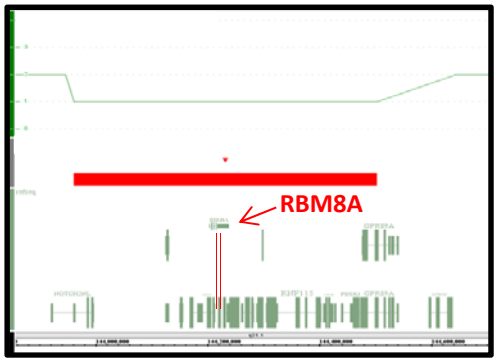
41. Patient 42 in in Supplemental Table 3. *SLC25A38* c.560G>A



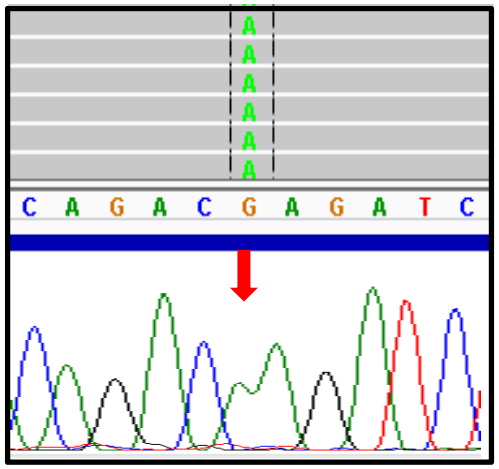
42a. Patient 43 in in Supplemental Table 3. *RBM8A* c.-21G>A (compound het)



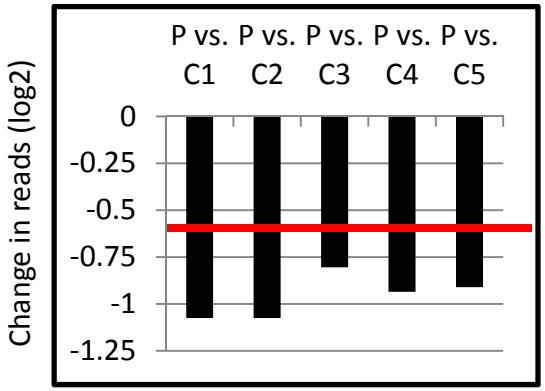
42b. Patient 43 in in Supplemental Table 3. *RBM8A* one copy number deletion as indicated by log2< -0.6 when patient calls are compared to controls (compound het) (C, control; P, patient.)



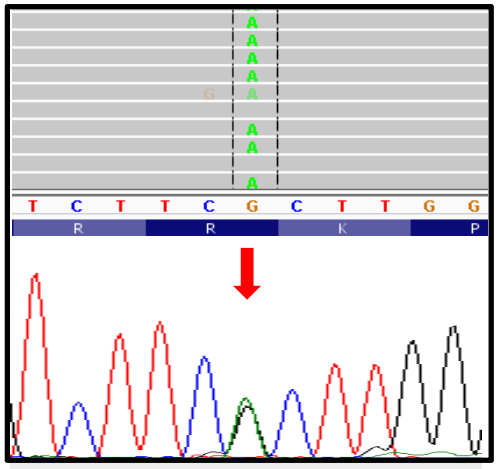
42c. Patient 43 in Supplemental Table 3. One copy number deletion that included *RBM8A* was found by Affymetrix SNP6.0 array. The red arrow points to the location of *RBM8A*. The red vertical lines show its approximate borders .



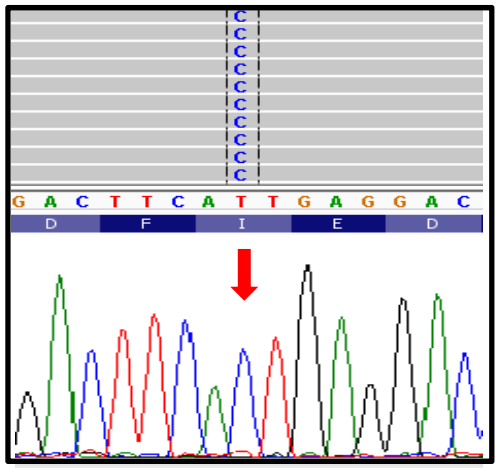
43a. Patient 44 in in Supplemental Table 3. *RBM8A* c.-21G>A (compound het)



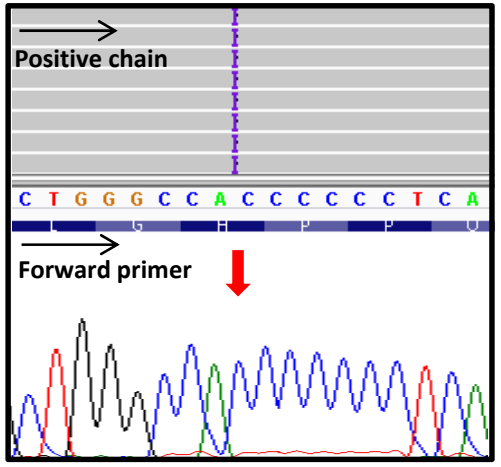
43b. Patient 44 in in Supplemental Table 3. *RBM8A* one copy deletion as indicated by log2< -0.6 when patient calls are compared to controls(compound het) (C, control; P, patient.)



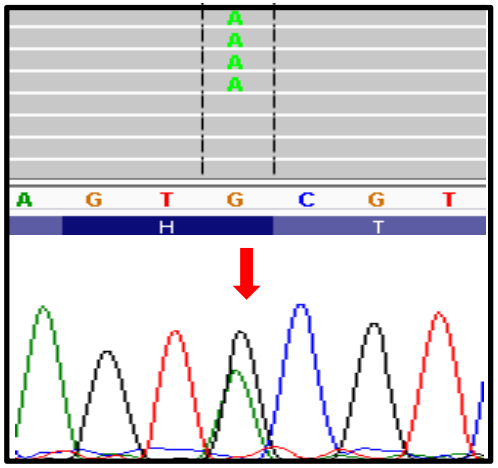
44. Patient 1 in Supplemental Table 4. *GATA2* c.1009C>T



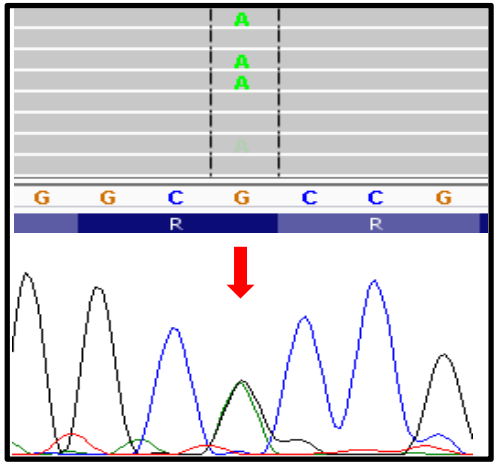
45. Patient 2 in Supplemental Table 4. *WAS* c.881T>C (hemizygous)



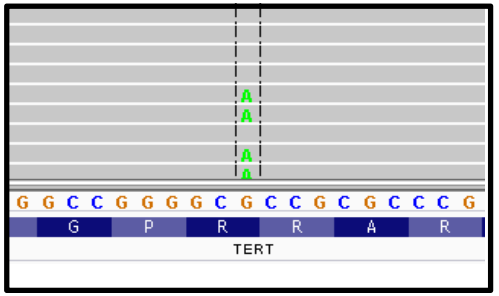
46. Patient 3 in Supplemental Table 4. *G6PC3* c.911dupC (hom)



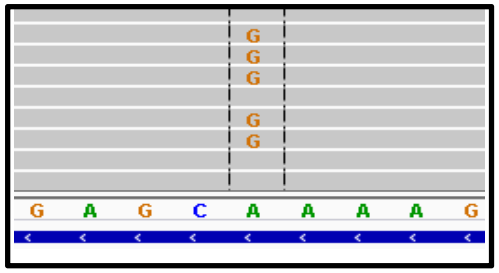
47. Patient 4 in Supplemental Table 4. *TERT* c.1234C>T



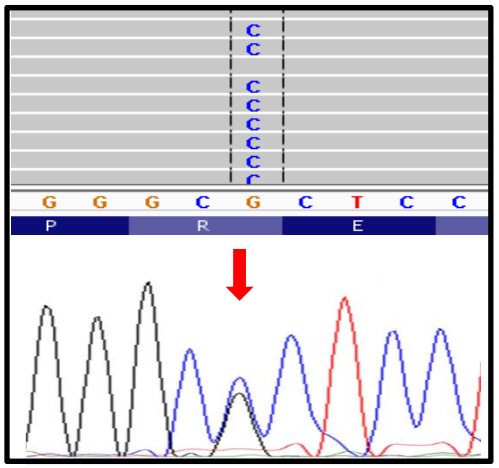
48. Patient 5 in Supplemental Table 4. *TERT* c. 2014C>T



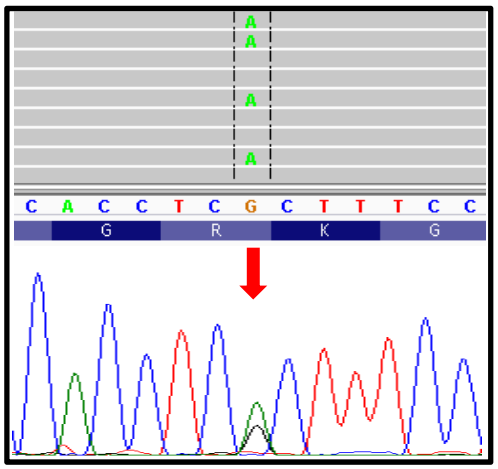
49. Patient 6 in Supplemental Table 4. *TERT* c. 2014C>T (Mutation was Validated in a clinical lab)



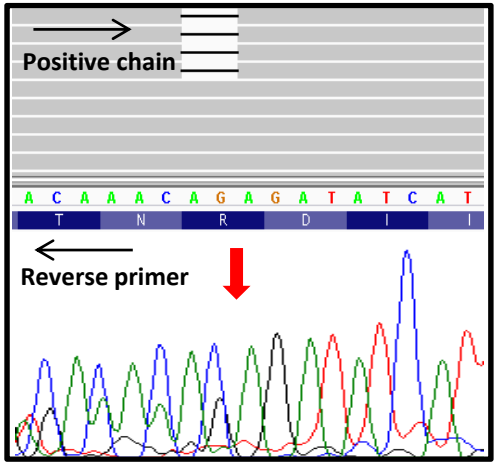
50. Patient 7 in Supplemental Table 4. *TERC* n.83T>C (Mutation was Validated in a clinical lab)



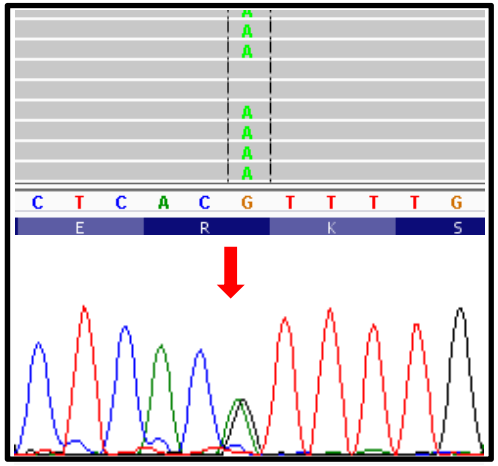
51. Patient 8 in Supplemental Table 4. *TINF2* c. 844C>G



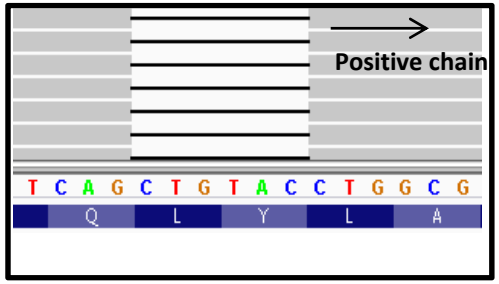
52. Patient 9 in Supplemental Table 4. *CXCR4* c.1000C>T



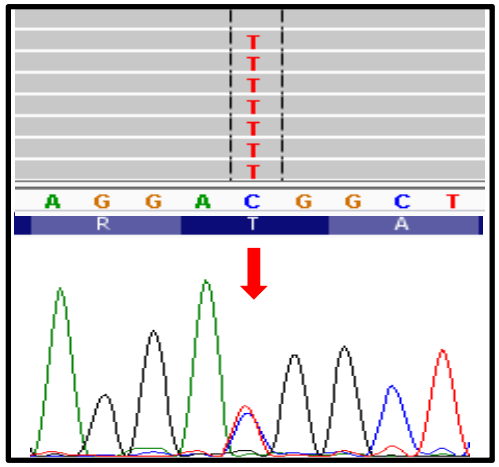
53. Patient 10 in Supplemental Table 4. *RPL5* c.174_175delAG



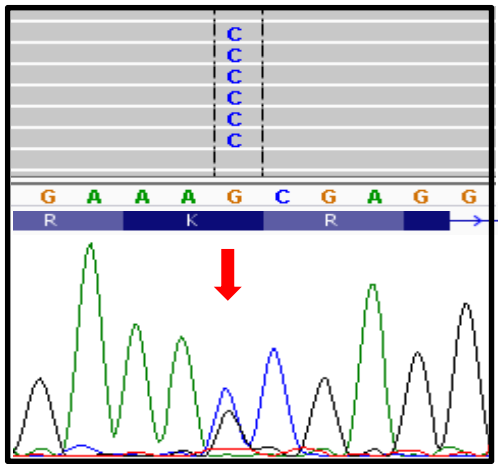
54. Patient 11 in Supplemental Table 4. *MYH9* c.3493C>T



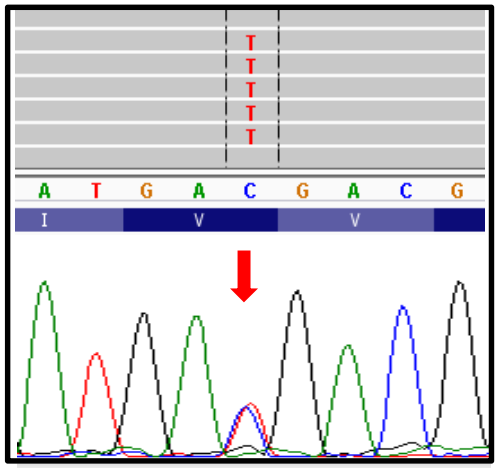
55. Patient 12 in Supplemental Table 4. *WAS* c.157_162delCTGTAC (hemizygous) (Mutation was Validated in a clinical lab)



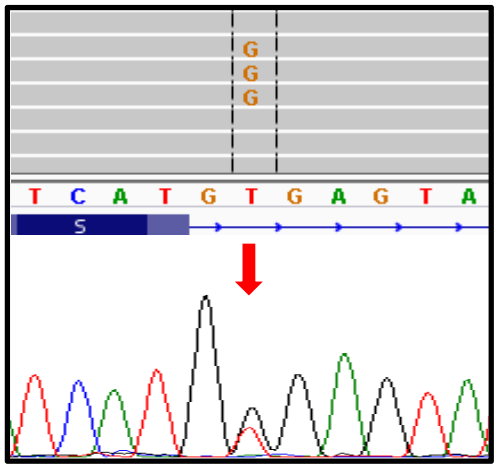
56a. Patient 13 in Supplemental Table 4.
RTEL1 c.1373C>T



56b. Patient 13 in Supplemental Table 4.
RTEL1 c.1416G>C

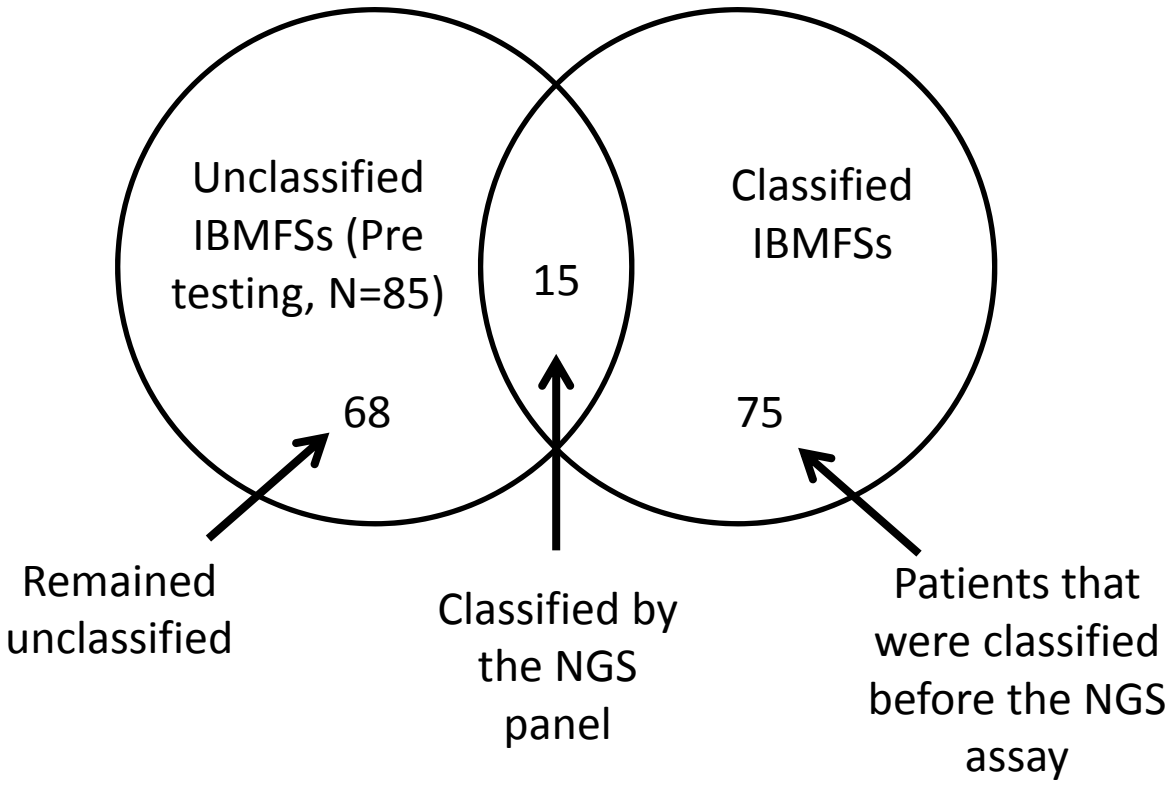


57. Patient 14 in Supplemental Table 4.
TERT c.2371 G>A



58. Patient 16 in Supplemental Table 4.
MASTL c.811+2 T>G

Figure 59. A Venn diagram showing how many unclassified patients were added to the classified pool after being tested by the NGS gene panel assay (15), and how many patients whose diagnosis remained unclassified after being tested by the assay (68).



Supplementary Tables

Improving diagnostic precision, care and syndrome definitions using comprehensive next generation sequencing for the inherited bone marrow failure syndromes

Ibrahim Ghemlas, Hongbing Li, Bozana Zlateska, Robert Klaassen, Conrad V Fernandez, Rochelle A Yanofsky, John Wu, Yves Pastore, Mariana Silva, Jeff H Lipton, Josse Brossard, Michon Bruno, Sharon Abish, MaCregor Steele, Roona Sinha, Mark Belltrutti, Vicky Breaky, Lawrence Jardine, Lisa Goodyear, Lillian Sung, Santhosh Dhanraj, Emma Reble, Amanda Wagner, Joseph Beyene, Peter Ray, Stephen Meyn, Michaela Cada, Yigal Dror

Supplementary Table 1: A list of 72 inherited bone marrow failure syndrome genes that were included in next generation sequencing panel assay and their coverage. We designed a custom NGS IBMFS Gene Panel Assay that allows the discovery of mutations in a comprehensive panel of 72 known IBMFS genes. The assay is based on a hybridization oligonucleotide pool, which covers coding regions, 50bp flanking intronic regions that include splicing sites, 3'-untranslated regions that include potential translation regulatory elements, and upstream promoter regions. The oligonucleotide size was set at 150 mers for read length with 3x tiling and a maximum of 10bp overlap between oligonucleotides. The design was submitted to the Agilent HaloPlex Design Wizard program (http://www.halogenomics.com/haloplex/custom-reagent-kits).

Gene	Regions	Coverage (%)	High Coverage (>= 90%)	Low Coverage (< 90%)
ABCB7	15	100	15	0
AK2	11	100	11	0
ALAS2	12	100	12	0
ANKRD26	37	99.7	37	0
BTHS	5	100	5	0
CDAN1	25	99.29	25	0
CTC1	14	100	14	0
CXCR4	3	100	3	0
DKC1	14	100	14	0
ELANE	5	100	5	0
FANCA	35	98.61	34	1
FANCB/FAAP95	10	100	10	0
FANCC	21	100	21	0
FANCD1/BRCA2	27	99.31	27	0
FANCD2	45	97.15	40	5
FANCE	10	100	10	0
FANCF	1	100	1	0
FANCG	11	100	11	0
FANCI	37	99.98	37	0
FANCJ/BRIP1	24	98.09	23	1
FANCL	14	100	14	0
FANCM	25	99.57	24	1
FANCO/RAD51C	11	100	11	0
FANCP/PALB2	14	99.69	14	0
FECH	11	98.25	11	1
G6PC3	37	100	37	0
GATA1	6	100	6	0

GATA2	10	100	10	0
GFI1	10	98.98	10	0
GLRX5	3	100	3	0
GP1BA	2	100	2	0
HAX1	3	100	3	0
HOXA11	12	100	12	0
KLF1	3	100	3	0
LIG4	4	99.94	4	0
MASTL	12	99.96	12	0
MPL	12	98.17	12	0
MYH9	46	99.97	46	0
NBEAL2	34	99.93	34	0
NHP2	3	100	3	0
NOP10	2	98.81	2	0
PALB2	15	100	15	0
PUS1	5	100	5	0
RBM8A	4	81.41	2	2
RMRP	1	100	1	0
RPL11	6	100	6	0
RPL27	4	100	4	0
RPL35A	7	99.24	7	0
RPL5	7	99.44	7	0
RPS10	7	100	7	0
RPS19	6	100	6	0
RPS24	8	100	8	0
RPS27	2	100	2	0
RPS26	3	99.34	3	0
RPS29	4	94.86	4	0
RPS7	2	96.87	2	0
RTEL1	33	100	33	0
RUNX1	26	99.34	26	0
SBDS	5	90.61	2	3
SEC23B	20	100	20	0
SLC19A2	6	100	6	0
SLC25A38	8	100	8	0
SLC37A4	2	100	2	0
SMARCAL1	21	95.97	20	1
SRP72	17	99.16	17	0
TERC	1	100	1	0
TERT	16	100	16	0

TINF2	3	94.86	2	1
***USB1/* C16orf57**	8	93.33	7	1
WAS	11	99.57	11	0
WRAP53	7	100	7	0
XRCC2	4	95.62	4	0

[*] *FANCQ/ERCC4* was identified as an IBMFS gene after January 2013, and was added to the panel when the second batch of patients was tested.

The gene list is modified and updated from Dror Y. Genetic Basis of Inherited Bone Marrow Failure Syndromes. InTech Open Access Publisher 2011: pp 357-392.

Supplementary Table 2: List of genes and mutation in previously genotyped patients which were validated with NGS IBMFS gene panel

Patient	Gene	Nucleotide change	Protein change	Mutation type
1*	*SBDS* (hom)	c.258+2T>C	Splicing	Splicing
2	*RPL5* (het)	c.83delC	p.Thr28Metfs*10	Frameshift
3	*ELANE* (het)	c.597+5 G>A	Splicing	Splicing
4	*FANCA* (het)	c.3788-3790 delTCT	p.F1263SFS*194	Frameshift
5	*TINF2* (het)	c. 845G>A	R282H	Missense
6*	*cMPL* (hom)	c.304C>T	R102C	Missense
7	*RPS19* (het)	c.250_251delAG	p.R84Lfs*69	Frameshift
8*	*DKC1* (com hem)	c.112delA; c.116InsC	I38S ; K39T	Frameshift
9	*TERT* (het)	c.2383-15C>T	Splicing	Splicing
10	*RPL11* (het)	c.158-1G>C	Splicing	Splicing

*Two different mutations that were present in one allele (combined mutations) or two identical mutations that were found in both alleles (homozygous mutations) were counted twice. Thus, the total number of mutations (*i.e.* mutant alleles) in these 10 patients is 13.
Com, two mutations combined on the same allele; hem, hemizygous; het, heterozygous; hom, homozygous; NA, not applicable

Supplemental Table 3: List of identified damaging mutations in patients with classified IBMFSs without known genes

Number	Clinical Diagnosis	Gene	Mode	Nucleotide change	Protein change	Mutation type	Previous reporting
1	DBA	*RPS26* (het)	AD	c. 243delC	p.Ser81Argfs*3	Indel/Frameshift	Novel[2]
2	DBA	*RPS26* (het)	AD	c.1A>G	p.Met1?	Start code lost	Reported
3	DBA	*RPS26* (het)	AD	c.4-32_21 delGTTTTCCTAACA	Splicing change	Splicing	Novel[2]
4	DBA	*RPS19* (het)	AD	c.10_13 delAGTT	p.Val4Leufs*2	Indel/Frameshift	Reported
5	DBA	*RPS19* (het)	AD	c. 3G>T	p.Met1?	Start code lost	Reported
6	DBA	*RPL11* (het)	AD	c.60_61delCT	p.Cys21Serfs*33	Indel/Frameshift	Reported
7	DBA	*RPL11* (het)	AD	c.60_61delCT	p.Cys21Serfs*33	Indel/Frameshift	Reported
8	DBA	*RPS24* (het)	AD	c.1A>G	p.Met1?	Start code lost	Reported
9	DBA	*RPS24* (het)	AD	c.4-2A>G	Splicing change	Splicing	Novel[2]
10	DBA	*RPL35A* (het)	AD	c.78_80delTCT	p. Leu28del	Indel/Inframe	Reported
11	DBA	*RPL11* (het)	AD	c.372C>G	p.Ile124Met	Missense	Novel[2]
12	DBA	*RPS29* (het)	AD	c.63-3 C>A	Splicing change	Splicing	Novel[2]
13	DBA	*RPS19* (het)	AD	c.185G>A	p.Arg62Gln	Missense	Reported
14	DBA	*RPS19* (het)	AD	c.16delG	p.Val6*	Nonsense	Novel[2]
15	DBA	*RPS7* (het)	AD	c.398T>C	p.Leu133Ser	Missense	Novel[2]

16	DBA	*SBDS* (combined)	AR	c.127G>T c.258+2T>C	p.Val43Leu Splicing change	Missense Splicing	Novel[2] Reported
17	FA	*FANCE* (hom)	AR	c. 1111C>T	p.Arg371Trp	Missense	Reported
18	FA	*FANCA* (hom)	AR	c.1115_1118delTTGG	p.Val372Alafs*42	Indel/Frameshift	Reported
19	FA	*FANCA* (hom)	AR	c.1645C>T	p.Gln549*	Nonsense	Reported
20	FA	*FANCA* (combined)	AR	c.2851 C>T c.1470G>A	p.Arg951Trp p.Gln490Gln	Missense Splicing	Reported Reported
21	FA	*FANCA* (hom)	AR	c.2830_2831Ins GAAATTCAACCTGAAGCTG	p.Asp944Glyfs*5	Indel/Frameshift	Reported
22	FA	*BRIP1* (hom)	AR	c.2392C>T	p.Arg798*	Nonsense	Reported
23	FA	*BRIP1* (hom)	AR	c.2392C>T	p.Arg798*	Nonsense	Reported
24	FA	*ERCC4* (hom)	AR	c.2065C>A	p.Arg689Ser	Missense	Reported
25	FA	*TINF2* (het)	AD	c.734C>A	p.Ser245Tyr	Missense	Reported
26	DC	*RTEL1* (combined)	AR	c.49C>T c.3442delC	p.Pro17Ser p.Gln1148Argfs*96	Missense Indel/Frameshift	Novel[2] Novel[2]
27	DC	*TERC* (combined)	AD	n.37A>G n.216_229del GGCGGGTCGCCTGC	NA NA	ncRNA ncRNA	Reported Reported

28	SCN	*ELANE* (het)	AD	c.466T>G	p.Trp156Gly	Missense	Novel[2]
29	SCN	*ELANE* (het)	AD	c. 452G>C	p.Cys151Ser	Missense	Novel[2]
30	SCN	*ELANE* (het)	AD	c.176 T>C	p.Leu59Pro	Missense	Novel[2]
31	CN	*ELANE* (het)	AD	c.377C>T	p.Ser126Leu	Missense	Reported
32	CN	*ELANE* (het)	AD	c.182C>T	p.Ala61Val	Missense	Reported
33	CN	*ELANE* (het)	AD	c.574_581dupGGCCGGCA	p.Val197Argfs*18	Indel/Frameshift	Novel[2]
34	SCN	*HAX1* (hom)	AR	c. 131InsA	p.Trp44*	Nonsense	Reported
35	CDA	*CDAN1* (combined)	AR	c.2015C>T c.2081C>T	p.Pro672Leu p.Pro694Leu	Missense Missense	Reported Novel[2]
36	CDA	*CDAN1* (combined)	AR	c.2015C>T c.2081C>T	p.Pro672Leu p.Pro694Leu	Missense Missense	Reported Novel[2]
37	SDS	*SBDS* (combined)	AR	c.120delG c.258+2T>C	p.Ser41Alafs*17 Splicing change	Indel/Frameshift Splicing	Novel[2] Reported
38	FT	*MYH9* (het)	AD	c. 4562A>G	p.His1521Arg	Missense	Novel[2]
39	FT	*ANKRD26* (het)	AD	c.4976dupA	p.Ile1659Tyrfs*3	Indel/Frameshift	Novel[2]
40	FT	*TERT* (het)	AD	c.2383-15T>C	Splicing change	Splicing	Novel[2]
41	FT	*TERT* (het)	AD	c.2383-15T>C	Splicing change	Splicing	Novel[2]

42	CSA	*SLC25A38* (hom)	AR	c.560G>A	p.Arg187Gln	Missense	Novel[2]
43	TAR	*RBM8A*[1]	AR	c. -21G>A/ Large deletion[1]	Reduced translation Large deletion[1]	5'- UTR Large deletion[1]	Reported Reported
44	TAR	*RBM8A*[1]	AR	c. -21G>A/ Large deletion[1]	Reduced translation Large deletion[1]	5'- UTR Large deletion[1]	Reported Reported

AD, autosomal dominant; AR, autosomal recessive; CDA, congenital dyserythropoietic anemia; CSA, congenital sideroblastic anemia; CN, cyclic neutropenia; DBA, Diamond-Blackfan anemia; DC, Dyskeratosis congenita; FA, Fanconi anemia; FT, familial thrombocytopenia; ncRNA, non-coding RNA; SCN, severe congenital neutropenia; SDS, Shwachman–Diamond syndrome; TAR, Thrombocytopenia with Absent Radii

[1]Analysis of the NGS data by the SureCall CNV algorithm identified a large deletion on the allele without the mutation (Supplementary Fig 42, 43). This confirmed a compound heterozygosity state, which is the commonest genotype combination in this disease.

[2]See information in Supplementary Table 5 about damage prediction of this mutation. The criteria for calling a variant a novel/most likely damaging mutation are provided in Supplementary Table 5 and in the Methods Section (in the paragraph "Variant analysis and filtering strategy").

Supplementary Table 4: List of patients with unclassified IBMFSs who were Genotyped and diagnosed based on this study.

Number	Clinical phenotype	Gene	Nucleotide change	Protein change	Mutation type	Previous reporting	Diagnosis based on this study
1	Neutropenia, MDS, mother with neutropenia. Negative for *ELA2* mutations.	*GATA2* (het)	c.1009C>T	p.Arg337*	Nonsense	Reported	Familial MDS
2	Neutropenia, solitary kidney, maternal grandfather and granduncle with neutropenia and AML. Negative screens for FA, DC and mutations in common neutropenia genes.	*WAS* (hemi)	c.881T>C	p.Ile294Thr	Missense	Reported	SCN
3	Neutropenia, atrial septal defect, negative for mutations in common neutropenia genes.	*G6PC3* (hom)	c.911dupC	p.Gln305Serfs*82	Indel/Frameshift	Novel[1]	SCN
4	Chronic pancytopenia from the age of 2 years; hypocellular bone marrow, brother with neutropenia and failure to thrive; short telomeres	*TERT* (het)	c.1234C>T	p.His412Tyr	Missense	Reported	DC
5	Pancytopenia from the age of 2.5 years. Decreased marrow cellularity, and reduced erythropoiesis and megakaryopoiesis. Red blood cell transfusion until the age of 18 years. Spontaneous elevation of chromosome fragility (stimulation with cross linking agents were not done), but repeat testing with mitomycin C and diepoxybutane in	*TERT* (het)	c. 2014C>T	p.Arg672Cys	Missense	Novel[1]	DC

	adulthood was normal. Telomere testing has not been done.						
6	Moderate to severe anemia, moderate neutropenia, mildly hypoplastic thumbs, developmental delay; semilobar holoprosencephaly; hypertonus and contractures; mildly hypocellular marrow, short telomeres, high eADA.	*TERT* (het)	c. 2014C>T	p.Arg672Cys	Missense	Novel[1]	DC
7	Chronic moderate pancytopenia, hypocellular marrow; paternal grandmother with thrombocytopenia; short telomeres	*TERC* (het)	n.83T>C	NA	ncRNA	Reported	DC
8	SAA, developmental delay, diabetes mellitus, pyloric stenosis, cerebral calcifications	*TINF2* (het)	c. 844C>G,	p.Arg282Gly	Missense	Novel[1]	DC
9	Severe neutropenia, anemia, thrombocytopenia; marrow active without dysplasia; reduced T & B lymphocytes, low IgG	*CXCR4* (het)	c.1000C>T	p.Arg334*	Missense	Reported	WHIM syndrome
10	Hydrops fetalis, severe anemia that persisted, early moderate thrombocytopenia, intermittent neutropenia; marrow at the age 2 months showed increased celluarity, normal erythropoiesis, reduced granulopoiesis, reduced megakaryopoiesis,	*RPL5* (het)	c.174_175delAG	p.Arg58Argfs*53	Indel/Frameshift	Novel[1]	DBA
11	Congenital thrombocytopenia, persistent neutropenia, large platelets.	*MYH9* (het)	c.3493C>T	p.Arg1165Cys	Missense	Reported	MYH9- related disorder

12	Thrombocytopenia and anemia from early childhood, arthritis and vasculitis (low T and B cells while on prednisone). Active marrow.	*WAS* (hemi)	c.157_162delCTGTAC	p.Leu53_Tyr54del	Indel/Inframe	Novel[1]	WAS
13	SAA; short telomeres; no response to IST	*RTEL1* (comb)	c.1373C>T c.1416G>C	p.Thr458Met p.Lys472Asn	Missense	Novel[1] Novel[1]	DC
14	SAA; no response to IST	*TERT* (het)	c.2371G>A	p.Val791Ile	Missense	Reported	DC
15	SAA; no response to IST	*MASTL* (het)	c.811+2 T>G	Splicing change	Splicing	Novel[1]	*MASTL* associate disorder

AD, autosomal dominant; AR, autosomal recessive; DBA, Diamond Blackfan anemia; DC, Dyskeratosis congenita; eADA, adenosine deaminase; IgG, Immunoglobulin G ; IST, immunosuppressive therapy; MDS, myelodysplastic syndrome; ncRNA, non-coding RNA; SAA, severe aplastic anemia; SCN, severe congenital neutropenia; TL, telomere length ; WAS, Wiskott-Aldrich syndrome ; WHIM, Warts-Hypogamaglobulinemia-Infection-Myelokathexis.

[1]See information in Supplementary Table 5 about damage prediction of this mutation. The criteria for calling a variant a novel/most likely damaging mutation are provided in Supplementary Table 5 and in the Methods Section (in the paragraph “Variant analysis and filtering strategy”).

Supplementary Table 5: Bioinformatic data related to novel/most likely causal mutations. The table shows mutation information, as well as prediction of conservation and damaging effect of the mutations on protein by several different softwares. Variants were considered novel / most likely causal mutations if they fulfilled all the following: 1) they appeared in allelic dosage that was consistent with the known inheritance mode of the disease, 2) the MAF was <0.001, 3) evolutionary conserved amino acid/s are affected, 4) the variant was considered damaging by at least 2 of the following prediction software programs: PolyPhen2, SIFT/SIFT-Indel, Provean, MutationTaster and Human Splicing Finder. In this paper we referred to both, previously published mutations and novel mutations as "causal mutations".

Patient	**Gene**	**Nucleotide change**	**Protein change**	**Mutation type**	**MAF (EVS)**	**Conser-vation[1]**	**MutationTaster[2]**	**Provean[3]**	**Polyphen2[4]**	**SIFT[5]/ SIFT-Indel**	**Human Splicing Finder**
1 (Patient 1 SuppTable 3)	*RPS26*	c. 243delC	p.Ser81Argfs*3	Indel/ Frameshift	NR	NA	Disease causing	ID	ID	Damaging	NA
2 (Patient 3 SuppTable 3)	*RPS26*	c.4-32_21 delGTTTTCCTA ACA	Splicing change	Splicing	NR	NA	Splice site changes	ID	ID	ID	Splicing branch point broken[9]
3 (Patient 14 SuppTable 3)	*RPS19*	c.16delG	p.Val6*	Indel/ Nonsense	NR	NA	Disease causing	ID	ID	Damaging	NA
4 (Patient 10 SuppTable 4)	*RPL5*	c.174_175delAG	p.Arg58Argfs*53	Indel/ Frameshift	NR	NA	Disease causing	ID	ID	Damaging	NA
5 (Patient 11 SuppTable 3)	*RPL11*	c.372C>G	p.Ile124Met	Missense	NR	5.94	Disease causing	Deleterious (-2.60)	Probably damaging (0.944)	Intolerant (0)	NA
6 (Patient 9 SuppTable 3)	*RPS24*	c.4-2A>G	Splicing change	Splicing	NR	NA	Disease causing	ID	ID	ID	Site broken
7 (Patient 12 SuppTable 3)	*RPS29*	c.63-3 C>A	Splicing change	Splicing	NR	NA	Disease causing	ID	ID	ID	Site broken

8 (Patient 15 SuppTable 3)	*RPS7*	c.398T>C	p.Leu133Ser	Missense	NR	4.6	Disease causing	Deleterious (-5.462)	Probably damaging (0.99)	Intolerant (0.01)	NA
9 (Patient 40 SuppTable 3)	*TERT*	c.2383-15T>C[6]	Splicing change	Splicing	0.0005	NA	Splice site changes	ID	ID	ID	Site broken
10 (Patient 5 SuppTable 4)	*TERT*	c. 2014C>T[7]	p.Arg672Cys	Missense	0.000082	4.67	Protein might be affected	Deleterious (-3.317)	Probably damaging (0.99)	Intolerant (0.022)	NA
11 (Patient 6 SuppTable 4)	*TERT*	c. 2014C>T[7]	p.Arg672Cys	Missense	0.000082	4.67	Protein might be affected	Deleterious (-3.317)	Probably damaging (0.99)	Intolerant (0.022)	NA
12 (Patient 26 SuppTable 3)	*RTEL1*	c.3442delC	p.Gln1148Argfs *96	Indel/ Frameshift	NR	NA	Disease causing	ID	ID	Damaging	NA
		c.49C>T	p.Pro17Ser	Missense	NR	4.86	Disease causing	Deleterious (-6.73)	Probably damaging (0.998)	Intolerant (0.02)	NA
13 (Patient 13 SuppTable 4)	*RTEL1*	c.1416G>C	p.Lys472Asn	Missense	NR	4.5	Disease causing	Deleterious (-2.94)	Probably benign (0.094)	Tolerated (0.076)	NA
		c.1373C>T	p.Thr458Met	Missense	0.000539	4.34	Splice site changes; Protein might be affected	Neutral (-1.29)	Probably damaging (0.98)	Intolerant (0.05)	ESE site is broken, and Creates a new ESS site
14 (Patient 8 SuppTable 4)	*TINF2*	c. 844C>G,	p.Arg282Gly	Missense	NR	5.16	Disease causing	Deleterious (-3.60)	Possibly damaging (0.839)	Intolerant (0.000)	NA

15 (Patient 16 SuppTable 3)	*SBDS*	c.127G>T	p.Val43Leu	Missense	NR	5.19	Disease causing	Neutral (-1.632)	Probably benign (0.005)	Tolerated (0.12)	Site broken
16 (Patient 37 SuppTable 3)	*SBDS*	c.120delG	p.Ser41AlaFs*17	Indel/ Frameshift	NR	NA	Disease causing	ID	ID	Damaging	NA
17 (Patient 28 SuppTable 3)	*ELANE*	c.466T>G	p.Trp156Gly	Missense	NR	4.42	Disease causing	Deleterious (-11.79)	Probably damaging (0.999)	Intolerant (0.006)	NA
18 (Patient 29 SuppTable 3)	*ELANE*	c. 452G>C	p.Cys151Ser	Missense	NR	4.42	Disease causing	Deleterious (-9.55)	Probably damaging (1.0)	Intolerant (0.000)	NA
19 (Patient 30 SuppTable 3)	*ELANE*	c.176 T>C	p.Leu59Pro	Missense	NR	3.24	Disease causing	Deleterious (-6.51)	Probably damaging (1.0)	Intolerant (0.000)	NA
20 (Patient 33 SuppTable 3)	*ELANE*	c.574_581dupGGCCGGCA	p.Val197Argfs*18	Indel/ Frameshift	NR	NA	Disease causing	ID	ID	Damaging	NA
21 (Patient 3 SuppTable 4)	*G6PC3*	c.911dupC	p.Gln305Serfs*82	Indel/ Frameshift	NR	NA	Disease causing	ID	ID	Damaging	NA
22 (Patient 35 SuppTable 3)	*CDAN1*	c.2081C>T[8]	p.Pro694Leu	Missense	NR	5.77	Disease causing	Deleterious (-9.02)	Probably damaging (0.999)	Intolerant (0.003)	NA
23 (Patient 36 SuppTable 3)	*CDAN1*	c.2081C>T[8]	p.Pro694Leu	Missense	NR	5.77	Disease causing	Deleterious (-9.02)	Probably damaging (0.999)	Intolerant (0.003)	NA
24 (Patient 41 SuppTable 3)	*SLC25A38*	c.560G>A	p.Arg187Gln	Missense	0.000077	4.89	Disease causing	Deleterious (-3.78)	Probably damaging (0.999)	Intolerant (0.02)	NA

25 (Patient 15 SuppTable 4)	*MASTL*	c.811+2 T>G	Splicing change	Splicing	NR	5.82	Disease causing	ID	ID	ID	Site broken
26 (Patient 39 SuppTable 3)	*ANKRD 26*	c.4976dupA	p.Ile1659Tyrfs*3	Indel/ Frameshift	NR	NA	Disease causing	ID	ID	Damaging	NA
27 (Patient 38 SuppTable 3)	*MYH9*	c. 4562A>G	p.His1521Arg	Missense	NR	5.25	Disease causing	Deleterious (-6.53)	Probably damaging (1.0)	Intolerant (0.01)	NA
28 (Patient 12 SuppTable 4)	*WAS*	c.157_162delCTGTAC	p.Leu53_Tyr54del	Indel/ Inframe	NR	NA	Protein might be affected	Deleterious (-10.896)	ID	Damaging	NA

[1]The MasterTaster software program evaluates the effect of variants on both, protein function/structure as well as on splicing.
[2]Conservation ranges from -12.3 to +6.17, with +6.17 being the most conserved.
[3]Provean scores: deleterious <-2.50; neutral >-2.50
[4]PolyPhen 2 scores: probably damaging (>0.85–1); possibly damaging (>0.15–0.84); probably benign (< 0.14)
[5]SIFT scores: intolerant (0.00-0.05); potentially intolerant (0.051-0.10); borderline (0.101-0.20), or tolerant (0.201-1.00)
[6,7,8]Patients have recurrent mutations in this study. [6]The same mutation was found in another patient in our registry who had aplastic anemia and very short telomeres and responded to androgen therapy. This mutation was used for validation of the NGS assay (Supplementary Table 2, Patient 9).
[9]The deleted fragment GTTTTCCTAACA contains the only YURAC splicing branch point consensus sequence in intron 1 of *RPS26*, which in this case it is CTAAC. Splicing branch points are typically located 20-50 nucleotide upstream of the acceptor site, similar to the present sequence. Deletion of the branch point abolishes binding of splicing factor 1 and assembly of the spliceosome.
ESE, exonic splicing enhancer; ESS, Exonic splicing silencer; EVS, exome variant server database (http://evs.gs.washington.edu/EVS/). MAF in other databases was analyzed as indicated in Table 1, but not provided herein; ID, indeterminate by the software; MAF, minor allele frequency; NA, not applicable; NR, not reported in this database; SuppTable, Supplementary Table.