Supplementary Materials

Somatic reversion impacts myelodysplastic syndromes and acute myeloid leukemia evolution in the short telomere disorders

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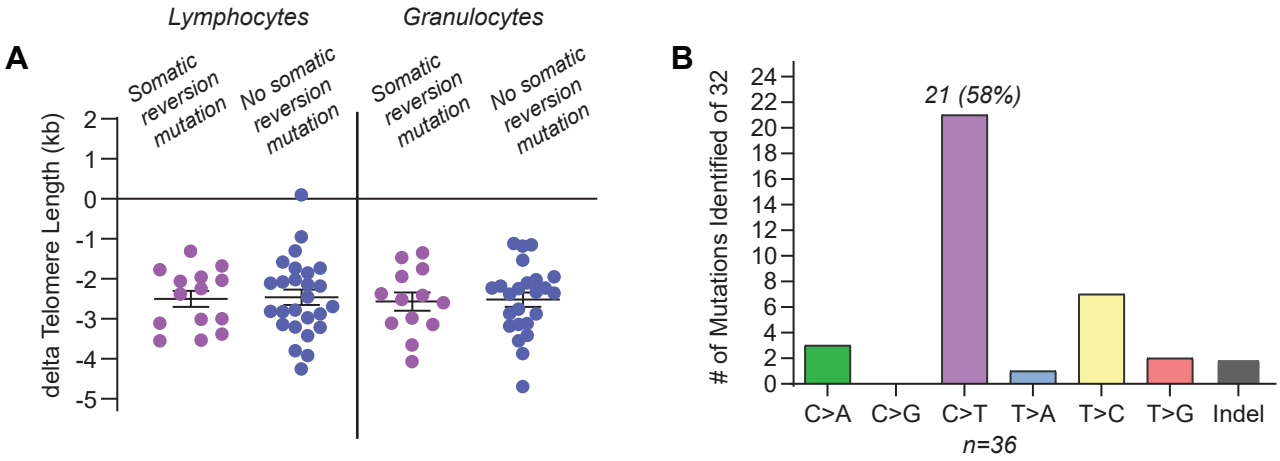
Supplementary Figure 1



Mutation types detected and their clone size and effect on telomere length. A. Comparison of telomere length graphed as the difference from the age-adjusted 50th percentile in lymphocytes and granulocytes. Data are stratified by the presence or absence of a somatic reversion telomere-related mutation. The mean is shown and error bars refer to standard error of the mean. **A.** Mutation types identified in cohorts (controls, short telomere and short telomere with MDS/AML).

Supplementary Table 1. Genes and regions (n=17) targeted for Haloplex sequencing

Gene Name	NM_ID/Coordinates
Telomerase Core Promoters	
TERT promoter	chr5:1295085-1295385
TR promoter and TR gene	chr3:169482378-169483348
Shelterin	
POT1	NM_015450
TINF2	NM_001099274
TERF2IP	NM_018975
ACD/TPP1	NM_001082486
TERF1	NM_017489
TERF2	NM_005652
RNA Exosome and RNA Processing	
MTREX/SKIV2L2	NM_015360
RBM7	NM_001286045
DIS3	NM_014953
PAPBN1	NM_004643
TENT4B/PAPD5	NM_001040284
ZC3H18	NM_001294340
ALT	
ATRX	NM_000489
DAXX	NM_001141970
Cell Cycle Checkpoint	
TP53	NM_000546

Supplementary Table 2. Germline mutations in telomere or telomerase genes not previously reported*

Mutant Gene	Coding Variant	Protein	Prior report
DKC1	c.942G>A	p.Lys314Lys	Gaysinskaya 2020[1]
	c.915+10G>A**	-	-
NAF1	c.950A>T	p.Asp317Val	-
PARN	c.543_544insTT	p.Asp182Leufs*5	-
	NC_000016.9:g.(?_14725823)_(14643928_?)del	Exon 1-21 deletion***	Feurstein 2020[2]
	c.(?_-135_(c.1480+1_1481-1)del	Exon 1-21 deletion***	-
TERT	c.345C>G	p.Phe115Leu	-

*Remaining mutations have been reported in Alder *et al.* 2018 and Schratz *et al.* 2020[3, 4].

**This mutation was identified in association with a more common *RTEL1* variant of unknown significance but because of rarity, was felt to be the more likely culprit.

***These two individuals shared a deletion of the *PARN* exons but it is unclear whether they shared the same breakpoints.

Supplementary Table 3. Somatic Mutations by Ultra-Deep Targeted Sequencing*

Disease Classification	Chr	Start	Stop	Reference	Variant	Gene Symbol	Mutation Type	Coding Position	Amino Acid Change	Total Read Count	Unique Count	Ref Count	Var Count	VAF
MDS-free	chr5	1295228	1295228	G	A	TERT	3_prime_UTR	c.-124 C>T	NULL	784	146	144	2	0.0137
MDS-free	chr5	1295228	1295228	G	A	TERT	3_prime_UTR	c.-124 C>T	NULL	892	124	122	2	0.0161
MDS-free	chr5	1295228	1295228	G	A	TERT	3_prime_UTR	c.-124 C>T	NULL	513	72	70	2	0.0278
MDS-free	chr5	1295228	1295228	G	A	TERT	3_prime_UTR	c.-124 C>T	NULL	524	62	58	4	0.0645
MDS-free	chr5	1295228	1295228	G	T	TERT	3_prime_UTR	c.-124 C>A	NULL	230	21	16	5	0.2381
MDS-free	chr5	1295228	1295228	G	A	TERT	3_prime_UTR	c.-124 C>T	NULL	720	108	80	28	0.2593
MDS-free	chr5	1295228	1295228	G	A	TERT	3_prime_UTR	c.-124 C>T	NULL	203	26	14	12	0.4615
MDS-free	chr5	1295228	1295228	G	A	TERT	3_prime_UTR	c.-124 C>T	NULL	778	68	30	38	0.5588
MDS-free	chr5	1295250	1295250	G	A	TERT	3_prime_UTR	c.-146 C>T	NULL	209	26	24	2	0.0769
MDS-free	chr5	1295250	1295250	G	A	TERT	3_prime_UTR	c.-146 C>T	NULL	1370	252	102	150	0.5952
MDS-free	chr5	54640968	54640968	G	A	MTREX	missense	c.G1052A	p.R351Q	13287	823	817	6	0.0073
MDS-free	chr5	54701332	54701332	G	A	MTREX	missense	c.G2561A	p.R854H	46701	891	885	6	0.0067
MDS-free	chr7	124464108	124464108	A	T	POT1	missense	c.T1813A	p.C605S	12812	952	943	9	0.0095
MDS-free	chr7	124493099	124493099	G	A	POT1	missense	c.C796T	p.H266Y	1172	181	170	11	0.0608
MDS-free	chr7	124503475	124503475	T	C	POT1	missense	c.A475G	p.M159V	1885	213	182	31	0.1455
MDS-free	chr7	124511096	124511096	C	T	POT1	splicing	c.125-1G>A	NULL	4541	299	284	15	0.0502
MDS-free	chr7	124532323	124532323	T	C	POT1	missense	c.A121G	p.T41A	2280	120	104	16	0.1333
MDS-free	chr7	124532347	124532347	T	C	POT1	missense	c.A97G	p.K33E	35810	1437	1423	14	0.0097
MDS-free	chr11	114278186	114278186	A	-	RBM7	frameshift_del	c.461delA	p.Met155Cysfs*49	6770	754	735	18	0.0239
MDS-free	chr13	73345938	73345938	C	T	DIS3	missense	c.G1600A	p.E534K	46204	900	697	203	0.2256
MDS-free	chr13	73346338	73346338	C	T	DIS3	missense	c.G1462A	p.D488N	3728	160	121	39	0.2438
MDS-free	chr16	75690321	75690321	A	C	TERF2IP	missense	c.A1012C	p.K338Q	11127	940	927	13	0.0138
MDS-free	chr17	7572948	7572952	TGTCT	-	TP53	frameshift_del	c.1157_1161del	p.Lys386Cysfs*4	1964	144	113	30	0.2083
MDS/AML	chr17	7576897	7576897	G	A	TP53	nonsense	c.C949T	p.Q317X	10226	302	282	20	0.0662
MDS/AML	chr17	7577022	7577022	G	A	TP53	nonsense	c.C916T	p.R306X	18247	2283	355	1928	0.8445
MDS-free	chr17	7577094	7577094	G	A	TP53	missense	c.C844T	p.R282W	41925	3685	3542	143	0.0388
MDS-free	chr17	7577144	7577144	A	G	TP53	missense	c.T794C	p.L265P	13653	1893	1801	92	0.0486
MDS-free	chr17	7577517	7577517	A	C	TP53	missense	c.T764G	p.I255S	20001	1237	1229	8	0.0065
MDS-free	chr17	7577548	7577548	C	T	TP53	missense	c.G733A	p.G245S	15979	2877	2711	166	0.0577
Control	chr17	7577568	7577568	C	T	TP53	missense	c.G713A	p.C238Y	36554	2634	2608	26	0.0099
MDS/AML	chr17	7578190	7578190	T	C	TP53	missense	c.A659G	p.Y220C	15861	2318	2195	123	0.0531
MDS/AML	chr17	7578419	7578419	C	A	TP53	nonsense	c.G511T	p.E171X	38047	4459	4304	155	0.0348

*Includes variants detected by Haloplex custom-designed platform. Additional variants (n = 4) were identified using a clinically validated platform with overall unique mean coverage 443x.

Supplementary References

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