A novel germline *SAMD9L* mutation in a family with ataxia-pancytopenia syndrome and pediatric acute lymphoblastic leukemia

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Supplementary Materials and Methods

Ethics

Clinical information and samples were collected with informed consent from all subjects and approval through institutional human ethics review board-approved protocols from the Australian Familial Haematological Cancer Study (AFHCS) (Royal Adelaide Hospital (RAH) #091203 and #100702, and Children, Youth and Women's Health Service #REC1542/12/12, Adelaide, South Australia, Australia), and conducted in accordance with the Declaration of Helsinki.

Patients and Samples:

Blood and hair samples were obtained with consent from all living descendants of the family as part of the Australia Familial Haematological Cancer Study (AFHCS).

Sanger Sequencing of *SAMD9L* gene

gDNA was isolated from hair bulbs, peripheral blood mononuclear cells (PBMNC) or lymphoblastic cell lines (LCL). PBMNC or LCL DNA for WES and Sanger sequencing and hair DNA for Sanger sequencing only. SAMD9L coding regions were Sanger sequenced to confirm WES variants and for segregation in other relatives.

Primer name	**Sequence**
SAMD9L S1473N F	5'- AACTCCGAGAGGTCTTGCAATTTGT -3'
SAMD9L S1473N R	5'- CTTGCCTTCAGCCTGACCAGTTAGA -3'
SAMD9L E461Vfs F	5'- CTCATAGGAAACCGAGACTCACTGGA -3'
SAMD9L E461Vfs R	5'- CCAGCTGGGCTGTTGGTAAAGATT -3'
SAMD9L S608Tfs F	5'- GAAAGCCCAGGAGATCCACTCATT -3'
SAMD9L S608Tfs R	5'- CCAGTGCAGTCAAGACATCCTCTTTC -3'

Whole Exome Sequencing (WES)

WES was performed on DNA from three affected individuals, III-1 (LCL), IV-1 (LCL) and IV-2 (PBMNC) using the Illumina HiSeq2500. Reads were mapped against human genome build GRCh37/hg19. WES was performed and analysed at the ACRF Cancer Genomics Facility as previously described.[1]

Investigation of variants present in both individuals in heterozygosity utilised population databases identifying variants which were rare (<0.1% in ExAC), predicted damaging (CADD>10), with good conservation (GERP>3). This variant filtration identified 19 variants.

Cytogenetic Evaluation

Copy Number Variation (CNV) was assessed in three affected individuals (III-1, IV-1, IV-2) using high density SNP array (Illumina 850K assay chip). Data was analysed and visualized using BlueFuse Multi Software (Illumina).

Long Range PCR Clonal Analysis

Isolated LCL gDNA from individual IV-1 was amplified using Long Amp Taq DNA Polymerase (New England Biolabs) and Sanger sequencing primers: SAMD9L S1473N R (5'-CTTGCCTTCAGCCTGACCAGTTAGA-3') and SAMD9L S608Tfs F (5'-GAAAGCCCAGGAGATCCACTCATT-3'). Amplified regions were cloned into pGEM-T (Promega) and transduced into supplied XL-10 competent cells. Positive transduced cells were cultured, lysed and Sanger sequenced to determine genotype of cloned fragment.

Supplementary References

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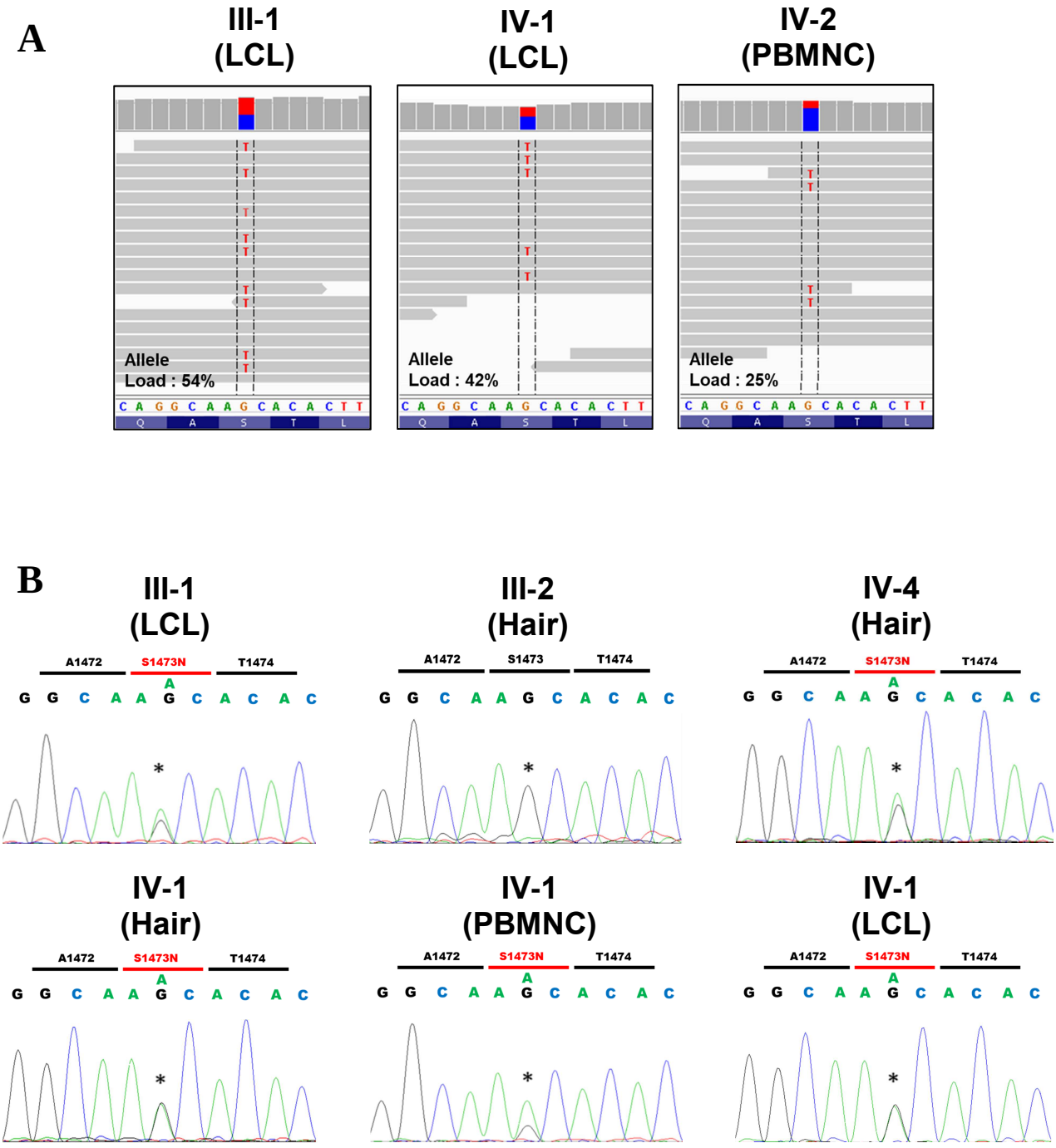
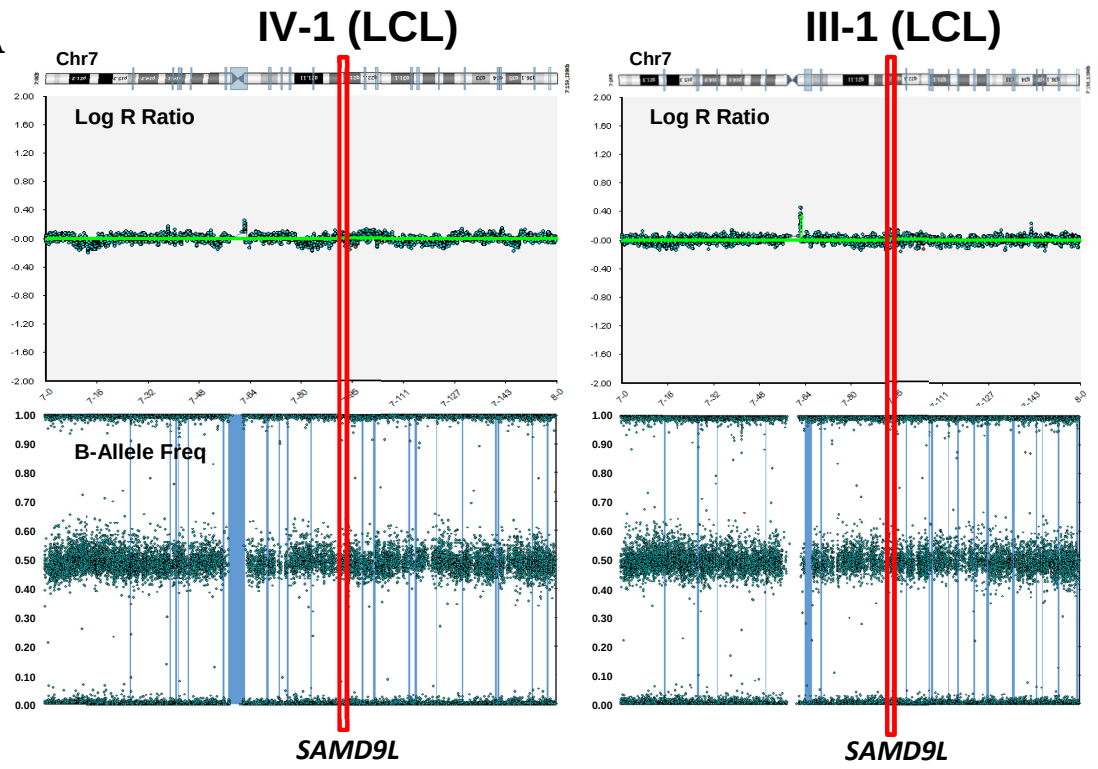
Supplementary Figures

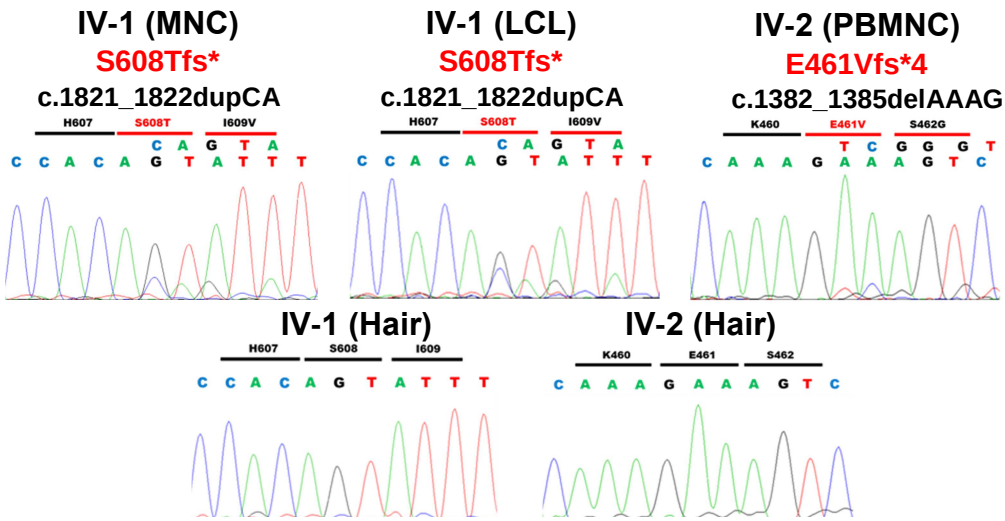


Supplementary Figure 1. Germline p.S1473N mutation identified in individuals with hematological diseases. (A) Whole exome sequencing of germline missense mutation SAMD9L S1473N (g.chr7:92760867C>T; c.4418G>A [NM_152703.4]; p.Ser1473Asn [NP689916.2]) in individuals III-1, IV-1 and IV-2. A reduced variant allele frequency is seen for IV-2 with 25% mutant allele. (B) Sanger sequence confirmation of heterozygous germline SAMD9L S1473N mutant allele in affected individuals and wildtype genotype in unaffected mother.

A



B



C

	Germline		Somatic	Somatic
Genomic Configuration	Wildtype	Mutant	*In cis*	*In trans* #
Genotype	S1473 /S608	**S1473N** /S608	**S1473N** /**S608Tfs*6**	S1473 /**S608Tfs*6**
# of clones	**7**	**2**	**7**	3
Interpretation	Wildtype allele	Germline but no somatic mutation	Germline and somatic mutation on the same allele	Likely PCR artefact due to incomplete extension

Supplementary Figure 2. Clonal reversion mechanisms of germline inherited mutations. (A) SNP array of Chromosome 7 in III-1 and IV-1. No chromosomal abnormalities seen compare to IV-2 in Figure 1D. (B). Sanger sequence confirmations of acquired SAMD9L mutations. (C) Clonal analysis of PCR amplified genomic DNA across *SAMD9L* mutation regions from IV-1 LCL. Genotyped clone proportions suggest germline p.S1473N mutation and upstream p.S608Tfs*6 frameshift mutations are *in cis* configuration. $^{\#}$*In trans* configuration clonal analysis proposes that incomplete PCR extension occurred in 15% of clones. Bold= Colonies consistent with somatic mutation occurring on germline mutated allele with V.A.F from WES and SNP array.

Supplementary Table 1. Individual IV-1 Transplant Conditioning Regime

Day	Treatment	Dose
- 6	Cyclophosphamide	60mg/m^2
	Anti-thymocyte Globulin	15mg/kg twice a day
- 5	Cyclophosphamide	60mg/m^2
	Anti-thymocyte Globulin	15mg/kg twice a day
- 4	Anti-thymocyte Globulin	15mg/kg twice a day
	Total Body Irradiation	
- 3	Total Body Irradiation	
- 2	Total Body Irradiation	
- 1	Rest Day	
0	Cord Blood Transplant	
1	Methotrexate	15mg/m^2
3	Methotrexate	10mg/m^2
6	Methotrexate	10mg/m^2
11	Methotrexate	10mg/m^2

Supplementary Table 2. Shortlist of predicted pathogenic segregating variants after WES data filtering

CHROM	POS	Gene	EFFECT_SNPEFF	AA_CHANGE_SNPEFF	ExAC_%	CADD	GERP	OMIM_phenotypes
6	107008769	AIM1	missense_variant	p.Arg1575Cys/c.4723C>T	0.007	32.00	5.02	
6	101090505	ASCC3	missense_variant	p.Ile1285Val/c.3853A>G	0.021	23.20	5.15	
2	179736230	CCDC141	missense_variant	p.Ala710Val/c.2129C>T	0.05	27.00	4.00	
17	46051389	CDK5RAP3	missense_variant	p.Arg93Trp/c.277C>T	0.000	35.00	3.28	
18	13087608	CEP192	missense_variant	p.Phe1986Leu/c.5956T>C	0.001	29.20	5.76	
1	60366747	CYP2J2	missense_variant	p.Ala407Val/c.1220C>T	0.002	33.00	5.72	
16	70365692	DDX19B	missense_variant	p.Ser364Asn/c.1091G>A	0.082	25.40	4.89	
16	72132927	DHX38	missense_variant	p.Arg289His/c.866G>A	0.034	18.82	3.92	
1	167096611	DUSP27	missense_variant	p.Ser748Leu/c.2243C>T	0.005	18.18	4.82	
2	63206344	EHBP1	missense_variant	p.Arg863Trp/c.2587C>T	0.027	34.00	5.86	Prostate cancer, hereditary, 12
1	92732036	GLMN	missense_variant	p.Leu385Ser/c.1154T>C	0.000	31.00	5.82	Glomuvenous malformations
12	102811750	IGF1	missense_variant	p.Lys145Met/c.434A>T	0.006	27.90	5.54	Growth retardation with deafness and mental retardation due to IGF1 deficiency
6	129691121	LAMA2	missense_variant	p.Glu1649Gln/c.4945G>C	0.007	23.00	5.98	Muscular dystrophy, congenital, due to partial LAMA2 deficiency :: Muscular dystrophy, congenital merosin-deficient
1	209797298	LAMB3	missense_variant	p.Thr675Met/c.2024C>T	0.009	11.12	3.06	Epidermolysis bullosa, junctional, non-Herlitz type :: Amelogenesis imperfecta, type IA :: Epidermolysis bullosa, junctional, Herlitz type
12	6626546	NCAPD2	missense_variant	p.Arg401Cys/c.1201C>T	0.001	35.00	4.55	
2	26688865	OTOF	missense_variant	p.Arg1527His/c.4580G>A	0.000	33.00	5.58	Auditory neuropathy, autosomal recessive, 1 :: Deafness, autosomal recessive 9
22	21742598	RIMBP3B	missense_variant	p.Arg1484Lys/c.4451G>A	0.000	20.60	3.01	
7	**92760867**	**SAMD9L**	**missense_variant**	**p.Ser1473Asn/c.4418G>A**	**0.000**	**17.47**	**3.23**	**Ataxia-pancytopenia syndrome**
1	153750994	SLC27A3	missense_variant	p.Arg518Gln/c.1553G>A	0.058	28.20	5.01	

Heterozygous variants predicted to affect protein function , present in all affected individuals, and ExAC<0.1%, CADD>10, GERP>3.

Supplementary Table 3. SAMD9L Variant reference list

Germline	Paired Somatic	Reference
S626L		2
M840K		3
H880G		7
H880Q	Q569P	10
H880Q	S1317Rfs*21	11
I891T	K768*	6
T951Nfs*3		3
R986C/T233N		6
R986C		11
R986C	R223X	10
R986C	E276X	10
R986C	L402Rfs*8	6
R986C	Y568C	10
R986C	R843W	10
R986C	F1092L	10
R986C	A1113T	3
R986C	R1524H	10
R986H		11
D1034Y		3
S1143G		3
D1148H		3
W1180R		2
C1196S		7
R1281K		2
R1281K	V19E	10
R1281K	C228F	10
R1281K	A260T	10
R1281K	R285W	10

Germline	Paired Somatic	Reference
R1281K	R359Q	10
R1281K	V361Efs*10	10
R1281K	N499S	10
R1281K	E562K	10
R1281K	R589X	10
R1281K	E776Gfs*13	10
R1281K	R843W	10
R1281K	R986L	10
R1281K	T1053I	10
R1281K	F1092V	10
R1281K	T1122S	10
R1281K	T1122_G1124delinsS	10
R1281K	L1153delL	10
R1281K	D1171N	10
R1281K	Q1183X	10
R1281K	S1397P	10
R1281K	K1408Nfs*9	10
R1281K	L1455X	10
S1473N	S608Tfs*6	#
S1473N	E461fs*43	#
I1493V		3
V1512A	L409fs	10
V1512A	Q780X	10
V1512A	K1265N	10
V1512M	R1188X	11
L1521V		3
S1550P		3

Refer to “Supplementary References” for indicated reference number. #: Identified in our study
Black: Germline mutation; Red: Somatic mutation

Supplementary Table 4. SAMD9 Variant reference list

Germline	Paired Somatic	Reference
R459Q		8
Y469C	V853I	3
Y469S		3
K676E		10
R685Q		8
A722E	R685*	4
T728A		3
D769N		9
D769G	Q39*	4
Q776K	D682G	3
T778I		2
N834Y		9
E974K		9
R982H		8
R982C	R1533fs*	8
R982C	K27fs*	8
I983S	N765Tfs*13	8
I983V		3
E1136Q	F583I	5
E1136Q	R221fs*	5
R1187Vfs*2		3
A1195V		9
P1280L		9
Q1286K		9
R1293W		9
R1293Q		8
L1569N	R344fs*	8

Refer to “Supplementary References” for indicated reference number.
Black: Germline mutation; Red: Somatic mutation