In trans early mosaic mutational escape and novel phenotypic features of germline SAMD9 mutation

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Running title : Novel features in a family with SAMD9 mutation

Word count 998

Mutations in *SAMD9* or *SAMD9L* are found in patients with familial predisposition to myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) associated with MIRAGE syndrome (Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes, and Enteropathy) and ATXPC (ataxia-pancytopenia syndrome) (1-3) respectively. The wild-type (WT) function of SAMD9 and SAMD9L in haematopoietic cell lines is antiproliferative. Germline mutations are thought to be gain-of-function (GOF) mutations exacerbating the antiproliferative effects of the WT gene(4-6). Functional somatic reversion has been described in affected individuals through loss of heterozygosity (-7 or 7q-) or uniparental disomy (UPD), or through acquisition of loss of function (LOF) mutations. Thus far LOF mutations have been described *in cis* for *SAMD9*, but rarely in *trans* for SAMD9L(7,8). Here we present a four-generation pedigree affected by AML and MDS with novel disease features and a reversion mutation in *trans* consistent with germline mosaicism.

The proband (III-1) presented in 1986 aged 10 with a history of excessive bruising. She was one of 4 siblings, 2 of which were stillborn. She was also noted to have an extensive family history of MDS and AML, specifically, her mother (II-1) had MDS, aunt (II-4) died of AML age 11 following MDS, cousin (III-5) died of AML age 8 and mother's cousin (II-8) died of leukaemia (Figure 1A). Her haematological parameters at presentation were normal, however she had abnormal platelet function tests. In 1998 (age 22) she developed tri-lineage cytopenias. Bone marrow aspirate (BMA) at this time showed MDS with 7q- in 5/30 metaphases (46,XX,del(7)(q22q33)[5]/46,XX[25]). Additional multisystem abnormalities were identified over her 20 year follow-up; imaging undertaken for urinary tract infections, adenomyosis and an iliofemoral DVT in 2002 (age 26) revealed duplex left kidney and ureters, intermediate situs, including right sided stomach and spleen, multiple small splenenculi, reversed pancreas, midline liver, mispositioned transverse colon and abnormal anatomy of venous drainage.

Follow-up BMA in 2004(age 28) showed MDS with normal karyotype (NK) with similar findings in in 2015(age 39). Illumina Trusight myeloid panel showed an MDS-associated *ZRSR2* variant (pGly438_Ser439insSerArg).

Her daughter, IV-1 was born in 1996 at 28 weeks due to severe IUGR. Blood count at birth showed thrombocytopenia (90×10^9/l), presumed to be due to prematurity. However, aged 2 she was noted to have anaemia (Hb 9.1g/dl) and thrombocytopenia (65×10^9/l). BMA showed MDS with NK. She underwent an unrelated donor transplant age 4. Pre-transplant assessment noted that she had global developmental delay. She died 2 months post-

transplant due to EBV-related disease. III-1 had 5 additional pregnancies. IV-III was complicated by severe polyhydramnios and the pregnancy terminated because of concerns regarding congenital anomalies. IV-5 is alive and well age 10.

Informed consent was obtained according to UCL/UCLH Biobank for health and disease. Whole exome sequencing was undertaken on stored bone marrow DNA of proband (III-1) from 2015 and identified 2 non-synonymous base changes in *SAMD9* c.2958C>G resulting in S986C and c.1447C>G resulting in p.L483V (Figure 1B). *In silico* analyses of these variants suggested both mutations were likely to be pathogenic (Figure 1C).

To determine if the mutations found in the proband were present in other family members we undertook targeted deep sequencing of the regions surrounding the SAMD9 variants in IV-1 and the probands mother (II-1) along with additional available samples from IV-5 and III-1 (Figure 1D). III-1, IV-1 and II-1 carried L483V at allele frequencies consistent with germline origin. The S986C variant was present only in samples from III-1. Here it was present at an allele frequency of 21% in the peripheral blood (2001) and 30.4% in the BMA from 2015. Matched buccal swab (2015) also showed this variant but at a lower allele frequency (17%). To determine whether this was due to blood contamination we obtained nail clippings from III-1. This revealed the presence of the S986C mutation at a low frequency of 3.6%. This suggests that the proband underwent this reversion mutation in a germline mosaic manner early in development. BMA from III-1 in 2015 was also analysed using SNP array which showed no abnormalities. Specifically, we could not detect any evidence of UPD on chromosome 7 to account for the apparent reversion to 100% WT copies of chromosome 7 after 1998. However, as only 16% of cells in the 1998 sample carried this deletion this would most likely be below the level of detection of the SNP array (20%).

To identify if the 2 mutations found in *SAMD9* in the proband were *in cis* or *in trans*, a PCR amplicon of *SAMD9* encompassing both mutations (1701bp) cloned and colonies sequenced. 33/65 (50.7%) carried L483V as expected, while 16/65 (24.6%) carried S986C (Figure 2B). 94% of colonies carrying S986C were WT for L483V indicating that these mutations occurred in trans on opposite alleles. 2 colonies carried both mutations most likely representing cross-over events. This is in contrast to previously described double mutations in SAMD9 where mutations have only been described *in cis*(5).

In this report we describe an 4 generation pedigree of *SAMD9* associated phenotypes including AML, MDS and developmental anomalies. We highlight a novel germline *SAMD9* mutation confirmed in 3 of the affected family members. The proband in this case also demonstrates a hitherto undescribed pattern of multiorgan involvement and congenital defects not previously associated with *SAMD9* or *SAMD9L* mutations.

The proband in this case displays genetic reversion from her inherited *SAMD9* mutation by two different mechanisms; an *in trans* mutation of the other allele of SAMD9 that appears to have occurred during development in a germline mosaic manner, and may also explain the more extensive range of developmental anomalies observed in this case. In addition the proband showed transient interstitial loss on chromosome 7q in 16% of cells early in the course of her MDS. Without the support of functional studies, it is tempting to speculate that the S986C mutation confers dominant-negative activity against the putative gain-of-function L483V mutation and has clonal advantage over those with loss of 7q (Figure 2C).
In summary we describe a pedigree of *SAMD9* mutated familial leukaemia predisposition with novel clinical and genetic features.

References

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Legends

Figure 1

A - 4 generation family tree. Affected members in black. B – Schematic of SAMD9 structure showing location of mutations (structure adapted from (9)). C – Analysis of analysis of SAMD9 variants using 1000 genome browser (1000G); ExAC (Exome Aggregation Consortium) and ClinVar (NCBI clincal variant analysis)(to determine whether described as SNPs), and PolyPhen2 (D=probably damaging); SIFT (Sorting intolerant from intolerant)(D=Deleterious); MutAss (Mutation Assess) (M=moderate; N=Neutral); CADD(Combined Annotation Dependent Depletion) (Scaled Score >20=top1% scores most

likely damaging) – to assess pathogenicity. D – Allele frequency of variants in III-1, IV-1 and II-1 in the noted cell types and time points (nd=not done) *indicates sample on which WES was undertaken.

Figure 2

A – Sanger sequencing traces from TOPO-cloned PCR fragment of SAMD9. B – Colony analysis of mutations to define *cis* or *trans* (dark boxes – mutation present, light boxes absent). C – Proposed scheme of clonal evolution/reversion of SAMD9 mutant clone in proband III-1

FIGURE 1

A
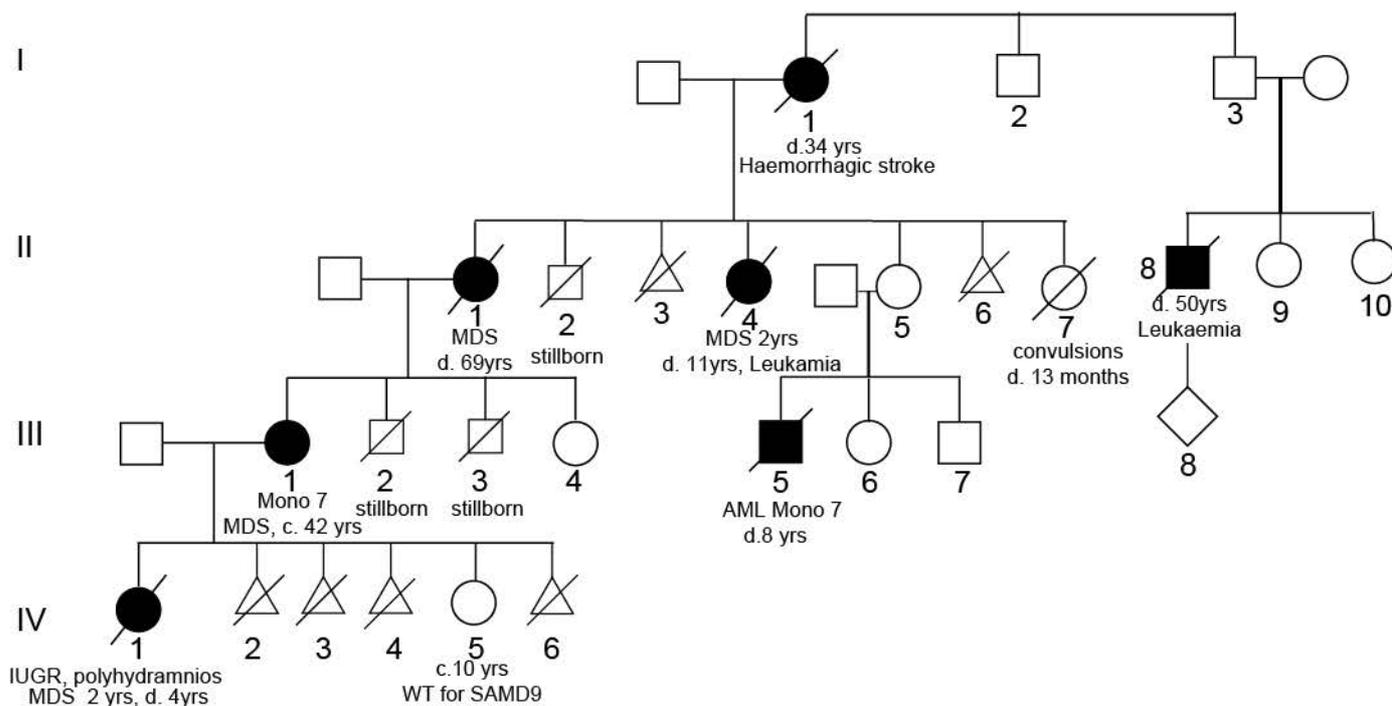
B
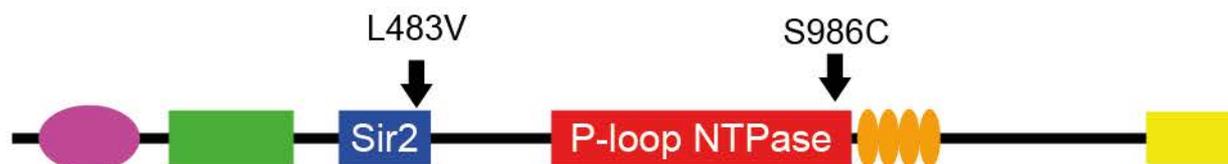
C

	1000G	ExAC	ClinVar	polyPhen2	SIFT	MutAss	CADD
L483V	no	no	no	D	D	M	21.5
S986C	no	no	no	D	D	N	21.1

D
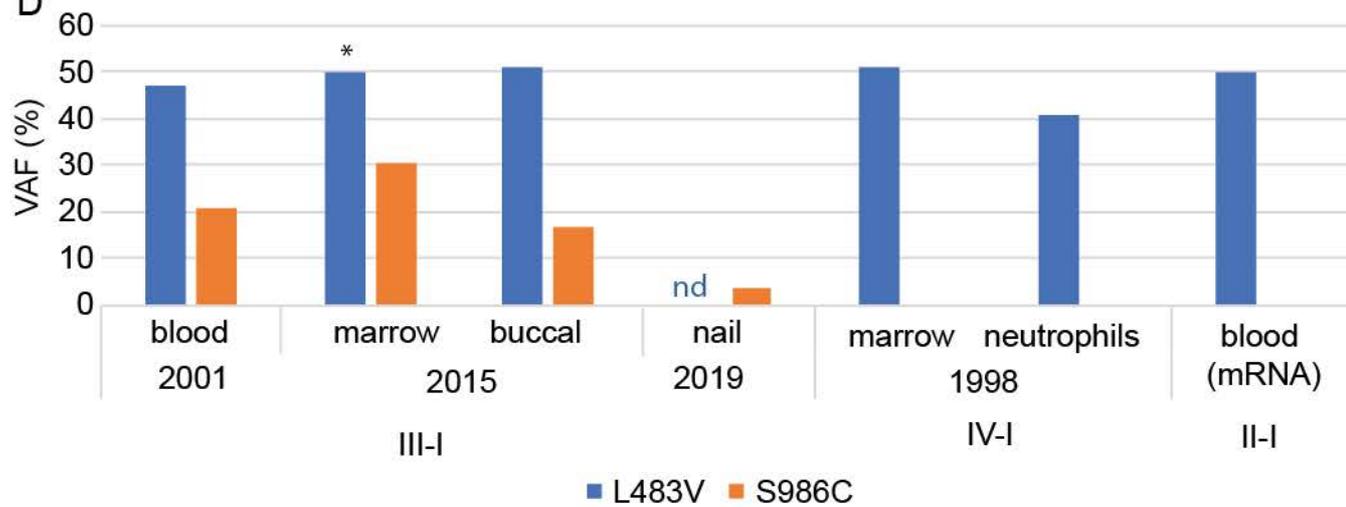
A   Sequence indivudal colonies from TOPO cloned
     SAMD9 amplicon carrying both mutation locations

L483V

A T T T C T A C T C T A A A T C T T T A C
I   S   T   L   N   L   Y

A T T T C T A C T G T A A A T C T T T A C
I   S   T   V   N   L   Y

S986C

A T C A T T C A C T C T T T G A T T G C A
I   I   H   S   L   I   A

A T C A T T C A C T G T T T G A T T G C A
I   I   H   C   L   I   A

B

| L483V | | 50.7% |
| S986C | | 24.6% |
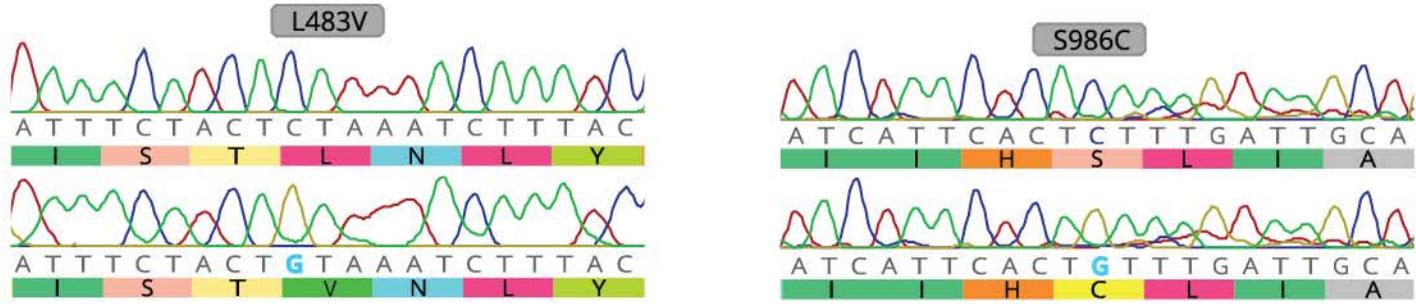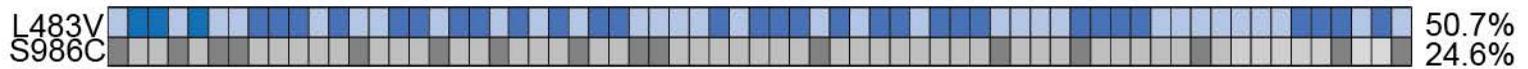
L483V/S986C	3.076923077
L483V/WT or WT/S986C	70.76923077
WT/WT	27.69230769

C

TIME

L483V/WT   L483V/S986C   Del 7(q22-q33)